Strål
säkerhets
myndigheten
Swedish Radiation Safety Authority

Authors: Jean Emeny

Research

2009:24

UV-radiation induced disease – roles of UVA and UVB

Report number: 2009:24 ISSN: 2000-0456
Available at www.stralsakerhetsmyndigheten.se

Title: UV-radiation induced disease – roles of UVA and UVB.
Report number: 2009:24.
Authors: : Jean Emeny.
University of Leicester, UK .
Date: July 2009.

SSM Perspective

This international workshop was recommended by the authority's scientific UV board. The workshop collected scientists from various disciplines within the field of UV radiation, and its impact on the human body. The workshop was unique since researchers from these disciplines rarely meet, as there are no other conferences or workshops which span the entire field.

Background

There is a large uncertainty concerning the impact of UVA and UVB radiation on the skin and eye. For example, UVA radiation used to be considered low risk since it was believed that DNA damage could only be caused by UVB radiation. However, recent studies show that UVA and UVB can give rise to similar DNA damages in human skin. Because of the potentially greater exposure to UVA while using sunbeds or UVB-blocking sunscreens, further information on the role of UVA is crucial. It constitutes a necessary foundation for the authority for its recommendations for prevention as well as regulations. In particular, the new findings may have a vast impact on the risk estimation and regulations of sunbeds.

Objectives of the project

Exposure to ultraviolet (UV) radiation is a dominating risk factor underlying skin cancer, but major uncertainties remain concerning its biological effects and cellular defence mechanisms, hindering implementation of effective preventive measures. A conference sponsored by the Swedish Radiation Protection Authority and the Swedish Cancer Society and held at Karolinska Institutet, Stockholm in October, 2007, brought together scientists studying different aspects of the biological impact of UV-radiation to present and discuss current knowledge of this area of research with special attention to the relative importance of short (UVB) and long (UVA) wavelength UV-radiation. This report is based on the evidence presented at that meeting.

Results

The project has resulted in a deeper understanding of the biological effects of UV radiation. In particular, the UVA radiation was shown to give DNA damage similar to UVB-induced lesions, and mechanisms for this were suggested. In addition, it was shown that the cellular response was

different for UVA and UVB radiation, which may explain why UVA induces more DNA mutations than UVB per initial DNA damage. Mutations of different genes typical for skin cancers were discussed. Melanomas were found to have different mutations depending on whether they arise at chronically sun exposed sites or intermittently exposed sites of the body. These different types of melanoma have different prognosis and mean age of incidence.

For those aged less than 30, UV exposure involves greater risk because naevus development is still active.

Still many uncertainties remain. Despite extensive effort, the mechanism of UVA-induced genotoxicity remains to be clarified. The nature of the melanocyte, and more information about melanin forms and precursors and their possible role as photosensitisers in UV-induced genotoxicity is desirable. The consequences of different spectral balances are also not properly understood and it may be necessary to change the design of sunbeds.

Effect on SSM supervisory and regulatory task

The executive summary includes recommendations both for further studies and for public health. Prevention recommendations include sunscreen use and limiting of sun exposure, informing the public about the ineffectiveness of tanning as UV protection, promotion of UV protection of eyes and recommended avoidance of sunbeds for those less than 18 years, or possibly aged less than 30.

Project information

The workshop was organised by professors Rune Toftgård, Dan Segerbäck and Johan Hansson at Karolinska Institutet. They engaged a science writer, Jean Emeny, to write a brief summary, which was published in the Journal of Investigative Dermatology (vol. 128, p.1875–1877, 2008), as well as this extended summary for the former Swedish Radiation Protection Authority, now the Swedish Radiation Safety Authority.

Preface

Organising Committee

Dr Rune Toftgård, Department of Biosciences and Nutrition, Karolinska Institutet, Novum, Huddinge, Sweden;
Dr Johan Hansson, Department of Oncology-Pathology, Karolinska Institutet, Karolinska University Hospital Solna, Stockholm, Sweden;
Dr Dan Segerbäck, Department of Biosciences and Nutrition, Karolinska Institutet, Novum, Huddinge, Sweden.

Conference Coordinator

Mairon Sandin Department of Biosciences and Nutrition, Karolinska Institutet, Novum, Huddinge, Sweden.

Rapporteur

Dr Jean Emeny, Cancer Biomarkers and Prevention Group, University of Leicester, UK.

Sponsors

Swedish Radiation Protection Authority;
Swedish Cancer Society.

Executive summary

Exposure to ultraviolet (UV)-radiation is a dominating risk factor underlying skin cancer, but major uncertainties remain concerning its biological effects and cellular defence mechanisms, hindering implementation of effective preventive measures. A conference sponsored by the Swedish Radiation Protection Authority and the Swedish Cancer Society and held at Karolinska Institutet, Stockholm in October, 2007, brought together scientists studying different aspects of the biological impact of UV-radiation to present and discuss current knowledge of this area of research with special attention to the relative importance of short (UVB) and long (UVA) wavelength UV-radiation. This report is based on the evidence presented at that meeting.

DNA damage, repair and mutagenesis

DNA is considered to be the main target for UV-induced carcinogenesis, although the DNA damage that is induced varies with wavelength across the solar spectrum. UVB induces pyrimidine dimers in DNA between adjacent thymine and/or cytosine bases. UVA is thought to induce photosensitisation, resulting in oxidative damage to DNA, but has also been found to induce the formation of significant amounts of pyrimidine dimers. Experiments designed to resolve the mechanism of UVA-induced genotoxity using human fibroblasts, transgenic mouse cells or Chinese hamster ovary (CHO) cells have given conflicting results and the role of UVA-induced DNA damage is still unclear. Future studies in a model that replicates human skin more closely might allow elucidation of this problem.

Knowledge of repair of UV-induced DNA damage is extensive since this model has been used to study nucleotide excision repair. More recently it has been realised that a whole new family of polymerases plays an important role in the cellular processing of UV-induced DNA damage and a role for polymerase iota (ι) has been suggested in the antimutagenic bypass of specific UV-induced lesions.

Cellular response to UV irradiation

The response of different skin cell types to UV irradiation may determine whether DNA damage results in tumorigenesis. Early S-phase arrest was observed after UVA irradiation of human fibroblasts, but late S-phase arrest after UVB irradiation. Prominent and longer-lasting activation of p53 was found following UVB treatment of both human fibroblasts and keratinocytes compared with UVA treatment. These differences in response might explain why UVA induces more mutations per initial dimer load.

The cell cycle response to UV-induced DNA damage also differs in melanocytes and melanoma cells with marked G1 arrest being seen in primary human melanocytes, especially after UVA irradiation, but not in melanoma cells. Evidence suggests that melanoma cells have lost the ability to regulate the cell cycle at the G1/S checkpoint.

UVA-radiation induces the haem catabolic enzyme haem oxygenase 1 (HO-1) in human skin fibroblasts and melanocytes but not in epidermal keratinocytes. Induction of HO-1 is a general response to oxidative stress in mammalian cells and understanding of its regulation may help in elucidation of the cellular response to UVA-radiation.

Repetitive UVA irradiation also causes mitochondrial DNA mutagenesis in human dermal fibroblasts in vitro and in human skin in vivo, resulting in changes known to be involved in photoageing and carcinogenesis. The role of transcription-coupled nucleotide excision repair in this process is of interest.

An in vitro keratinocyte/melanocyte co-culture system promises to provide valuable information on cellular interactions in skin carcinogenesis. In this system, melanocytes can be rescued from UVB-induced apoptosis by the presence of keratinocytes. Resistance to apoptosis, a mechanism for eliminating cells with irreparable DNA damage, is a hallmark of most malignancies, including melanoma. Determining the role of apoptosis signalling pathways and cellular interactions in response to UV-radiation might enable strategies to be developed to prevent or eliminate tumours arising from melanocytes. The rescue of melanocytes from UVB-induced apoptosis by prior induction of heat shock protein 70 (hsp70) might represent a potential target for cancer therapy.

The underlying genetic and transcriptional events occurring during tumour development are beginning to be unravelled by analyses of mutations in micro-dissected cell clones. A direct link between actinic keratoses, squamous cell carcinoma (SCC) in situ and invasive SCC has been suggested. In addition, gene expression differences have been found between normal basal cells and basal cell carcinoma (BCC) cells.

Skin carcinogenesis in experimental models

Hybrids in the fish *Xiphophorus* shows an unexpectedly high efficacy for induction of melanomagenesis by UVA as well as UVB. If also true for humans, this has major implications for prevention and protection strategies for melanoma. In *Xiphophorus,* the presence of reactive melanin radicals parallels the fish action spectrum for melanoma, suggesting an involvement of melanin in carcinogenesis. There is some evidence from studies with mouse cells for a role for melanin in photosensitisation via damage to melanosomes and formation of bulky DNA adducts in addition to direct radical damage to DNA.

A number of mouse models of melanoma have been developed that mirror the human situation and promise to throw light on the process of melanomagenesis. In the hairless mouse model, equally carcinogenic doses of UVA induce fewer cyclobutane pyrimidine dimers (CPDs), suggesting that other, non-dimer, kinds of DNA damage are involved. In this strain, UVB- and UVA-radiation induce differing cell cycle and apoptotic responses. In an hepatocyte growth factor/scatter factor (HGF/SF) transgenic mouse model developed from the FVB albino mouse strain, UVB- but not UVA-irradiated neonates develop melanoma. In crosses of nucleotide excision repair (NER)-deficient mouse strains with C57Bl/6-HGF/SF transgenics, melanomas developed more rapidly after neonatal UV treatment in some crosses, supporting a role in melanoma for UVB lesions which are repaired by NER. Crosses of the HGF/SF mouse with a new mouse model with melanocyte-specific inducible green fluorescent protein promise to aid the identification of genes involved in melanomagenesis. Mouse strains carrying *Hras* or *Nras* mutations develop melanoma after a single neonatal UVB exposure, and also show increased proliferation and migration of melanocytes to the epidermal basal layer, perhaps contributing to their increased susceptibility. Evidence indicates that the Scf/Kit signalling pathway, which mediates keratinocye/melanocyte interaction, plays a role in melanoma development. A line derived from a similar inducible *Hras* mouse model that develops melanomas of which 20% metastasize holds promise for identification of a metastasis-specific gene expression signature.

Other experimental models include growth factor-overexpressing newborn and adult human skin grafted to SCID mice, producing melanoma-like lesions; for newborn foreskin grafts, this requires UVB. Additionally, cultures of human multi-potent cells have been derived that can be induced to differentiate into melanocyte-like cells and can incorporate into synthetic skin in the same way as epidermal melanocytes. These models have potential for determination of the processes involved in the transformation of melanocytes to tumour cells.

Skin carcinogenesis in humans

Analysis of mutations in the *PTCH* gene, which are frequently found in sporadic BCCs, has revealed a high frequency of UV-related mutations, supporting the role of UV-radiation in the development of BCCs.

Most human melanomas show activating mutations in the *NRAS* or *BRAF* proto-oncogenes and significant differences are found between the two types, including gene-expression differences. Sequencing of melanoma genomes and expression analysis should allow a better understanding of the role of UV in skin mutagenesis and carcinogenesis.

Melanomas have been classified into mucosal; acral; non-chronic sun damage (CSD) associated; and CSD associated types, which differ in the frequency of occurrence of chromosomal aberrations, and *NRAS*, *BRAF* and *KIT* mutations. Distinct histopathological features allow the generation of classification trees that enable good prediction of the *BRAF* versus *NRAS* status of tumours and may allow better assessment of prognosis.

Sub-erythemal exposure is more typical of human exposure but repeated sub-erythemal exposure results in an accumulation of erythema in skin types I and II. Evidence, although conflicting, suggests that UVA is more immunosuppressive than erythemogenic. Hence, good UVA protection is needed to ensure that sunscreens provide comparable sun protection and immunoprotection.

Solar UV-radiation is the most important preventable cause of cataract. Increased understanding of the pathophysiology of UV-induced cataract might allow development of cheap pharmacological intervention methods, especially useful in geographical areas where surgery is inacessible.

Population studies

Analysis of familial mutations predisposing to melanoma will allow determination of individual risk. Germline *CDKN2A* mutations are found in 40% of melanoma-prone families and inheritance of variants of the melanocortin receptor gene, *MC1R*, also increases the risk of melanoma. The mutations found differ geographically and phenotype varies with mutation type, e.g. association with pancreatic cancer.

Increased numbers of melanocytic naevi is the most potent risk factor for melanoma. Naevus number is predominantly genetically determined so that naevus genes when identified are likely low penetrance melanoma susceptibility genes.

Recent epidemiological studies have shown that sunburn and solarium use increase risk of melanoma, and childhood, adolescence and early adulthood have been discussed as the most sensitive age periods.

An IARC meta-analysis of published studies revealed an increased risk of melanoma for ‘ever use’ of indoor tanning facilities, with a greater risk associated with sunbed use starting in adolescence or young adulthood. Some evidence was also found for increased risk of SCC associated with first use of sunbeds before 20 years of age.

The Genes and Environment in Melanoma study, motivated by the observation that sun exposure prior to the diagnosis of melanoma appears to enhance *survival*, is compiling data on more than 3000 melanoma patients to determine survival in relation to solar exposure. If the protective effect of solar exposure is replicated, this will have important implications for the directions of future research regarding the mechanism underlying such an effect.

The apparent protective effects of sunlight against infections and some cancers might result from an effect of vitamin D on the immune system. There is recent evidence from meta-analyses for a role of polymorphisms in the vitamin D receptor gene (*VDR*) in melanoma susceptibility.

Exposure assessment

Quantitation of population exposure to solar UV is important in predicting the risk of skin disease. Analysis of urinary UV-induced dimers is non-invasive and gives a good estimation of total body UV dose, showing promise for use in population studies. A behavioural model of UV exposure, which generates estimates that agree well with data obtained in other studies, could be rapidly adapted to different populations and changes in behaviour so that the effects of these can be anticipated. The COST726 action, founded in 2004, aims to advance understanding of UV-radiation distribution under various meteorological conditions in Europe and to assess changes so that action can be taken if necessary to reduce skin cancer risk in populations.

Discussion

Despite extensive effort, the mechanism of UVA-induced genotoxicity remains to be clarified. Human melanocytes or keratinocytes or human skin transplanted onto mice promise to be suitable models for experimentation. Experimental models are useful but care is necessary when extrapolating results to humans.

The lesions observed in tumours are not always representative of the initial spectrum of genotoxic events and are not necessarily UVA or UVB specific. Sequencing of melanoma genomes should help to clarify the situation.

The nature of the melanocyte that is the target for UV-induced genotoxicity and carcinogenicity in humans is of interest as is understanding melanocyte differentiation and migration. More information about melanin forms and precursors and their possible role as photosensitisers in UV-induced genotoxicity is desirable. Transgenic pigment models may be useful in understanding the role of human genes.

A biological action spectrum for melanoma and other skin cancers in mammals is crucial. The identification of people who are at greater risk of the deleterious effects of UV exposure, whether because of genetics, age or exposure pattern, is important for public health reasons. In addition, a reliable method for classification of melanomas has implications for prognosis and treatment and work on the mutational status of melanomas is encouraging.

Determination of levels of exposure is important in determining risk of skin disease. A non-invasive urinary exposure analysis method being developed shows promise. Methods for modelling exposure will also be useful in predicting the effects of climate change. Calibration of the radiometers used in exposure monitoring is crucial if measurements for different times and locations are to be reliably compared.

Because of the potentially greater exposure of the population to UVA while using UVB-blocking sunscreens or during use of modern sunbeds, further information on the role of UVA is crucial. It is also important to determine whether high irradiance is more carcinogenic per joule because sunbed regulations limit irradiance. The consequences of different spectral balances are also not properly understood and it may be necessary to change the design of sunbeds. Prevention recommendations include the use of sunscreens, UV protection of eyes, and the avoidance of sunbeds for those less than 18 years old. For those aged less than 30, UV exposure involves greater risk because naevus development is still active; however, it may not be possible to be prescriptive about the behaviour of those over 18. The influence of latitude should be considered when making recommendations for Northern Europe.

Science should be communicated clearly and honestly to decision-makers and the public, including the contribution of sunbed use and sunny holidays to UV exposure and the risks this represents. Sound advice is needed as is co-operation between science and industry in the design of sunbeds to minimise the risks of UV exposure.

Recommendations

Knowledge gaps and areas of concern identified during the meeting were used to generate a list of recommendations for future work and for public health advice.

Further studies

- Continue development of models that mirror the human situation, e.g. human melanocytes or keratinocytes in vitro, mouse models, or human skin grafts in a mouse model, to determine the role of UVB vs UVA in skin carcinogenicity.
- Determine which melanocyte (immature or stem cell progenitor) is the target for UV-induced genotoxicity and carcinogenicity in humans with the aim of developing early detection methods or because different therapeutic methods might be more effective.
- Investigate the different melanin forms and precursors and their possible role as photosensitisers in UV-induced genotoxicity.
- Establish a reliable method for classification of melanomas to aid prognosis and effective targeted treatment.
- Establish a biological action spectrum for melanoma and other skin cancers in mammals.
- Determine the effect of UV irradiance on carcinogenic effects per joule with respect to both melanoma and squamous cell carcinoma of the skin (sunbed regulations limit irradiance).
- Develop non-invasive total exposure methods to identify high risk subpopulations.
- Investigate pathophysiology of UV-induced cataract to enable development of cheap pharmacological intervention methods, for use in the developing world.

Public health

- Regulate the calibration of radiometers used in exposure monitoring to ensure that measurements can be compared for different times and locations.
- Use exposure modelling to predict the results of lifestyle and climate change.
- Promote skin ‘awareness’ to lower risk of death from melanoma.
- Promote sunscreen use and limiting of sun exposure and inform public about ineffectiveness of tanning in providing protection against UV.
- Promote UV protection of eyes.
- Improve methodology for individual assessment of risk contributions from sunbathing and use of sunbeds.
- Recommend avoidance of sunbeds for those less than 18 years old, and possibly aged less than 30.

- Evaluate the design and regulation of sunbeds, in close association with industry, in the light of emerging evidence about the effects of UVA and establish effective policing of adherence to regulations.
- Establish clear lines of communication with policy makers, industry and the public.

Sammanfattning

UV-strålning är en dominerande riskfaktor för utveckling av hudcancer, men det råder fortfarande stor ovisshet kring dess biologiska påverkan och cellulära försvarsmekanismer, något som förhindrar genomförandet av effektiva förebyggande åtgärder. I oktober 2007 hölls en konferens vid Karolinska Institutet, Stockholm, med ekonomiskt stöd av Strålsäkerhetsmyndigheten och Cancerfonden. Konferensen sammanförde forskare som studerar olika aspekter av biologisk påverkan av UV-strålning. Syftet var att presentera och diskutera aktuell kunskap inom detta forskningsområde, med särskilt beaktande av den relativa betydelsen av kortvågig (UVB) och långvågig (UVA) UV-strålning. Denna rapport baseras på de vetenskapliga fakta som presenterades under mötet.

DNA-skador, deras reparation och inducerade mutationer

DNA antas vara huvudmålet för UV-inducerad cancer, men olika våglängder av UV-strålning inducerar olika typer av DNA-skador. UVB orsakar sk pyrimidindimerer i DNA, medan UVA via andra mekanismer kan ge upphov till delvis samma dimerer men också till sk oxidativa skador i DNA. I experiment som utförts för att lösa mekanismen bakom UVA-inducerade mutationer har experiment med olika typer av celler gett motstridiga resultat och rollen av UVA-inducerade DNA-skador är fortfarande oklar. Framtida studier i en modell som kopierar mänsklig hud kan kanske kasta ljus över detta problem.

Kunskapen om reparation av UV-inducerade DNA-skador är omfattande, eftersom UV är standardmodellen för att studera mekanismen för DNA-reparation. På sista tiden har man förstått den viktiga rollen en helt ny familj av polymeraser för cellens hantering av UV-inducerade DNA-skador och mekanismer för denna interaktion har föreslagits.

Cellens reaktion på UV-bestrålning

Olika typer av hudcellers reaktion på UV-bestrålning kan kanske vara avgörande om DNA-skador skall ge upphov till tumörer. Man har observerat att UVA och UVB skiljer sig med avseende på deras effekt på cellcykeln och i aktivering av tumörprocessorn P53. Man har även iakttagit skillnader mellan normala melanocyter och celler från melanom avseende på hur de reagerar på bestrålning med UVA, vilket kanske kan förklara att tumörceller förlorar förmågan att reglera cellcykeln.

Upprepad UVA-bestrålning inducerar mutationer i mitokondrier och sådana förändringar vet man att de är involverade i åldrande av huden och i hudcancer. Hudcellstyperna melanocyter och keratinocyter kan också interagera med varandra och på så sätt påverka programmerad celldöd (apoptos). Ett fastställande av dessa mekanismer kan kanske ge möjlighet för att utveckla strategier som kan förebygga eller eliminera tumörer uppkomna från melanocyter. De underliggande cellulära händelser som pågår under tumörutvecklingen är på väg att lösas genom analys av mutationer i mikrodissekerade cellkloner.

Hudcancer i experimentella modeller

Vissa framavlade genetiska varianter av fisken *Xiphophorus* är känsliga för induktion av melanom från UVA- liksom från UVB-strålning. Om detta gäller även för människan så har denna observation stora implikationer för prevention och skyddsstrategier mot melanom. Även reaktiva melaninmolekyler kan kanske vara involverade i tumörutveckling.
Ett antal musmodeller för melanom som speglar humansituationen har utvecklats och som verkar lovande för att belysa processen för induktion av melanom. I en modell med hårlösa möss som används för hudcancerstudier så inducerar UVA färre dimerer i DNA än den dos av UVB som är lika cancerframkallande. Dessa resultat pekar mot att andra DNA-skador än UV-specifika dimerer är betydelsefulla för tumörinduktion och i denna musstam så inducerar dessutom UVA och UVB olika reaktioner från cellen.

Musstammar som har mutationer i vissa onkogener utvecklar melanom efter en enstaka neonatal dos UVB och visar också på ökad proliferation och migration av melanocyter till det epidermala basalcelllagret, vilket kanske bidrar till deras ökade känslighet.
Andra experimentella modeller inkluderar nyfödda möss som överuttrycker tillväxtfaktorer och hud från vuxna människor som transplanterats till möss och ger där upphov till melanomliknade skador. Dessutom har kulturer av humana multipotenta celler tagits fram och som kan induceras för att utvecklas till melanocytliknande celler och därefter inkorporeras i syntetisk hud på samma sätt som epidermala melanocyter. Dessa modeller har potential för att bestämma de processer som är involverade i transformationen av melanocyter till tumörceller.

Hudcancer och andra UV-relaterade sjukdomar hos människan

Analys av mutationer i en viss gen, som ofta hittas i sporadiska basalcellscarcinom (BCC), har avslöjat en hög frekvens av UV-relaterade mutationer, vilket stödjer rollen av UV-strålning vid utvecklingen av BCC. De flesta humana melanom visar aktiverande mutationer i onkogener och signifikanta skillnader hittas mellan olika gener. En sekvensering av genomet och analys av utryckta gener bör medföra en bättre förståelse för rollen som UV spelar vid induktion av hudcancer.

Melanom kan delas in i olika klasser, vilka skiljer med avseende på frekvens kromosomabberationer och mutationer i vissa gener. Genom analyser av dessa förändringar kan man förhoppningsvis förbättra prognosbedömning.

Exponering som inte ger upphov till erytem är vanligast för människan, men upprepade sådana exponeringar resulterar i en ackumulation av erytem. Data, om även inte samstämmiga, indikerar att UVA framför allt har en effekt på immunsystemet snarare än att den orsakar erytem. Skydd mot erytem är därför en dålig indikator på solskyddsmedels effektivitet mot UVA och solljus, vilket har följdeffekter för märkning av konsumentprodukter.
UV-strålning från solen är den viktigaste orsaken till katarakt och som går att begränsa. Ökad förståelse för mekanismerna bakom UV-inducerad katarakt kan kanske leda till utveckling av billiga farmakologiska interventionsmetoder som kan vara speciellt användbara i geografiska områden där kirurgi inte är tillgänglig.

Populationsstudier

Analys av ärftliga mutationer som orsakar en ökad benägenhet till att utveckla melanom tillåter bestämning av individuell känslighet. En sådan muterad gen hittades i 40 % av melanombenägna familjär.

Ökat antal födelsemärken av en speciell typ är den största riskfaktorn för melanom. Antalet födelsemärken är ärftligt och de gener som ligger bakom detta (ännu ej identifierade) är antagligen gener med en låg penetrans för ökad cancerrisk. Epidemiologiska studier har visat att om man bränner sig i solen eller använder sig av solarium så ökar risken för att få melanom och detta gäller speciellt om detta sker i barndomen, i tonåren eller som ung vuxen. Ett stöd för ökad risk för skivepitelcancer fanns också om solarium användes första gången före 20 års ålder. En ökad relativ risk för melanom är också associerat med intermittent solexponering, medan intermediär kronisk solexponering verkar vara milt skyddande.

Den eventuellt skyddande effekten av solljus mot viss typ av cancer, i första hand på andra ställen än huden, kan orsakas av en effekt av vitamin D på immunsystemet. Vid en meta-analys av olika epidemiologiska studier har man funnit en effekt av polymorfism i vitamin D receptorgenen när det gäller melanomkänslighet. Ett större EU-projekt motiveras av observationen att solexponering före melanomdiagnosen verkar öka överlevnad. Man sammanställer i projektet data över mer än 3000 melanompatienter för att bestämma överlevnad i relation till solexponering. Om den skyddande effekten kan upprepas så kommer det att ha viktig följd för forskningsinriktningen när det gäller mekanismen bakom en sådan effekt.

Exponeringsuppskattning

Kvantifiering av populationers exponering för UV från solen är en viktig del av riskuppskattningen för hudcancer. Analys av UV-inducerade DNA-skador i urin medför inga problem när det gäller att insamla prover för analys. Metoden ger en god uppskattning av totalkroppsdos av UV och är därför lovande för användning i populationsstudier. En beteendemodell för UV-exponering, som genererar uppskattningar som är i överensstämmelse med data från andra studier, skulle snabbt kunna adapteras till olika populationer och förändringar i beteenden så att effekter av dessa kan förutses. COST726-aktionen, som bildades 2004, har som målsättning att öka förståelsen av UV-strålnings distribution vid olika metrologiska förhållanden i Europa och att uppskatta förändringar så att åtgärder kan vidtagas om nödvändigt.

Diskussion

Trots omfattande forskning så är mekanismerna för UVA-inducerad genotoxicitet inte helt klarlagda. Att använda mänskliga melanocyter eller keratinocyter, samt att transplantera hud från människa till mus är lovande forskningsmodeller. Generellt är experimentella metoder användbara, men stor hänsyn måste tas när man extrapolerar från djur till människa.

DNA-förändringar i tumörer är inte alltid representativa för det initiala spektrat av genotoxiska händelser och är inte nödvändigtvis specifika för UVA- eller UVB-exponering. Gensekvensering av melanom kan ytterligare klarlägga läget.

Karaktären av melanocyter, målcellen för UV-inducerat melanom i människa, är viktig, liksom förståelsen av hur dessa celler reagerar. Ytterligare information om olika former av melanin, förstadier till melanin, samt vilken eventuell fotosensibiliserande inverkan de har på UV-inducerad genotoxicitet är önskvärd. Pigmentmodeller i transgena möss kan vara användbara för att förstå de mänskliga genernas roll.

Det biologiska aktionsspektrat för melanom och andra hudcancerformer hos däggdjur är viktigt, både för enstaka våglängder och för blandningar av våglängder, detta gäller även aktionsspektrum för nedtryckning av immunsystemet.

Identifieringen av individer som är mer mottagliga för de skadliga effekterna av UV-exponering, vare sig dessa beror på genetiska förutsättningar, ålder eller exponeringsmönster, är viktigt av folkhälsoskäl. Dessutom så har en pålitlig metod för klassificering av melanom följder för prognos och behandling. Vidare så ger arbete med mutationsstatus för melanom uppmuntrande resultat för framtida insatser på folkhälsoområdet.

Bestämning av exponeringsnivåer är viktigt för riskuppskattning. En icke invasiv metod med analys av urinprover efter exponering är utvecklad och visar lovande resultat. Exponeringsmodeller är också användbara för förutsägelser om effekter av klimatförändringar. En noggrann kalibrering av exponeringsmätare är nödvändig för att mätningar gjorda på olika platser och vid olika tidpunkter skall vara jämförbara.

Eftersom människor potentiellt utsätts för större mängder UVA genom användning av UVB-blockerande solskyddskrämer, samt genom att moderna solarier endast ger mycket små mängder UVB, så är ytterligare studier av vilken roll UVA spelar av centralt intresse. Det är också viktigt att fastställa om hög irradians är mer carcinogen per joule eftersom solarier har gränsvärden baserade på irradians. Konsekvenserna av skiftande spektral balans är inte helt klarlagda, och det kan visa sig vara nödvändigt att förändra designen av solarier.

Rekommendationer för prevention innefattar användningen av solskyddskrämer, UV-skydd för ögonen, samt undvikande av solande i solarier för ungdomar under 18 år. För människor yngre än 30 år pågår fortfarande bildningen av födelsemärken, och UV exponering innebär då

en större risk, dock kanske det finns sämre möjligheter att införa begränsningar för människor över 18 år. Hänsyn bör tas till betydelsen av latitud vid rekommendationer för norra Europa.

Framtagna vetenskapliga fakta skall klart och tydligt rapporteras till beslutsfattare och allmänhet, innefattande bidraget av solarier och resor utomlands och motsvarande risker. Förnuftig rådgivning behövs, liksom samarbete mellan vetenskap och industri vid konstruktionen av solarier, för att minska riskerna med UV-exponering.

Rekommendationer

Kunskapsluckor och områden av speciellt intresse som identifierades under mötet nedtecknades, och utifrån dessa skapades en lista med rekommendationer för vidare studier och råd för folkhälsan.

Vidare studier

- Använd humana melanocyter eller keratinocyter, eller modeller där hud transplanteras från människa till mus för att klarlägga inverkan av UVA respektive UVB
- Klarlägg vilken melanocytform (omogen eller stamcellsförstadium) som är målet för UV-inducerad genotoxicitet och carcinogenicitet hos människa med målsättningen att utveckla tidiga detektionsmetoder
- Undersök olika former av melanin och förstadier till dessa, samt vilken roll de kan ha som fotosensibiliserande faktorer i UV-inducerad genotoxicitet.
- Etablera en pålitlig metod för att klassificera melanom i syfte att förbättra prognos och effektiv målinriktad behandling.
- Fastställa biologiskt aktionsspektrum för immunosuppression i människa och för skivepitelcancer i pigmenterade möss
- Klarlägga betydelsen av UV irradians för carcinogena effekter per joule med avseende på såväl melanom som skivepitelcancer i huden (regler för solarier är baserade på irradians)
- Utveckla icke invasiva modeller för helkroppsexponering och därmed förbättra exponeringsanalysen
- Undersöka patofysiologin vid UV-inducerad katarakt för att underlätta utvecklingen av billiga behandlingsmetoder i utvecklingsländer.

Råd för folkhälsan

- Reglera kalibrering av exponeringsmätare för att säkerställa jämförbarhet vid mätningar som utförs vid olika tidpunkter och på olika ställen
- Använd exponeringsmodellering för att kunna förutsäga effekter av livsstil och klimatförändringar
- Uppmana till medvetenhet om risker vid hudexponering för att medverka till att sänka risken för död i melanom
- Uppmana till användning av solskyddsmedel (med UVA skydd för att säkerställa att immunförsvaret inte påverkas) och begränsa solexponering, samt informera allmänheten om hur ineffektivt solbränna skyddar mot UV
- Uppmana till användning av UV-skydd för ögonen
- Förbättra metodologin för individuellt riskbidrag från solbadande och användning av solarium
- Rekommendera att man undviker exponering i solarium för ungdomar under 18, samt möjligen under 30 år
- Utvärdera konstruktion och reglering av solarier, i nära samarbete med industrin, i och med nya fakta angående effekterna av UVA-exponering
- Etablera öppna informationskanaler med beslutsfattare, industri och allmänhet

1. Introduction

Exposure to UV-radiation is a dominating risk factor underlying skin cancer, which is the most common form of cancer in the Western world and, moreover, increasing in incidence. Additionally, UV-radiation may have detrimental effects on the eye and affect the immune system. Although the biological effects of UV-radiation and the cellular defence mechanisms have been intensively investigated, major uncertainties remain. This lack of knowledge also hinders implementation of effective preventive measures.

At a conference sponsored by the Swedish Radiation Protection Authority and the Swedish Cancer Society held at Karolinska Institutet, Stockholm on 18–20 October, 2007, scientists studying different aspects of the biological impact of UV-radiation were brought together to present and discuss current knowledge of this area of research[1]. Special attention was focused on the relative importance of short (UVB) and long (UVA) wavelength UV-radiation. This report is based on the evidence presented at that meeting[2].

[1] Emeny J, Hansson J, Toftgård R, Segerbäck D. 2008. Report of the conference on UV-radiation-induced disease: roles of UVA and UVB. J Invest Dermatol 128:1875-7.

[2] Since the meeting, an IARC report, 'IARC Working Group Report 5: Vitamin D and cancer' (25 November, 2008) has been published. This report has been discussed by Grant WB. 2009. A critical review of IARC report 5, Dermato-Endocrinology 1:1-9.

2. Role of UV-radiation in skin carcinogenesis

Solar ultraviolet (UV) radiation is an established environmental physical carcinogen, which has been implicated in the etiology of human skin cancer [Pfeifer, Annex III.6]. UVC (wavelength <280 nm) is absorbed by stratospheric oxygen, the majority of UVB (280–320 nm) is blocked by ozone, but UVA (320–400 nm), owing to its high penetrating efficiency, passes through the atmosphere and reaches the surface of the earth. Terrestrial sunlight UV therefore comprises ~95% UVA; the remainder is UVB. However, because of the high energy and potent photocarcinogenicity of UVB it accounts for the majority of solar UV-associated neoplasia. UVA is estimated to contribute to 10–20% of sunlight-induced carcinogenesis.

2.1. DNA damage, repair and mutagenesis

DNA is considered to be the main target for UV-induced carcinogenesis because its modification can lead to mutagenesis and tumour initiation [Douki, Annex III.1]. However, the kind of DNA damage that is induced varies with wavelength across the solar spectrum.

Different initial DNA lesions result in different mutations, e.g. pyrimidine dimers give rise to C to T and CC to TT transitions, whereas 8-oxodG (7,8-dihydro-8-deoxoguanine) gives rise to G to T and A to C transversions. Whether or not mutations result from UV-induced lesions will depend on the relative repair rates for different kinds of lesion and the efficiency of DNA repair pathways in individual cells.

2.1.1. UVB-radiation-induced DNA damage

UVB (wavelength 280–320 nm) is well absorbed by DNA and induces pyrimidine dimers between adjacent thymine and/or cytosine bases [Douki, Annex III.1]. Two types of lesion, cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (64PPs), can be formed at each of the four possible bipyrimidine dinucleotides (TT, TC, CT and CC); however, TT and TC are much more photoreactive than CT and CC. The same effects are found with isolated DNA, cultured mammalian cells and human skin, although 20-fold protection is afforded by the shielding effect of skin. This suggests that external factors have little effect on the process. Background levels of 8-oxodGuo (8-oxo-7,8-dihydro-2′-deoxyguanosine) are also found but CPDs are 100-fold more frequent.

The photoproducts induced by UVB differ in the efficiency with which they are repaired. Recent experiments in primary cultures of human keratinocytes have shown that 64PPs are rapidly repaired. Cyclobutane pyrimidine dimers are, in general, repaired much more slowly, although C-T dimers are well repaired and C-C dimers show an intermediate level; T-T dimers are least well repaired. The same relative repair rates were also observed in fibroblasts and skin.

2.1.2. UVA-radiation-induced DNA damage

UVA-radiation (wavelength 320–400 nm) is much more abundant in solar radiation than UVB [Douki, Annex III.1]. Because UVA is much less well absorbed by DNA, the underlying genotoxic mechanism is thought to be photosensitisation, involving unknown endogenous chromophores, resulting in oxidative damage to DNA. Photosensitisation may result in the formation of reactive oxygen species or electron abstraction, leading to the production of 8-oxodGuo, or via Fenton chemistry to hydroxyl radicals, generating strand breaks and oxidised bases.

Analysis of UVA-induced oxidative DNA damage in CHO (Chinese hamster ovary) cells and human monocytes has shown that much greater amounts of 8-oxodGuo than strand breaks or oxidised pyrimidines are formed [Kielbassa, et al. 1997; Pouget, et al. 2000; Douki, et al.

2003]. This suggests that singlet oxygen plays a major role in lesion formation. This was confirmed by ^{18}O labelling of cultured human monocytes.

The first study of UVA induction of dimers, in bacterial DNA, took rigorous precautions to exclude short wavelength light and included experimental proof that these wavelengths were not involved [Tyrrell 1973]. Despite this, early observations of UVA induction of CPDs by UVA in bacteria, rodent cells and whole skin had been attributed to contamination of the light sources with UVB. However, the early results have been confirmed in UVA-irradiated CHO cells in which many T-T CPDs and a few C-T and C-C CPDs have been detected but no 64PPs [Douki et al. 2003]. The amounts of T-T CPDs found were greater than 8-oxodGuo. Similar results have been found in human skin fibroblasts, keratinocytes and skin. The lesser amounts of 8-oxodGuo cannot be explained by repair during irradiation as they are not increased in *OGG1* knock-out cells, which lack 8-oxoguanine glycosylase I. An important observation that may account for descrepancies in the literature is that culture medium very efficiently photosensitizes the formation of 8-oxodGuo [T. Douki, personal communication]. During exposure to a full spectrum, UVA may also be implicated in the photoisomerisation of UVB-induced 64PPs into poorly repaired and highly mutagenic Dewar photoproducts [Douki, et al. 2003].

2.1.3. Genotoxicity of UVB vs UVA

The mechanism of genotoxicity of UVB and UVA was examined in human fibroblasts by quantifying CPDs, 64PPs and 8-oxodG in the overall genome [Pfeifer, Annex III.6]. UVB-irradiated cells showed substantial amounts of CPDs, dependent on radiation dose. UVA-irradiated cells showed lower, but significant levels of CPDs, again dependent on dose. Dose-related 64-PPs were also induced by UVB, but not appreciably by UVA. Elevated levels of 8-oxodG were found in UVA- but not UVB-irradiated cells [Besaratinia, et al. 2005].

Analysis of polymerase-blocking lesions induced by UVB in the *p53* tumour suppressor gene, implicated in squamous (SCC) and basal cell carcinoma (BCC), revealed that these were formed almost exclusively at pyrimidine-rich sequences. UVA on the other hand induced lesions at purine- and pyrimidine-containing sequences along the gene. Mapping of specific types of DNA damage by ligation-mediated polymerase chain reaction (LM-PCR) analysis showed formation of CPDs along the *p53* gene but these differed in UVB- vs UVA-irradiated cells. Fpg-sensitive sites (oxidised and ring-opened purines) were found exclusively in UVA-treated cells and mapped mainly to guanines, consistent with the presence of 8-oxodG in the overall genome [Besaratinia, et al. 2005].

Determination of the nature of UVA-induced mutagenicity in the overall genome and the *cII* transgene in BigBlue® mouse cells showed that it was mediated by oxidative DNA damage [Besaratinia, et al. 2007]. Addition of photosensitisers, riboflavin or δ-aminolevulinic acid, together with UVA treatment increased DNA damage and mutagenesis but the antioxidant vitamin C inhibited it. In contrast to the pattern of mutagenicity found for UVA, sunlight-induced mutagenicity is dominated by single and tandem C to T transitions at dipyrimidine sites, suggesting a major role for UVB-, not UVA-induced effects.

Similar experiments compared the genotoxicity of UVB, and UVA and solar-simulated light (SSL) in the overall genome of transgenic mice and damage repair. Equilethal doses of UVB and SSL, but not UVA, induced CPDs and 64PPs in this system; CPDs persisted for at least 24 hours, whereas 64PPs were repaired within six hours. The oxidised purines induced by UVA and SSL were repaired within 30 minutes [Besaratinia, et al. 2008].

These results were paralleled for the *cII* gene. Whereas UVB and SSL were extremely mutagenic as measured by mutant frequency in the *cII* gene, UVA was only weakly-mutagenic. The mutations induced by UVB and SSL were dominated by C to T transitions at

dipyrimidines, which accounted for 85% and 80%, respectively, of their mutagenicity. The G to T transversions induced by UVA in the *cII* gene contributed only 3.3% of the SSL mutation load. The differences in the rates of repair of the lesions induced by UVB and UVA may explain their different contributions to the mutagenicity of solar radiation. Mutation data for different genes in human skin cancer show a preponderance of C to T transitions, supporting the conclusions from the transgenic mouse studies.

Mutation formation per DNA photoproduct (dimer) increases with wavelength, from UVB to UVA [Enninga, et al. 1986], perhaps because of the occurrence of non-pyrimidine dimer damage [Rünger, Annex III. 5]. Physiological doses of both UVA and UVB were found to produce dose-dependent mutations in an *hprt* mutagenesis assay in human neonatal skin fibroblasts. Sequencing of the *hprt* gene in the mutants, showed, unexpectedly, a similar distribution of mutations for UVB and UVA, with C to T transitions predominating [Kappes, et al. 2006].

To understand why a UVA-induced DNA photoproduct is more mutagenic than that induced by UVB, given that the kinds of mutations found were similar [Rünger 2008; Rünger and Kappes 2008], it was hypothesised that the cellular response to DNA damage is important. Activation of the tumour suppressor gene *p53* was found to be less after UVA than UVB treatment in skin fibroblasts and keratinocytes. Analysis of the cell cycle by fluorescence-activated cell sorting (FACS) showed early S-phase arrest after UVA, and late arrest after UVB treatment. Activation of neither *p95* nor *p53* was involved in the UVA effect. No G1/S-phase arrest was found in synchronised cells after UVA or UVB irradiation but in synchronised cells 24 hours after release from serum and irradiation, arrest was seen only with UVB. Longer-lasting induction of the DNA repair enzyme XPC was found after UVB, although DNA repair was not improved after either type of irradiation. In contrast to UVB, UVA treatment does not result in activation of *FANCD2, BRCA1, RAD51* or *H2AX* [Dunn, et al. 2006], which mediate recombinational DNA repair. As a consequence of the lesser cellular response after UVA treatment, it is thought that mutation formation and survival of mutated cells are more likely, hence explaining why UVA appears to induce more mutations per dimer load. It may thus not be necessary to postulate non-dimer DNA damage to explain the effects of UVA. It may also mean that pure UVA sources may be more mutagenic than mixed sources, with implications for sunbed use and hopes for antioxidant protection against skin cancer.

2.1.4. The role of DNA repair polymerases

In normal cells, the CPD and 64PP lesions induced by UV-radiation are repaired by the nucleotide excision repair (NER) system [Sarasin, Annex III.2]. This involves recognition of the lesion, opening of the DNA helix, demarcation of the lesion, dual excision of the damaged DNA strand, error-free resynthesis of the gap and ligation of the new strand [Stary, et al. 2003]. Translesion synthesis (TLS) is normally carried out by DNA polymerase eta (pol η) with few mistakes. However, xeroderma pigmentosum variant (XPV) patients, who develop numerous UV-induced skin cancers, lack this polymerase. The mutational spectra of genes such as the *p53* tumour suppressor gene in these patients will represent the consequences of error-prone TLS in the absence of pol η.

The error rates and types of mutations generated by replication of UVC-irradiated SV40-based shuttle vectors were determined in an XPV cell line lacking pol η, in stable pol η-complemented clones of these cells and in normal cells. Complementation with pol η resulted in a strong decrease in UV-induced base substitutions in the vector, especially in UV-irradiated host cells. Two- to eight-fold protection is seen at both TA and GC base pairs although mutations at the *lacZ'* target gene on the vector are dominated by CG to TA transitions.

Other polymerases that might be involved in error-prone TLS in the absence of pol η are pol ι, which interacts with pol η, and pol ζ, which can bypass T-T dimers in vitro and is important for UV mutagenesis in vivo. To determine the contribution of these polymerases to UV mutagenesis, a series of knock-out BL2 cell lines was developed, lacking the genes for pol ι, Rev3 (the catalytic subunit of pol ζ), or both pol ι and pol η. The UV-sensitivity of the cell lines was: wild type = POLι <POLη <POLη/ι <<REV3. Mutant frequencies on the *lacZ'* target gene on the vector were increased 50% over wild type in the POLη$^{-/-}$ line, no different in the POLι$^{-/-}$ line and decreased five- to ten-fold in the REV3$^{-/-}$ line.

The mutational spectra in UV-irradiated shuttle vectors were determined in these cell lines and a database of the sequences of more than 1200 mutations was generated. Analysis of the appearance or disappearance of UV-induced mutational hotspots in the *lacZ'* gene of the vector suggested that some lesions could not be copied without pol ζ, and that pol ι can error-free bypass some specific lesions and, in a pol η-deficient background, can error-prone bypass others [Gueranger, et al. 2008]. Elucidation of the role of TLS DNA polymerases in UV-induced mutagenesis may allow a better understanding of skin carcinogenesis.

The mechanism of mutagenesis induced by UVA, as compared with UVC, has been further investigated in CHO cells proficient or deficient in NER, transcription-coupled repair only or base excision repair (BER) in order to understand the early observations that UVA is more mutagenic per NER incision [Jenssen, Annex III.26]. UVA-induced mutagenicity in NER-proficient and -deficient cells differed less than for UVC-induced mutagenicity, indicating a lesser involvement of pyrimidine dimers in UVA-induced mutagenicity. The absence of 64PP formation after UVA could not explain the differences. A CPD-specific endonuclease assay showed that CPDs were formed after UVA irradiation. However, at equal amounts of CPD formation, UVA exposure induces higher mutation levels. T-T CPDs, which are the predominant CPDs produced after UVA were found to be less mutagenic than C-containing CPDs, hence other mutagenic lesions, not repairable by NER, appear to be responsible for the higher mutagenic yield of UVA-radiation. Deficiency in recombinational repair enhances the toxicity of UVA, so a multiple damage site is suggested as the candidate lesion.

2.2. Cellular response to UV irradiation

Human skin comprises three layers: the epidermis, dermis and hypodermis. Keratinocytes and melanocytes are the main constituents of the epidermis; melanocytes are attached to the basement membrane that separates the epidermis from the dermis. Melanin is synthesised in the melanocytes and transferred via dendrites to keratinocytes where it plays a critical role in photoprotection [Costin and Hearing 2007].

The acute effects of exposure to sunlight are damage to cutaneous DNA and proteins and modification of cell membranes, resulting in erythema and sunburn [Tyrrell, Annex III.3]. Chronic effects are photoageing and carcinogenesis. UVB is thought to play a major role in causing these effects; the contribution of UVA is less well understood. The cutaneous target of the UVB component of solar radiation is restricted mainly to the epidermal layer whereas the UVA component of sunlight penetrates deep into the dermis.

2.2.1. Mitochondrial DNA damage and photoageing

In addition to damage to nuclear DNA, UV-radiation causes damage to mitochondrial (mt)DNA [Krutmann, Annex III.4]. Mutations of mtDNA have been reported to play a causative role in neurodegeneration, normal ageing, premature ageing of skin (photoageing) and several types of tumour. A 4977 bp mtDNA deletion is increased 10-fold in photoaged skin. It is thought that mitochondrial function decreases as mtDNA mutations increase, setting up a vicious circle in which an increasingly defective respiratory chain leads to less oxygen consumption and functional/structural changes.

Repetitive UVA irradiation causes mtDNA mutagenesis in human dermal fibroblasts in vitro and in human skin in vivo [Berneburg, et al. 2004, 2005] and sunbed use is associated with the generation of mtDNA mutations [Reimann, et al. 2008]. Induction of the common deletion in normal human fibroblasts by UVA is paralleled by a reduction in oxygen consumption, mitochondrial membrane potential and ATP content and an increase in matrix metalloproteinase-1, known to be involved in photoageing and carcinogenesis. Repetitive irradiation of fibroblasts in the presence of creatine completely abolishes induction of the common deletion by UVA [Berneburg, et al. 2005].

Human skin equivalent models (dermal equivalents) of Kearns-Sayre syndrome patients, who carry the mtDNA common deletion, show early and increased contraction in collagen gels due to the greater production of reactive oxygen species compared with normal human fibroblasts. Later effects are degradation of the extracellular matrix and neovascularisation. Cockayne syndrome fibroblasts, which are defective in the *CSA* and *CSB* genes, involved in transcription-coupled NER, are more susceptible to mtDNA mutation. Treatment with the antioxidant vitamin E reduces their susceptibility. The role of the *CSB* and *CSA* genes is being studied in $CSB^{-/-}$ hairless mice and in epidermal and dermal specific *CSB* knockouts. In addition, human fibroblasts with differing degrees of expression of *CSB* or that lack mtDNA are being studied in dermal equivalents in order to elucidate the mechanism of photoageing and its prevention.

2.2.2. Haem oxygenase and the anti-inflammatory response

UVA-radiation generates a major oxidative stress in cells, exacerbated by the release of free iron and haem [Tyrrell, Annex III.3]. At biologically relevant doses of UVA, the haem catabolic enzyme haem oxygenase 1 (HO-1) is induced in human skin fibroblasts and melanocytes but not epidermal keratinocytes. The induction of this enzyme is a general response to oxidative stress in mammalian cells; its negative regulation is crucial to maintaining cellular homeostasis under stress-free conditions.

Haem oxygenase 1 is regulated at the transcriptional level by Nrf2/MafK activation complexes and Bach-1/MafK suppressor complexes at upstream *cis*-acting elements of the *HO-1* gene. In human skin fibroblasts, UVA irradiation causes accumulation of Nrf2 via haem release from microsomal membranes and stabilisation of the protein and to a lesser extent via transcriptional activation of Nrf2. Inhibition of haem synthesis by succinyl acetate suppresses UVA-induced Nrf2 accumulation in human skin fibroblasts. Haem binding to Bach-1 results in its removal from DNA, export from the nucleus and consequent up-regulation of HO-1 transcription. In Bach-1-transfected cells, UVA induction of HO-1 is suppressed. Refractoriness to re-induction of HO-1 by UVA develops as a result of removal of haem by HO-1 and de novo synthesis of Bach-1.

Human keratinocytes, which are much less sensitive to UVA-induced damage, express HO-2 constitutively. The HO-2 expression appears to dampen the HO-1 response in these cells. Silencing of HO-2 expression in an immortalised human skin keratinocyte cell line increases UVA-induced HO-1 expression, supporting the idea that haem oxygenase itself regulates its expression.

2.2.3. Cell cycle response of melanocytes and melanoma cells to UV irradiation

The cell cycle response to DNA damage can be highly sensitive, hence the effect of UV wavelength on this response was examined in primary human melanocytes and in melanoma cells [Kowalczuk, Annex III.27]. Cell cycle distribution was analysed by FACS and G1/S checkpoint-related cell cycle proteins by western blot analysis. Exposure of melanocytes caused marked G1 arrest, especially after UVA irradiation (cf. results described by Rünger in fibroblasts and keratinocytes; Section 2.1.3). This was not seen in melanoma cells. Melanoma cells showed a lack of p16 expression, suggesting that these cells have lost the ability to regu-

late the cell cycle at the G1/S checkpoint. In melanoma cells, p27 was increased following UVA exposure but decreased after exposure to UVC, perhaps in response to oxidative DNA damage in the former case.

2.2.4. Apoptosis

Apoptosis provides a mechanism for eliminating cells with irreparable DNA damage and resistance to apoptosis is a hallmark of most malignancies, including melanoma [Rosdahl, Annex III.24]. UV triggers several apoptosis signalling pathways in many cell systems.

The regulation of apoptosis in human skin cells was studied in an in vitro keratinocyte/melanocyte co-culture system. Melanocytes express a high basal level of Bcl-2 (an anti-apoptotic protein of the mitochondrial pathway) compared with keratinocytes but no difference in expression of Bax (a pro-apoptotic protein) is found. mRNA of both Bcl-2 and Bax was up-regulated after UVB irradiation (>305 nm) but only a slight increase in apoptosis was found. An increase in UVB of wavelength 280–305 nm resulted in greater apoptosis and upregulation of Bcl-2 but not Bax mRNA. No change in protein levels was found; the translocation of proteins within the cell was found to be more important for accelerating apoptosis. The presence of keratinocytes rescued melanocytes from UVB-induced apoptosis [Bivik, et al. 2005].

Apoptosis mediated by the mitochondrial pathway involves release of cytochrome c and caspase 3 and the outcome depends on a balance of anti- and pro-apoptotic regulators. Both UVA and UVB induce Bax, Bid and Bcl-X_L translocation from the cytosol to mitochondria. Bcl-2, which was thought to be attached only to membranes, was found in the cytosol in melanocytes and was also translocated to the mitochondria after UVB or UVA irradiation. The pro-apoptotic lysosomal proteases Cathepsin B and D were released from lysosomes to the cytosol. However, the presence of cathepsin inhibitors reduced Bax translocation and apoptosis. Micro-injection of cathepsin B induced apoptotic cell death. No involvement of caspase-8 was found when examined up to 8 hours after irradiation, hence the death receptor pathway of apoptosis was not involved [Bivik, et al. 2006].

Heat shock protein 70 (Hsp70), which is expressed in many tumours, is the main stress-induced heat shock protein involved in folding and transport of proteins; it also protects cells against apoptosis. Exposure of human melanocytes to heat and then UVB significantly increased the level of Hsp70 and prevented cathepsin and cytochrome c release and Bax translocation, thus rescuing the cells from apoptosis. Hsp70 small interfering (si)RNA eliminated the anti-apoptotic effect. Hsp70 therefore represents a potential target for cancer therapy [Bivik, et al. 2007].

Unravelling the role of apoptosis signalling pathways in response to UV-radiation might enable strategies to be developed to prevent or eliminate tumours arising from melanocytes and allow identification of individuals at risk of developing melanoma.

2.2.5. Clonal analysis of skin cancers

Skin cancer provides an excellent model to study the the process of carcinogenesis from single malignantly transformed cell to tumour [Asplund, Annex III.8]. The three main cancer types, BCC, SCC and malignant melanoma, are well characterised morphologically. Microdissection of histologically defined normal and malignant cell populations allows the underlying genetic and transcriptional events occurring during tumour development to be determined.

In chronically sun-exposed skin, clones of morphologically normal cells with nuclear accumulations of immunoreactive p53 are found; up to 70% of these carry a mutated *p53* gene. Alterations of the *p53* tumour suppressor gene are common in non-melanoma skin cancer and dysregulation of p53 pathways appears to be an early event in the development of both BCC

and SCC. Mutational analysis of p53 clones in actinic keratoses and SCC in situ and invasive SCC suggests that there is a direct link between these lesions. Similar analysis of p53 clones in BCC has indicated the development of subclones in tumours with additional *p53* mutations [Bäckvall, et al. 2005].

Microdissection together with RNA extraction, amplification and cDNA microarray analysis has been used to examine gene expression in specific cell populations of normal basal cells and BCC. Upregulation of 202 genes and downregulation of 161 genes was found in BCC. The genes involved included *Frizzled homolog 8, cyclooxygenase, Gli1,* and a number of new genes of interest. The microarray results are being validated using antibodies from the Human Proteome Resource Project (http://www.proteinatlas.org/object.php).

2.3. Skin carcinogenesis in experimental models

Of the melanoma animal models that are available, the most accessible to experimentation are the fish *Xiphophorus* and the marsupial opossum *Monodelphis domestica*. Melanomas historically were difficult to initiate in the mouse [Noonan, et al. 2003] but recent models have been developed (see below).

Xiphophorus develops melanomas spontaneously and also in response to UV irradiation. Progression from naevus to metastasis occurs but the melanomas originate in dermal melanophores, which differ from skin melanocytes. Homologues of genes involved in human melanoma have been found in *Xiphophorus*. The action spectrum for melanoma development in response to irradiation showed a high efficacy for UVB but also an unexpectedly high efficacy for UVA. The phylogenetic distance between fish and human may limit the applicability of this model.

The first mammalian model for melanoma was in a non-placental mammal, the opossum, *M. domestica*. Chronic UV irradiation initiates melanomas that metastasise and have dermal pathology. Unlike the situation in *Xiphophorus*, UVA has only been shown to initiate focal melanocytic hyperplasia, not melanoma, in this model, although recent analysis [G. Timmins, personal communication] suggests UVA may require longer exposures. Homologues of genes involved in human melanoma have also been found in *M. domestica*; however, genetic studies may be limited by the inability to derive inbred lines of this species.

Melanomas in the mouse are typically dermal melanomas that are not similar histopathologically to human melanomas. Adult murine melanocytes are confined to hair follicles except in areas such as the ear and tail where they are epidermally located. In neonatal mice, melanocytes are located in the dermal/epidermal junction as well as the hair follicle. Mouse melanocytes are very resistant to UV induction of melanoma. In an early UV-responsive mouse model, expression of an SV40 T-antigen transgene, which perturbs multiple cellular signals, was targeted to melanocytes by the tyrosinase promoter. Neonatal UV irradiation resulted in cutaneous malignant melanoma. Subsequently developed models include *Ink4a/arf*-deficient strains with activated Ras and crosses of these into a *p53*-null background.

2.3.1. Wavelength dependence of melanoma in a mouse model

The wavelength composition of sunlight varies with global factors such as latitude, season and time of day and also with local factors such as air pollution, reflectance of surroundings and cloud cover (see Section 2.6) [De Fabo, Annex III.11]. Knowledge of relative melanoma effectiveness as a function of wavelength is therefore important for estimation of melanoma effective dose received from sunlight or artificial sources.

Non-melanoma skin cancer has traditionally been thought to be initiated by UVB, which is absorbed by several important biomolecules, causing sunburn, suntanning, skin and corneal damage, pre-vitamin D formation and alterations to the immune system. UVB is also

mutagenic and stimulates multiple cellular signalling pathways. Melanoma, however, can occur on infrequently exposed sites and is associated with sporadic burning doses of sunlight, not chronic exposure. Also no consensus UVB signature lesions are found in melanoma, unlike non-melanoma skin cancer. UVA is also biologically active, via photosensitisation rather than direct absorption, and can also stimulate multiple signalling pathways. No mammalian action spectrum for melanomagenesis is available but data from the fish *Xiphophorus* show an unexpectedly high efficacy for UVA in addition to the high efficacy of UVB. Because UVA is more abundant than UVB in sunlight, if the action spectrum is similar in humans, this has major implications for prevention and protection strategies for melanoma.

An hepatic growth factor/scatter factor (HGF/SF) transgenic mouse model has been developed from the FVB albino mouse strain which produced junctional melanomas in response to neonatal UV irradiation [Noonan, et al. 2001]. Melanocytes in this strain are located in the dermis, at the dermal/epidermal junction, in the basal layer of the epidermis and also, as normal, in the base of the hair follicle. UV-irradiated neonates of this strain develop melanoma after 6 to 9 months. Irradiation of adults increases the multiplicity of melanomas in neonatally irradiated mice but does not, by itself, initiate melanoma. The histopathology of melanoma development in this strain closely resembles that of human melanoma. This model mirrors the human situation in which childhood sunlight exposure is critical for melanoma but adult exposure is also important.

The albino HGF/SF-FVB mouse model has been used to determine which wavelengths initiate melanoma using specialised optical sources that emit highly resolved UVB or UVA wavebands, a combination of these or solar-simulated radiation (SSR). Only UVB initiated melanoma in this model; UVA was ineffective, even at physiologically relevant doses that were 30-fold higher than melanomagenic UVB doses [de Fabo, et al. 2004].
The role of DNA repair was examined in crosses of NER-deficient mouse strains with C57Bl/6-HGF/SF transgenics. After neonatal UV treatment, melanomas developed more rapidly in some crosses, supporting a role in melanoma for UVB lesions which are repaired by NER.

A new mouse model with inducible melanocyte-specific expression of green fluorescent protein (GFP) promises to enable identification of melanoma induction pathways when crossed with HGF/SF mice.

2.3.2. Melanin as a photosensitiser

The wavelengths in sunlight that cause melanoma are unclear [Timmins, Annex III.13]. Evidence that suggests a role for UVA in melanoma includes prevalence across latitude, the presence of only few dimer signature mutations, and the rarity of melanoma in albinos. Melanin photosensitisation and UVA may thus play major roles in human melanoma causation. Experimental models to examine this should ideally be pigmented, exhibit UVA effects and multistage carcinogenicity. Hybrid *Xiphophorus* fish fulfil some of the requirements for a good experimental model of melanoma, especially for action spectrum determination. The *Xiphophorus* action spectrum for melanoma is still the only one available, with important implications if similar to humans [Setlow, et al. 1993; Mitchell, et al. 2007]. To examine the role of melanin as a photosensitiser, electron paramagnetic resonance (EPR) measurements of reactive melanin radicals (RMRs) in *Xiphophorus* have been compared with the action spectrum for melanoma; these are identical from 303 to 434 nm, spanning UVB and UVA [Wood, et al. 2006].

It was hypothesised that melanin has two photosensitising roles in the melanosome after UV irradiation: direct radical damage of DNA; and via formation of bulky quinone-DNA adducts resulting from leakage of tyrosinase-derived quinones from RMR-damaged melanosomes. Testing of this hypothesis in SSL-irradiated B16 mouse melanoma cells showed that inhibition of tyrosinase prevented loss of viability whereas the addition of tyrosine decreased it.

Labelling experiments with ^{13}C-tyrosine suggested the formation of DNA adducts. Such adducts might generate T to A transversions and be implicated in the formation of the *BRAF*^{V600} mutation (T1799A) frequently found in melanomas.

2.3.3. Differential effects of UVA and UVB on keratinocytes and melanocytes

Most early studies of the effects of UV-radiation were carried out with fibroblasts rather than keratinocytes; the latter are more relevant to skin carcinogenesis and differ from fibroblasts in antioxidant defences and repair proficiency [de Gruijl, Annex III.15].

The kinetics of cell cycle response differ after UVA and UVB irradiation of the epidermis of hairless mice (cf. cellular effects described in Sections 2.1.3 and 2.2.3) An immediate maximum suppression of DNA replication was seen after UVA, compared with around 6 hours for UVB, and S-phase arrest resolved more quickly after UVA than after UVB irradiation. Expression of p53, and apoptosis, increased faster after UVA than UVB (see Section 2.2.4). In contrast, dermal melanocytes of hairless mice do not show apoptosis after UVB irradiation but do show proliferation 4 days later; this is not seen after UVA exposure.

The action spectrum for induction of skin carcinoma in hairless mice peaks at 293 nm and falls by orders of magnitude towards the UVA band. Equally carcinogenic doses of UVA induce fewer CPDs, suggesting that other kinds of damage are involved (cf. Sections 2.1.3 and 2.1.4). Chronic UVA exposure induces benign papillomas initially, followed by a higher rate of carcinomas. UVA- and UVB-induced carcinomas show a different mutational profile: UVB induces CPD-related signature mutations in the *p53* gene, whereas UVA irradiation results in few *p53* mutations, other than at some UVB-like hotspots, and a lack of oxidation-related mutations, i.e. G to T transversions (cf. Section 2.1.3). The *p53* gene is therefore not a dominant target in UVA carcinogenesis and UVA-induced membrane damage may play a role in tumorigenesis. Interestingly, low-level chronic UVB exposure, inducing little or no hyperplasia, caused accumulation of CPDs in epidermal stem and progenitor cells [Nijhof, et al. 2006].

Naevi were induced in the skin of hairless mice by intermittent overexposure to UVB, but not UVA; neonatal UVB exposure also induced naevi. However, very few melanomas resulted, even in mice defective in NER or the tumour suppressor genes *p16Ink4a/p19Arf*. However, induction of melanoma has been reported in *Xpc*$^{-/-}$ *Ink4a/Arf*$^{-/-}$ mice carrying a *Kras* mutation after a single neonatal exposure [Yang, et al. 2007]; this result shows promise for a mouse model of human melanoma.

2.3.4. Melanocyte proliferation and migration

Exposure of skin to UV-radiation causes DNA damage in epidermal keratinocytes. Signalling molecules released by these cells cause melanocytes to activate pigmentation pathways [Walker, Annex III.12]. This activation also results in melanocyte proliferation. Melanocyte activation is a protective (tanning) response, increasing melanin levels in keratinocytes as protection against subsequent exposure. Excessive melanocyte proliferation may, however, increase melanoma risk. Most mouse models of melanoma carry mutations that result in melanocyte hyperproliferative phenotypes and this may, in part, explain their proneness to melanoma.

Mouse models that carry melanocyte-specific *Hras* (*Tpras*) or *Nras* mutations develop melanoma after a single neonatal UVB exposure. The additional presence of an Rb or p53 pathway defect increases susceptibility although the respective mechanisms responsible may differ. Neonatal, but not adult, UVB exposure of wild-type mice results in migration of melanocytes to the epidermal basal layer. Melanocyte numbers peak at 5 days post-exposure; the cells seem to have migrated from the hair follicles via the outer root sheath as a result of signals from UV-damaged keratinocytes. The melanocyte proliferative response was induced by high

dose UVB, but not high dose UVA, suggesting that pyrimidine dimers play the major role. The response was greater in *Hras* and *Nras* mice than in wild type. No deficiency in ability to remove pyrimidine dimers was found in the mutant melanocytes, so this cannot explain their increased susceptibility to transformation.

Melanocyte migration to the epidermal basal layer is mediated by the stem cell factor (Scf)/Kit signalling pathway. Scf signals from keratinocytes, which are increased after UV irradiation, influence the distribution of melanocytes via the Kit receptor on the latter. In another transgenic mouse strain (*Mt-Ret*) that develops spontaneous melanoma with high penetrance, specific antibody blocking of the melanocyte Kit receptor during the first few days after birth (but not later) almost completely prevented subsequent melanoma development [Kato, et al. 2004]. Thus, the presence in neonates of Kit-sensitive melanocytes that have a high propensity for proliferating after UVR exposure seems critical for melanoma development in mice.

2.3.5. Melanocyte stem cell signature

Melanocyte proliferation in normal skin comprises a series of steps: decoupling, division, migration and repositioning, re-coupling, dendrite extension and growth control [Herlyn, Annex III.14]. Each of these steps involves a variety of accessory factors, e.g. E-cadherin, SCF, $\alpha 6\beta 1$. In stress situations, i.e. sunburn, CCN3 secures anchorage of melanocytes through DDR1-mediated collagen IV adhesion. In melanoma, the homeostatic mechanisms break down and keratinocytes do not communicate with melanocytes (see Section 2.2.4 for further results on cellular interactions).

The role of UVA/B in induction of melanoma was investigated in newborn and adult human skin, overexpressing growth factors, grafted to SCID mice. Overexpression of growth factors (bFGF, SCF, ET-3) induced melanoma-like lesions in adult skin without UVB; for newborn foreskin grafts, UVB was required. Lesions induced by growth factors alone do not develop autonomy.

Certain genes in signalling pathways are commonly altered in melanoma, e.g. *BRAF* (60–70%), *p16*, *PTEN* and *AKT*. In normal melanocytes, the oncogene $BRAF^{V600E}$ induces senescence; *p53* knockdown inhibits this and induces proliferation and malignant transformation.

Traditionally, it has been hypothesised that cancer results from the stepwise accumulation of genetic mutations that confer proliferative advantages. An alternative hypothesis is that stem cells (tumour-initiating cells) exist that are self-renewing and can re-populate a tumour with various cell types; the tumour microenvironment, including fibroblasts and endothelial cells, is also involved in tumour progression [Lee and Herlyn, 2007]. Multi-potent stem cell-like cells have been isolated from the dermis of human foreskin. In culture, these cells can form three-dimensional spheres. Neural crest-like cells isolated in this way can be induced to differentiate into melanocyte-like cells, expressing appropriate markers. They can incorporate into synthetic skin in the same way as epidermal melanocytes if the microenvironmental conditions are right. Their response to the presence of $BRAF^{V600E}$ and *p53* knockdown will be of interest. Differentiated dermal sphere cells and cells from melanoma cell lines and fresh tumour specimens will be investigated by microarray analysis to determine a melanocyte/melanoma stem cell signature.

2.3.6. Melanoma metastasis gene signature

Genetically engineered mouse models have been derived that express an inducible $Hras^{V12G}$ allele in the context of *Ink4a/Arf* deficiency (iHRAS) [Heffernan, Annex III.10]. These develop melanomas with high penetrance and short latency but, in contrast to human melanomas, these do not metastasize. The melanomas regress after removal of the doxycyline (DOX) inducer, indicating a role for Ras in melanoma initiation and maintenance.

A second line, iNRAS, with inducible *Nras*^{Q61R} directed to the melanocyte compartment of *Ink4a/Arf*-deficient mice, develops melanomas, 20% of which metastasize to lymph nodes and lung. These melanomas were dependent on Ras for maintenance as they regressed on withdrawal of DOX; however, cyclical induction of Ras expression led to the generation of 'escapers'. UV irradiation of neonatal pups reduced tumour latency, suggesting cooperation beween Nras and UV in melanomagenesis.

If metastatic potential is determined by differentially expressed genes in primary melanomas, comparative gene expression analysis of these two mouse lines may lead to the identification of a metastasis gene signature. Comparison with genomic hybridisation profiles of human melanoma will allow candidate metastasis genes to be identified. A similar approach has identified *NEDD9* as such a gene [Kim, et al. 2006].

2.4. Skin carcinogenesis in humans

Basal cell carcinoma, the most common form, accounts for 90% of all skin cancers. It originates in the basal cells, at the bottom of the epidermis (outer skin layer), and is caused by long-term exposure to sunlight. Squamous cell carcinoma is the second most common type of skin cancer. It originates in the epidermis, eventually penetrating the underlying tissue if not treated. In a small percentage of cases, this cancer spreads (metastasizes) to other parts of the body. Malignant melanoma is a form of skin cancer that is currently affecting more and more people: each year, more than 53,000 cases are diagnosed in the US. Melanoma originates in moles or other growths on normal skin. It is a very serious type of skin cancer, but the cure rate is quite good if it is diagnosed and removed early.

A number of cellular pathways are involved in susceptibility to skin cancer and development of BCC, SCC and melanoma. These pathways control: cell cycle progression, such as *CDK4* and the *CDKN2a* locus, *INK4A* and *ARF*; growth factor signalling, such as the MAPK pathway encompassing *RAS*, *RAF* and *ERK*; *PTEN* and *AKT* survival signalling, including *P13K*; the DNA damage response, including *ARF* and *p53* [Miller and Mihm 2006].

The mutational spectra observed in skin tumours show a predominance of bipyrimidine sites, indicating a role for DNA damage induced by the UVB component of sunlight [Giglia-Mari and Sarasin 2003] [Douki, Annex III.1]. The contribution of UVA is less clear-cut because it induces a variety of lesions. Although the yield of lesions is lower than for UVB, the higher intensity of UVA may compensate in part.

2.4.1. UV-related mutational pattern of the *PTCH* gene in basal cell carcinoma

UV-radiation is a major etiological risk factor for BCC, one of the most common cancers in western countries [Lindström, Annex III.29]. Mutations in the *PTCH* gene, a transmembrane receptor gene involved in the hedgehog signalling pathway and thought to be a tumour suppressor gene, are frequently found in sporadic BCCs. Analysis of data from the PTCH Mutation Database (http://www.cybergene.se/PTCH/) revealed a high frequency of UV-related mutations, CC->TT or C->T, in *PTCH*. 50% of *PTCH* mutations in sporadic BCCs and 77% of mutations in BCCs in xeroderma pigmentosum patients were of this type [Lindstrom, et al. 2006]. This supports the role of UV-radiation in the development of BCCs. Mutations were clustered in the two predicted large extracellular loops and the large intracellular loop of the PTCH protein, indicating their probable functional importance.

2.4.2. Somatic *NRAS* and *BRAF* mutations in human melanoma

Somatic mutations of genes involved in cell signalling pathways or regulation of the cell cycle are common in human melanomas [Hansson, Annex III.22]. The majority show activating mutations in the *NRAS* or *BRAF* proto-oncogenes involved in growth factor signalling, in particular *NRAS*^{Q61R}, *NRAS*^{Q61K} and *BRAF*^{V600E}. The *BRAF* and *NRAS* mutations are mutually

exclusive and occur early. The mutation in the primary tumour is maintained in multiple metastases. A large screening of melanomas and metastases showed *BRAF* in 54% of patients, *NRAS* in 27% and both together in only 1%; 18% of tumours were wild type for these genes. Mutations in metastases were identical to those in the primary tumour in the large majority of cases.
Some significant differences were found between *NRAS* and *BRAF* melanomas: age at diagnosis was higher for *NRAS* than for *BRAF* tumours; a higher Clark level of invasion was found for *NRAS* vs *BRAF* tumours; the presence of a preexisting naevus was more likely for *BRAF*; and lymphocyte infiltration was greater for BRAF. However, overall *survival* showed no association with *NRAS* or *BRAF*. Gene expression profiling of melanoma metastases showed that 79 genes were significantly upregulated in *BRAF* cf *NRAS* mutant tumours, with clustering on chromosome 7, on which *BRAF* is located. No difference was found between *NRAS* and *BRAF* mutant tumours in the expression of phosphorylated ERK, part of the mitogen-activated protein kinase (MAPK) signalling pathway.

The commonly found $NRAS^{Q61}$ mutations are consistent with UV-induced induction of dipyrimidine dimers. However, the $BRAF^{V600E}$ mutation is not and may arise by an indirect mechanism (see Section 2.3.2). In a study of patients with familial melanoma carrying germline mutations in the cyclin-dependent kinase inhibitor 2A gene *CDKN2A*, 95% of tumours carried $NRAS^{Q61}$ mutations. The predisposition to melanoma of families with this genotype may also be associated with hypermutability [Eskandarpour, et al. 2003] and is consistent with observations in knock-out mice. This study is being extended to families with other germline mutations within the GenoMEL network (see Section 2.5.3).

A review of *NRAS/BRAF* mutations in melanoma, histopathology and UV exposure found the highest frequencies of these mutations in superficial spreading melanomas and nodular melanomas from continuously and intermittently exposed body sites. Lower frequencies occurred in lentigo maligna melanoma and acral lentiginous melanoma and the lowest were observed in melanomas on non-UV exposed mucosal membranes. *NRAS* mutations are more common in melanomas from chronically exposed than intermittently exposed sites; *BRAF* mutations show the opposite pattern. *NRAS/BRAF* mutational status promises to be a significant indicator in the molecular classification of melanoma.

2.4.3. Genotype-phenotype correlations in melanoma

Although exposure to UV light is an important etiological factor in melanoma, certain melanoma types, mucosal and acral, arise in the complete absence of UV or on areas of the body that are well protected from exposure, respectively [Bastian, Annex III.21]. The incidence of mucosal and acral melanoma is independent of ethnicity and latitude. The melanomas that occur on sun-exposed skin in Caucasians may not be wholly attributable to their lesser degree of pigmentation as albinos of African descent are not significantly more susceptible to melanoma but do have an increased incidence of SCC. Subtypes of melanoma may thus be categorised by body site, UV exposure and genetics.

Four melanoma types were proposed for comparative genetic studies: mucosal; acral; non-chronic sun damage (CSD) associated; CSD associated. Array-based comparative genomic hybridisation (CGH) showed a greater degree of chromosomal aberrations in mucosal or acral melanomas, including gains, losses and amplifications, although these involved different genomic regions in the two types of tumour. When the MAPK pathway was looked at in detail, mutations in *BRAF* were found to be significantly more common in the non-CSD group; frequent loss of chromosome 10 was also found in this group. CSD-related melanomas had infrequent *BRAF* mutations but frequent increases in *CCND1* copy number. In all groups, mutations in *RAS* genes were in *NRAS* and did not occur in association with *BRAF* mutations (see Section 2.4.2). Fewer copies of *PTEN*, carried on chromosome 10 and part of the phosphatidylinositol-3-kinase (PI3K) pathway, were found in samples with *BRAF* mutations than those with *NRAS* mutations. This is consistent with a requirement for a second mutation (loss of the

negative regulator *PTEN*) to activate the PI3K pathway in addition to a *BRAF* mutation, which would only activate the MAPK pathway. Activation of both pathways appears to be necessary for melanomagenesis.

The higher frequency of *BRAF* mutations in sun-exposed individuals without CSD suggested that a genetic susceptibility factor might be involved in these cases, especially one found in Caucasians, such as the highly polymorphic melanocortin-1 receptor gene (*MC1R*). Sequencing of the germline *MC1R* gene and exon 15 of the *BRAF* gene in melanomas revealed an association between *BRAF* mutations and variant *MC1R* genes in non-CSD cases [Landi, et al. 2006].

Further analysis of the CGH data revealed mutations or copy number increases in the *KIT* genomic region in 40% of acral and mucosal melanomas and 30% of CSD melanomas [Curtin, et al. 2006]. *KIT* encodes a receptor tyrosine kinase that binds stem cell factor (SCF) and is involved in activation of multiple signalling pathways, including PI3K and MAPK (see Section 2.3.4). Melanomas with *KIT* mutations rarely showed *BRAF* or *NRAS* mutations. Treatment of patients with these melanomas with imatinib may be possible because *KIT* mutations are found in other types of cancer that are imatinib responsive.

Melanomas can be categorised by a number of morphological and other features: pigmentation, scatter, nesting, degree of circumscription, cell shape and size, age of patient, and degree of solar elastosis. Genotype-phenotype correlation analysis of 302 primary melanomas showed that *BRAF* mutations are associated with distinct histopathological features. Classification trees can be generated that allow a good prediction of the *BRAF* versus *NRAS* status of tumours.

2.4.4. Sub-erythemal exposure, sunscreen use and melanoma

Sub-erythemal exposure is a dose of UV radiation that is below that required to produce erythema or repeated individual doses that are not erythemogenic per se [Young, Annex III.23]. It can be expressed as a fraction of minimal erythema dose (MED) or standard erythema dose (SED) or as J/cm^2. It is more typical of human exposure, which is a series of acute sub-erythemal exposures, resulting in tanning, *stratum corneum* thickening and tolerance. These factors may influence susceptibility to skin cancer.

Skin can be categorised into six types ranging from type I, which is white, sunburns easily, tans very poorly if at all and is associated with a high cancer risk, to type VI, which is black, does not easily burn, tans very well and has a low cancer risk. MED increases with skin type from I to VI but there is considerable overlap. It is a more useful indicator of skin response than skin type. The differences found are not due to pigmentation as areas of vitiligo have only slightly lower MEDs than normal skin in the same individual. 3 MED SSR fractionated over 4 days has the same erythemal effect as a single 3 MED dose in skin types I and II. Daily sub-erythemal exposure of skin type I or II over three weeks results in an accumulation of marked erythema. Higher white skin types (e.g. III and IV) show better resolution of erythema.

Sunscreens are categorised by their sun protection factor (SPF), which indicates protection against erythema. However, SPF is a poor indicator of protection against UVA and sunscreens differ considerably in their efficacy against UVA. IARC [2001] concluded that there is *inadequate evidence* for the cancer-preventive activity of topical use of sunscreen formulations against cutaneous malignant melanoma or BCC of the skin and *limited evidence* for protection against SCC of the skin.

Daily sub-erythemal exposure results in an accumulation of pigmentation, i.e. tanning, and epidermal changes. Acute SSR exposure results in global DNA damage (T-T dimers) that is a function of dose not skin type. Daily sub-erythemal SSR exposure also results in an accumu-

lation of DNA damage; however, it may induce DNA repair in skin types that tan well and can protect against DNA photodamage and erythema after a 2 MED challenge. The application of a sunscreen can protect against T-T dimer formation induced by sub-erythemal exposure.

The cellular response to DNA damage may be repair, apoptosis or mutation. Daily sub-erythemal SSR exposure results in accumulation of p53 although, interestingly, no apoptosis, and sunscreen prevents this. Sub-erythemal exposure also has immunosuppressive effects: loss of $CD1a^+$ Langerhans cells and a suppression of the contact hypersensitivity (CHS) response to dinitrochlorobenzene (DNCB). Low SPF sunscreen protects against the former. Erythema is not a useful indicator of immunosuppression, hence SPF does not represent immunoprotection factor (IPF). Evidence, although conflicting, suggests that UVA is immunosuppressive, more so than erythemogenic. Hence good UVA protection is needed to ensure that SPF = IPF. A recent review of the evidence [Norval, et al. 2008] shows that SSR has a cumulative effect on immunosuppression and repeated sub-erythemal exposure does not result in photoadaptation.

Photoageing results in a number of histological and biochemical changes in the dermis and epidermis. Repeated experimental sub-erythemal exposure to UVB+UVA or to UVA alone can reproduce these effects but *not* elastosis, the major change found in photoaged skin.

2.4.5. UV-induced cataract

Cataract, opacification of the lens, is the leading cause of blindness in the world and its incidence is expected to increase as populations age [Söderberg, Annex III.16]. Surgery is often not accessible in developing countries; hence, cataract represents an important health and economic burden. Solar UV-radiation is the most important preventable cause of cataract.

The human cornea transmits almost 50% of UV radiation of wavelength 400–320 nm but the transmittance drops quickly and is negligible below 290 nm. Almost all UV that penetrates the cornea is attenuated in the ocular lens. The dose–response function for UV-induced cataract in experimental models is continuous. A statistically defined threshold, the Maximum Tolerable Dose ($MTD_{2.3:16}$) has been defined: there is a 16% probability that an individual exposed to 1 $MTD_{2.3:16}$ kJ/m^2 will express more light scattering after the exposure than is expected in 2.3% of normal lenses in individuals with unexposed eyes. Studies have shown that previous assumptions that doses more than 24 hours apart are additive may be incorrect. Sensitivity to UV is greater in the lenses of young individuals and species vary considerably in response.

The lens is formed from ectodermal tissue and contains epithelial cells that give rise to lens fibres throughout life. Cataracts represent a change in the refractive index of the lens. UV-radiation causes a cation shift, resulting in cell disruption and damage caused by water being pulled into the lens. In vivo exposure to UV-radiation depletes glutathione, thought to be involved in the defence against photooxidation and the formation of reactive oxygen species in the lens. Supplementation with the antioxidant α-tocopherol protects against UV-induced cataract via protection against glutathione depletion. Experiments in thiol transferase knock-out mice also support a role for glutathione in protection against UV-induced toxicity.

UV-radiation induces unscheduled DNA synthesis in the lens and morphological changes suggestive of apoptosis. Significant increases in p53 and caspase-3 transcription were found in UV-irradiated lenses [Ayala, et al. 2007]; Terminal deoxynucleotidyl transferase biotin-dUTP nick end labelling (TUNEL) also indicated DNA degradation. These results are consistent with the morphological evidence of apoptosis, perhaps indicating disposal of irreparably damaged cells. Increased understanding of the pathophysiology of UV-induced cataract might

allow development of cheap pharmacological intervention methods, which would be especially useful in geographical areas where surgery is inacessible.

2.5. Population studies

Population studies can throw light on behavioural and environmental factors that influence disease risk that cannot be detected in small-scale studies. However, the design of population studies has to take into account, among other factors, the time needed to obtain a result and the resources available. Case–control studies have the advantage of shorter timespans but results may be compromised by recall bias of exposure. Prospective studies avoid this disadvantage but the time taken to acquire sufficient numbers of cases may be excessive. Other designs include the case–cohort study where controls are selected from the initial cohort as cases arise.

2.5.1. Solar and artificial UV exposure and risk of melanoma

Solar UV exposure is the major risk factor for melanoma. Sunburn and intermittent exposure are associated with increased risk, and childhood and the teen years have been discussed as critical periods [Veierød, Annex III.20]. The role of UVA versus UVB radiation is unclear as is information on dose–response in humans and the lag period involved.

The Norwegian–Swedish Women's Lifestyle and Health Cohort Study [Veierød, et al. 2003] was the first prospective study to examine associations between pigmentation, sun exposure and malignant melanoma. Over 100,000 women aged 30–50 in 1991/2 were recruited. A questionnnaire was used to determine history of sunburn, sunbathing holidays and solarium use at ages 0–9, 10–19, 20–29, 30–39 or 40–49 years as well as physical characteristics, such as weight and height, hormonal factors, diet and social class. Linkage of the database to the national registries of Norway and Sweden allowed follow-up.

Early results showed that sunburn and solarium use increase risk of melanoma. Adolescence and early adulthood appeared to be the most sensitive age periods. A follow-up through to December 2005 (14 years), by which time 412 cases of melanoma had been recorded, showed that sunburns in childhood, teens and early adulthood increase melanoma risk. Adult solarium use also increases melanoma risk.

Prospective studies allow exposure to be recorded before diagnosis, thus avoiding recall bias at recruitment. Follow-up was complete in this study and tumours confirmed histopathologically. The study is limited by the lack of exposure data after 1991–2 and follow-up time for adult exposure.

Interpretation of solarium results should take into account the fact that regulations have changed over time, e.g. in Norway and Sweden both in 1982–3 and 1991–2 [Nilsen, Annex III.30]. A Norwegian survey has shown that the UVA to UVB ratio has changed over the last 20 years and that strict regulations are essential but insufficient if not followed by inspections [Nilsen, et al. 2008].

2.5.2. Artificial UV sources and skin cancer

A meta-analysis of 23 published studies on skin cancer and UV radiation by an IARC working group revealed an increased risk of melanoma for 'ever use' of indoor tanning facilities [IARC, 2006] [Autier, Annex III.19]. Analysis of seven studies that looked at age of use showed a greater risk associated with sunbed use starting in adolescence or young adulthood. Some evidence was also found for increased SCC risk for first use of sunbeds before 20 years of age. Studies published since the meta-analysis include those on the University of Lund cohort and the Swedish-Norwegian cohort (see above). These showed an increase in melanoma risk with sunbed use and increasing risk with exposure in the 1980s.

Animal and human studies indicate that susceptibility to UV is more important during childhood and adolescence. At an individual level, the total number of naevi or total number of large-sized naevi are the best predictors of individual risk of melanoma. Naevi are markers of sun exposure and genetic factors. Hereditary factors, skin type and colour, hair and eye colour, have a strong influence on naevus development. The role of sun exposure is illustrated by the greater number of naevi in children up to three years of age growing up in Queensland (19°S) than in Scotland (55°N). The anatomical distribution of naevi in children does not match that in adults; however, it does mimic the gender-specific distribution of melanoma in adults. It is hypothesised that there are two types of melanoma: melanoma associated with naevi, mainly in young subjects, and on trunk and lower limbs; and melanoma associated with older subjects, associated with solar skin lesions rather than naevi, and mainly on head and neck. Different kinds of UV exposure may be involved, i.e. UVA at younger ages.

Because long latency periods can be expected between sunbed exposure and skin cancer, the magnitude of the association may not yet be detectable. Hence, it would be prudent to recommend avoidance of sunbed use at age less than 30 years. Evidence also suggests that sunscreen use prolongs sun exposure [Autier, et al. 2007], with implications for recommendations on use.

2.5.3. Melanoma susceptibility genes and interaction with sun exposure

CDKN2A mutations are found in 40% of melanoma families (three or more cases) [Newton Bishop, Annex III.17]. Melanoma families carrying a *CDKN2A* mutation can be identified by the presence of multiple cases, early age of onset, family members with multiple primaries and pancreatic cancer. The *CDKN2A* locus codes for two different proteins, p16 and $p14^{ARF}$, which control cellular proliferation and senescence. The mutations found differ geographically: single founder mutations are predominant in Sweden and the Netherlands. France, Spain and Italy have the same most frequent mutation and, not surprisingly given the historical importance of emigration, Australia and the UK have the same most common mutations. Phenotype is found to vary with mutation type, e.g. association with pancreatic cancer.

The penetrance of *CDKN2A*, estimated by the Melanoma Genetics Consortium (GenoMEL), is 0.30 by age 50, and 0.67 by age 80. An effect of living in an area with a high incidence of melanoma was found, with higher penetrance seen in Australia by age 80 than in Europe; this probably reflects a greater degree of sun exposure in the former. The pattern of sun exposure that is important for development of melanoma in *CDKN2A* families is currently under investigation.

Differences in the proportion of melanoma families with the *CDKN2A* mutation are also found in different geographical regions, varying from 20% in Australia to 57% in Europe. This may reflect the relative contribution to the total of genes of low penetrance, e.g. the melanocortin receptor gene *MC1R*, that show greater penetrance in regions of high sunlight exposure and would otherwise not be identified so frequently.

Inheritance of variants of the melanocortin receptor gene, *MC1R*, which encodes the melanocyte-stimulating hormone receptor and is implicated in the pigmentation process, increases the risk of melanoma. The presence of *MC1R* variants increases the penetrance of *CDKN2A* mutations. Interactions between the two mutations vary with country and may reflect interaction with other pigment genes, behavioural differences or sun exposure.

The presence of increased numbers of melanocytic naevi is the most potent risk factor for melanoma [Gandini, et al. 2005a]. Twin studies have shown that genetic variance accounts for 66% of the total; 14% is attributable to eye and hair colour and skin type, leaving 52% unaccounted for [Wachsmuth, et al. 2004). Sun exposure on hot holidays contributed to one-third of the environmental influence in this twin study.

The atypical mole syndrome (AMS), which is a phenotype indicative of an expanded population of atypical melanocytes, naevi that fail to mature normally, and naevi that are more frequent on the trunk, is more common in *CDKN2A* mutation carriers. However, not all cases can be explained by the presence of the *CDKN2A* mutation. This suggests that other co-segregating genes exist, possibly differing for 'banal' and atypical naevi.

A study of naevus counts and sun exposure in young healthy UK women showed higher relative risks of increased total naevus numbers on the trunk and leg for those who had ever holidayed abroad. Having *lived* abroad, by contrast, reduced relative risk of atypical naevi, especially if this was early in life [dos Santos Silva 2009].

The incidence of melanoma increases with age; this is especially striking in sunny countries with an outdoor lifestyle such as Australia. However, no dose–response curve for sun exposure and melanoma exists for humans. A meta-analysis of published studies [Gandini, et al. 2005b] has shown an increased relative risk of melanoma for intermittent sun exposure and a history of sunburn; intermediate levels of chronic sun exposure appear to be mildly protective. This has been hypothesised to result from vitamin D synthesis, among other possibilities. Vitamin D has effects on cell growth, differentiation, apoptosis and tumour–immune system interaction and there is recent evidence from meta-analyses for a role of polymorphisms in the vitamin D receptor gene (*VDR*) in melanoma susceptibility [Mocellin and Nitti 2008; Gandini, et al. 2009].

2.5.4. UV-induced vitamin D and cancer prevention – a hypothesis

The primary source of vitamin D in humans is that induced by solar UVB irradiation [Grant, Annex III.28]. In addition to the calcemic effect of vitamin D, which helps reduce fractures resulting from falls, the vitamin may protect against bacterial and viral infections through the induction of cathelicidin. This is supported by the observation of higher infectious disease rates in winter when UVB exposure is lowest. It has been hypothesised that UVB and vitamin D could also reduce the risk of autoimmune diseases and cancers linked to viral infections by the same mechanism [Grant 2008a, b]. The geographical variations in autoimmune diseases, such as multiple sclerosis, and certain cancers, such as prostate cancer, are consistent with this hypothesis. Also, a recent meta-analysis has found that vitamin D reduces mortality rates. This hypothesis requires further evaluation but suggests that the health benefits of UVB irradiance may balance the health risks.

2.5.5. Sun exposure and mortality from melanoma

Several meta-analyses and numerous studies of sun exposure have found associations between intermittent sun exposure and risk of melanoma [Berwick, Annex III.25]. Chronic sun exposure appears to result in no or decreased risk. Melanomas differ in growth rate, with some, such as lentigo maligna melanoma, appearing indolent in contrast to the rapidly growing types such as nodular melanoma. Analysis of mutations is beginning to provide information that will enable distinction between different types of melanoma.

The incidence of melanoma is increasing but mortality less so. This increase seems to be in indolent melanomas, perhaps resulting from changes in diagnosis for medico-legal reasons. Early detection is not associated with reduced mortality. However, in the few survival studies that have been undertaken, the presence of 'solar elastosis' (breakdown of elastin in the dermis) is associated with better prognosis[3].

Five-year survival was followed in a population-based case–control study in which patients were interviewed about self-examination practices, counts of naevi were made and 96% of all

[3] This has been explored further by Grant WB. 2008. Skin aging from ultraviolet irradiance and smoking reduces risk of melanoma: epidemiological evidence. Anticancer Res 28:4003-8.

biopsies were reviewed by a dermatopathologist. Sun exposure was significantly inversely associated with risk of death from melanoma: individuals who had ever been severely sunburned or who had had high levels of intermittent sun exposure were less likely to die from melanoma than those who had never been severely sunburned or who had had low levels of intermittent exposure [Berwick, et al. 2005]. The presence of solar elastosis was also inversely associated with death from melanoma. Skin 'awareness', although not skin self-examination or physician skin examination, was associated with a lower risk of death from melanoma. The Helios-I multicentre case–control study [Rosso, et al. 2008] also found an inverse correlation between death from melanoma and number of beach holidays.

The better survival of sun-exposed patients might result from better vitamin D status, induction of DNA repair capacity or the induction of indolent lesions; genetic factors might be responsible for the more aggressive lesions.

The role of genetic factors in melanoma is being explored in the Genes and Environment in Melanoma (GEM) study. This international study comprises over 3500 subjects enrolled in 2000. At baseline, original biopsies were reviewed and sun exposure evaluated. *CDKN2A* and *MC1R* alleles were sequenced and NER single nucleotide polymorphisms (SNPs) genotyped. Survival analysis will be complete at the end of 2008; *VDR* SNPs and relevant interacting genes will be genotyped.

2.6. Exposure assessment

Together with understanding the hazards asssociated with UV irradiation, knowledge of population exposure to solar UV is important in predicting the risk of skin disease. In everyday life, people are exposed to UV-radiation from a variety of sources and these vary in their properties.

Use is frequently made of questionnaires to determine exposure, as, for example, in the Norwegian–Swedish Women's Lifestyle and Health Cohort Study [Veierød, et al. 2003]. For a prospective study such as this, recall bias is less of a problem than for case–control studies. Estimates of personal exposure are also obtained using UV-sensitive film badges or electronic dosimeters. This requires high compliance by many people over a long period of time. For estimation of total body dose, assay of urinary dimers shows promise (see below) and is non-invasive. Mathematical modelling has the advantage of ability to predict how a system will behave without the need to undertake expensive, time-consuming or impractical experiments.

2.6.1. UV-induced pyrimidine dimers from human skin and urine

High levels of pyrimidine dimers, detectable by ^{32}P-postlabelling, are formed in human skin after exposure to a single dose of SS-UV irradiation, equivalent to an hour's summer exposure in Stockholm [Segerbäck, Annex III.9]. The levels exceed those of DNA adducts induced by a variety of chemical carcinogens by orders of magnitude. Dose–response relationships are found over a wide range of doses. Substantial inter-individual variation is found for the same UV dose but the cause is unknown. Sunscreen use is highly protective against dimer formation but tanning has little effect [Hemminki, et al. 2001].

Removal of DNA lesions is fast at first but slower later. 6-4 photoproducts are repaired much faster than CPDs and TT-C is repaired faster than TT-T (see Section 2.1.1). Large inter-individual differences are found in repair rates. Patients with BCC are less well able to repair TT-C and TT-T than controls. However, no difference was found for melanoma patients.

The use of skin biopsies for large-scale studies of the human response to UV is impractical and the procedure invasive and unsuitable for use with children. Analysis of UV-induced dimers excreted in urine is non-invasive and, importantly, gives a better estimation of total body dose. Solarium studies have shown that urinary dimer excretion correlates with UV dose

[Kotova, et al. 2005]. Background levels were found in adults during the summertime but not winter. A study of life guards in southern Sweden revealed high levels of urinary dimers. Sun bathing also results in high levels in adults and children. Large inter-individual differences were found in urinary dimer levels; this observation, together with the differences found in dimer levels in skin, protection by sunscreen and DNA repair rates among individuals has important implications for risk of developing skin cancer.

2.6.2. Ratio comparisons of spectrally differing UV source effects calculated with action spectra for different endpoints

The general population is exposed to a variety of natural and artificial UV-radiation sources, involuntarily or deliberately, e.g. solar UV, solaria, medical treatment [Wester, Annex III.31]. Ultraviolet-radiation emissions from different sources vary widely in spectral composition and irradiance level. For example, sunlight has a limited range of irradiance levels and UVB/UVA ratios, solaria have strong UVA but some or no UVB, and general illumination is weak with wide spectral variation. Standard action spectra have been published by a number of authorities for endpoints such as general UV hazard, erythema, NMSC (non-melanoma skin cancer) or previtamin D3 production in human skin. The SSI has measured different UV-radiation sources spectroradiometrically over many years. A logarithmic plot of vitamin D3- versus NMSC-effective irradiance falls on a straight line. Sunbed exposure is no guarantee of vitamin D induction. General lighting is orders of magnitude weaker than solar UV but falls on the same dose–response line. Illumination techniques can differ by 1 to 2 orders of magnitude.

2.6.3. UV-radiation weighted with action spectra against total ozone and solar zenith angle

Variations in UV-radiation weighted with previtamin D3 or SCUP-H (skin cancer action spectrum in mouse corrected for human skin) in comparison with erythemal were determined as functions of total ozone and solar zenith angle using the model libRadtran v.1.1 [Biszczuk, et al. 2008] [Biszczuk, Annex III.33]. The ratio of biologically effective UV (UVBE) to erythemal was calculated. Up to 70° solar zenith angle, doses of UV-radiation weighted with previtamin D3 action spectrum are higher compared with the erythemal dose. At all solar zenith angles, the intensity of SCUP-H-weighted UV-radiation is higher than the erythemal dose.

The variations in UVBE over Poland during the summer months of 1999-2001 were calculated using a UV reconstruction model formulated by A. Curylo as part of COST726 (see below). Higher values of UVBE were found in southern Poland and these were greatest in June and July.

2.6.4. Long-term changes and climatology of UV-radiation over Europe

Solar UV-radiation has a wide variety of effects on humans and the environment [Litynska, Annex III.32]. To determine its impact requires knowledge of UV climatology and changes that have occurred in the past. The COST726 action (http://www.cost726.org), founded in 2004, has the main objective of advancing the understanding of UV-radiation distribution under various meteorological conditions in Europe and to assess changes. Knowledge of UVBE doses and their geographical distribution is crucial for the European population. The initiative has a number of practical objectives and is organised into four working groups responsible, respectively, for: data collection, UV modelling, requirements for biological effects, and developing quality control recommendations and procedures. Among the final outcomes will be a European UV climatology atlas.

2.6.5. Modelling population exposure to solar radiation

A behavioural model has been developed that uses a random sampling technique to look at the variability of exposure at different times of year [Diffey 2008] [Diffey, Annex III.18].

Input data include ambient UV, obtained from satellite data and mean cloud cover, the fraction of solar UV received on the face, and time spent outdoors. Time spent outdoors was determined from a questionnaire, hosted on the Cancer Research UK website during the summer of 2007. It was found that weekday and summer exposure data could be fitted to a log-normal distribution whereas holiday exposure fitted a normal distribution.

Taking northern Europe (50°N) and Florida (28°N) as examples, 1000-fold and 200-fold differences, respectively, were found in exposure over the year. Annual facial exposure was ~150 SEDs for northern Europe and ~400 SEDs for Florida. Holiday exposure accounted for one-third and one-quarter, respectively, of the annual dose. The exposures obtained by modelling agreed well with data obtained by Danish personal monitoring studies and for indoor workers at 34°N in an American study. The annual UVA facial exposures in Florida and northern Europe were estimated to be approximately 1200 J/cm^2 and 700 J/m^2, respectively, giving a ratio of 1.71. This is appreciably lower than the ratio of erythemal facial exposures in these two locations (400:150 or 2.7), reflecting the fact that ambient UVA radiation shows a smaller dependence on latitude than erythemal (i.e. largely UVB) exposure.

Exposure levels are expected to increase as cheap flights make long-haul holidays available to more people and climate change results in changed behaviour. The model described can be rapidly adapted to different populations and and changes in behaviour so that the effects of these can be anticipated.

3. Discussion

This section summarises the main points made during the general discussion that followed the presentations.

3.1. Cell studies

Despite considerable efforts to determine the mechanism of UVA-induced genotoxicity, this has still not been clarified and conflicting results have been obtained. In UVA-irradiated Chinese hamster ovary (CHO) cells, greater amounts of cyclobutane pyrimidine dimers (CPDs) than 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo) were found [Douki, et al. 2003]. Similar results were obtained in primary cultures of skin cells and in whole skin [Mouret, et al. 2006]. In human fibroblasts, UVA induced significant levels of CPDs and elevated levels of 7,8-dihydro-8-deoxoguanine (8-oxodG) [Besaratinia, et al. 2005]. Analysis of lesions in the *p53* gene revealed CPD formation but the sites differed for UVB and UVA; oxidative damage sites were only found in UVA-treated cells. In BigBlue mouse cells, overall genomic and *cII* transgene UVA mutagenicity were mediated by oxidative damage [Besaratinia, et al. 2007]. In the *hprt* gene in human fibroblasts, C to T transitions were the predominant mutation for both UVA and UVB [Kappes, et al. 2006].

To resolve these differences, human melanocytes or keratinocytes would be suitable cell types for experimentation although results from keratinocytes could not be transposed to melanocytes and vice versa. Whether DNA damage or induction of mutations is the most suitable endpoint is unclear. Genotype, e.g. for DNA repair, must also be taken into account. It is important to study the mutagenic outcome of UVA and UVB in vivo, and human skin transplanted onto mice promises to be a suitable model. Wavelengths are necessarily tested separately but must also be combined to establish whether they behave differently in this situation.

There is likely selection of rare types of lesion in carcinogenesis so the lesions observed in tumours are not necessarily representative of the initial genotoxic events. Signature mutations are not necessarily UVA or UVB specific. G to T is very common but not necessarily the result of formation of 8-oxodG. *BRAF* mutations in melanoma have one main site, probably not due to UV, and may not be a good example of UV-induced carcinogenesis in humans. It is also unclear whether *BRAF* mutations seen early in life are the result of sun exposure. Dimer lesions do appear to be involved in *NRAS* mutations, hence these are probably UV induced. Care should be taken when using data from cell culture and extrapolating to the human situation. The sequencing of melanoma genomes should allow a better understanding of the role of UV in skin mutagenesis and carcinogenesis.

3.2. Experimental models

Experimental models have different advantages and disadvantages: some may be more convenient for acute exposure experiments whereas others may be more amenable for studying chronic exposures or in large-scale screening for toxic environmental agents. Experimental models have a role in establishing as much information as possible when similar experiments cannot be done in humans. The use of multiple models promises greater progress although caution must always be exercised in judging what is relevant for humans.

Good differentiation between wavelengths is necessary when carrying out experiments. For solar effects, solar-simulated light can be used. For other purposes, e.g. to replicate sunbed exposure, UVA- or UVB-enriched radiation may be used. Contaminating wavelengths can be a problem and the filters necessary to exclude unwanted wavelengths are expensive, hence, improvements are needed in this area. Nothing can simulate sun and so caution is needed in extrapolating to the human situation.

Little attention has been focussed on which melanocyte is the target for UV-induced genotoxicity and carcinogenicity in humans, e.g. an immature or susceptible stem cell progenitor. Stem cells in other systems are more resistant to DNA damage. Mutation formation is replication/S-phase dependent but it is unclear whether stem cells are proliferating or not. It is clearly important to analyse the most appropriate cell type.

Understanding melanocyte differentiation and migration in the (neonatal) mouse and the location of melanin in this system may be informative. Transgenic pigment models may be useful in understanding the role of human genes.

Very little is known about human pigmentation and how it is regulated and its interaction with melanoma. Melanins and their location differ with skin type, e.g different forms of melanin are found in black people and melanin is mostly located in keratinocytes. More information about melanin forms and precursors and their possible role as photosensitisers in UV-induced genotoxicity is desirable, given the differing susceptibility to melanoma seen amongst human skin types.

3.3. Human studies

There is a need for a biological action spectrum for melanoma and other skin cancers in mammals. In addition, a reliable method for classification of melanomas has implications for prognosis and treatment and work on the mutational status of melanomas is encouraging.

Determination of levels of exposure is important in determining risk of skin disease. A non-invasive urinary analysis method being developed shows promise. Methods for modelling exposure also promise to be useful in predicting the effects of climate change. Calibration of the radiometers used in exposure monitoring is important so that measurements can be compared for different times and locations.

Population studies indicate that chronic (e.g. occupational) exposure is less melanomagenic than intermittent exposure and may even be protective. Acquired naevi are a predictor of risk for melanoma. The identification of people who are at greater risk of the deleterious effects of UV exposure, whether because of genetics, age or exposure pattern is important for public health reasons. The sensitivity of children is an important issue; some early German reports suggested that children are less sensitive than adults [reviewed by Ellinger 1935, communicated by FR de Gruijl]. With age the distribution of melanoma changes with increases in melanoma on the trunk.

Because of the potentially greater exposure of the population to UVA while using UVB-blocking sunscreens or during use of modern sunbeds, further information on the role of UVA is crucial. It is also important to determine whether high irradiance is more carcinogenic per joule because sunbed regulations limit irradiance. The consequences of different spectral balances are also not properly understood. It may be necessary to change the design of sunbeds. Regulations should be policed to ensure their effectiveness.

The human race has been exposed to UV for thousands of years and, given that most cancers occur after reproductive age, humans can be said to be adapted. However, lighter skin types are not adapted to travelling to sunnier locations. The level of exposure in Sweden is high. Because of the uncertainties surrounding the length of time between exposure and occurrence, the levels of skin disease to be expected in the future are unclear.

Prevention recommendations include the use of sunscreens, UV protection of eyes, and to avoid sunbeds if less than 18 years old. For those aged less than 30, UV exposure involves greater risk because naevus development is still active but 18 is the age of majority. It should

be borne in mind that most studies have been carried out at higher latitudes when making recommendations for Northern Europe.

Science should be communicated clearly and honestly to decision-makers and the public, including the contribution of sunbed use and sunny holidays to UV exposure and the risks this represents. Sound advice is needed and co-operation between science and industry in the design of sunbeds to minimise the risks of UV exposure.

4. Recommendations

Knowledge gaps identified by presenters or during discussions at the meeting were used to generate a list of recommendations for future work. Recommendations for public health advice were similarly compiled. The recommendations were refined in the light of comments received from presenters when reviewing the draft manuscript.

4.1. Further studies

- Continue to develop models that mirror the human situation, e.g. human melanocytes or keratinocytes in vitro, mouse models, or human skin grafts in a mouse model, to determine the role of UVB vs UVA in skin carcinogenicity.
- Determine which melanocyte (immature or stem cell progenitor) is the target for UV-induced genotoxicity and carcinogenicity in humans with the aim of developing early detection methods or because different therapeutic methods might be more effective.
- Investigate the different melanin forms and precursors and their possible role as photosensitisers in UV-induced genotoxicity.
- Establish a reliable method for classification of melanomas to aid prognosis and effective targeted treatment.
- Establish a biological action spectrum for melanoma and other skin cancers in mammals.
- Determine the effect of UV irradiance on carcinogenic effects per joule with respect to both melanoma and squamous cell carcinoma of the skin (sunbed regulations limit irradiance).
- Develop non-invasive total exposure methods to identify high risk subpopulations.
- Investigate pathophysiology of UV-induced cataract to enable development of cheap pharmacological intervention methods, for use in the developing world.

4.2. Public health

- Regulate the calibration of radiometers used in exposure monitoring to ensure that measurements can be compared for different times and locations.
- Use exposure modelling to predict the results of lifestyle and climate change.
- Promote skin ‘awareness’ to lower risk of death from melanoma.
- Promote sunscreen use and limiting of sun exposure and inform public about ineffectiveness of tanning in providing protection against UV.
- Promote UV protection of eyes.
- Improve methodology for individual assessment of risk contributions from sunbathing and use of sunbeds.
- Recommend avoidance of sunbeds for those less than 18 years old, and possibly aged less than 30.
- Evaluate the design and regulation of sunbeds, in close association with industry, in the light of emerging evidence about the effects of UVA and establish effective policing of adherence to regulations.

- Establish clear lines of communication with policy makers, industry and the public.

5. References

Autier P, Boniol M, Doré J-F. 2007. Sunscreen use and increased duration of intentional sun exposure: Still a burning issue. Intl J Cancer 1212:1-5.

Ayala M, Strid H, Jacobsson U, Söderberg PG. 2007. p53 expression and apoptosis in the lens after ultraviolet radiation exposure. Invest Ophthalmol Visual Sci 48(9):4187-91.

Bäckvall H, Asplund A, Gustafsson A, Sivertsson Å, Lindeberg J, Ponten F. 2005. Genetic tumor archeology: microdissection and genetic heterogeneity in squamous and basal cell carcinoma. Mutat Res 571:65-79.

Berneburg M, Plettenberg H, Medve-König K, Pfahlberg A, Gers-Barlag H, Gefeller O, Krutmann J. 2004. Induction of the photoaging-associated mitochondrial common deletion *in vivo* in normal skin. J Invest Dermatol 122:1277-83.

Berneburg M, Gremmel T, Kürten V, Schroeder P, Hertel I, von Mikecz A, Wild S, Chen M, Declercq L, Matsui M, Ruzicka T, Krutmann J. 2005. Creatine supplementation normalizes mutagenesis of mitochondrial DNA as well as functional consequences. J Invest Dermatol 125:213-20.

Berwick M, Armstrong BK, Ben-Porat L, Fine J, Kricker A, Eberle C, Barnhill R. 2005. Sun exposure and mortality from melanoma. J Natl Cancer Inst 97(3):195-9.

Besaratinia A, Synold TW, Chen H-H, Chang C, Xi B, Riggs AD, Pfeifer GP. 2005. DNA lesions induced by UV A1 and B radiation in human cells: Comparative analyses in the overall genome and in the p53 tumor suppressor gene. Proc Natl Acad Sci USA 102(29):10058-63.

Besaratinia A, Kim S-I, Bates SE, Pfeifer GP. 2007. Riboflavin activated by ultraviolet A1 irradiation induces oxidative DNA damage-mediated mutations inhibited by vitamin C. Proc Natl Acad Sci USA 104(14):5953-8.

Besaratinia A, Kim S-I, Pfeifer GP. 2008. Rapid repair of UVA-induced oxidized purines and persistence of UVB-induced dipyrimidine lesions determine the mutagenicity of sunlight in mouse cells. FASEB J 22:2379-92.

Biszczuk J, Litynska Z, Curylo A. 2008. UV radiation influence on human health – action spectra: erythemal, previtamin D3, SCUP-H. Proceedings Electrotechnical Institute [Prace Instytutu Elektrotechniki ISSN 0032-6216] 234:205-16.

Bivik CA, Andersson EB, Rosdahl IK. 2005. Wavelength-specific effects on UVB induced apoptosis in melanocytes. A study of Bcl-2/Bax expression and keratinocyte rescue effects. Melanoma Res 15:7-13.

Bivik CA, Larsson PK, Kågedal KM, Rosdahl IK, Ollinger KM. 2006. UVA/B-induced apoptosis in human melanocytes involves translocation of cathepsins and Bcl-2 family members. J Invest Dermatol. 126(5):1119-27

Bivik C, Rosdahl I, Öllinger K. 2007. Hsp70 protects against UVB induced apoptosis by preventing release of cathepsins and cytochrome *c* in human melanocytes. Carcinogenesis 28(3):537-44.

Costin G-E, Hearing VJ. 2007. Human skin pigmentation: melanocytes modulate skin color in response to stress. FASEB J 21:976-94.

Curtin JA, Busam K, Pinkel D, Bastian BC. 2006. Somatic activation of KIT in distinct subtypes of melanoma. J Clin Oncol 24(26):4340-6.

De Fabo EC, Noonan FP, Fears T, Merlino G. 2004. Ultraviolet B but not ultraviolet A radiation initiates melanoma. Cancer Res 64:6372-6.

Diffey B. 2008. A behavioural model for estimating population exposure to solar ultraviolet radiation. Photochem Photobiol 84:371-5.

dos Santos Silva Isabel, Higgins Craig D, Abramsky Tanya, Swanwick Maureen A, Frazer Jacqueline, Whitaker Linda M, Blanshard Margaret E, Bradshaw John, Apps John M, Bishop D Timothy, Newton-Bishop Julia A, Swerdlow Anthony J. 2009. Overseas sun exposure, nevus counts, and premature skin aging in young English women: A population-based survey. J Invest Dermatol 129:50-9.

Douki T, Reynaud-Angelin A, Cadet J, Sage E. 2003. Bipyrimidine photoproducts rather than oxidative lesions are the main type of DNA damage involved in the genotoxic effect of solar UVA radiation. Biochemistry 42(30):9221-6
Dunn J, Potter M, Rees A, Rünger TM. 2006. Activation of the Fanconi Anemia/BRCA pathway and recombination repair in the cellular response to solar ultraviolet light. Cancer Res 66(23):11140-7.
Ellinger F. 1935. Die biologischen Grundlagen der Strahlenbehandlung. Berlin and Vienna, Urban & Schwarzenberg.
Enninga IC, Groenendijk RTL, Filon AR, van Zeeland AA, Simons JWIM. 1986. The wavelength dependence of u.v.-induced pyrimidine dimer formation, cell killing and mutation induction in human diploid skin fibroblasts. Carcinogenesis 7:1829-36
Eskandarpour M, Hashemi J, Kanter L, Ringborg U, Platz A, Hansson J. 2003. Frequency of UV-inducible NRAS mutations in melanomas of patients with germline CDKN2A mutations. J Natl Cancer Institute 85(11):790-8.
Gandini S, Sera F, Cattaruzza MS, Pasquini P, Abeni D, Boyle P, Francesco Melchi C. 2005a. Meta-analysis of risk factors for cutaneous melanoma: I. Common and atypical naevi. Eur J Cancer 41:28-44.
Gandini S, Sera F, Cattaruzza MS, Pasquini P, Picconi O, Boyle P, Francesco Melchi C. 2005b. Meta-analysis of risk factors for cutaneous melanoma: II. Sun exposure. Eur J Cancer 41:45-60.
Gandini S, Raimondi S, Gnagnarella P, Doré JF, Maisonneuve P, Testori A. 2009. Vitamin D and skin cancer: a meta-analysis. Eur J Cancer. 45(4):634-41
Giglia-Mari G, Sarasin A. 2003. TP53 mutations in human skin cancers. Hum Mutat 21:217-28.
Grant WB. 2008a. Hypothesis - ultraviolet-B irradiance and vitamin D reduce the risk of viral infections and thus their sequelae, including autoimmune diseases and some cancers. Photochem Photobiol 84:356-65.
Grant WB. 2008b. Response to comments by Norval and Woods to my hypothesis regarding vitamin D viral infections and their sequelae. Photochem Photobiol 84:806-8.
Gueranger Q, Stary A, Aoufouchi S, Faili A, Sarasin A, Reynaud CA, Weill JC. 2008. Role of DNA polymerases eta, iota, and zeta in UV resistance and UV-induced mutagenesis in a human cell line. DNA Repair 7:1551-62.
Hemminki K, Xu G, Le Curieux F. 2001. Ultraviolet radiation-induced photoproducts in human skin DNA as biomarkers of damage and its repair. IARC Sci Publ 154:69-79.
IARC. 2001. Sunscreens. In: Handbooks of Cancer Prevention Vol. 5; Lyon, IARC, WHO.
IARC. 2006. The association of use of sunbeds with cutaneous malignant melanoma and other skin cancers: A systematic review. Int J Cancer 120:1116–22.
Kappes UP, Luo D, Potter M, Schulmeister K, Rünger TM. 2006. Short- and long-wave UV light (UVB and UVA) induce similar mutations in human skin cells. J Invest Dermatol 126:667-75.
Kato M, Takeda K, Kawamoto Y, Twuzuki T, Hossain K, Tamakoshi A, Kunisada T, Kambayashi Y, Ogino K, Suzuki H, Takahashi M, Nakashima I. 2004. c-Kit-targeting immunotherapy for hereditary melanoma in a mouse model. Cancer Res 64:801-6.
Kielbassa C, Roza L, Epe B. 1997. Wavelength dependence of oxidative DNA damage induced by UV and visible light. Carcinogenesis 18:811-6.
Kim M, Gans JD, Nogueira C, Wang A, Paik J-H, Feng B, Brennan C, Hahn WC, Cordon-Cardo C, Wagner SN, Flotte TJ, Duncan LM, Granter SR, Chin L. 2006. Comparative oncogenomics identifies NEDD9 as a melanoma mestastasis gene. Cell 125:1269-81.
Kotova N, Hemminki K, Segerbäck D. 2005. Urinary thymidine dimer as a marker of total body burden of UV-inflicted DNA damage in humans. Cancer Epidemiol Biomarkers Prev 14(12):2868-72.
Landi MT, Bauer J, Pfeiffer RM, Elder DE, Hulley B, Minghetti P, Calista D, Pinkel D, Kanetsky PA, Bastian BC. 2006. MC1R germline variants confer risk for BRAF-mutant melanoma. Science 313:512.

Lee JT, Herlyn M. 2007. Microenvironmental influences in melanoma progression. J Cell Biochem 101:862-72.
Lindström E, Shimokawa T, Toftgård R, Zaphiropoulos PG. 2006. PTCH mutations: distribution and analyses. Hum Mutat 27:215-19.
Miller AJ, Mihm MC. 2006. Mechanisms of disease: Melanoma. N Engl J Med 355:51-65.
Mitchell D, Paniker L, Sanchez G, Trono D, Nairn R. 2007. The etiology of sunlight-induced melanoma in Xiphophorus hybrid fish. Mol Carcinog 46(8):679-84
Mocellin S, Nitti D. 2008. Vitamin D receptor polymorphisms and the risk of cutaneous melanoma: a systematic review and meta-analysis. Cancer 113(9):2398-407.
Mouret S, Baudouin C, Charveron M, Favier A, Cadet J, Douki T. 2006. Cyclobutane pyrimidine dimers are predominant DNA lesions in whole human skin exposed to UVA radiation. Proc Natl Acad Sci USA 103:13765-70.
Nijhof JGW, Braun KM, Giangreco A, van Pelt C, Kawamoto H, Boyd RL, Willemze R, Mullenders LHF, Watt FM, de Gruijl FR, van Ewijk W. 2006. The cell-surface marker MTS24 identifies a novel population of follicular keratinocytes with characteristice of progenitor cells. Development 133:3027-37.
Nilsen LT, Hannevik M, Aalerud TN, Johnsen B, Friberg EG, Veierød MB. 2008. Trends in UV irradiance of tanning devices in Norway: 1983-2005. Photochem Photobiol 84(5):1100-8
Noonan FP, Recio JA, Takayama H, Duray P, Anver MR, Rush WL, Lindner G, De Fabo EC, Merlino G. 2001. Neonatal sunburn and melanoma in mice. Nature 413:271-2.
Noonan FP, Dudek J, Merlino G, De Fabo E. 2003. Animal models of melanoma: an HGF/SF transgenic mouse model may facilitate experimental access to UV initiating events. Pigment Cell Res 16:16-25.
Norval M, McLoone P, Lesiak A, Narbutt J. 2008. The effect of chronic ultraviolet radiation on the human immune system. Photochem Photobiol 84:19-28.
Pouget J-P, Douki T, Richard M-J, Cadet J. 2000. DNA damage induced in cells by γ and UVA radiation as measured by HPLC/GC-MS and HPLC-EC and comet assay. Chem Res Toxicol 13(7):541-9.
Reimann V, Krämer U, Sugiri D, Schroeder P, Hoffmann B, Medve-Koenigs K, Jöckel KH, Ranft U, Krutmann J. 2008. Sunbed use induces the photoaging-associated mitochondrial common deletion. J Invest Dermatol 128(5):1294-7
Rosso S, Sera F, Segnan N, Zanetti R. 2008. Sun exposure prior to diagnosis is associated with improved survival in melanoma patients: Results from a long-term follow-up study of Italian patients. Eur J Cancer 44(9):1275-81.
Setlow RB, Grist E, Thompson K, Woodhead AD. 1993. Wavelengths effective in induction of malignant melanoma. Proc Natl Acad Sci USA 90(14):6666-70.
Stary A, Kannouche P, Lehmann A, Sarasin A. 2003. Role of DNA polymerase eta in the UV mutation spectrum in human cells. J Biol Chem 278:18767-75.
Rünger TM. 2007 How different wavelengths of the ultraviolet spectrum contribute to skin carcinogenesis - role of the cellular damage response. J Invest Dermatol 127:2103-5
Rünger TM. 2008. C to T transition mutations are not solely UVB-signature mutations, because they are also generated by UVA. J Invest Dermatol 128:2138-40
Rünger TM, Kappes UP. 2008. Mechanisms of mutation formation with long-wave ultraviolet light (UVA). Photodermatol Photoimmunol Photomed 24:2-10
Tyrrell RM. 1973. Induction of purimidine dimers in bacterial DNA by 365 nm radiation. Photochem Photobiol 17:69-73.
Veierød MB, Weiderpass E, Thörn M, Hansson J, Lund E, Armstrong B, Adami H-O. 2003. A prospective study of pigmentation, sun exposure, and risk of cutaneous malignant melanoma in women. J Natl Cancer Inst 95(20):1530-8.
Wachsmuth RC, Turner F, Barrett JH, Gaut R, Randerson-Moor JA, Bishop DT, Newton Bishop JA. 2004. The effect of sun exposure in determining nevus density in UK adolescent twins. J Invest Dermatol 124:56-62.
Wood SR, Berwick M, Ley RD, Walter RB, Setlow RB, Timmins GS. 2006. UV causation of melanoma in *Xiphophorus* is dominated by melanin photosensitized oxidant production. Proc Natl Acad Sci USA 103(11):4111-5.

Yang G, Curley D, Bosenberg MW, Tsao H. 2007. Loss of *Xeroderma Pigmentosum C* (*Xpc*) enhances melanoma photocarcinogenesis in *Ink4a-Arf*-deficient mice. Cancer Res 67(12):5649-57.

Annex I. Programme

UV-radiation induced disease - Roles of UVA and UVB
October 18-20, 2007

October 17 **18.00 Welcoming Reception**
Location: Vin-& Sprithistoriska museet (The Historical Museum of Wines and Spirits) Dalagatan 100, Stockholm

October 18 **Opening Day – Nobel Forum**
08:00-09:00 **Registration – Nobel Forum**
09.00-09.15 **Welcome Address** – Rune Toftgård (Chairman of the Organizing Committee)
Session 1: **Cellular Effects of UV-Radiation**
Chairpersons: Alain Sarasin and Dan Segerbäck
09.15-09.45 Thierry Douki (France) *UVA-induced DNA damage in cells: oxidative lesions and pyrimidine dimers*
09.45-10.15 Alain Sarasin (France) *Molecular mechanisms of UV-induced mutagenesis in human cells*
10.15-10.45 Coffee break – Press Conference
10.45-11.15 Rex Tyrrell (UK) *UVA, a strong inducer of the anti-inflammatory stress response involving heme oxyenase 1*
11.15-11.45 Jean Krutmann (Germany) *UVA radiation-induced mt DNA mutagenesis*
12.00-13.00 Lunch
13.00-13.30 Thomas Rünger (USA)*Role of cellular DNA damage responses for the mutagenic outcome of exposures to UVA and UVB*
13.30-14.00 Gerd Pfeifer (USA) *Mechanisms of UV- and sunlight-induced mutagenesis*
14.00.14.30 Tim Bowden (USA) *UVA Mediated signaling pathways in human keratinocytes*[4]
14.30-15.00 Coffee break
15.00-15.30 Anna Asplund (Sweden) *Morphology-based analysis of skin carcinogenesis*
15.30-16.00 Dan Segerbäck (Sweden) *^{32}P-Postlabelling analysis of UV induced pyrimidine dimers from human skin and urine*
Session 2: **Experimental Models of UV-Induced Disease**
Chairpersons: Meenhard Herlyn and Rune Toftgård
16.00-16.30 Timothy Heffernan (USA) *Mouse models of melanoma*

Short talks
16.30-17.30

October 19 – Day 2
Session 2: **Experimental Models of UV-Induced Disease**
Chairpersons: Meenhard Herlyn and Rune Toftgård
08.30-09.00 Edward De Fabo (USA) *Role of UVB and UVA in melanoma*
09.00-09.30 Graeme Walker (Australia) *Induction of melanoma in mice: the role of UVR-induced melanocyte proliferation*
09.00-10.00 Graham Timmins (USA) *Animal models of melanoma*
10.00-10.30 Coffee break
10.30-11.00 Meenhard Herlyn (USA) *Human melanocyte transformation*
11.00-11.30 Frank de Gruijl (The Netherlands) *Differential effects of UVB and UVA1 radiations on keratinocytes and melanocytes in murine skin carcinogenesis*

[4] This presentation did not take place

11.30-12.00 Per Söderberg (Sweden) *Ultraviolet radiation induced cataract - what did we learn from experiments?*

12.00-13.00 Lunch

Session 3: Human Studies of UV-Induced Disease

Chairpersons: Marianne Berwick and Johan Hansson

13.00-13.30 Julia Newton Bishop (UK) *Melanoma susceptibility genes and interaction with sun exposure*

13.30-14.00 Brian Diffey (UK) *A behavioural model for estimating population exposure to solar ultraviolet radiation*

14.00-14.30 Philippe Autier (France) *Indoor tanning and skin cancer*

14.30-15.00 Coffee break

15.00-15.30 Marit Bragelien Veierød (Norway) *Exposure to solar and artificial ultraviolet radiation and the risk of cutaneous malignant melanoma – The Norwegian-Swedish Women´s Lifestyle and Health Cohort Study*

Short talks

15.30-16.30

19.00 Conference Dinner at Stallmästaregården

October 20 - Day 3

Session 3: Human Studies of UV-induced Disease

Chairpersons: Marianne Berwick and Johan Hansson

08.30-09.00 Boris Bastian (USA) *Distinct patterns of geneticGenetic and phenotypic alternationsPhenotypic Alterations in melanoma that depend on anatomic site and degree of UV exposure*

09.00-09.30 Johan Hansson (Sweden) *NRAS and BRAF mutations in human cutaneous melanoma*

09.30-10.00 Antony Young (UK) *Effects of sub-erythemal exposure on human skin in vivo*

10.00-10.30 Coffee break

10.30-11.00 Inger Rosdahl (Sweden) *Signaling sathways in UVA and UVB induced apoptosis in human MC*

11.00-11.30 Marianne Berwick (USA) *Solar exposure as a prognostic factor in melanomas*

11.30-12.15 General Discussion and Recommendations

12.15-12.30 Closing of the meeting

Annex II. Participants

Name	Affiliation
Asp, Helene	Swedish Radiation Protection Authority, Sweden
Asplund, Anna	Uppsala Universitet, Sweden
Autier, Philippe	Jules Bordet Institute, Brussels, Belgium
Backenhorn, Katja	Lund University, Sweden
Bastian, Boris	University of California, San Francisco, USA
Beitner, Harry	Karolinska University Hospital, Sweden
Berne, Berit	Akademiska Hospital, Uppsala, Sweden
Berwick, Marianne	Imperial College, London, UK
Biszczuk, Julita	Institute of Meteorology and Water Management, Poland
Bivik,Cecilia	Linköping University, Sweden
Bragelien Veierød, Marit	University of Oslo, Norway
De Fabo, Edward	George Washington University, USA
De Gruijl, Frank	Leiden University Medical Center, The Netherlands
Delinassios, George	St John's Institute of Dermatology, UK
Diffey, Brian	University of Newcastle upon Tyne, UK
Douki, Thierry	CEA-Grenoble, France
Emeny, Jean	University of Leicester, UK
Galichanin, Konstantin	St Erik's Hospital, Sweden
Grant, William	Sunlight, Nutrition and Health Research, USA
Håkansson, Daniel	Stockholm University, Sweden
Hansson, Johan	Karolinska Universitet, Sweden
Heffernan, Timothy	Dana Farber Cancer Institute, Boston, USA
Herlyn, Meenhard	Wistar Institute, Philadelphia, USA
Jenssen, Dag	Stockholm University, Sweden
Johansson, Fredrik	Stockholm University, Sweden
Kowalczuk, Christine	Health Protection Agency, Radiation Protection Division, UK
Krutmann, Jean	Heinreich-Heine University, Dusseldorf, Germany
Krynitz, Britta	Karolinska University Hospital, Sweden
Laurent, Roland	Ultra Tan AB, Sweden
Lindström, Erika	Karolinska Institutet, Sweden
Litynska, Zenobia	Institute of Meteorology and Water Management, Poland
Lucas, Robyn	Australian National University, Canberra, Australia
Newton Bishop, Julia	St James' University Hospital, Leeds, UK
Nilsen, Lill Tove	Norwegian Radiation Protection Authority, Norway
Nylén, Per	Swedish Work Environment Authority, Sweden
Öllinger, Karin	Linköping University, Sweden
Pastila, Riikka	Radiation and Nuclear Safety Authority, Finland
Paulsson, Lars-Erik	Swedish Radiation Protection Authority, Sweden
Pfeifer, Gerd	Beckman Research Institute, Duarte, California, USA
Rannug, Agneta	Karolinska Institutet, Sweden
Richarz, Frank	JK Holding GmbH, Germany
Rodvall Ylva	Stockholm County Council, Sweden
Rosdahl, Inger	Linköping University, Sweden
Rünger, Thomas	Boston University School of Medicine, USA
Sandberg, Tove	Karolinska Institutet, Sweden
Sarasin, Alain	CNRS, Institut Gustave Roussy, France
Segerbäck, Dan	Karolinska Institutet, Sweden
Söderberg, Per	Karolinska Insitutet, Sweden

Thunell, Lena	Linköping University, Sweden
Timmins, Graham	University of New Mexico, USA
Tyrrell, Rex	University of Bath, UK
Walker, Graeme	University of Abertay Dundee, UK
Wester, Ulf	Swedish Radiation Protection Authority, Sweden
Westermark, Karin	Swedish Radiation Protection Authority, Sweden
Wäster, Petra	Linköping University, Sweden
Young, Antony	Kings College, University of London, UK

Annex III. Abstracts[5]

III.1. UVA-induced DNA damage in cells: oxidative lesions and pyrimidine dimers

Thierry Douki
Laboratoire Lésions des Acides Nucléiques, DRFMC/SCIB UMR-E N°3 CEA/UJF; CEA-Grenoble, France

The link between skin carcinogenesis and exposure to solar ultraviolet radiation is unambiguously established. DNA has been identified as a main target because modification of its chemical structure may lead to mutations and initiate tumour progression. However, the nature of the DNA damage varies from one part of the solar spectrum to the other. The UVB (280-320 nm) photochemistry of DNA is now well understood but much more questions remain for the UVA range (320-400 nm).

DNA exhibits an absorption maximum around 270 nm. Therefore, it is a major cellular chromophore for UVB radiations. Excitation of the DNA bases by the absorbed photons leads to the formation of the well known pyrimidine dimers between adjacent thymine and/or cytosine. Two types of lesions, namely cyclobutane pyrimidine dimers (CPD) and pyrimidine (6-4) pyrimidone photoproducts (64PP), can be formed at each of the four possible bipyrimidine dinucleotides (TT, TC, CT and CC). However, all of these photoproducts are not produced in the same yield [Douki and Cadet 2001]. TT and TC are much more photoreactive than CT and CC. In addition, the ratio between the yields of CPD and 64PP greatly depends on the two pyrimidines involved. Interestingly, the distribution of bipyrimidine photoproducts is the same in isolated DNA, in cultured mammalian cells and in human skin. This observation emphasizes that UVB photochemistry of DNA only results from direct absorption with very little influence of external factors. However, a noticeable result is the 20-fold protection afforded by skin as the result of shielding by the upper layers of the epidermis [Mouret, et al. 2006].

UVA photons are only very poorly absorbed by DNA. However, UVA has been shown to be mutagenic in cultured cells. UVA-induced chemical modifications of DNA are likely to be triggered by photosensitization process. In these pathways, UVA radiation is absorbed by endogenous chromophores (yet still unidentified) that then transfer their energy to molecular oxygen leading to the formation of singlet oxygen. An alternative but less likely pathway involves one-electron abstraction from DNA by the excited sensitizer. With both photosensitization processes, guanine is the predominant target within cellular DNA. However, UVA is also known to induce the cleavage of the DNA backbone. Small amounts of oxidation products of pyrimidines bases are also detected [Pouget, et al. 2000]. These observations show that unspecific and highly reactive hydroxyl radicals are also produced, although to a lesser extent than singlet oxygen.

The distinction between UVA- and UVB-induced DNA damage is not so clear. Indeed, CPDs have also been observed in the DNA of UVA-irradiated cells and skin. These results have often been disregarded because contaminant UVB in the light source was suspected. However, recent more extensive studies showed that the distribution of UVA-induced dimeric photoproducts in cells was drastically different from that triggered by direct absorption [Douki, et al. 2003; Courdavault, et al. 2004]. No 64PP was found and an almost exclusive formation of TT CPD was observed. The photochemical process leading to CPD upon UVA irradiation remains unknown, although a photosensitized triplet energy transfer could be involved. Interestingly, the yield of the most frequent guanine oxidation product (8-oxo-7,8-dihydroguanine) was found to be 3 to 6 times-lower than that of TT CPD. This observation

[5] Abstracts in this Annex are reproduced as provided, except that full details of papers cited have been added where necessary.

shows that oxidative damage is not the main pathway involved in the genotoxicity of UVA. The same observations were made in human biopsies [Mouret, et al. 2006]. Skin afforded a much more limited protection against UVA- than UVB-induced DNA damage.

The role of UVB-induced DNA photoproducts in skin carcinogenesis clearly appears in the mutation spectra determined in skin tumours where mutational events at bipyrimidine sites predominate. The contribution of UVA to solar mutagenesis is more difficult to evaluate. First, a larger variety of DNA damage is produced. In addition, the yield of lesions is lower than with UVB although it is partly compensated by the higher intensity of UVA radiation. Data are thus needed to identify the most deleterious DNA lesions in the UVA range in order to evaluate the consequences of increase in exposure to UVA (sunbathing, artificial tanning equipment) and to better assess UVA photoprotection of modern sunscreens.

References

Courdavault S, Baudouin C, Charveron M, Favier A, Cadet J, Douki T. 2004. Larger yield of cyclobutane dimers than 8 oxo-7,8-dihydroguanine in the DNA of UVA-irradiated human skin cells. Mutat Res 556:135-42.

Douki T, Cadet J. 2001. Individual determination of the yield of the main-UV induced dimeric pyrimidine photoproducts in DNA suggests a high mutagenicity of CC photolesions. Biochemistry 40:2495-501.

Douki T, Reynaud-Angelin A, Cadet J, Sage E. 2003. Bipyrimidine photoproducts rather than oxidative lesions are the main type of DNA damage involved in the genotoxic effect of solar UVA radiation. Biochemistry 42:9221-6.

Mouret S, Baudouin C, Charveron M, Favier A, Cadet J, Douki T. 2006. Cyclobutane pyrimidine dimers are predominant DNA lesions in whole human skin exposed to UVA radiation. Proc Natl Acad Sci USA 103:13765-70.

Pouget J-P, Douki T, Richard M-J, Cadet J. 2000. DNA damage induced in cells by gamma and UVA radiations as measured by HPLC/GC-MS, HPLC-EC and comet assay. Chem Res Toxicol 13:541-9.

III.2. Molecular mechanisms of UV-induced mutagenesis in human cells

A. Sarasin[1], A. Stary[1], Q. Gueranger[2], C.-A. Reynaud[2], J.-C. Weill[2]
[1]CNRS FRE 2939 CNRS, Institut Gustave Roussy, 94805 Villejuif, France
[2]INSERM U783, Faculté Necker, 75730 Paris, France

Several patients exhibit UV-sensitivity caused by some type of DNA repair deficiency. Classical xeroderma pigmentosum (XP) patients do not excise UV-induced DNA lesions. Mutations observed in UV-irradiated XP cells are, therefore, a direct image of error-prone translesion synthesis (TLS) across all types of unrepaired UV-induced damage. XP variant patients (XPV) are deficient in the TLS polymerase eta (Pol eta), the unique TLS polymerase able to replicate in vitro efficiently opposite TT dimer photoproducts with few mistakes if any. Mutations observed in XPV cells reflect the TLS activity of polymerases other than DNA polymerase eta. All these patients develop numerous UV-induced skin cancers. Mutation spectra observed in some key genes isolated from these tumours, such as the p53 tumour suppressor gene, reflect the in vivo consequences of TLS across the lesions. Analysis of these tumours confirms that UV-induced mutagenesis is basically characterized by C to T transitions localized at dipyrimidine sites. CC to TT tandem mutations represent the signature of UV-induced mutagenesis. Their frequencies are exacerbated in classical XP cells or tumours, probably due to an increase of cytosine deamination with time, leading to uracil that is replicated by Pol eta as a T. In XPV tumours, increased mutagenesis is observed, as compared to normal cells, because the relatively error-free Pol eta is absent and replaced by another TLS polymerase more error-prone, especially on T-containing dimers. The level of C to T transitions decreases as compared to classical XP as well as the tandem frequency.

To evaluate the contribution of Pol eta to UV mutagenesis in vivo, we compared the UV-induced mutation spectra produced in the Pol eta-deficient XPV cell line (XP30RO) and in stable Pol eta-complemented clones derived from these cells. We assessed by DNA sequencing the error rates and the types of mutations generated by replication of UVC-irradiated SV40-based shuttle vectors in unirradiated or UVC-irradiated human host cells. Because replication of UV-damaged plasmids requires the human cellular replication machinery, the level and the kind of mutagenic TLS are dependent on the presence of Pol eta in host human cells and can be modulated by the localization of Pol eta in replication foci after UV radiation. UV-irradiation of host cells prior to shuttle vector transfection strongly increases the mutation frequency on undamaged templates. This increase is independent of Pol eta because we found roughly the same level of mutations in the Pol eta-complemented clones. It suggests that UV-insult to host cells can lead to abnormal replication of unirradiated vectors in a Pol eta-independent manner. Irradiation of both shuttle vectors and host cells leads to the highest mutation frequency in the XPV cell line. Complementation by Pol eta reduces strongly the mutation frequencies by 4-fold with a similar decrease for mutations at C:G and T:A base pairs to that observed in wild type cells. XPV cells are therefore less likely than normal cells to incorporate dAMP and dGMP opposite UV photoproducts containing T and C respectively. Thus, Pol eta is required in vivo not only to the accurate bypass photoproducts containing thymine residue, in agreement with biochemical data, but also contributes to error-free bypass of UV lesions containing a cytosine residue.

It was interesting to consider which TLS polymerases might be responsible for the error-prone bypass of UV damage in Pol eta-deficient XPV cells. One candidate is Pol iota, which physically interacts with Pol eta and colocalized with Pol eta at replication foci after UV irradiation. Another candidate is Pol zeta, which can bypass TT dimers in vitro and is required for a large proportion of UV mutagenesis in vivo. To analyze the role of this two TLS DNA polymerases in UV-induced mutagenesis, Burkitt's lymphoma BL2 cells have been engineered to specifically knock-out, by homologous recombination, genes coding for Pol iota, Rev3 (the catalytic sub-unit of Pol zeta) and both Pol iota and Pol eta. Analysis of the mutation spectra, using UV-irradiated shuttle vectors replicating in these three types of cells, reveals the role and properties of these two TLS polymerases in vivo during the bypass of UV-induced DNA lesions. The large database, we obtained by sequencing more than 1200 mu-

tants, allowed us a detailed analysis of UV-induced mutations through the identification of hotspot positions whose occurrence reaches statistical significance. In the absence of Rev3, there was a marked increase in the proportion of C to T transitions targeted at the 3' C of TC and CC sites, correlated with the presence of five hotspot positions unique to this cell line and occurring in this context. Conversely mutations at the 3' T of TT and CT sites dropped down considerably in the absence of Rev3, suggesting that such lesions could not be copied without Pol zeta. Specific mutation hotspots were only observed in the Pol eta-deficient cell lines. These mutations, which disappeared in the Pol eta/Pol iota-deficient cell line, were concentrated on TT sites (3 out of 5), and may thus be due to a back-up mutagenic bypass of TT CPDs by Pol iota. Several other hotspots, present in the Pol iota deficient background, suggested in contrast an error-free bypass by Pol iota, either alone or in combination with Pol zeta. These results strengthen the suggestion that Pol iota is involved in error-prone bypass of UV-induced lesions in the absence of Pol eta and suggest for the first time the general involvement of Pol iota in vivo in the antimutagenic bypass of some precise UV-induced lesions.

These results allow us to better understand the molecular mechanism of UV-induced mutagenesis and data concerning the properties of several TLS DNA polymerases may shed light also on the origin of other types of cancers.

References

Giglia-Mari G, Sarasin A. 2003. TP53 mutations in human skin cancers. Hum Mutat 21:217-28.
Gueranger Q, Stary A, Aoufouchi S, Faili A, Sarasin A, Reynaud C-A, Weill J-C. 2008. Role of human DNA polymerases eta, iota and zeta in UV-resistance and UV-induced mutagenesis. DNA Repair 7:1551-62.
Stary A, Kannouche P, Lehmann AR, Sarasin A. 2003. Role of DNA polymerase eta in the UV mutation spectrum in human cells. J Biol Chem 278:18767-75.

III.3. UVA and the role of the heme oxygenase 1 stress response

R.M. Tyrrell
Department of Pharmacy and Pharmacology, University of Bath, Bath, BA2 7AY, UK

The UVA component of sunlight generates a major oxidative stress in cells [Tyrrell 1991] which is further exacerbated by the release of the pro-catalytic factors, free iron and heme. About 20 years ago it was observed that biologically relevant fluences of UVA radiation induced a 32Kd stress protein in human cells which we identified [Keyse and Tyrrell 1989] as the heme catabolic enzyme, heme oxygenase 1 (HO-1). Following this initial observation we established that this was a general response to oxidative stress in mammalian cells and the levels of activation remain the highest for any oxidant-inducible gene expression observed to date. Furthermore, in the case of UVA, the response is mediated via singlet oxygen [Basu-Modak and Tyrrell 1993]. Evidence also soon emerged that this was a key protective enzyme in cells [Vile, et al. 1994]. However, a major impetus to the field was the discovery that the stress response is a major anti-inflammatory mechanism in mammals and the enzyme has now been implicated in many disease states [Otterbein and Zuckerbraun 2005]. Understanding the mechanism of action of this pathway will be a key to developing new therapies including those reversing endothelial dysfuntion. Using UVA as the model oxidant in skin cells still provides a powerful experimental system to understand the regulatory pathways underlying activation of this gene.

Much has been learnt about regulation of gene expression and homeostatic mechanisms by observing how key stress genes designed to respond to abrupt changes in the environment are maintained in a quiescent state under stress-free conditions. Mammalian cells respond to a large range of stresses including heat, toxic agents (e.g. heavy metals) and oxidants. The HO1 response is currently the most studied of these responses and it is now understood that the negative regulation of this anti-inflammatory oxidant stress protein is crucial to maintaining cellular homeostasis under stress-free conditions [see Tyrrell in Otterbein and Zuckerbraun 2005]. The clearest molecular model to emerge for regulation of HO-1 at the transcriptional level involves the dynamic exchange between Nrf2/MafK transcriptional activation complexes and Bach1/MafK suppressor complexes at the pair of cis-acting elements (MARE, StREs, AP-1) located in the mouse and human HO-1 promoter upstream region. For the environmental oxidant, UVA radiation, we have examined the role of heme, Nrf2, and Bach1 in HO-1 regulation. Changes in heme status and Nrf2 activity are clearly involved in the up-regulation by UVA and both heme and Bach1 are involved in maintaining low expression. Under acute UVA stress, heme is released and Nrf2 accumulates in the nucleus while Bach1 binds to heme, loses its DNA binding and is exported from the nucleus allowing transcriptional up-regulation of HO-1. The removal of heme by the induced HO-1 and de novo synthesis of Bach1 leads to the development of refractoriness to further induction by UVA and heme. Removal of heme by the novel constitutive expression of HO-2 in human keratinocytes appears to dampen the HO1 responses in these crucial cells and the response also involves changes in nuclear expression and transport of Bach1. These in vitro models, together with organotypic skin cultures and skin biopsies will eventually define the role of the heme oxygenases in this crucial organ [see Reeve and Tyrrell 2007] and provide greater insight as to the crucial role played by this enzyme generally in maintaining cellular homeostasis, protecting organs and preventing disease.

References

Basu-Modak S, Tyrrell RM. 1993. Singlet oxygen. A primary effector in the UVA/near visible light induction of the human heme oxygenase gene. Cancer Res 53:4505-10.
Keyse SM, Tyrrell RM. 1989. Heme oxygenase is the major 32-kDa stress protein induced in human skin fibroblasts by UVA radiation, hydrogen peroxide and sodium arsenite. Proc Natl Acad Sci USA 86:99-103.
Otterbein L, Zuckerbraun BS. eds. 2005. Heme oxygenase: The elegant orchestration of its products in medicine, pp 333-49, New York, Nova Science Publishers.

Reeve V, Tyrrell R. 2007. UVA and inducible protection. In: Giacomoni P. ed. Biophysical and physiological effects of solar radiation on human skin, pp 293-310, Cambridge, UK, RSC Publications.
Tyrrell RM. 1991. UVA (320-380 nm) radiation as an oxidative stress. In: Sies H. ed. Oxidative stress: oxidants and antioxidants, pp 57-83, London, Academic Press.
Vile GF, Basu-Modak S, Waltner C, Tyrrell RM. 1994. Heme oxygenase 1 mediates an adaptive response to oxidative stress in human skin fibroblasts. Proc Natl Acad Sci USA 91:2607-10.

III.4. UVA radiation – induced mt DNA mutagenesis

Jean Krutmann
Heinrich-Heine Universität, Dusseldorf, Germany

Abstract not available.

III.5. Role of cellular DNA damage responses for the mutagenic outcome of exposures to UVA and UVB

Thomas M. Rünger
Department of Dermatology, Boston University, USA

Long-wave ultraviolet light (UVA) is able to damage DNA, to cause mutations, and to induce skin cancer, but the exact mechanisms of UVA-induced mutation formation remain a matter of debate. While DNA photoproducts (pyrimidine dimers) are well established to mediate mutation formation with shortwave ultraviolet light (UVB), other types of DNA damage, such as oxidative base damage have long been thought to be the premutagenic lesions for UVA mutagenesis. However, DNA photoproducts can also be generated by UVA, and there are several lines of evidence from spectra of UVA- and UVB-induced mutations in human skin cells that photoproducts are the most important premutagenic lesions not only for UVB, but also for UVA.

Previous findings on UVA-mutagenesis, in particular that UVA induces more mutations per photoproduct than UVB, and that the action spectrum of UV-induced skin cancers in mice is characterized by a second peak in the UVA range without a parallel increase in photoproduct formation appear to support a prominent role of a non-photoproduct type of DNA lesion in UVA mutagenesis and to be incompatible with DNA photoproducts being the main premutagenic lesions for UVA.

We have developed a hypothesis that reconciles all those data. The fate of a given type of DNA damage, in essence, the question whether it induces a mutation or not, depends not only on the DNA damage itself, but also on the cellular reaction to the DNA damage, including cell cycle arrest, induction of DNA repair, and apoptosis. We hypothesize that a weaker anti-mutagenic cellular response is responsible for a higher mutation rate per DNA photoproduct with UVA, as compared to UVB. When comparing roughly equi-mutagenic and equi-toxic doses of UVA and UVB, we have consistently found much less prominent anti-mutagenic responses in skin fibroblasts to UVA than to UVB, including p53- and p95-activation, cell cycle arrests, and DNA recombination repair. These findings indicate that exposures to UVA from a pure UVA source may be more mutagenic/carcinogenic than UVA from a mixed UVA/UVB source, in which UVB-induced protective cellular responses may better protect against the mutagenic properties of the few UVA-induced DNA photoproducts.

References

Dunn J, Potter M, Rees A, Rünger TM. 2006. Activation of the Fanconi anemia/BRCA pathway and recombination repair in the cellular response to solar ultraviolet light. Cancer Res 66:11140-7.
Kappes UP, Luo D, Potter M, Schulmeister K, Rünger TM. 2006. Short- and long-wave ultraviolet light (UVB and UVA) induce similar mutations in human skin cells. J Invest Dermatol 126:667-75.
Rünger TM. 2007. How different wavelengths of the ultraviolet spectrum contribute to skin carcinogenesis – role of the cellular damage response. J Invest Dermatol 127:2103-5.
Rünger TM, Kappes UP. 2007. Mechanisms of mutation formation with long-wave ultraviolet light (UVA). Photodermatol Photoimmunol Photomed 24:2-10.

III.6. Mechanisms of UV- and sunlight-induced mutagenesis

Gerd P. Pfeifer, Sang-In Kim, Ahmad Besaratinia
Division of Biology, Beckman Research Institute of the City of Hope, Duarte, CA 91010, USA

Sunlight ultraviolet (UV) radiation is an established environmental physical carcinogen, which has been implicated in the etiology of human skin cancer. Of the UV spectrum of sunlight, the UVC (<280 nm) is entirely absorbed by stratospheric oxygen (O_2). Ozone (O_3) blocks the majority of UVB (280-320 nm). Owing to its high penetrating efficiency, UVA (320-400 nm) can mostly pass through the various atmospheric layers and reach the surface of the earth. Thus, the terrestrial sunlight UV is mainly comprised of UVA (~95%) and the remainder is unabsorbed UVB (~5%). Despite the low presence of UVB in sunlight, its high energy and potent photocarcinogenicity accounts for the majority of solar UV-associated neoplasia. Nonetheless, UVA is estimated to contribute to 10-20% of sunlight-induced carcinogenesis.

The different ultraviolet (UV) wavelength components, UVA (320-400 nm), UVB (280320 nm), and UVC (200-280 nm), have distinct mutagenic properties. A hallmark of UVC, UVB and sunlight mutagenesis is the high frequency of transition mutations at dipyrimidine sequences containing cytosine and particularly 5-methylcytosine. This mutagenic specificity has been linked to the formation of cyclobutane pyrimidine dimers (CPDs) containing either cytosine or 5-methylcytosine, which effectively can undergo deamination when present within the pyrimidine dimer lesions. (6-4) photoproducts also form but are thought to be less mutagenic than CPDs due to rapid repair kinetics. The deaminated CPDs, now containing uracil from deamination of cytosine or thymine from deamination of 5-methylcytosine, are highly mutagenic intermediates that may be replicated with incorporation of adenine opposite the lesions thus leading to a C to T or 5mC to T transition mutation of the original sequence.

On the other hand, UVA also can induce CPDs at high doses, mostly at 5'-TT sequences, but these are not highly mutagenic due to correct replication bypass by DNA polymerase eta. In addition, UVA induces oxidative DNA damage at guanines through a singlet oxygen pathway leading to the formation of 7,8-dihydro-8-oxo-2'-deoxyguanosine (8-oxo-dG) [Besaratinia, et al. 2005].

Transgenic rodent mutagenesis systems are invaluable models for correlation studies of DNA damage and mutagenesis at both genomic and single nucleotide level. Using transgenic BigBlue® mouse embryonic fibroblasts, we have recently shown typical oxidative-DNA damage mediated mutagenicity of UVA in the *lacI* and *cII* transgenes. We have also demonstrated that addition of photosensitizers, i.e., riboflavin and delta-aminolevulinic acid, can intensify the observed UVA-induced DNA damage and mutagenesis, whereas inclusion of the antioxidant vitamin C can counteract the induced effects [Besaratinia, et al. 2007]. The characteristic oxidative-DNA damage derived mutagenicity of UVA is, however, not prevalent in sunlight-induced mutagenesis, wherein single and tandem C to T transitions at dipyrimidine sites dominate the spectrum of induced mutations. The latter points to an overruling involvement of dipyrimidine lesions induced by UVB in solar mutagenesis. Mechanistically, however, the superseding role of UVB over UVA in sunlight-induced mutagenesis has not been explained yet.

We investigated the kinetics of repair for UVA- and UVB-induced lesions in relation to mutagenicity in transgenic mouse embryonic fibroblasts irradiated with equilethal doses (70% cell survival) of UVA and UVB in comparison to simulated sunlight (SSL). Cleavage assays with specific DNA repair enzymes revealed that both UVB- and simulated sunlight irradiation, but not UVA-irradiation, induced CPDs and (6-4)PPs, in the genome overall of irradiated cells. Whereas the induced (6-4)PPs were repaired within 6 h after both UVB- and SSL-irradiation, the CPDs remained persistent for at least 24 h. Also, UVA- and SSL-irradiation alike formed significant levels of oxidized purines in the genome overall of irradiated cells; however, the induced lesions were completely repaired within 30

min post-irradiation. Footprinting of DNA damage/repair at sequences along the *cII* transgene confirmed these findings. Determination of *cII* mutant frequency showed that UVA-irradiation was weakly but significantly mutagenic, whereas SSL- and UVB-irradiation were extremely mutagenic. The induced mutation spectra by UVB- and SSL-irradiation were both characterized by a significant increase in the relative frequency of C to T transitions at dipyrimidines. This type of mutations accounted for 85% and 80% of the observed mutagenicity of UVB- and SSL-irradiation, respectively. The G to T transversions induced by UVA-irradiation, however, contributed only to 3.3% of the mutation load imposed by SSL-irradiation. We concluded that the prevailing role of UVB over UVA in solar mutagenesis is due to different kinetics of repair for lesions induced by the respective irradiation.

References

Besaratinia A, Synold TW, Chen HH, Chang C, Xi B, Riggs AD, Pfeifer GP. 2005. DNA lesions induced by UV A1 and B radiation in human cells: comparative analyses in the overall genome and in the p53 tumor suppressor gene. Proc Natl Acad Sci USA 102:10058-63.

Besaratinia A, Kim SI, Bates SE, Pfeifer GP. 2007. Riboflavin activated by ultraviolet A1 irradiation induces oxidative DNA damage-mediated mutations inhibited by vitamin C. Proc Natl Acad Sci USA 104:5953-8.

III.7. UVA mediated signaling pathways in human keratinocytes[6]

G. Tim Bowden, Jack Zhang, Michael Bachelor, Amy Silvers
The Arizona Cancer Center, The University of Arizona Cancer Center, Tucson, Arizona 85724, USA

The major risk factor for the development of non-melanoma skin cancer is exposure to sunlight. The UV spectrum is subdivided into three ranges: UVC <290 nm, UVB 290-320 nm and UVA 320-400 nm. Because stratospheric ozone completely absorbs UVC and a large portion of UVB, 90-99% of solar radiation at the earth's surface is UVA. Although a more prominent role for UVA in skin carcinogenesis has recently emerged, the precise signaling pathways that are activated in response to UVA have not been fully elucidated. Using cultured human keratinocytes we have demonstrated that UVA irradiation induces activator protein-1 (AP-1) DNA binding and AP-1 transactivation [Silvers and Bowden 2002]. We found that a strong correlation existed between UVA-induced AP-1 activity and accumulation of c-Fos, c-Jun and Fra-1 proteins. We found that activation of both JNK and p38 MAP kinase play critical roles in UVA-mediated AP-1 transactivation and c-Fos expression in human keratinocytes. Another downstream target in UV skin carcinogenesis is cyclooxygenase-2 (COX-2). We discovered that UVA-induced expression of COX-2 is post-transcritionally regulated by p38 MAP kinase through message stabilization and AU-rich elements (AREs) in the 3' UTR of the COX-2 gene [Bachelor, et al. 2002]. We have also investigated an anti-apoptotic signaling pathway in human keratinocytes mediated through p38 MAP kinase. We have evidence that UVA induces the expression of the anti-apoptotic protein, Bcl-X_L through a p38 MAP kinase pathway that again results in the stabilization of the BCL-X_L mRNA [Bachelor and Bowden 2004]. We have mapped functional ARE(s) to the 3'UTR of the Bcl-X gene. To understand the mechanism by which Bcl-X_L message is stabilized, we used a synthetic Bcl-X_L 3' UTR to capture RNA-binding proteins. Nucleolin was identified as one of the binding proteins as determined by MALDI-MS analysis. Further study showed that nucleolin specifically recognized the AU-rich elements (AUUUA) on the 3'-UTR of Bcl-X_L mRNA and could stabilize the mRNA in vitro. Interestingly, nucleolin physically interacted with Poly(A) Binding Protein and its stabilizing effect on Bcl-X_L mRNA was dependent on the presence of the poly(A) tail. Based on these data we propose a model in which nucleolin protects Bcl-X_L mRNA from nuclease degradation by enhancing the stability of the ribonucleoprotein (RNP) loop structure.

In conclusion, our data support the idea that UVA mediates multiple signal transduction pathways through activation of stress-activated kinases, p38 MAP kinase and JNK. UVA-activation of these pathways results in activation of the transcription factor AP-1, increased expression of an enzyme involved in the generation of prostaglandins, COX-2, and increased expression of the anti-apoptotic protein Bcl-X_L. We propose UVA-mediated increases in AP-1 and COX-2 may play a role in skin tumor promotion and progression through increases in interleukin-8 and vascular endothelial growth factor [Bachelor and Bowden 2004b]. In addition we propose that UVA induced expression of Bcl-X_L plays a role in clonal expansion of keratinocytes that have sustained initiating p53 tumor suppressor gene mutations [Zhang and Bowden 2007].

References

Bachelor MA, Bowden GT. 2004a. Ultraviolet A-induced modulation of Bcl-X_L by p38 MAPK in human keratinocytes. J Biol Chem 279:42658-68.
Bachelor MA, Bowden GT. 2004b. UVA-mediated activation of signaling pathways involved in skin tumor promotion and progression. Semin Cancer Biol 14:131-8.
Bachelor MA, Silvers AL, Bowden GT. 2002. The role of p38 in UVA-induced cyclooxygenase-2 expression in the human keratinocyte cell line, HaCaT. Oncogene 21:7092-9.
Silvers AL, Bowden GT. 2002. UVA irradiation-induced activation of activator protein-1 is correlated with induced expression of AP-1 family members in the human keratinocyte cell line HaCaT. Photochem Photobiol 75:302-10.

[6] This work was not presented at the meeting

Zhang J, Bowden GT. 2007. Targeting Bcl-X_L for prevention and therapy of skin cancer. Mol Carcinog 46:665-70.

III.8. Morphology-based analysis of skin carcinogenesis

Anna Asplund, Fredrik Pontén
Dept of Genetics and Pathology, Rudbeck laboratory, Uppsala University, Uppsala, Sweden

Carcinogenesis involves a multi-step process of somatic genetic events. The complexity of this multi-hit process makes it difficult to determine the sequential order and the outcome of single events. The analysis of various mutations and altered gene expression patterns are crucial to determine links between phenotypes and molecular events, and may also play an important role in the early detection and accurate diagnosis of a tumor. Such analyses require not only sensitive and accurate methods but also well defined starting material of high quality. Skin cancer provides an advantageous model for studying cancer development, since detectable lesions occur early during tumor progression, facilitating molecular analysis of cell populations from both pre-neoplastic and neoplastic lesions. We have employed morphological assessment and microdissection in combination with robust and reliable techniques in attempts to describe mutations and altered transcription profiles in defined lesions implicated in skin carcinogenesis. Alterations of the p53 tumor suppressor gene are common in non-melanoma skin cancer, and dysregulation of p53 pathways appear to be an early event in the development of both basal cell (BCC) and squamous cell carcinoma (SCC).

Microdissection-based studies have provided insight into the normal organization of epidermal proliferative units and links between normal cells and different stages of cancer, including epidermal p53 clones. Using p53 mutations as clonality markers, our data suggested a direct link between actinic keratosis, SCC in situ and invasive SCC. We have also shown that different parts of individual BCC tumors can share a common p53 mutation yet differ with respect to additional alterations within the p53 gene, suggesting a development of subclones within this unusually stable form of cancer. Futhermore, we have shown that microdissection in combination with robust strategies for RNA extraction, amplification and cDNA microarray analysis allow for reliable transcript profiling of specific cell populations defined in a tissue section. To validate data from transcriptional profiling experiments also on a protein level, we have employed an antibody-based proteomic strategy where specific antibodies directed towards the human proteome can be used to verify and further analyze the consequences of altered gene expression.

III.9. ^{32}P-Postlabelling analysis of UV induced pyrimidine dimers from human skin and urine

D. Segerbäck[1], T. Sandberg[1], N. Kotova[1], A. Blomqvist[2], S. Luoto[2], V. Bykov[1], E. Snellman[3], G. Xu[1], C. Zhao[1], F. le Curieux[1], M. Strozyk[1], P.C. Turner[4], C.P. Wild[4], A. Sylla[5], M.S. Diallo[5], K. Hemminki[1,6]
[1]Department of Biosciences and Nutrition, Karolinska Institute, Huddinge, Sweden
[2]Department of Occupational and Environmental Health, Central Hospital, Halmstad, Sweden
[3]Päijät-Häme Hospital, Lahti, Finland
[4]Leeds Institute for Genetics Health and Therapeutics, University of Leeds, Leeds, UK
[5]Institut Pasteur de Guinée, Kindia, Republic of Guinea
[6]Currently; German Cancer Research Institute, Heidelberg, Germany

Ultraviolet irradiation (UV) is considered to be a major causative factor in human skin cancer. The major products induced by UV in DNA are dipyrimidinic lesions, which are likely to be important in the pathogenesis of skin cancer. In our studies of human volunteers exposed to solar simulated UV radiation we excised skin biopsies after exposure and analysed UV dimers in DNA by a sensitive ^{32}P-postlabelling assay. The normal procedure was that volunteers were exposed to a single UV dose on buttock skin and punch biopsies taken at different time points. DNA was extracted, digested enzymatically, ^{32}P-labelled and analysed by HPLC equipped with an online radioisotope detector. The obtained data showed that high levels of dimers are formed in human skin in situ after just a single dose of solar simulated UV light corresponding to what will be obtained during one hour in summer time Stockholm. These levels are orders of magnitude higher than levels of DNA adducts from chemical carcinogens found in various target tissues, showing the potency of UV light to induce DNA damage in human skin. Dimer levels showed substantial inter individual variations, even when using same UV dose. The cause of these variations is unknown and could only partly be explained by skin type. Use of sun screens was shown to be highly protective against formation of UV dimers, whereas tanning had only marginal effects.

When studying repair of those lesions a mixed time kinetic was observed, i.e. an initial fast removal followed by a slower phase. 6-4 Photoproducts were repaired much faster than cyclobutane dimers and the TC cyclobutane dimer in the sequence TTC was repaired faster than the TT dimer in the sequence TTT. Large inter individual differences were observed also in repair of DNA damage. When analysing repair of dimers in the skin of malignant melanoma or with basal cell carcinoma patients we found a reduced capacity to remove UV damage among the basal cell carcinoma cases, but not the melanoma cases.

More recently we developed a postlabelling assay for detection of UV dimers present in urine as a result of DNA repair. The assay is very sensitive (only 10 µl of urine is needed) and has the advantage that it is non-invasive and can thus also be applied to studies in children. So far carried out studies showed that the amount of dimer excreted into urine correlated to the applied UV dose when using a solarium and that high levels of urinary dimers are found in adults, as well as in children, following brief sun exposure. More recently we have been studying life guards at a beach in southern Sweden and found that they had very high levels of dimers in their urine. Furthermore, even when not spending time in outdoor activities, T=T could be detected as background levels in adults during the summer time, but not in winter. In conclusion, our studies have shown that DNA damage by UV light can be analysed in humans following doses that everyone will receive just by spending a few hours outside and that there are inter individual differences that may have implications for who will develop cancer.

References

Kotova N, Hemminki K, Segerbäck D. 2005. Urinary thymidine dimer as a marker of total body burden of UV-inflicted DNA damage in humans. Cancer Epidemiol Biomarkers Prev 14:2868-72.

Xu G, Hemminki K, Le Curieux F. 2001. Ultraviolet radiation-induced photoproducts in human skin DNA as biomarkers of damage and its repair. IARC Sci Publ, vol 154, pp 69-79.

III.10. Mouse models of melanoma

Timothy P. Heffernan, Lynda Chin
Dana Farber Cancer Institute, Boston, MA, USA

Malignant melanoma is a highly metastatic disease that is notorious for its resistance to therapy and for its extremely aggressive clinical behavior. The rapid rise in the incidence of melanoma during the past decade is an alarming phenomenon that calls for an interdisciplinary approach to identify novel targets for therapeutic development, and to develop appropriate pre-clinical models for target validation, drug testing, and response prediction [Chin 2003; Chin, et al. 2006].

Genetically engineered mouse (GEM) models have contributed extensively to the field of melanoma research. We have utilized GEM models to investigate the contribution of signature genetic events observed in human melanoma, such as loss of the CDKN2A locus and somatic mutations in RAS. Mice engineered to express an inducible $HRAS^{V12G}$ allele in the context of INK4a/ARF deficiency (iHRAS) developed melanomas with high penetrance and short latency. These tumors were amelanotic, invasive, and highly vascularized, resembling human nodular melanomas [Chin, et al. 1999]. However, in contrast to nodular melanomas in humans, tumors derived from iHRAS mice failed to metastasize. To more accurately model human metastatic melanoma in the mouse we have generated a tetracycline inducible $NRAS^{Q61R}$ mouse line, in which NRAS expression is directed to the melanocyte compartment of INK4a/ARF deficient mice (iNRAS). Upon postnatal doxycycline administration, iNRAS mice developed cutaneous melanomas with 60-80% penetrance and latency of ~24 weeks. In contrast to iHRAS-driven melanomas, nearly 20% of iNRAS-induced melanomas metastasized to lymph nodes and lung. In addition, these cutaneous melanomas were dependent on NRAS for tumor maintenance, as withdrawal of doxycycline resulted in regression. Readministration led to recurrence with repeated ON/OFF cycles of transgene expression resulting in the generation of doxycyline-insensitive escapers. Moreover, UV irradiation of neonatal pups significantly reduced tumor latency suggesting possible cooperation between NRAS and UV in melanomagenesis.

Primary melanomas from iHRAS and iNRAS mice differ in their ability to metastasize and thus, may differ at the genomic level. Integrated approaches that encompass genomic characterization of staged iHRAS/iNRAS mouse and human melanomas can be utilized to identify a “metastasis gene signature.” Genome-wide expression profiles of iHRAS-driven and iNRAS-driven melanomas will identify a list of genes that differ between non-metastatic and metastatic mouse melanomas. Cross specie analysis with high-resolution array-CGH profiles of human melanoma will further filter candidates resulting in a list highly amenable to low complexity genetic screens. A similar cross specie comparative oncogenomic approach led to the identification of NEDD9 as a bonafide melanoma metastasis gene [Kim, et al. 2006]. Thus, mouse models of human melanoma serve as robust gene discovery engines for the identification of melanoma-related genes.

References

Chin L. 2003. The genetics of malignant melanoma: lessons from mouse and man. Nat Rev Cancer 3(8):559-70.
Chin L, Tam A, Pomerantz J, Wong M, Holash J, Bardeesy N, Shen Q, O'Hagan R, Pantginis J, Zhou, H, Horner JW 2nd, Cordon-Cardo C, Yancopoulos GD, DePinho RA. 1999. Essential role for oncogenic Ras in tumour maintenance. Nature 400(6743):468-72
Chin L, Garraway LA, Fisher DE. 2006. Malignant melanoma: genetics and therapeutics in the genomic era. Genes Dev 20(16):2149-82
Kim M, Gans JD, Nogueira C, Wang A, Paik JH, Feng B, Brennan C, Hahn WC, Cordon-Cardo C, Wagner SN, Flotte TJ, Duncan LM, Granter SR, Chin L. 2006. Comparative oncogenomics identifies NEDD9 as a melanoma metastasis gene.Cell 125(7):1269-81.

III.11. Role of UVB and UVA in melanoma

Edward De Fabo
Environmental and Occupational Health, School of Public Health and Health Services, The George Washington University, Washington, DC, USA

Cutaneous malignant melanoma (CMM) is one of the fastest increasing cancers and is associated with sunlight exposure of susceptible populations. The incidence of melanoma shows a latitude gradient and is highest in locations where fair-skinned populations live in areas of high sunlight exposure. Childhood exposure appears to be important – migration from a low sunlight to a high sunlight environment as a child confers a significantly higher risk than emigration as an adult although epidemiologic studies indicate that adult sunlight exposure does also play a role. Since sunlight is the major carcinogen for melanoma, investigations of the wavelengths responsible and the mechanism(s) by which melanoma is initiated are fundamental not only to understanding mechanism but also for accurate risk assessment and development of effective prevention strategies. The spectrum (wavelength composition) of sunlight as well as the total irradiance levels that we receive vary widely according to global factors such as latitude, season and time of day and also according to local factors like air pollution, reflectance (albedo) of surroundings and cloud cover. Thus estimation of melanoma effective doses received from sunlight or artificial sources requires knowledge of the relative melanoma effectiveness as a function of wavelength.

For non-melanoma skin cancer (NMSC) the consensus is that it is initiated by UVB wavelengths (280-320nm). UVB is very biologically active as it is absorbed by several important biomolecules, initiating sunburn, suntanning, skin and corneal damage, skin pre-Vitamin D formation and alterations to the immune system in addition to its carcinogenic properties. UVB is also mutagenic and stimulates multiple cellular signaling pathways. For melanoma, however, the picture is not so clear. CMM can occur on infrequently sun-exposed sites and is associated with sporadic burning doses of sunlight, not with chronic exposure. There are no consensus UVB signature lesions in melanoma as there are for NMSC. There is no mammalian action spectrum for melanoma and investigations of the wavelengths responsible have until recently been hampered by a lack of suitable animal models. In part because of this ambiguity, it has been proposed that UVA (320-400nm) is important in the initiation of melanoma. UVA is also biologically active, usually through photosensitization reactions rather than direct absorption as for UVB and can stimulate multiple signaling pathways in cells including melanocytes. The only action spectrum currently available for melanoma was carried out in the *Xiphophorus* fish with the provocative finding that, although the most effective wavelengths at melanomagenesis were in the UVB, UVA had an unexpectedly high efficacy. Because there is about 10-15 fold more UVA than UVB in sunlight, this finding predicted that the major carcinogen in sunlight is UVA. This conclusion had some major implications for prevention and protection strategies for melanoma.

We have developed a mouse model of UV-induced melanoma which has several major strengths as a model for human disease. Transgenic hepatocyte growth factor/scatter factor (HGF/SF) mice UV irradiated as neonates develop cutaneous melanoma 6-9 months later. Adult UV irradiation is ineffective at initiating melanoma but does increase melanoma multiplicity in animals which were UV irradiated as neonates. Thus, this model recapitulates the epidemiologic finding that childhood sunlight exposure is critical for melanoma and indicates it has a biologic basis but is also consistent with the concept that adult sunlight exposure is important. The UV-induced melanomas obtained in these animals showed loss of function of a major locus known to play a role in human melanoma, *ink4a* and HGF/SF transgenics crossed with *ink4a* genetically deficient animals developed UV-induced melanomas very rapidly, further confirming the relevance of this model. Most surprisingly and uniquely among mouse models to date, the melanomas arising after neonatal UV closely recapitulate human melanoma with pagetoid, nodular and pleiomorphic histopathologies. The full progression from early melanocytic lesion through to metastasis was observed. In our initial studies this model was derived in

an albino FVB strain but we have subsequently also derived melanomas with similar characteristics in pigmented HGF/SF transgenics in response to neonatal UV irradiation.

We have used this mouse model to address the question of which wavelengths initiate melanoma. We have used specialized optical sources, emitting combined or isolated, highly resolved, UVB or UVA wavebands or solar simulating radiation and the albino HGF/SF-FVB mouse model. Only UVB containing sources initiated melanoma. In contrast, irradiation with the isolated UVA waveband (>99.9% 320-400nm) produced no melanomas. Further, in separate experiments, irradiation with a broadband emitting F40 sunlamp filtered to remove more than 96% of the UVB prevented the induction of UV melanomas. Taken together, we concluded that UVB is responsible for the induction of mammalian CMM in this animal model system. UVA was ineffective even at doses considered physiologically relevant (150 kJ/m^2) and which were 30-fold higher than doses of UVB which were melanomagenic. This finding has major implications for evaluating risk exposure to UVB radiation from sunlight and artificial sources, for developing protection strategies against melanoma induction by UVB radiation, and for basic mechanism studies since UVB initiates DNA damage, cell signaling pathways, and immune alterations differently from UVA.

Further supporting a critical role for UVB, we have investigated the role of DNA repair in UV-induced melanoma using four well characterized mouse strains with deficiencies in nucleotide excision repair (NER), XPA, XPC, TTD (XPD), and CSB. NER is responsible for repair of UVB induced DNA damage. Each strain was crossed with C57BL/6-HGF/SF transgenics. After neonatal UV, multiple melanomas but no other malignant skin tumors arose in all HGF/SF groups with a high frequency (>80%) but not in NER deficient animals lacking HGF/SF. Analysis revealed a critical role for global genome repair, with XPA (P= 0.002) and XPC (P=0.01) but not CSB animals (P=0.3) developing melanomas more rapidly than repair competent mice. Melanoma was also significantly accelerated in TTD mice (P=0.02), consistent with observations that XPD polymorphisms are associated with melanoma susceptibility in humans. The findings further model human melanoma in that Xeroderma pigmentosum (XP) but not Cockayne syndrome (CSB) patients have enhanced susceptibility to skin cancer including melanoma.

Recently, we have developed a new and robust experimental animal model that will allow us to exploit our information on wavelength dependence to identify the critical genetic pathways for melanoma induction by UV radiation. Using a newly derived mouse with inducible melanocyte-specific GFP expression, highly purified (>95%) melanocytes have been obtained from disaggregated mouse skin by FACS sorting. Gene expression by microarray analysis in melanocytes from UVB or UVA treated neonates revealed UVB-specific persistent gene expression, absent from UVA treated animals, consistent with our in vivo melanomagenesis data. In studies currently underway, the GFP mouse will be crossed with HGF/SF transgenic mice. Gene expression comparisons between melanocytes from neonatally UVB and UVA irradiated animals, with and without the HGF/SF transgene, are expected to identify a subset of genes unique to UVB initiation of melanoma. We further anticipate this methodology to be applicable to several major outstanding questions in melanoma initiation by UV radiation and to result in derivation of novel therapeutic approaches for CMM.

References

De Fabo EC, Noonan FP, Fears T, Merlino G. 2004. Ultraviolet B but not ultraviolet A radiation initiates melanoma. Cancer Res 64:6372-6.

Noonan FP, Recio JA, Takayama H, Duray P, Anver MR, Rush WL, Lindner G, De Fabo EC, Merlino G. 2001. Neonatal sunburn and melanoma in mice. Nature 413:271-2.

Recio Juan A, Noonan Frances P, Takayama Hisashi, Anver Miriam R, Duray Paul, Rush Walter L, Lindner Gerd, De Fabo Edward C, DePinho Ronald A, Merlino Glenn. 2002. Ink4a/Arf deficiency promotes ultraviolet radiation-induced melanomagenesis. Cancer Res 62:6724-30.

III.12. Induction of melanoma in mice: the role of UVR-induced melanocyte proliferation and migration

Graeme Walker[1], Michael Kimlin[2], Sugandha Ravishankar[1], Elke Hacker[1], H. Konrad Muller[3], Nicholas Hayward[1]
[1]Queensland Institute of Medical Research, Queensland, Australia
[2]Australian Sun and Health Research Laboratory, QUT, Queensland, Australia
[3]Dept Pathology, University of Tasmania, Australia

Sun exposure is a strong risk factor for melanoma development, although the relationship is complex. Exposure of the skin to ultraviolet radiation (UVR) induces DNA damage in epidermal keratinocytes, which in turn release signalling molecules that cause melanocytes to activate pigmentation pathways. This melanocyte activation also results in melanocyte proliferation, and increased numbers of epidermal melanocytes can be observed in human and mouse skin after UVR, particularly after repeated exposures. Melanocyte activation is essentially a protective response, increasing melanin levels in epidermal keratinocytes in case of subsequent exposures. But it has been suggested that this proliferative response may also play a role in increasing melanoma risk, at least in a subset of melanoma cases. In most mouse models of melanoma the animals have been genetically engineered to carry mutations in melanocytes or adjacent keratinocytes that result in a melanocyte-hyperproliferative phenotype. This is presumably at least part of the reason for their vastly increased propensity for melanoma development compared with wild type mice.

In mice, a single exposure of 3- to 5- day-old newborn pups to UVR leads to melanoma development in *Mt-Hgf* transgenic mice much more effectively than acute or chronic exposures of adult transgenics [Noonan, et al. 2001]. Thus neonatal UVR treatment has become de rigueur for inducing melanomas in mice. The reason for the sensitivity of murine neonatal but not adult melanocytes to UVR-induced transformation is unclear, but it may depend in part on the immaturity of the newborn immune system. In addition, while in adult mouse skin virtually all melanocytes are located within the hair follicle, in neonates migrating melanocytes are present in the suprabasal epidermis during the first week after birth. These immature pigment cells are undoubtedly damaged by the UV rays.

We have shown that mice carrying a melanocyte-specific *Hras* mutation (*Tpras*) are highly susceptible to the development of melanoma after a single neonatal UVB exposure [Hacker, et al. 2006]. Animals also carrying an Rb pathway defect (*Cdk4*$^{R24C/R24C}$*/Tpras*) exhibited increased penetrance, decreased age of onset, and increased aggressiveness of lesions [Hacker, et al. 2006]. p53 pathway-defective *p53*$^{-/-}$*/Tpras* and *Arf*$^{-/-}$*/Tyr-Hras* mice also develop melanoma, although possibly via a different mechanism to those carrying an Rb pathway defect [Kannan, et al. 2003]. Given that a single neonatal exposure initiates melanoma tumorigenesis in our models, we have the opportunity to study the actual UVR exposure that initiates the neoplastic process. We have assessed melanocyte proliferation in the skin of the mice post neonatal exposure to determine whether it may be an important factor in their heightened susceptibility to UVR-induced melanoma.

In wild type neonates we observed a surprising UVR-induced melanocyte migration to the epidermal basal layer that does not to occur in adult wild type mice after an equivalent exposure. At 3d post UVR, when most DNA damage was repaired, dendritic melanocytes were visible at the dermo-epidermal junction, their numbers peaking at around 5d, then diminishing over time. These cells appear to migrate from the hair follicles via the outer root sheath, in response to paracrine signals from UVR-damaged epidermal keratinocytes. Epidermal melanocyte numbers at 5d post-UVR were greater in melanoma-prone *Nras* and *Hras*-expressing animals than wild type. These activated transgenic melanocytes remained within the basal layer for longer, were generally larger, and sat on the epidermal basement membrane with dendrites extending up into the epidermis, such that after UVR exposure the mouse skin was very reminiscent of human skin. The mutant melanocytes in the melanoma-prone mice removed pyrimidine dimers, so a repair defect cannot explain their increased melanoma

susceptibility. Surprisingly, epidermal melanocyte count in animals carrying the Cdk4-R24C mutation was no greater than that for wild type mice. Epidermal melanocyte count post-UVR was lowest in p53-null mice, most likely a result of the inability of p53-null keratinocytes to produce α-MSH in response to UVR.

It is well known that blocking stem cell factor (Scf) signalling though its receptor (c-Kit) can prevent melanocyte proliferation after UVR exposure. Evidence from melanoma-prone *Mt-Ret* transgenic mice suggests that inactivating Scf/Kit in neonates protects the animals from developing melanoma [Kato, et al. 2004]. We believe that melanoma development initiated in neonatal mice may be at least in part determined by the propensity of the neonatal melanocytes to proliferate and migrate, whether the UVR-stimulated cells emanate from activation of pre-existing immature epidermal melanocytes, or from the hair follicles.

In summary, after neonatal UVR exposure mouse melanocytes migrate to the epidermis as part of a tanning response, driven by signals from damaged epidermal keratinocytes. We believe that this hyperactivation of melanocytes may prime them for UVR-induced transformation, at least in some circumstances.

References

Hacker E, Muller K, Gabrielli B, Lincoln D, Pavey S, Broome-Powell M, Malumbres M, Barbacid M, Hayward N, Walker G. 2006. Spontaneous and UVR-induced multiple metastatic melanoma in *Cdk4*$^{R24C/R24C}$*/TPras* mice. Cancer Res 66:2946-52.

Kannan K, Sharpless NE, Xu J, O'Hagan RC, Bosenberg M, Chin L. 2003. Components of the Rb pathway are critical targets of UV mutagenesis in a murine melanoma model. Proc Natl Acad Sci USA 100:1221-5.

Kato M, Takeda K, Kawamoto Y, Tsuzuki T, Hossain K, Tamakoshi A, Kunisada T, Kambayashi Y, Ogino K, Suzuki H, Takahashi M, Nakashima I. 2004. c-Kit-targeting immunotherapy for hereditary melanoma in a mouse model. Cancer Res 64:801-6.

Noonan FP, Recio JA, Takayama H, Duray P, Anver MR, Rush WL, De Fabo EC, Merlino G. 2001. Neonatal sunburn and melanoma in mice. Nature 413:271-2.

III.13. Animal models of melanoma

Graham Timmins
College of Pharmacy, University of New Mexico, Albuquerque NM 87131, USA

Melanoma is generally increasing worldwide, with 60,000 cases and 8000 deaths yearly in the US alone. Yet, the most crucial data to effective prevention- which wavelengths in sunlight cause melanoma, or how these wavelengths act, remains unclear and highly controversial. Since this cannot be directly obtained in humans, animal models must be used, but controversy has resulted from animal models giving very different results on the relative importance of UVA and UVB. This is further complicated, as each of these models each has strengths and weaknesses in their relevance to human melanoma.

We will argue that human evidence on melanoma such as: i) its prevalence across latitude; ii) the paucity of UVB signature gene mutations and; iii) the enormous rarity of melanoma in albinos compared to non-melanoma skin cancers, suggests that melanin photosensitization and UVA play major roles in human melanoma causation, and conversely that UVB may not. Furthermore, human melanomagenesis is a complex multistage process. Thus, animal models should ideally be pigmented, exhibit UVA effects, and exhibit multistage carcinogenicity.

We will then discuss the relative merits of key *Xiphophorus* [Setlow, et al. 1993], transgenic mouse [Noonan, et al. 2001], and *Monodelphis domestica* [Ley, et al. 1989] models to study human melanoma etiology based upon their similarity to the human case, and argue that while all have important roles to play, the results of thorough wavelength dependence studies in *M. domestica* are likely to most closely model the human case. We will also present new data on the mechanisms by which melanin acts as a photosensitizer within the melanocyte.

References

Ley RD, Applegate LA, Padilla RS, Stuart TD. 1989. Ultraviolet radiation-induced malignant melanoma in *Monodelphis domestica*. Photochem Photobiol 50:1-5.
Noonan FP, Recio JA, Takayama H, Duray P, Anver MR, Rush WL, De Fabo EC, Merlino G. (2001). Neonatal sunburn and melanoma in mice. Nature, 413: 271-2.
Setlow RB, Grist E, Thompson, K, Woodhead AD. 1993. Wavelengths effective in induction of malignant melanoma. Proc Natl Acad Sci USA 90:6666-70.

III.14. Are melanocyte stem cells the target for transformation in human skin?

Meenhard Herlyn, Ling Li, Susan Zabierowski, Nga Nguyen, Jennifer Marmion, Katrin Sproesser, Mizuho Fukunaga-Kalabis
The Wistar Institute, Philadelphia, PA 19104, USA

Adult stem cells are potentially the cell of origin for many human cancers. Neural crest stem cells have been previously characterized by their expression of the intermediate filament nestin and the low-affinity nerve growth factor receptor NGFRp75. We describe the isolation of multi-potent stem cell-like cells from the dermis of human foreskin. Initially, single cells from dermis were grown in human embryonic stem cell-based media. After 10 to 14 days in culture, the cells formed three-dimensional spheres. Nineteen of 24 (79%) samples formed spheres and the sphere-forming efficiency of single samples was approximate 0.085%. The sphere-forming neural crest-like cells (SC-D) expressed nestin, NGFRp75, and the embryonal stem cell marker OCT4. SC-D displayed extensive self-renewal capacity. The stem cell properties of sphere cells were further characterized by induction of differentiation into several cell lineages. Using growth factors, we could induce SC-D cells into melanocytes, neuronal cells, smooth muscle cells, chondrocytes, and adipocytes. Cells derived from single-cell clones could also differentiate into the same lineages. The SC-D cells differentiating into melanocyte-like cells expressed MITF, TYRP-1, TYRP-2, and HMB-45. They incorporated into synthetic skin in the same manner as epidermal melanocytes by firmly localizing in the basement membrane zone. Our recent experiments suggest that as few as two genetic 'hits' can induce malignant transformation of melanocytes if the microenvironmental conditions are supporting cells to survive the initial crisis. Transfection of melanocytes with $BRAF^{V600E}$, that is found in over 65 % of melanomas, leads to cell senescence. Similarly, overexpression of n-Ras, mutated at codon 61, leads to senescence. However, co-expression of mutant BRAF with knockdown of p53 using an shRNA in a lentiviral vector leads to continuous growth, including in soft agar and immunodeficient animals. Whether melanocyte stem cells respond similarly to oncogenes when compared to differentiated cells is currently being investigated.

III.15. Differential effects of UVB and UVA1 radiations on keratinocytes and melanocytes in murine skin carcinogenesis

Frank R. de Gruijl
Dermatology, Leiden Univ. Med. Ctr., The Netherlands

In the 1930s the germicidal and mutagenic effects of UV, mostly UVC at 254 nm (a mercury line), were well documented. The action spectra were found to resemble the absorption spectrum of DNA, and not that of proteins. Following tradition, many of the cellular in vitro studies were, and still are, performed with UVC radiation, but the wavelength bands, UVB (280 – 315 nm) and UVA (315 – 400 nm), that we are exposed to in sunlight did finally receive due attention. With the availability of high-power UVA1 sources in the 1980s, it became feasible to study the effects of this radiation that is abundantly present in sunlight. Thus, it became clear that the parallel in wavelength dependence between mutagenesis and lethality broke down in the UVA range, where killing is more prominent per mutational event [Enninga, et al. 1986]; possibly due to a significant contribution from lipid and membrane damage. Moreover, and in contrast to mutagenesis with UVC or UVB radiation, mutagenesis with UVA1 radiation proved to be highly oxygen dependent [Applegate, et al. 1992]. Comparative analysis of wavelength dependencies of various types of DNA damage showed that 8-oxo-guanine (Fapy sites) and single strand breaks became relatively more important when compared to cyclobutane pyrimidine dimers (CPDs detected as T4endoV sites) moving from the UVB to the UVA band [Kielbassa, et al. 1997]. Most of these experiments were performed on fibroblasts (or more exotic cells) instead of keratinocytes which are more relevant to skin carcinogenesis. Although DNA damages are principally comparable in both types of cells, they do differ in constitutive antioxidant defences and repair proficiencies [D'Errico, et al. 2007].

Reflecting possible differences in damage and repair rates, we found different kinetics in the cell cycle responses after comparable sunburn exposures of UVB and UVA1 radiations: a maximum suppression of DNA replication was immediate with UVA1 irradiation and the S phase arrest appeared resolve more rapidly than with UVB exposure, where suppression of DNA synthesis was more profound and reached a maximum around 6 hours after exposure [de Laat, et al. 1997a]. Also, p53 expression increased slightly faster after UVA1 (max 12-24 h) than after UVB irradiation (max at 24 h). In line with these response differences, apoptosis was found to evolve faster after UVA1 than after UVB irradiation [Godar, et al. 1994]. In stark contrast to keratinocytes, (dermal) melanocytes in skin of hairless mice showed no apoptosis upon UVB overexposure (4 – 6 MEDs), but we could detect a significant proliferative response 4 days after the exposure, which was not detected after an overexposure to UVA1 radiation [van Schanke, et al. 2005].

Chronic UV exposure leads to induction of squamous cell carcinomas and actinic keratoses as precursor lesions. These tumors carry CPD-related 'UVB signature' mutations in p53 genes with a bias toward associations with dipyrimidine sites on the non-transcribed DNA strand [Dumaz, et al. 1997], corresponding with a low global genome repair of CPDs in mice. Such mutations occur very early on and can already be found in microscopic clusters of p53 overexpressing cells long before the occurrence of the eventual tumors [Rebel, et al. 2005]. Interestingly, low level chronic UVB exposure inducing no or hardly any hyperplasia was found to cause accumulation of CPDs specifically in epidermal stem and progenitor cells [Nijhof, et al. 2007].

We have established the action spectrum for the induction of skin carcinomas in hairless mice and found it to peak at 293 nm and drop by 4 orders of magnitude toward the UVA1 band [de Gruijl, et al. 1993]. Replacing UVB in part by equally carcinogenic UVA radiation lowered the load of CPDs in the skin while inducing carcinomas at equal rates [Berg, et al. 1995], i.e., with UVA radiation other types of (DNA) damage appeared to substitute for the UVB-induced CPDs. Chronic UVA1 exposure led to an initial preponderance of benign papillomas which were subsequently rapidly outnumbered by carcinomas occurring at much higher rate [Kelfkens, et al. 1991]; the dose dependency of these

carcinomas different somewhat from that found for UVB-induced carcinomas [de Laat, et al. 1997b]. While UVB-induced carcinomas showed an abundance of UVB signature mutations in the p53 gene [Rebel, et al. 2005], the UVA1-induced carcinomas showed a striking lack of p53 mutations – aside from a few UVB-like hotspot mutations -, i.e., an overall lack of expected oxidation-related mutations, e.g., G to T transversions [vn Kranen, et al. 1997]. Defects in transcription coupled nucleotide excision repair (TCR) led to a loss of the strand bias in UVB-induced p53 mutations [van Zeeland, et al. 2005]. Interestingly, a defect in TCR led to greatly enhanced induction of benign papillomas with H-ras mutations in codon 12 (with CC in the transcribed strand) [de Vries, et al. 1998]. Benign papillomas with H-ras mutations (codon 61) were also predominantly induced with chemical two stage DMBA/TPA skin carcinogenesis [Pazzaglia, et al. 2001]. Like the tumor promoter TPA (12-O-tetradecanoylphorbol-13-aceate), UVA1 radiation causes arachidonic acid release from membrane lipids, inflammation, hyperplasia through eicosanoids, and an increase in PKC [de Gruijl 2000]. These membrane-related effects together with the induction of papillomas, reveal tumor promotional mechanisms triggered by UVA1 radiation, similar to ATP.

Nevi were most efficiently induced in the skin of hairless mice by intermittent overexposure to UVB radiation [van Schanke, et al. 2006]; UVA1 radiation proved to be ineffective – fully in line with the exposure regimen that induced melanocytes proliferation most effectively. Neonatal UVB overexposure also contributed to induction of nevi. Despite an abundance of nevi in these experiments with either wild type mice or mice defective in nucleotide excision repair (Xpa) and/or defective in the tumor suppressors p16Ink4a/p19Arf, we only found very few melanomas (which did not carry the N-ras or B-raf mutations found in human melanomas). An experiment with intermittent overexposure of DNA repair-defective Xpc-/- mice had to be discontinued prematurely because the skin of these animals did not recuperate sufficiently between overexposures, in contrast to the skin of wild type animals. Recently, Yang et al [Yang, et al. 2007] reported on the induction of cutaneous melanoma in Xpc-/- Ink4a/Arf-/- mice by a single neonatal UVB exposure; these tumors carried K-ras mutations in codon 61. Hence, the experiments on UV-induced melanoma genesis are progressing to the stage that they emulate the pathogenesis of human melanoma.

References

Applegate LA, Lautier D, Frenk E, Tyrrell RM. 1992. Endogenous glutathione levels modulate the frequency of both spontaneous and long wavelength ultraviolet induced mutations in human cells. Carcinogenesis. 13:1557-60.

Berg RJ, de Laat A, Roza L, van der Leun JC, de Gruijl FR. 1995. Substitution of equally carcinogenic UV-A for UV -B irradiations lowers epidermal thymine dimer levels during skin cancer induction in hairless mice. Carcinogenesis 16(10):2455-9.

de Gruijl FR. 2000. Photocarcinogenesis: UVA vs UVB. Methods Enzymol. 319:359-66.

de Gruijl FR, Sterenborg HJ, Forbes PD, Davies RE, Cole C, Kelfkens G, van Weelden H, Slaper H, van der Leun JC. 1993. Wavelength dependence of skin cancer induction by ultraviolet irradiation of albino hairless mice. Cancer Res 53(1):53-60.

de Laat A, Kroon ED, de Gruijl FR. 1997a. Cell cycle effects and concomitant p53 expression in hairless murine skin after longwave UVA (365 nm) irradiation: a comparison with UVB irradiation. Photochem Photobiol 65(4):730-5.

de Laat A, van der Leun JC, de Gruijl FR. 1997b. Carcinogenesis induced by UVA (365-nm) radiation: the dose-time dependence of tumor formation in hairless mice. Carcinogenesis 18(5):1013-20.

de Vries A, Berg RJ, Wijnhoven S, Westerman A, Wester PW, van Kreijl CF, Capel PJ, de Gruijl FR, van Kranen HJ, van Steeg H. 1998. XPA-deficiency in hairless mice causes a shift in skin tumor types and mutational target genes after exposure to low doses of U.V.B. Oncogene 16(17):2205-12.

D'Errico M, Lemma T, Calcagnile A, Proietti De Santis L, Dogliotti E. 2007. Cell type and DNA damage specific response of human skin cells to environmental agents. Mutat Res 614(1-2):37-47.

Dumaz N, van Kranen HJ, de Vries A, Berg RJ, Wester PW, van Kreijl CF, Sarasin A, Daya-Grosjean L, de Gruijl FR, et al. 1997. The role of UV-B light in skin carcinogenesis through the analysis of p53 mutations in squamous cell carcinomas of hairless mice. Carcinogenesis. 18:897-904. .

Enninga IC, Groenendijk RTL, Filon AR, van Zeeland AA, Simons JWIM. 1986. The wavelength dependence of u.v.-induced pyrimidine dimer formation, cell killing and mutation induction in human diploid skin fibroblasts. Carcinogenesis 7:1829-36
Godar DE, Miller SA, Thomas DP. 1994. Immediate and delayed apoptotic cell death mechanisms: UVA versus UVB and UVC radiation. Cell Death Differ 1:59-66.
Kelfkens G, de Gruijl FR, van der Leun JC. 1991. Tumorigenesis by short -wave ultraviolet A: papillomas versus squamous cell carcinomas. Carcinogenesis 12(8):1377-82.
Kielbassa C, Roza L, Epe B. 1997. Wavelength dependence of oxidative DNA damage induced by UV and visible light. Carcinogenesis 18:811-6.
Nijhof JG, van Pelt C, Mulder AA, Mitchell DL, Mullenders LH, de Gruijl FR. 2007. Epidermal stem and progenitor cells in murine epidermis accumulate UV damage despite NER proficiency. Carcinogenesis 28:792-800.
Pazzaglia S, Mancuso M, Primerano B, Rebessi S, Biozzi G, Covelli V, Saran A. 2001. Analysis of c-Ha-ras gene mutations in skin tumors induced in carcinogenesis-susceptible and carcinogenesis-resistant mice by different two-stage protocols or tumor promoter alone. Mol Carcinog 30(2):111-8.
Rebel H, Kram N, Westerman A, Banus S, van Kranen HJ, de Gruijl FR. 2005. Relationship between UV-induced mutant p53 patches and skin tumours, analysed by mutation spectra and by induction kinetics in various DNA-repair-deficient mice. Carcinogenesis. 26(12):2123-30.
van Kranen HJ, de Laat A, van de Ven J, Wester PW, de Vries A, Berg RJ, van Kreijl CF, de Gruijl FR. 1997. Low incidence of p53 mutations in UVA (365-nm)-induced skin tumors in hairless mice. Cancer Res 57(7):1238-40.
van Schanke A, Jongsma MJ, Bisschop R, van Venrooij GM, Rebel H, de Gruijl FR. 2005. Single UVB overexposure stimulates melanocyte proliferation in murine skin, in contrast to fractionated or UVA-1 exposure. J Invest Dermatol 124:241-7.
van Schanke A, van Venrooij GM, Jongsma MJ, Banus HA, Mullenders LH, van Kranen HJ, de Gruijl FR. 2006. Induction of nevi and skin tumors in Ink4a/Arf Xpa knockout mice by neonatal, intermittent, or chronic UVB exposures. Cancer Res 66(5):2608-15.
van Zeeland AA, Vreeswijk MP, de Gruijl FR, van Kranen HJ, Vrieling H, Mullenders LF. 2005. Transcription-coupled repair: impact on UV-induced mutagenesis in cultured rodent cells and mouse skin tumors. Mutat Res 577(1-2):170-8.
Yang G, Curley D, Bosenberg MW, Tsao H. 2007. Loss of xeroderma pigmentosum C (Xpc) enhances melanoma photocarcinogenesis in Ink4a-Arf-deficient mice. Cancer Res 67(12):5649-57.

III.16. Ultraviolet radiation induced cataract, what did we learn from experiments?

Per Söderberg
St. Erik's Eye Hospital, Karolinska Institutet, Stockholm, Sweden

It is estimated that world wide there are 16-17 million individuals bilaterally blind from cataract making cataract the leading cause of blindness [Asbell, et al. 2005], and that the prevalence of cataract will double by year 2020 due to the increasing and rapidly aging world population. It is predicted that the health and economic burden of cataract on societies will escalate, particularly in the developing countries, where cataract occurs at an earlier age and cataract surgery is often inaccessible. Epidemiology has demonstrated that ultraviolet radiation (UVR) from the sun is the most important preventable cause of cataract [McCarty, et al. 2000].

The human cornea transmits almost 50 % of UVR in the wavelength region 400-320 nm. Below 320 nm, the transmittance quickly drops to a few percent at around 300 nm and essentially nothing below 290 nm. Almost all UVR that penetrates the cornea is attenuated in the ocular lens. Experimental data, using the endogenous chromophore lactate dehydrogenase, has demonstrated that in vivo UVR around 300 nm is attenuated within 0.5 mm ($1/e^2$) from the anterior surface of the lens.

Experimental in vivo exposure of animals has demonstrated that the dose-response function for UVR induced cataract is continuous. This finding has triggered development of a strategy to establish a statistically defined threshold, the Maximum Tolerable Dose-2.3:16 ($MTD_{2.3:16}$) [Söderberg, et al. 2002]. There is a 16 % probability that an individual exposed to $MTD_{2.3:16}$ kJ/m^2 expresses more light scattering after the exposure than is expected in 2.3 % of normal lenses in individuals with not exposed eyes. Using this strategy, earlier qualitative data indicating a maximum in vivo sensitivity of the lens at around 300 nm has been confirmed quantitavely. It was further demonstrated that the in vivo sensitivity is almost 5 times higher for very young individuals than for elderly individuals. This has led to the suggestion that biological efficient irradiance for the lens should be weighted for age as well as wavelength. Adopting the $MTD_{2.3:16}$ strategy, it was recently experimentally estimated that after sub-threshold dose, approximately 20 % of the damage is repaired by the lens with a repair rate (1/e) of 8 days. This contrasts with the assumption in current safety standards that all doses more than 24 hrs apart are to be considered additive.

In vivo experiments designed with the $MTD_{2.3:16}$ strategy have also demonstrated that there is a considerable species variation in sensitivity that grossly exceeds the variation expected due to species variation of attenuation in the cornea. This, indicates that evolution has led to biological adaptation and implicates that there are possibilities to pharmacologically modify the sensitivity.

In vivo experimental exposure of the lens to supra-threshold UVR induces light scattering that increases asymptotically declining. It was demonstrated that the light scattering is associated with a transient cation shift that osmotically induces edema and mitochondrial swelling.

It is believed that UVR causes photooxidation in the lens by primary absorption in N-formylkynurenine or one of its derivatives, either directly or after energy transfers to oxygen so that reactive oxygen species are formed. Reduced glutathione that can be recycled is believed to be one of the principle intrinsic defense mechanisms against oxidation insult in the lens.

It was demonstrated that in vivo exposure to UVR depletes the glutathione content, primarily in the anterior portion of the lens. It was further experimentally demonstrated that peroral supplementation with the antioxidant vitamin E-analogue, α-tocopherol, dose dependently protects against in vivo UVR cataract and that this protection is associated with protection against glutathione depletion. It

was also demonstrated in vivo that lack of the thiol transferase Glutaredoxin 1, that catalyzes glutathione mediated reduction of protein disulfide bonds, increases the toxicity of UVR.

Morphologically, experimental in vivo exposure to supra-threshold UVR dose induced apoptotic morphology. It was also demonstrated that in vitro exposure of the lens to UVR causes unscheduled DNA-synthesis. Further, it was recently shown that in vivo exposure to double threshold dose of UVR induces expression of message and product of the apoptosis controlling gene p53 [Ayala, et al. 2007]. It was also demonstrated with TUNEL labeling that in vivo UVR exposure to just above threshold dose induces apoptotic DNA degradation and that message and product for the apoptosis associated enzyme caspase-3 is expressed.

Blindness due to cataract is threatening the socioeconomic development of a large fraction of the world population and is not believed to be possible to solve with cataract surgery alone. Atmospheric changes may increase the exposure of the eye to the epidemiologically proven most important preventable cause of cataract, UVR. It is anticipated that recently increased knowledge on the dose-response function for in vivo UVR cataract, factors impacting on the in vivo lens sensitivity to UVR, and the kinetics of sub-threshold damage will improve the rational for preventive measures against UVR induced cataract. Further, it is my hope that the recently increased understanding of the pathophysiology of UVR induced cataract may provide clues to cheap pharmacological intervention that can prevent or at least delay cataract.

References

Asbell PA, Dualan I, Mindel J, Brocks S, Ahmad M, Epstein S. 2005. Age related cataract. Lancet 365:599-609.

Ayala MN, Strid H, Jacobsson U, Söderberg PG. 2007. p53 expression and apoptosis. In: The lens after ultraviolet radiation exposure. Invest Ophthalmol Visual Sci 48:4187-91.

McCarty CA, Nanjan MB, Taylor HR. 2000. Attributable risk estimates for cataract to prioritize medical and public health action. Invest Ophthalmol Visual Sci 41:3720-5.

Söderberg PG, Löfgren S, Ayala M, Dong X, Kakar M, Mody V. 2002. Toxicity of ultraviolet radiation exposure to the lens expressed by maximum tolerable dose (MTD). Dev Ophthalmol 35:70-5.

III.17. Melanoma susceptibility genes and interaction with sun exposure

Julia A Newton Bishop for the Melanoma Group
Division of Biostatistics and Epidemiology, Leeds Institute of Molecular Medicine, University of Leeds, UK

CDKN2A mutations explain susceptibility to melanoma in the majority of families in which hereditary mutations have been identified. Overall around 40% of 3 or more case families have mutations [Goldstein, et al. 2007]. In families studied by the Melanoma Genetics Consortium (GenoMEL, http://www.genomel.org), 2% have germline CDK4 mutations and 2% have splice site variants or deletions which impact only on the p14ARF product of the CDKN2A locus [Goldstein, et al. 2006]. In the remaining high-risk families without, to date, identified susceptibility genes, there is evidence of linkage to 1p36 [Bale, et al. 1989] and 1p22 [Gillanders, et al. 2003]. That CDKN2A mutations are found in the majority of the families with high numbers of melanoma cases suggests that other susceptibility genes are likely to have a lower penetrance. Within melanoma families overall, the presence of multiple cases, early age of onset, family members with multiple primaries and pancreatic cancer predict the likelihood of finding a mutation [Goldstein, et al. 2007], although the predictive value varies considerably between continents. This variation may result from differences in the dominant CDKN2A mutations between populations or may represent gene/environment interaction.

CDKN2A penetrance has been estimated by GenoMEL [Bishop, et al. 2002]. The 80 analyzed families contained 402 melanoma patients, 320 of whom were tested for mutations and 291 were mutation carriers. We also tested 713 unaffected family members for mutations and 194 were carriers. Overall, CDKN2A mutation penetrance was estimated to be 0.30 (95% confidence interval (CI) = 0.12 to 0.62) by age 50 years and 0.67 (95% CI = 0.31 to 0.96) by age 80 years. Penetrance was not statistically significantly modified by gender or by whether the CDKN2A mutation altered p14ARF protein. However, there was a statistically significant effect of residing in a location with a high population incidence rate of melanoma (P =0.003). By age 50 years CDKN2A mutation penetrance reached 0.13 in Europe, 0.50 in the United States, and 0.32 in Australia; by age 80 years it was 0.58 in Europe, 0.76 in the United States, and 0.91 in Australia. Thus CDKN2A mutation carriers were more likely to develop a melanoma if they had been exposed to higher levels of sun exposure; indicative of gene/environment interaction.

The proportion of 3 case families moreover, with CDKN2A mutations varies between continents, ranging from 20% (32/162) in Australia to 45% (29/65) in North America to 57% (89/157) in Europe [Goldstein, et al. 2007]. The explanation for this variation is currently under investigation, but a likely explanation is interaction between susceptibility genes and sun exposure. At lower latitudes, exposure to the sun is more marked and is hypothesized to increase the penetrance of less intrinsically penetrant susceptibility genes such as the melanocortin receptor MC1R. Thus where sun exposure is greater, then considerable clustering may occur as a result of people with sun sensitive phenotypes, sharing life styles associated with melanoma risk: whereas in less sunny countries such clustering would likely be attributable to inheritance of CDKN2A mutations.

The pattern of sun exposure, which is most important in families with CDKN2A mutations, is still under investigation. In sporadic melanoma, sun sensitivity, sunburn and vacational sun exposure are consistently shown to predispose to melanoma, and there is little evidence for a role for chronic sun exposure overall (except for melanoma on the head and neck). It will be interesting to see if this is also found in CDKN2A families.

The most potent phenotypic risk factor for melanoma is the presence of increased numbers of melanocytic naevi [Gandini, et al. 2005]. Twin studies provide good evidence that these naevi are genetically determined with a small effect of shared environment including sun exposure [Wachsmuth, et al. 2005]. We carried out a twin study in the UK in adolescent twins. By including

phenotypic variables and reported sun exposure into the heritability analysis, we concluded that 66% of the total variance of naevus count is attributable to genetic effects: 7% associated to eye colour, 6% to hair colour, and 1% to reported skin type, which leaves 52% as to yet unidentified genetic factors. Of the 25% of variation attributable to environmental influences, one-third is estimated to be because of sun exposure on hot holidays. Thus putative naevus genes interact with sun exposure in terms of expression of the phenotype. A recent study in patients with the atypical mole syndrome suggests that this interaction also moderates melanoma risk as would be expected [C. Bertram, et al., personal communication].

References

Bale SJ, Dracopoli NC, Tucker MA, Clark WH Jr, Fraser MC, Stanger BZ, et al. 1989. Mapping the gene for hereditary cutaneous malignant melanoma-dysplastic nevus to chromosome 1p. N Engl J Med 320:1367-72.

Bishop DT, Demenais F, Goldstein AM, Bergman W, Bishop JN, Bressac-de Paillerets B, et al. 2002. Geographical variation in the penetrance of CDKN2A mutations for melanoma. J Natl Cancer Inst 94:894-903.

Gandini S, Sera F, Cattaruzza MS, Pasquini P, Abeni D, Boyle P, et al. 2005. Meta-analysis of risk factors for cutaneous melanoma: I. Common and atypical naevi. Eur J Cancer 41:28-44.

Gillanders E, Hank Juo SH, Holland EA, Jones M, Nancarrow D, Freas-Lutz D, et al. 2003. Localization of a novel melanoma susceptibility locus to 1p22. Am J Hum Genet 73:301-13.

Goldstein AM, Chan M, Harland M, Gillanders EM, Hayward NK, Avril MF, et al. 2006. High-risk melanoma susceptibility genes and pancreatic cancer, neural system tumors, and uveal melanoma across GenoMEL. Cancer Res 66:9818-28.

Goldstein AM, Chan M, Harland M, Hayward NK, Demenais F, Bishop DT, et al. 2007. Features associated with germline CDKN2A mutations: a GenoMEL study of melanoma-prone families from three continents. J Med Genet 44:99-106.

Wachsmuth RC, Turner F, Barrett JH, Gaut R, Randerson-Moor JA, Bishop DT, et al. 2005. The effect of sun exposure in determining nevus density in UK adolescent twins. J Invest Dermatol 124:56-62.

III.18. A behavioural model for estimating population exposure to solar ultraviolet radiation

Brian Diffey
Emeritus Professor of Photobiology School of Clinical and Laboratory Sciences University of Newcastle, UK

Daily ambient erythemal ultraviolet (UV) radiation shows a clear-sky summer to winter ratio of about 20:1 in temperate latitudes (~50°), falling to about 3:1 in tropical latitudes (~30°), with day-to-day perturbations superimposed on this annual cyclic pattern as a result of cloud cover. However the UV exposure of an individual living at a specific location will exhibit much greater fluctuations than ambient variation due to differences in time spent outdoors and proximity to shade on different days throughout the year. Furthermore, the UV dose absorbed by the skin is further modified by the use of photoprotective agents such as hats, clothing and sunscreens.

Estimates of personal exposure are normally obtained by direct measurement using UV sensitive film badges [Diffey 1989] or electronic dosimeters [Thieden, et al. 2004a]. There is a great deal of heterogeneity in published dosimetric studies concerning factors such as numbers of subjects monitored, geographical location, period of study (ranging from a few days to sampling different periods throughout the year), anatomical site of dosimeter placement and data presentation.

An alternative approach is to model the variables that affect personal exposure and this is the basis of the method that will be reported, which uses a random sampling technique to explore variability of exposure at different times of the year by habitués.

The input data to the model are ambient erythemal UV throughout the year at the appropriate location, the fraction of solar UV received on the face relative to ambient, and the frequency distribution of time spent outdoors at different periods of the year. To provide data on the form of the distribution and the parameters describing it for various exposure periods, an online survey, hosted on the website of Cancer Research UK[7], was carried out during the summer of 2007.

Exemplar results will be presented for indoor workers living in either northern Europe or Florida. It is clear from the results that there are large seasonal variations in personal erythemal exposure, especially for indoor workers in northern Europe, which are due not only to seasonal changes in ambient, but just as importantly to seasonal variation in behaviour. Not surprisingly, holiday and summer weekend exposure account for the largest daily UV doses, a conclusion reached from personal UV monitoring studies in Denmark [Thieden, et al. 2004a, b].

So whilst there is only a 20-fold difference in clear-sky daily erythemal UV from mid-winter to mid-summer at latitudes of about 50°N, there is something like a 1000-fold variation in daily personal dose throughout the year with a dose to the face of more than 2 SED (roughly equivalent to one minimal erythema dose (MED) in unacclimatized, sensitive white skin) on about 16 to 22 days of the year in northern Europe, with a corresponding figure for Florida of 40-50 days. For 7 to 8 months of the year in northern Europe an indoor worker can expect to receive a facial exposure of less than 0.2 SED (roughly equivalent to one-tenth of an MED), but for people living in Florida this exposure would be exceeded on about 80% of days per year.

The annual facial exposure for people living in Florida is around 400 SED and of this total, about 100 SED is the result of 2-week vacational exposure. Clearly if sunscreens or other forms of photoprotection are used, the absorbed dose to the skin will be less than this.

For indoor workers living in northern Europe, a typical annual exposure is estimated to be about 150 SED. About one-third of this exposure is received during the 2-week summer vacation when this is

[7] http://info.cancerresearchuk.org/healthyliving/sunsmart/about-sunsmart/campaignresearch/?a=5441

taken at home latitudes. When the calculations are repeated assuming northern Europeans spend their 2-week summer vacation in Florida (but retaining the same distribution of time spent outdoors in holiday), the facial exposure on vacation doubles to about 100 SED and the annual exposure increases from around 150 to 200 SED. This illustrates just how important sun protection measures should be during recreational exposure in areas of high insolation if the annual UV burden is to be sensibly controlled.

One advantage of mathematical modelling is to predict how a system will behave without the need to undertake expensive, time-consuming, impractical or even impossible experiments. The model that will be described is straightforward to implement using Excel spreadsheets, rapidly adapted to different populations and situations, such as duration and location of vacations or changes in lifestyle, and yields results that are consistent with dosimetric field studies with human subjects.

References

Diffey BL. 1989. Ultraviolet radiation dosimetry with polysulphone film. In: Diffey BL. ed. Radiation measurement in photobiology, pp 135-139. London, Academic Press.
Thieden E, Philipsen PA, Heydenreich J, Wulf HC. 2004a. UV radiation exposure related to age, sex, occupation, and sun behaviour based on time-stamped personal dosimeter readings. Arch Dermatol 140:197-203.
Thieden E, Philipsen PA, Sandy-Møller J, Heydenreich J, Wulf HC. 2004b. Proportion of lifetime UV dose received by children, teenagers and adults based on time-stamped personal dosimetry. J Invest Dermatol 123:1147-50.

III.19. Artificial ultraviolet sources and skin cancers

Philippe Autier
International Agency for Research on Cancer, Lyon, France

Most artificial tanning devices carry a cancer risk comparable to Mediterranean sunlight. Experiments in human volunteers conducted during the last decade have shown that commercial tanning lamps produce the types of DNA damage associated with exposure to the solar spectrum.

In 2005, the IARC convened a Working Group of international experts on skin cancer and UV radiation in order to perform a systematic review of the potential association between sunbed use and skin cancer. The Working Group undertook a series of actions including a meta-analysis of the available twenty-three published studies (22 case–control, one cohort) in light-skinned populations which investigated the association between indoor tanning use and melanoma risk. The summary relative risk for ever versus never use of indoor tanning facilities from the 19 informative studies was 1.14 (95% Confidence Interval (CI): 1.00–1.31). When the analysis was restricted to the nine population-based case–control studies and the cohort study, the summary relative risk was 1.17 (95% CI: 0.96–1.42). The Working Group identified 7 epidemiological studies that assessed the melanoma risk associated with sunbed use according to age. All these studies found melanoma risks ranging from 1.4 to 3.8 with sunbed use starting during adolescence or during young adulthood. The meta-analysis performed by the IARC Working Group using published results of these seven studies found an overall increase in the risk of melanoma of 75% (summary relative risk: 1.75, 95% CI: 1.35-2.26) when sunbed use started before 35 years of age. Studies on exposure to indoor tanning appliances and squamous cell carcinoma found some evidence for an increased risk for squamous cell carcinoma, especially when age at first use was below 20 years.

Long latency periods may be expected between sunbed exposure and skin cancer, and therefore the real magnitude of the association may not yet be detectable. A considerable body of experimental and epidemiological knowledge support the hypothesis that exposure during childhood and adolescence are the most crucial periods of life for the initiation of biological phenomenon involved in the genesis of melanoma that will usually be diagnosed during adulthood.

In conclusion, it would be logical to recommend avoidance of sunbed use before 30 years old.

III.20. Exposure to solar and artificial ultraviolet radiation and the risk of cutaneous malignant melanoma – The Norwegian-Swedish Women's Lifestyle and Health Cohort Study

Marit B. Veierød[1], Elisabete Weiderpass[2]
[1]Department of Biostatistics, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway
[2]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden

Objective
To study solar and artificial ultraviolet (UV) radiation and risk of cutaneous malignant melanoma (hereafter called melanoma) in a large cohort study.

Background
Over the recent decades, incidence and mortality rates of melanoma have increased in Europe. Solar UV exposure is the major established risk factor for melanoma. Host susceptibility factors and the amount and pattern of the solar UV exposure can influence melanoma risk. Analysis after 8 years of follow-up of the Norwegian-Swedish Women's Lifestyle and Health Cohort Study, suggested that use of a solarium, i.e. a sunbed or sunlamp that emits artificial UV light, was associated with melanoma risk [Veierød, et al. 2003]. A recent review of the available evidence on artificial UV exposure and skin cancer, concluded that indoor tanning during adolescence or early adulthood increases risk of melanoma [IARC 2006a, b]. Yet, the evidence regarding dose-response is limited and the prolonged lag period after UV exposure is unknown. Moreover, the role of UVA and UVB on melanoma risk needs to be clarified.

Material and methods
The Norwegian-Swedish Women's Lifestyle and Health Cohort Study included more than 100 000 women aged 30-50 years in 1991/92 [Veierød, et al. 2003]. UV exposure and other individual characteristics were collected at cohort enrolment through a self-administered questionnaire. The participants were asked about their life histories of sunburn, sunbathing vacations and use of a solarium when they were aged 0-9, 10-19, 20-29, 30-39 or 40-49 years. Follow-up was achieved by linkages of the study database to national registries in Norway and Sweden through 2005. Relative risks are estimated by Poisson regression.

Results
The study sample now consists of 106 366 women. During an average follow-up of 14 years, 412 incident cases of melanoma have been reported to the national Cancer Registries. Detailed results regarding individual susceptibility and UV exposure will be presented. Use of solarium at least once a month during the second, third and fourth decades of life seems to increase melanoma risk. An important strength of the new analysis as compared to the previous published results is the large number of cases, which gives more precise estimates and enables investigation of more aspects of the UV-melanoma association. Due to implementation of national regulations for indoor tanning devices, the UVB to UVA ratio has changed over the period since 1980 and calendar year of use of tanning device is of interest in the discussion of these and other epidemiologic study results [Lazovich, et al. 2004; IARC 2006a, b].

References
IARC. 2006a. Exposure to artificial radiation and skin cancer. Lyon, International Agency for Research on Cancer. IARC Working Group Reports, vol 1.
IARC. 2006b. The association of use of sunbeds with cutaneous malignant melanoma and other skin cancers: A systematic review. Int J Cancer 120:1116–22.
Lazovich D, Sweeney C, Weinstock MA, Berwick M. 2004. Re: A prospective study of pigmentation, sun exposure, and risk of cutaneous malignant melanoma in women. J Natl Cancer Inst 96:335.

Veierød M.B, Weiderpass E, Thörn M, Hansson J, Lund E, Armstrong B, Adami HO. 2003. A prospective study of pigmentation, sun exposure, and risk of cutaneous malignant melanoma in women. J Natl Cancer Inst 95:1530-8.

III.21. Distinct Patterns of Genetic and Phenotypic Alternations in Melanoma that Depend on Anatomic Site and Degree of UV exposure

Boris C. Bastian
Department of Dermatology and Pathology, University of San Francisco, CA, USA

Melanocytic neoplasms, both benign and malignant, display a kaleidoscope of phenotypic variation. Multiple categories of melanoma are distinguished in the current WHO classification of skin tumors [Leboit, et al. 2006] but once metastasis has occurred there is a very homogeneous and grim clinical course independent of the type of melanoma. The cytotoxic and immunological therapies that have been available, have not shown much therapeutic effectiveness for any melanomas. In addition, many of the classification criteria that have been proposed show considerable overlap, making reproducible categorization of cases difficult [Weyers, et al. 1999]. Thus melanoma is frequently regarded as a homogeneous disease, and only a small minority of investigative studies in the fields of epidemiology, cell biology, genetics, and therapy has emphasized potential differences among disease subgroups. Despite the impression of the uniformity of many disease characteristics, there are strong arguments supporting the existence of distinct melanoma types. Although exposure to UV light is considered an important etiologic factor in melanocytic neoplasia, its role is complex. Certain melanoma types such as melanomas on mucosal membranes arise in complete absence of UV irradiation. Similarly, melanomas can arise in sites such as glabrous skin i.e. the non hair-bearing skin of the palms and soles and areas under the nails that are well protected from UV radiation. These two forms of melanoma, mucosal and acral, arise with similar absolute incidences throughout world populations independent of ethnicity and latitude [Elwood, et al. 1989]. In Caucasians, these “non-UV” induced melanomas are outnumbered significantly by melanomas occurring on sun-exposed skin, but overall represent a significant fraction of the world melanoma burden. The apparent susceptibility of Caucasians to melanomas on sun-exposed skin may not be entirely attributed to differences in skin pigmentation, because albinos of African descent do not have a significantly increased incidence of melanomas, but rather an increase in squamous cell carcinoma [Streutker, et al. 2000]. These divergent patterns suggest fundamental differences of the role of UV-induction and body site distribution dependent on the underlying genetic predisposition. Moreover, the vast phenotypic differences among melanomas can be suspected of having a genetic basis, which could form the framework on which to base a useful classification scheme. We used a genetic approach to compare primary melanomas from different body sites and degrees of chronic sun induced damage of the surrounding skin. The differences we have found range from the nature of the genomic instability, the specific genomic regions that are gained and lost, the frequencies of mutations in specific genes, to the effect of germ line genetic variants on risks for melanomas with particular characteristics. E.g. we were able to demonstrate that melanomas on acral skin and mucosa have a type of genomic instability that results in the frequent occurrence of amplifications and deletions involving very small segments of the genome, while such events are much rarer in melanomas on sun-exposed skin. The genomic regions that are amplified differ in mucosal and acral melanomas. More generally, genomic aberrations can be used to distinguish melanoma types [Bastian, et al. 2000, 2003; Curtin, et al. 2005]. Furthermore, he found differences in the mutation frequency of BRAF. BRAF mutations occurred in ~ 60% of cases on skin subject to sun exposure but without chronic sun damage (CSD) as measured by solar elastosis. By contrast, BRAF mutation frequencies were much lower in melanomas arising without sun exposure or on skin with marked CSD [Maldonado, et al. 2003; Curtin, et al. 2005]. Similarly, true congenital nevi which arose in utero under complete UV-protection, also did not show BRAF mutations [Bauer, et al. 2007]. Based on our findings of we hypothesized that the high frequency of BRAF mutations in melanomas arising on sun-exposed skin without CSD were due to a germ line susceptibility factor resulting in UV-sensitivity [Curtin, et al. 2005]. In collaboration with Dr. Teresa Landi from the NIH we subsequently showed that germline variants of MC1R were a major component of this susceptibility [Landi, et al. 2006]. Further analysis our array CGH data suggested a genomic region harboring KIT to be important in melanomas on mucosa, acral skin and skin with CSD. We found oncogenic mutations or focused copy number increases in the gene in 40% of acral and mucosal melanomas, and 30% of CSD melanomas.

These results suggest that the existing drugs such as imatanib could be useful in certain melanoma types [Curtin, et al. 2006]. More recent studies correlating phenotypic alterations with the underlying genotype have shown that mutations in BRAF are associated with distinct histopathological features of the primary tumor that can be combined to simple algorithms that can predict the mutation status of BRAF with high accuracy.

References

Bastian BC, Kashani-Sabet M, Hamm H, Godfrey T, Moore II DH, Bröcker EB, LeBoit PE, Pinkel D. 2000. Gene amplifications characterize acral melanoma and permit the detection of occult cells in the surrounding skin. Cancer Res 60:1968-73.

Bastian BC, Olshen AB, LeBoit PE, Pinkel D. 2003. Classifying melanocytic tumors based on DNA copy number changes. Am J Pathol 163:1765-70.

Bauer J, Curtin JA, Pinkel D, Bastian BC. 2007. Congenital melanocytic nevi frequently harbor NRAS mutations but no BRAF mutations. J Invest Dermatol 127:179-182.

Curtin JA, Busam K, Pinkel D, Bastian BC. 2006. Somatic activation of KIT in distinct subtypes of melanoma. J Clin Oncol 24:4340-46.

Curtin JA, Fridlyand J, Kageshita T, Patel HN, Busam KJ, Kutzner H, Cho KH, Aiba S, Brocker EB, LeBoit PE, Pinkel D, Bastian BC. 2005. Distinct sets of genetic alterations in melanoma. N Engl J Med 353:2135-47.

Elwood JM. 1989. Epidemiology and control of melanoma in white populations and in Japan. J Invest Dermatol 92:214S-221S.

Landi MT, Bauer J, Pfeiffer RM, Elder DE, Hulley B, Minghetti P, Calista D, Pinkel D, Kanetsky PA, Bastian BC. 2006. MC1R germline variants confer risk for BRAF-mutant melanoma. Science 313:512-12.

LeBoit PE, Burg G, Weedon D, Sarasin A. 2006. Skin tumours. Pathology & genetics. Lyon, IAR-CPress.

Maldonado JL, Fridlyand J, Patel H, Jain AN, Busam K, Kageshita T, Ono T, Albertson D, Pinkel D, Bastian BC. 2003. Determinants of BRAF mutations in primary melanoma. J Natl Cancer Inst 95:1878-90.

Streutker CJ, McCready D, Jimbow K, From L. 2000. Malignant melanoma in a patient with oculocutaneous albinism. J Cutan Med Surg 4:149-52.

Weyers W, Euler M, Diaz-Cascajo C, Schill WB, Bonczkowitz M. 1999. Classification of cutaneous malignant melanoma: a reassessment of histopathologic criteria for the distinction of different types. Cancer 86:288-99.

III.22. NRAS and BRAF mutations in human cutaneous melanoma

Johan Hansson, Suzanne Egyházi, Anton Platz
Department of Oncology-Pathology, Karolinska Institutet, Cancer Center Karolinska, Karolinska University Hospital Solna, Stockholm, S-17176, Sweden

Mutational activation of components of the Ras-Raf-Mek-Erk signal transduction pathways, as well as alterations in genes regulating the cell cycle machinery are common among human melanomas. A majority of cutaneous melanomas show activating mutations in the *NRAS* or *BRAF* proto-oncogenes [Albino, et al. 1984; Davies, et al. 2002]. In cutaneous melanomas *NRAS* codon 61 mutations, mainly Q61R (CAA/ CGA) and Q61K (CAA/AAA) changes, are by far the most frequent *RAS* alterations. In *BRAF* the V600E mutation (GTG/GAG), accounts for over 90% of mutations in melanoma.

In investigations of primary and metastatic melanomas we and others have observed that both *NRAS* and *BRAF* mutations occur early, already in the radial growth phase of melanomas, and in a mutually exclusive manner [Omholt, et al. 2002, 2003]. In a large screening of *NRAS* or *BRAF* mutations in primary melanomas and metastases we analysed a total of 294 melanoma tumours from 219 patients [Edlundh-Rose, et al. 2006]. Mutations in *BRAF* exons 11 and 15 were identified in 156 (53%) tumours and *NRAS* exon 2 mutations in 86 (29%) tumours. Overall, mutations in *NRAS* or *BRAF* were found in 242 of 294 tumours (82%) and were mutually exclusive in all but two cases (0.7%). Multiple metastases were analysed in 57 of the cases and mutations were identical in all except 3, indicating that *BRAF* and *NRAS* mutations occur before metastasis. Association with pre-existing nevi was significantly higher in *BRAF* mutated tumours (P =0.014). In addition, tumours with *BRAF* mutations showed a significantly more frequent moderate to pronounced infiltration of lymphocytes (P = 0.013). *NRAS* mutations were associated with a significantly higher Clark level of invasion (P= 0.022) than *BRAF* mutations. Age at diagnosis was significantly higher in tumours with *NRAS* mutations than in those with *BRAF* mutations (P = 0.019). *NRAS* and *BRAF* mutations, however, did not influence the overall survival from time of diagnosis (P = 0.7).

A large body of data links the risk of melanoma to intermittent and/or chronic exposure to solar ultraviolet radiation (UV) and UV has been implicated in the induction of melanoma-associated *NRAS* and *BRAF* mutations. While the *NRAS* activating codon Q61 (CAA) is UV sensitive site where UV-induced di-pyrimidine dimers may occur in the non-coding DNA strand, the *BRAF* V600 codon (GTG) is not an obvious site for UV mutagenesis. However, the *BRAF* V600E mutation has been suggested to be induced by UV-induced indirect mechanisms such damage induced by reactive oxygen species, or by cyclobutane dimer formation in the vicinity of codon 600, followed by error prone repair via specialized DNA polymerases. In a study of primary familial melanomas from patients with germline *CDKN2A* mutations we found *NRAS* codon 61 mutations in 95% (20/21) of tumors [Eskandarpour, et al. 2003]. The majority of these individuals carried the *CDKN2A* p.112dupR founder mutation and we postulated that the high frequency of *NRAS* activation may reflect a hyper-mutability phenotype associated with this hereditary defect. These initial findings are now extended in a larger set of familial melanomas with different underlying germline *CDKN2A* mutations within the GenoMEL network of collaborating centers.

In a review of the published literature we found that superficial spreading melanomas and nodular melanomas from continuously as well as intermittently sun exposed body sites, show the highest frequencies of *NRAS* as well as *BRAF* mutations, with lower frequencies of such mutations in lentigo maligna melanomas and acral lentiginous melanomas. The lowest *NRAS* or *BRAF* mutation frequencies are observed in melanomas on non-UV exposed mucosal membranes. *NRAS* mutations are more frequent in tumors on continuously sun exposed body sites in comparison to intermittently sun exposed locations, while the pattern is the opposite for *BRAF* mutations.

In conclusion, activating mutations in *NRAS* and *BRAF* are very common in melanoma emphasizing that the Ras - Raf - Mek - Erk, as well as the Ras - PI-3-kinase - Akt pathways are promising targets

for melanoma therapy. While both *NRAS* and *BRAF* mutations are linked to UV exposure there are differences in exposure pattern as well as biological differences between melanomas with different mutations, indicating that *NRAS/BRAF* mutation status may be a significant parameter in a future molecular classification of cutaneous melanoma.

References

Albino AP, Le Strange R, Oliff AI, Furth ME, Old LJ. 1984. Transforming ras genes from human melanoma: a manifestation of tumour heterogeneity? Nature 308:69-72.

Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, et al. 2002. Mutations of the BRAF gene in human cancer. Nature 417:949-54.

Edlundh-Rose E, Egyházi S, Omholt K, Månsson-Brahme E, Platz A, Hansson J, Lundeberg J. 2006. NRAS and BRAF mutations in melanoma tumours in relation to clinical characteristics: a study based on mutation screening by pyrosequencing. Melanoma Res 16:471-8.

Eskandarpour M, Hashemi J, Kanter L, Ringborg U, Platz A, Hansson J. 2003. Frequency of UV-inducible NRAS mutations in melanomas of patients with germline CDKN2A mutations. J Natl Cancer Inst 95:790-8.

Omholt K, Karsberg S, Platz A, Kanter L, Ringborg U, Hansson J. 2002. Screening of N-ras codon 61 mutations in paired primary and metastatic cutaneous melanomas: mutations occur early and persist throughout tumor progression. Clin Cancer Res 8:3468-74.

Omholt K, Platz A, Kanter L, Ringborg U, Hansson J. 2003. NRAS and BRAF mutations arise early during melanoma pathogenesis and are preserved throughout tumor progression. Clin Cancer Res 9:6483-88.

III.23. Effects of Sub-erythemal Exposure on Human Skin in vivo

Antony R. Young
King's College London, St John's Institute of Dermatology, London, United Kingdom

The long-term effects of UVR on human skin have been largely determined by epidemiology and supported by animal experiments. Advice on preventing skin cancer by sunscreens is largely based on our knowledge from acute sunscreen studies. Furthermore, studies on the role of sunscreens in preventing skin cancer have, in general, given a disappointing outcome and the reasons for the lack of long-term photoprotection are poorly understood.

Acute studies of the effects of UVR on human skin, usually with erythemal exposures, are not able to provide information on how the skin responds and adapts to repeated sub-erythemal exposure that is more typical of "real life". This presentation will review the limited human data on the effects of repeated sub-erythemal UVR exposure on human skin, with a focus on biomarkers for DNA damage, immunosuppression and photoageing, and the possible role of sunscreens in preventing such effects.

References

Narbutt J, Lesiak A, Skibinska M, Wozniacka A, van Loveren H, Sysa-Jedrzejowska A, Lewy-Trenda I, Omulecka A, Norval M. 2005. Suppression of contact hypersensitivity after repeated exposures of humans to low doses of solar simulated radiation. Photochem Photobiol Sci 4:517-22.
Young AR, Orchard GE, Harrison GI, Klock JL. 2007. The detrimental effects of daily sub-erythemal exposure on human skin in vivo can be prevented by a daily-care broad-spectrum sunscreen. J Invest Dermatol 127:975-8.

III.24. Signaling Pathways in UVA and UVB induced Apoptosis in Human MC.

Cecilia Bivik, Petra Wäster, Karin Öllinger, Inger Rosdahl
Faculty of Health Sciences, Linköping University, Linköping, Sweden

Melanocytes (MCs) are target cells for ultraviolet (UV) irradiation, generating DNA damage and cellular defects, which subsequently might lead to malignant melanoma. Apoptosis provide a mechanism for eradicating cells with irreparable DNA damages and it is assumed that this process eliminates potential cancer cells. Resistance to apoptosis is a hallmark for most malignancies including melanoma. In many cell systems UV has been shown to trigger several signaling pathways to apoptosis, including DNA damage with activation of tumour suppressor gene P53, activation of death receptors or the mitochondrial pathway to apoptosis. However, the specific mechanisms underlying UV induced apoptosis in epidermal MC are not known. MCs originating from the neural crest have like nerve cells been considered to be relatively resistant to apoptosis and "sun-burn MCs" have not been described in the skin.

The mitochondrial pathway regulated by Bcl-2 family proteins, plays a key role in UV induced apoptosis. We have studied the regulation of apoptosis in human MCs irradiated with UVA and UVB in vitro. Different wavebands within the UVB spectrum had different apoptotic power and induced a diverse response pattern of Bcl-2 and Bax. Both Bcl-2 and Bax mRNA were up regulated to preserve protein levels and only a slight increase in apoptosis was noted after UVB (50 mJ/cm^2, λ>305). When the waveband 280-305 nm was increased the rate of apoptosis was amplified and Bcl-2 was upregulated, while Bax mRNA was unaltered. However, no change in Bcl-2 or Bax protein level was detected. A redistribution of these proteins from different compartments within the cell was found to be more important for accelerating apoptosis than a direct up regulation. The frequency of apoptosis was found to be significantly lower in MCs co-cultured with irradiated matched keratinocytes (KCs) than in MCs from pure cultures, indicating MCs to be protected from apoptosis by release of substance/s from the KCs. This rescue response concurred with a fast and significant increase in Bcl-2 mRNA level in MCs.

We have shown UVA and UVB to induce apoptosis in human MC in vitro by activating the mitochondrial pathway, by release of cytochrome c and caspase 3. The outcome of a death signal depends on the balance between positive and negative apoptotic regulators, such as members of the Bcl-2 family. UVA and UVB induced Bax, Bid and Bcl- X_L translocation from the cytosol to the mitochondria. Bcl-2 protein is generally thought to be attached only to membranes. In the MCs however, Bcl-2 was found to be localized also in the cytosol and translocation occurred to mitochondria following UVA/B, which was verified by cytosolic extraction. The lysosomal proteases cathepsin B and D, which may act as pro-apoptotic mediators were released from the lysosome to the the cytosol after UVA/B. The frequency of Bax translocation and apoptosis was markedly reduced, when using the cathepsin B and D inhibitors. Pro-apoptotic effect of these cathepsins was confirmed by microinjection of cathepsin B, which induced apoptotic cell death. In conclusion, both UVA and UVB activate the mitochondrial pathway of apoptosis in human MCs in vitro. Our results emphasize cathepsins to be pro-apoptotic mediators in MCs operating upstream of Bax.

Heat shock protein 70 (Hsp70) is highly expressed in many tumours and is the main stress-induced heat shock protein involved in folding and transport of proteins, but has also an anti-apoptotic function. Exposure of human MCs to heat and subsequent UVB significantly increases the level of Hsp70. The UVB induced apoptosis accompanied by lysosomal and mitochondrial membrane permeabilization, detected as release of cathepsin D and cytochrome c was prevented by heat pretreatment, an effect that was eliminated by Hsp70 siRNA transfection. In the surviving MCs immunofluorescence staining of Hsp70 showed the protein to be distributed in an organelle-restricted pattern. In purified fractions of mitochondria and lysosomes, recombinant Hsp70 was shown to attach to both lysosomal

and mitochondrial membranes. Moreover, in apoptotic cells Bax was translocated from a diffuse cytosolic location into mitochondrial structures, which was inhibited by Hsp70 induction. Hsp70 operating at several levels in this pathway makes it a very effective protein inhibiting apoptosis in MCs, and targeting Hsp70 might be a potential strategy for cancer therapy in the future.

Understanding the diverse action of various components in the apoptosis signaling pathways are of great importance to discover strategies to replace or restore functions thereby offering powerful and selective techniques to prevent or eliminate tumours arising from MCs and might also be of value to identify individuals at risk to develop melanoma.

III.25. Solar Exposure As A Prognostic Factor In Melanomas

Marianne Berwick
Department of Epidemiology and Public Health, Imperial College, London, UK

Solar exposure is the major etiologic factor known for the development of melanoma to date. In fact, intermittent exposure seems to be the class of solar exposure that contributes to the development of melanoma. Several meta-analyses and numerous studies of sun exposure in the etiology of melanoma have found moderate to strong associations with measures of intermittent solar exposure and the development of melanoma. Variables measured include reported sunburns, reported outdoor activities such as swimming, and "potential" exposure at latitude of residence. In these studies, the overall risk of "intermittent" exposure is around 1.5-1.7 for the development of melanoma. Conversely, measures of chronic sun exposure, such as occupation and daily and consistent outdoor exposure seem to have a decreased risk or no risk at all. Such a paradoxical relationship was highlighted by Gallagher et al. [Elwood, et al. 1985] who suggested that the decrease in outdoor occupations was associated with the parallel rise in indoor work and such an increase might have been partially responsible for the increase in melanoma incidence. A concomitant association is noted among countries that develop economically to the level of Western nations. In countries such as Spain and Portugal, we now see melanoma rates increasing dramatically.

New genetic tools, as we have heard, have allowed the characterization of melanomas by mutations or CGH copy number increases in the tumor. The identification of BRAF and NRAS mutations as potentially "necessary" but not sufficient steps in the development of melanoma as presented the research community with new insights into the development of melanoma. The identification of C-Kit mutations has also provided additional information that links the aggressive mucosal, acral and head and neck melanomas [Curtin, et al. 2006].

Further insight is gained by the study of growth rate in melanomas and the lack of association of early detection with improved mortality [Baade, et al. 2006; Berwick 2006]. Some melanomas appear to be indolent, particularly Lentigo Maligna Melanoma and some Superficial Spreading Melanomas, and others grow very rapidly, particularly Nodular Melanomas.

In the few survival studies conducted to date, there has been a surprisingly consistent finding that "solar elastosis"– the breakdown of elastin in the dermis associated with both aging and sun damage - or other markers of solar exposure, such as intermittent sun exposure and sunburns – are associated with better prognosis than those lesions which do not develop with such indications.

We followed 528 individuals for 5-year survival and found that those with any of the markers of solar exposure, but particularly solar elastosis, had a two-fold better survival than those who did not [Berwick, et al. 2005]. This analysis had the ability to simultaneously account for intensity of surveillance activity as well as traditional clinical prognostic factors such as Breslow thickness.

Several potential explanations exist. In the first place, it could be that those with higher levels of sun exposure had functionally better vitamin D status. Vitamin D is known to dampen cellular proliferation and increase apoptosis – both of which would tend to keep a lesion from progressing. In the second place, small repeated doses of UV are able to increase DNA repair capacity, and to the extent that DNA repair capacity keeps melanomas from further proliferation, this may be an important regulatory function. Thirdly, the possibility that solar exposure induces "indolent" lesions and inherited genetic factors are responsible for the more aggressive lesions, similar to the c-Kit distribution, is under active investigation.

Whether solar elastosis or sun exposure itself is actually responsible for the inhibition of melanoma lesions is at this point unknown. These factors may actually be proxies for other as yet unmeasured variables, such as solar-induced immune stimulation of host defenses.

References

Baade PD, English DR, Youl PH, Elwood JM, Aitken JF. 2006. The relationship between melanoma thickness and time to diagnosis in a large population-based study. Arch Dermatol 142(11):1422-7.
Berwick M, Armstrong BK, Ben-Porat L, Fine J, Kricker A, Eberle C, Barnhill R. 2005. Sun exposure and mortality from melanoma. J Natl Cancer Inst 97(3):195-9.
Berwick M. 2006. Pathways to the development of melanoma: a complex issue. J Invest Dermatol 126:1932-3.
Curtin JA, Busam K, Pinkel D, Bastian BC. 2006. Somatic activation of KIT in distinct subtypes of melanoma. J Clin Oncol 24:4340-6.
Curtin JA, Fridlyand J, Kageshita T, Patel HN, Busam KJ, Kutzner H, Cho K-H, Aiba S, Brocker E-B, LeBoit PE, Pinkel D, Bastian B. 2005. Distinct sets of genetic alterations in melanoma. N Engl J Med 353:2135.
Elwood JM, Gallagher RP, Hill GB, Pearson JC. 1985. Cutaneous melanoma in relation to intermittent and constant sun exposure--the Western Canada Melanoma Study. Int J Cancer 35(4):427-33.

Short talks/posters

III.26. Mutagenic and toxic DNA lesions induced by UVA

Anna Biverstål, Fredrik Johansson, Dag Jenssen, Klaus Erixon
Stockholm University, Department of Genetics, Microbiology and Toxicology. Svante Arrhenius våg 16, 106 91 Stockholm, Sweden

DNA repair deficient cells have been used to probe for differences in the mutagenicity and toxicity of DNA lesions induced by UVA irradiation in comparison to what we and others have found for UVC. Survival and gene mutations in the *hprt* locus were measured in Chinese hamster ovary (CHO) cells, proficient or deficient in nucleotide excision repair (NER), deficient only for transcription coupled repair or deficient in base excision repair (BER).

The difference between mutagenicity in NER deficient and NER proficient cells was less extenuated for UVA than for UVC, indicating that pyrimidine dimers contributed substantially less to UVA mutagenicity. This could not be explained by the absence of (6-4)PP formation by UVA indicated a difference in spectrum of mutagenic lesions. UVA induced a rapid BER activity but very little NER activity also indicating the absence of (6-4)PP formation. However, a detectable yield of CPDs was observed, using a CPD-specific endonuclease assay. On the other hand, exposure to UVA was found to induce a higher mutation level than found for UVC in NER proficient cells, when calculated at equal amounts of CPDs, suggesting the induction of mutagenic lesions by UVA that are not repaired by NER. Since UVA has been reported to induce predominately TT-CPDs, we investigated their mutagenicity relative to C-containing CPDs. We applied the photo-sensitizer acetophenon together with UVB to intact cells and measured the levels of mutations and CPDs. The results suggest that TT_CPDs are less mutagenic than C-containing CPDs.

Thus, the overall conclusions of this study is that in the presence of proficient NER, other mutagenic lesions(s), not repairable by NER, is responsible for a significant part of the mutagenic yield of UVA.

III.27. Wavelength dependence of cell cycle responses in human melanocytes and melanoma cells following exposure to ultraviolet radiation.

Christine Kowalczuk, Marisa Priestner, Rick Saunders, Simon Bouffler
Health Protection Agency, Radiation Protection Division, Chilton, UK

Animal studies have raised the possibility that long wavelength UVA may be more effective at inducing melanoma than DNA damage spectra would predict. Cell cycle responses to DNA damage can be highly sensitive; therefore, this study has examined the wavelength dependence of the effects of UVR on the cell cycle response of human melanocytes and melanoma cells.

Primary human melanocytes (isolated from juvenile foreskin tissue) and G361 human melanoma cells were exposed to 254 nm germicidal UVC radiation, 311 nm UVB radiation, or broadband UVA radiation (maximum output between 350 – 450 nm). Flow cytometry was used to monitor cell cycle distributions for up to one week post-irradiation and western blot analyses of G1/S checkpoint related cell cycle proteins (p16, p21, p27 and p53) were carried out.

Melanoma cells showed a sustained, dose-dependent G2/M block following exposure with all wavelengths; in addition, transit through S phase was slowed following UVA irradiation. There was no apparent block to G1 cells entering S at any wavelength. Exposure of melanocytes, on the other hand, caused a marked G1 arrest, particularly following UVA irradiation. Preliminary western blot analyses of melanoma cells showed a lack of p16 expression. There were transient increases in the expression of p21 and p53 proteins following UVA or UVC irradiation whilst p27 was increased follwing UVA exposure, but decreased after exposure to UVC.

Results show that G361 malignant melanoma cells have lost the ability to regulate the cell cycle at the G1/S checkpoint. This is supported by preliminary western blot analyses showing a lack of p16 expression in these cells. UVA nevertheless induced strong cell cycle delays in both melanocytes and G361 melanoma cells, indicating that UVA exposure can significantly affect genome metabolism. The wavelength dependence of the p27 response in melanoma cells could be indicative of the involvement of p27 in the response to UVA-induced oxidative damage. This result will be confirmed in further studies and western blot analyses will be extended to examine melanocyte responses.

References

Kowalczuk CI, Priestner MC, Pearson AJ, Saunders RD, Bouffler SD. 2006. Wavelength dependence of cellular responses in human melanocytes and melanoma cells following exposure to ultraviolet radiation. Int J Radiat Biol 82(11):781-92

III.28. The beneficial role of moderate ultraviolet-B irradiance greatly outweighs the adverse effects

William B. Grant
Sunlight, Nutrition, and Health Research Center (SUNARC), 115 Van Ness Ave., Suite 101, San Francisco, CA 94109, USA

Solar ultraviolet-B (UVB) irradiance is the primary source of vitamin D for most people on earth. There is a rapidly expanding body of scientific literature showing that vitamin D has many positive health benefits. The calcemic effect helps reduce the risk of falls and fractures. The non-calcemic effects are now being emphasized. The benefits in reducing the risk of many types of cancer are now well documented in ecological, observational, and prospective double-blind studies. More recently, the benefits of vitamin D in fighting both bacterial and viral infections through induction of human cathelicidin by 1,25-dihydroxyvitamin D are being studied. Low UVB and serum 25-hydroxyvitamin D (calcidiol) levels in winter explain in part why infectious disease rates are highest in winter. I have submitted a manuscript hypothesizing that this is the mechanism whereby UVB and vitamin D reduce the risk of autoimmune diseases and several types of cancer linked to viral infections. For example, multiple sclerosis and prostate cancer have geographical variations consistent with this hypothesis. A recent meta-analysis found that vitamin D reduces mortality rates. I am finishing a study showing that about 6% of the per direct health spending in the United States is linked to people not getting as much vitamin D as those living in the sunny Southwest. Thus, the health benefits of moderate solar UVB irradiance greatly outweigh the health risks from all solar UV irradiance in the United States.

III.29. UV-related mutational pattern of the PTCH gene in basal cell carcinomas (poster)

Erika Lindström, Rune Toftgård, Peter G. Zaphiropoulos
Department of Biosciences and Nutrition, Karolinska Institutet, SE-141 57 Huddinge, Sweden

UV-radiation is well known to be one of the most important etiological risk factors for the development of basa cell carcinomas (BCCs), the most common cancer in the western countries, the incidence of which is increasing rapidly [Miller and Weinstock 1994; Brash 1997; Boyle, et al. 2004]. Based on compiled data from our PTCH Mutation Database; http://www.cybergene.se/PTCH/, which is an annotated locus specific database (LSDB) containing all known information regarding the PTCH mutations/SNPs, a significantly high frequency of UV-related mutation, CC>TT or C>T transitions at dipyrimidine sites, was found in the transmembrane receptor PTCH, the human homolog of the Drosophila segment polarity gene patched [Lindström, et al. 2006]. Specifically, 50% (43/86) of the mutations in sporadic BCCs (SP-BCCs) and 77% (24/31) of the mutations in BCCs from patients with Xeroderma pigmentosum (XP-BCCs) were of such type. These mutations resulted into premature termination in 44% (19/43) of the SP-BCCs and in 29% (7/24) of the XP-BCCs. On the other hand, missense mutations were found in 49% (21/43) of the SP-BCCs and in 67% (16/24) of the XP-BCCs. The distribution pattern of these UV-related missense mutations on the predicted secondary structure of the PTCH protein revealed that 43% (9/21) of the SP-BCC mutations were located within the first large extracellular loop, compared to 25% (4/16) of the XP-BCC mutations. Another favored location of the UV-related SP-BCC mutations was the C-terminal region with 24% (5/21) compared to 12% (2/16) of the XP-BCC mutations. On the contrary, the large intracellular loop was found to harbor 38% (6/16) of the XP-BCC mutations but only 14% (3/21) of the SP-BCC mutations. These results are in agreement with our recent general analysis of PTCH mutational pattern where the SP-BCC missense mutations were found to be clustered within the first extracellular loop, the C-terminus, as well as the large intracellular loop. In the same study the XP-BCC missense mutations were found to be concentrated into the large intracellular loop, allowing the speculation that the DNA sequence corresponding to this region may be highly sensitive to UV radiation. Both the large extracellular loops and the C-terminal region are known to be functionally important, and moreover the large intracellular loop has also been reported to bind cyclin B1. In our analysis we have also detected specific hot spot regions, especially UV-related, with certain amino acids, p.R195, p.Q365, p.R770, and p.W926 as being highly overrepresented [Barnes, et al. 2001; Lindström, et al. 2006].

References

Barnes EA, Kong M, Ollendorf V, Donaoghue DJ. 2001. Patched1 interacts with cyclin B1 to regulate cell cycle progression. EMBO J 20:2214-23.
Boyle P, Doré JF, Autier P, Ringborg U. 2004. Cancer of the skin: a forgotten problem in Europe. Ann Oncol 15:5-6.
Brash DE. 1997. Sunlight and the onset of skin cancer. Trends Genet 13:410-4.
Lindström E, Shimokawa T, Toftgård R, Zaphiropoulos PG. 2006. PTCH mutations: distribution and analyses (Review). Hum Mutat 27(3):215-9
Miller DL, Weinstock MA. 1994. Nonmelanoma skin cancer in the United States: incidence. J Am Acad Dermatol 30(5):774-8.

III.30. Ultraviolet radiation from indoor tanning devices in Norway, 1983-2005

Lill Tove Nilsen[1], Merete Hannevik[1], Tommy Nakken Aalerud[1], Bjørn Johnsen[1], Eva Godske Friberg[1], Marit Bragelien Veierød[2]
[1]Department for Radiation Protection and Nuclear Safety, Norwegian Radiation Protection Authority, Østerås, Norway
[2]Department of Biostatistics, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway

Use of indoor tanning devices is widespread in Northern Europe and north America. It is associated with adverse health effects including increased risk of skin cancer. However, it is not clear what wavelengths and exposure patterns that are most important e.g. for cutaneous malignant melanoma, the most deadly form of skin cancer. Irradiance data has been collected from tanning models approved for cosmetic use in Norway since the first Norwegian regulation was implemented in 1983. Since 1983, all models needed an approval before being sold or used. UVA and ACGIH-weighted irradiance limits were stated in the Norwegian regulation until 1992, whereas UV type 3 limits were valid from late 1992 (<0.15 W/m^2 for CIE-weighted short (<320 nm) and long wave (320-400 nm) irradiances). All irradiance data were converted to CIE-weighted irradiances. In addition, irradiance were measured or estimated for tanning devices inspected in tanning studios in 1998-1999 and 2003.

Approval data showed that the mean CIE-weighted short wave irradiance was much lower than the limit in 1983-1992. Also the CIE-weighted long wave irradiance was lower. Both values increased in the period 1993-2005. The mean long wave irradiance was much higher than tropical sun in the whole period (1983-2005), whereas the short wave irradiance increased to the same level as summer sun in South Norway in the last period.

Inspection data from 2003 revealed that 6 out of 10 tanning devices were equipped with correct lamps, compared to 3 out ot 10 in 1998-1999. The short wave irradiances for devices in use in 1998-1999 were much higher than what was approved. This had decreased in 2003. The long wave irradiance differed less between inspected and approved devices. Due to decreased short wave, the total UV irradiance decreased from the first to the second survey in Norway whereas two studies from Scotland in 1997 and 2004-2005 showed the opposite trend [Moseley, et al. 1998, 1999; Oliver, et al. 2007]. There are no national regulations regarding use of tanning devices in Scotland.

Our results show that the UVB to UVA ratio has changed in the tanning devices over the last 20 years and also that strict regulations are essential but insufficient if not followed by inspections.

References

Moseley H, Davidson M, Ferguson J. 1998. A hazard assessment of artificial tanning units. Photodermatol Photoimmunol Photomed 14:79-87.
Moseley H, Davidson M, Ferguson J. 1999. Sunbeds and need to know. Br J Dermatol 141:589-90.
Oliver H, Ferguson J, Moseley H. 2007. Quantitative risk assessment of sunbeds: impact of new high power lamps. Br J Dermatol 157:350-6.

III.31. Ratio comparisons of spectrally differing UV sources effects calculated with action spectra for erythema, non-melanoma skin cancer, general hazard evaluation and vitamin D induction: NMSC risk vs Vitamin D effectiveness per SED of typical UV sources

Ulf Wester
Swedish Radiation Protection Authority (SSI)

Characteristics of ultraviolet radiation emissions from different kinds of UV sources can vary widely both in terms of spectral composition and irradiance level. In the general population people can be expsed to a wide assortment of natural and/or artificial UV sources – involuntarily or deliberately. Natural solar UV varies within a certain range of levels and spectral UVB/UVA ratios. Artificial UV sources for intense deliberate exposure sessions (e.g. solaria) can be many times more powerful and have widely different spectal compositions. Other artifical UV sources may give unintentional but prolonged low irradiance exposures of an even greater spectral variation.

Action spectra for a number of well-known biological effects are published and have become standards. Widely used is one action spectrum for general 'UV hazard' evaluation [The International Commission on Non-Ionizing Radiation Protection 2004], another for erythema effectiveness [Commission Internationale de l'Eclairage, 1998], a third for non-melanoma skin cancer induction [CIE 2000] and a fourth for the production of previtamin D3 in human skin [CIE 2006].

Different UV sources have been spectroradiometrically measured at the Swedish Radiation Protection Authority (SSI) over the years. Examples of how effective emissions compare and vary will be presented.

References

The International Commission on Non-Ionizing Radiation Protection (ICNIRP). 2004. Guidelines on limits of exposure to ultraviolet radiation of wavelengths between 180 nm and 400 nm (incoherent optical radiation). Health Physics 87(2):171-86
Commission Internationale de l'Eclairage: Erythema reference action spectrum and standard erythema dose. Standard CIE S 007/E-1998, Vienna Austria, CIE Central Bureau.
CIE TC 6-32 report: Action spectrum for photocarcinogenesis (non-melanoma skin cancers), CIE 138/2 in CIE Collection in Photobiology and Photochemistry 2000, CIE 138-2000
CIE Action Spectrum for the production of previtamin D3 in human skin. Technical Report CIE 174:2006

III.32. COST 726: Long term changes and climatology of UV radiation over Europe

Z. Litynska (Chair)[1], P. Koepke (Vice-chair and WG2)[2], H. de Backer (WG1)[3], A.W. Schmalwieser (WG3)[4], J. Gröbner (WG4)[5], +40 members
[1] Institute of Meteorology and Water Management, Legionovo, Poland
[2] Meteorological Institute, Department of Physics, L-M University Munich, Germany
[3] Royal Meteorological Institute of Belgium, Ukkel, Belgium
[4] Institute of Medical Physics and Biostatistics, University of Veterinary Medicine, Vienna, Austria
[5] Physikalisch-Meteorologisches Observatorium Davos, World Radiation Center (PMOD/WRC), Davos, Switzerland

The ultraviolet (UV) radiation reaching the ground is only a small portion of the radiation we receive from the sun. Nevertheless, UV radiation has a wide variety of effects on humans and the environment. Studies on the impact of UV radiation require knowledge of UV climatology and changes that have occurred in the past. It would be of special importance having the estimates of average and extreme characteristics of the UV impact on various biological systems (including human beings) as well as doses over different time periods. For this the COST 726 action was founded in 2004. The main objective of the Action is to advance the understanding of UV radiation distribution under various meteorological conditions in Europe in order to determine UV radiation climatology and assess UV changes over Europe. Since UV solar radiation plays an important role in many processes in the biosphere, including the influence on human organisms, and may be very harmful if UV exposure exceeds ‘safe; limits, the knowledge of biologically effective UV radiation doses and their geographical distribution and climatology in Europe is crucial for the European population, who will be addressed as the main end user of the Action. To achieve its general objective, the Action has the following practical objectives:

(i) to make an inventory of available solar radiation data sets, including UV data, spectral and broadband, ancillary data (ozone, clouds, sunshine etc.) and available satellite data,
(ii) to advance the understanding of UV reconstruction models for the calculations of UV climatology and assessment of UV changes,
(iii) to advance the understanding of biological UV radiation climatology and changes in Europe,
(iv) to advance the understanding of UV influence on ecosystem, oboth on the basis of climatology and changes of selected effective UV radiation doses in Europe,
(v) to use the advanced knowledge under the points above, in order to elaborate a comprehensive analysis and information basis addressed to beneficiaries,
(vi) Additionally, special attention should be paid to application of QC/QA procedures for the UV measurements with broadband instruments. To get homogeneity of the broadband data, an additional objective is to create a European reference group of broadband radiometers.

The COST726 action is organised in 4 working groups (WG). WG1 is responsible for data collection, WG2 for UV modelling, WG3 for the requirements for biological UV effects, WG4 for developing quality control recommendations and procedures.

The members of the Action come from 22 European countries and two international organizations. The major benefits of the Action will be a geographically broader and scientifically deeper knowledge of the climatology of UV radiation and of selected biologically effective UV radiation doses across Europe. The main beneficiaries will be the public, researchers in atmospheric and medical sciences as well as authorities and policy makers.

The progress of the Action and the outcome is presented at: www.cost726.org

III.33. UV radiation weighted with action spectra: erythemal, previtamin D3, SCUP-H against total ozone and solar zenith angle

Julita Biszczuk, Zenobia Litynska, Aleksander Curylo
Centre of Aerology, Institute of Meteorology and Water Management, Legionova, Poland

Introduction

The UV radiation plays a meaningful role in many processes in the biosphere. The knowledge of UV biologically effective (UVBE) radiation and its distribution is very important for the poopulation. In the frame of this work we will present the variations of UV radiation, weighted with Previtamin D3 (CIE, 2006) and SCUP-H (de Gruijl, 1994) action spectra, in comparison with erythemal (CIE, 1987) as functions of total ozone and solar zenith angle. The ratio was calculated: *UVBE/Erythemal*. The normalized action spectra were used: action spectrum for the production of previtamin D3 in human skin, normalized to 1 at 298 nm, skin cancer action spectrum in mice corrected for human skin, normalized to 1 at 299 nm. For the calculations the UV radiation transfer model *libRadtran v.1.1* was used.

Moreover, the variations of UVBE radiation over Poland from the period 1999-2001, for summer will be presented. To calculate the UV radiation the UV reconstruction model formulated by A. Curylo in the frame of COST Action 726 was used.

Results

The ratio of UV radiation weighted with Previtamin D3 action spectrum to erythemal as functions of solar zenith angle and total ozone is between 1.8 to 0.4. Higher values of UV radiation weighted with Previtamin D3 action spectrum against erythemal are observed at the lower solar zenith angles.

The ratio of UV radiation weighted with SCUP-H action spectrum to erythemal as functions of solar zenith angle and total ozone is between 2.1 to 1.2. Higher values of UV radiation weighted with SCUP-H action spectrum against erythemal are observed in all range of solar zenith angle.

In both cases bigger variations can be seen in the function of solar zenith angle than of total ozone.

Conclusions

Up to 70° solar zenith angle doses of UV radiation weighted with Previtamin D3 action spectrum are higher in comparison with doses of erythema. In all range of solar zenith angle the intensity of UV radiation weighted with SCUP-H action spectrum is higher than weighted with erythemal.

References

CIE. 1987. A reference action spectrum for ultraviolet induced erythema in human skin. See http://www.cie.co.at/publ/abst/s007.html
CIE. 2006. Action spectrum for the production of Previtamin D3 in human skin. See http://www.cie.co.at/publ/abst/174-06.html
De Gruijl FR, van der Leun KC. 1994. Estimate of the wavelength dependency of ultraviolet carcinogenesis in humans and its relevance to the risk assessment of a stratospheric ozone depletion. Health Physics 67(4):319-25.
McKenzie RL. 2007. UV radiation climatology and trends. In: Proceedings of the UV Conference 'One century of UV radiation research', 18-20 September 2007, Davos, Switzerland, Julian Gröbner (ed.), pp 13-14. Available (07/05/09) at:
http://www.pmodwrc.ch/uvconf2007/uvconf_proceedings_15082007.pdf

Annex IV. Acronyms

64PP	pyrimidine (6-4) pyrimidone photoproduct
8-oxodG	7,8-dihydro-8-deoxoguanine
8-oxodGuo	8-oxo-7,8-dihydro-2′-deoxyguanosine,
AMS	atypical mole syndrome
Bach-1	BRCA1-associated protein (DNA helicase)
Bax	BCL2-associated X protein (pro-apoptotic protein)
BCC	basal cell carcinoma
Bcl-2	B-cell CLL/lymphoma 2 (anti-apoptotic protein of the mitochondrial pathway)
BER	base excision repair
bFGF	basic fibroblast growth factor
Bid	BH3-interacting domain death agonist (activation of pro-apoptotic gene products)
BRAF	v-raf murine sarcoma viral oncogene homolog B1
CCND1	cyclin D1
CDKN2A	cyclin-dependent kinase inhibitor 2A gene
CHO	Chinese hamster ovary (cell line)
CPD	cyclobutane pyrimidine dimer
CS	Cockayne syndrome
CSD	chronic sun damage
DNCB	1-chloro-2,4-dinitrobenzene
DOX	doxycycline
FACS	fluorescence-activated cell sorting
GFP	green fluorescent protein
HGF/SCF	hepatocyte growth factor/scatter factor; SF
HO-1	haem oxygenase 1
HRAS	v-Ha-ras Harvey rat sarcoma viral oncogene homolog
Hsp70	heat shock protein 70
IPF	immunoprotection factor
KIT	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog
LM-PCR	ligation-mediated polymerase chain reaction
MAPK	mitogen-activated protein kinase (signalling pathway)
MC1R	melanocortin-1 receptor gene
MDS	multiple damage site
MED	minimal erythema dose
MM	malignant melanoma
MTD	Maximum Tolerable Dose
mtDNA	mitochondrial DNA
NEDD9	neural precursor cell expressed, developmentally down-regulated 9 gene
NER	nucleotide excision repair
NMSC	non-melanoma skin cancer
NRAS	neuroblastoma RAS viral (v-ras) oncogene homolog
Nrf2	nuclear factor (erythroid-derived 2)-like 2
OGG1	8-oxoguanine DNA glycosylase
p16	CDKN2A (cyclin-dependent kinase inhibitor 2A)
p16Ink4a/-19Arf	CDKN2A
p27	Cdkn1b (cyclin-dependent kinase inhibitor 1B)
PI3K	phosphatidylinositol-3-kinase (pathway)

PTCH	patched homolog 1 (Drosophila)
PTEN	phosphatase and tensin homolog
RMR	reactive melanin radicals
SCC	squamous cell carcinoma
Scf	stem cell factor (KITLG, KIT ligand)
SCUP-H	skin cancer action spectrum in mouse corrected for human skin
SED	standard erythema dose
siRNA	small interfering RNA
SNP	single nucleotide polymorphisms
SPF	sun protection factor
SSL	simulated sunlight
SSR	solar-simulated radiation
TLS	translesion synthesis
TTD	trichothiodystrophy
TUNEL	terminal deoxynucleotidyl transferase biotin-dUTP nick end labelling
UVBE	biologically effective UV
VDR	vitamin D (1,25- dihydroxyvitamin D3) receptor
XPV	xeroderma pigmentosum variant

Strålsäkerhetsmyndigheten
Swedish Radiation Safety Authority

SE-171 16 Stockholm
Solna strandväg 96

Tel: +46 8 799 40 00
Fax: +46 8 799 40 10

E-mail: registrator@ssm.se
Web: stralsakerhetsmyndigheten.se