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International conference on UV radiation-induced disease

— roles of UVA and UVB

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SSM perspective

Background

The annual incidence of skin cancer – the main cause of which is exposure to ultraviolet (UV) radiation – continues to rise. SSM is striving for a society that is safe from the harmful effects of radiation through measures designed to increase the level of safety and limit the risks that accompany radiation. An international conference sponsored by Swedish Radiation Safety Authority held at Karolinska Institutet in May 2012, explored the state of knowledge concerning ultraviolet radiation-induced disease and the roles of UVA and UVB. The conference was initiated by SSM:s scientific UV advisory board and researchers from various disciplines within the field of UV radiation were invited.

Objective

Exposure to ultraviolet 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. During recent years, we have seen the classification by International Agency for Research of Cancer of the whole solar UV spectrum as carcinogenic to humans, the introduction of BRAF inhibitors for treatment of melanoma and advances in understanding of the mechanisms of skin carcinogenesis and the risk factors involved. The conference was held to present and discuss progress in the field, with emphasis on the differential effects of UVA and UVB and links to disease.

Results

There is a large uncertainty concerning the impact of UVA and UVB radiation on the skin. For example, UVA radiation used to be considered low risk since it was believed that DNA damage could only be caused by UVB radiation. Because of the potentially greater exposure to UVA while using sunbeds or UVB-blocking sunscreens, further information on the role of UVA is important. The results presented at the conference give rise to a deeper understanding of the biological effects of UV radiation. In particular, 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 differences are associated with different prognosis and mean age of incidence. For those aged less than 30, UV exposure involves greater risk because naevus development is still active. Nevertheless, many uncertainties remain.

Need for further research

Despite extensive efforts, the mechanism of UVA-induced genotoxicity remains to be further 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.

Project information

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This report concerns a study which has been conducted for the Swedish Radiation Safety Authority, SSM. The conclusions and viewpoints presented in the report are those of the author/authors and do not necessarily coincide with those of the SSM.

Content

Preface

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1. Executive summary

In 2007, an international conference sponsored by the former Swedish Radiation Protection Authority and the Swedish Cancer Society and held at Karolinska Institutet explored the state of knowledge concerning ultraviolet (UV) radiation-induced disease and the roles of UVA and UVB. The following five years we have seen the classification of the whole solar UV spectrum by IARC as carcinogenic to humans, the introduction of BRAF inhibitors for treatment of melanoma and advances in understanding of the mechanisms of skin carcinogenesis and the risk factors involved. In May 2012, a second, follow-up, conference was held at Karolinska Institutet, funded by the Swedish Radiation Safety Authority, to present and discuss progress in the field, again with emphasis on the differential effects of UVA and UVB and links to disease. This report is based on the evidence presented at that meeting.

1.1. Cellular effects of UV radiation

The UV component of terrestrial sunlight is primarily UVA (320–400 nm) with UVB (280–320 nm) comprising only about 5%. UVB radiation induces cyclobutane pyrimidine dimers (CPDs), especially thymine dimer (T=T), and also (6-4) photoproducts (6-4PPs) by direct photochemical reaction with DNA. These photolesions are not evenly distributed along the DNA with methylation status, chromatin structure and chromosomal site affecting the outcome. Earlier emphasis was given to the induction by UVA radiation of oxidative damage to DNA, for example 8-oxo7,8-dihydroguanine (8-oxoGua), mediated by photosensitisers; however, it has become clear that CPDs are also induced by UVA radiation, although their distribution pattern differs from those induced by UVB. The ratio of CPDs to 8-oxoGua is approximately fivefold in most skin cell types but lower in melanocytes, perhaps reflecting greater oxidative stress in the latter. Few 6-4PPs are induced by UVA.

In human, nucleotide excision repair is the only repair pathway capable of removing CPDs and 6-4PPs. Recent studies have shown the contribution made to the DNA recognition step of global genomic repair (GGR) by polyADP-ribose polymerase. This polymerase plays a key role in the stabilisation of DDB2, the first protein that arrives at the UV-damaged chromatin, and the recruitment of chromatin remodellers to make the UV-damaged chromatin accessible for repair. In mice lacking transcription-coupled repair (TCR) the epidermis is more sensitive to UVB-induced erythema with an increase in apoptotic cells. In mouse embryonic stem cell lines with an inducible *hprt* reporter gene, UV-induced nucleotide substitutions were more frequent when the reporter gene was transcribed, but only from UV damage in the transcribed strand. This strand bias, also seen in *p53* mutations in sun-exposed human skin, was due to a rapid transcription-dependent deamination of cytosine at CPDs that could be prevented by TCR. These experiments highlight the role of transcription in sunlight-induced mutagenesis in the skin and the crucial role of TCR in counteracting this type of mutagenesis.

Heme oxygenase 1 (HO-1), important in the maintenance of heme and iron homeostasis, is induced in fibroblasts by oxidative stress, including UVA irradiation. Results suggest that the stabilisation of the transcription factor Nrf2 by UVA and the DNA binding of the transcriptional suppressor protein Bach1 and, consequently,

activation of HO-1 are mediated by heme. Prolonged expression of HO-1 is prevented by UVA induction of Bach1. Keratinocytes are less susceptible to UVA-induced membrane damage, possibly due to their constitutive expression of heme oxygenase 2 (HO-2) leading to low heme levels which prevent activation of HO-1. An understanding of the regulation of the anti-inflammatory enzyme HO-1 may lead to therapeutic interventions.

The possibility that activation of Nrf2, which induces expression of a number of reactive oxygen species (ROS)-detoxifying enzymes as well as HO-1, might have therapeutic benefits was tested in transgenic mice expressing a constitutively active mutant of Nrf2 in keratinocytes. UVB-induced apoptosis, ROS levels and p53-positive keratinocytes were reduced, but not formation of CPDs. Mice expressing higher levels of Nrf2 showed epidermal abnormalities resembling the phenotype of patients with ichthyosis. Microarray expression analysis revealed changes in the regulation of genes with a role in epidermal barrier formation. Caution is therefore needed in the use of Nrf2-activating compounds for chemoprevention of different insults, including UV damage.

In addition to DNA damage, UVB effects can be mediated by the arylhydrocarbon receptor (AhR) and epidermal growth factor receptor (EGFR). Recent experiments implicated 6-formyl[3,2-*b*]carbazole (FICZ), formed by UVB irradiation of tryptophan, in the AhR and EGFR responses. A reduction in CPDs in a UVB-irradiated keratinocyte cell line in the presence of the AhR antagonist 3'-methoxy-4'-nitroflavone (MNF) suggested that the AhR is involved in UVB-induced carcinogenesis. MNF also inhibited UVB induction in keratinocytes of the matrix metalloproteinase MMP-1, which breaks down collagen, perhaps implicating AhR in photoageing. An AhR antagonist has been developed that is effective in reducing UVB induction of matrix metalloproteinase (MMP-1) and other enzymes in human skin with potential anti-ageing effects. AhR was also found to be expressed in melanocytes. AhR knock-out mice (ko) showed reduced UVB-induced tanning and reduced melanocyte proliferation.

Wrinkling in photoaged skin is thought to result from changes in elastin synthesis and degradation. Extracellular elastases are well known, but in a search for intracellular enzymes the lysosomal cysteine protease cathepsin K (catK) was detected in neonatal human fibroblasts. Internalised elastin was degraded by catK in fibroblasts. Irradiation of skin explants by UVA, and to a lesser extent UVB, induced catK in fibroblasts in the upper dermis. The possibility that photoageing might represent a UV-induced acceleration of intrinsic ageing was suggested by the observation that progerin, a truncated pre-lamin A associated with premature ageing syndromes, is induced by repeated low-dose UVA in fibroblasts. A single UVA exposure induces progerin mRNA more effectively in fibroblasts aged in vitro and cells from older donors than in young fibroblasts.

Investigation of the possibility that rapid pigment darkening of human skin induced by UVA might be mediated by phototransduction uncovered a retinal-dependent Ca^{2+} mobilisation response in epidermal melanocytes. Melanocytes were found to express full-length rhodopsin and reduction of expression of this reduced the UV-induced Ca^{2+} response. The increased melanin levels seen in UVA-irradiated melanocytes in the presence of retinal were reduced by calcium depletion, suggesting a novel phototransduction pathway responsible for rapid pigment darkening.

1.2. Experimental models of UV-induced disease

The development of mouse models of UV-induced melanoma with 'humanized' skin, such as the transgenic HGF/SF mouse, represents an important advance for mechanistic studies. Only neonatal UVB initiates melanoma in FVB-HGF albino mice; however, testing of black and albino littermates from a cross with C57BL/6 mice revealed that UVA as well as UVB induced melanoma in the black mice. UVA induction of melanoma occurred via an oxidative process in the black mice, whereas UVB-induced disease resulted directly from DNA damage. These results highlight the complexity of the role of melanin in UV-induced skin disease.

UV irradiation also promotes outgrowth of UV-initiated melanomas. UV-irradiated HGF/SF neonatal skin grafted onto wild-type mice that have also been UV-irradiated neonatally produces melanomas faster than on non-irradiated recipients. UV irradiation does not increase neonatal immune tolerance but does mobilise melanocytes from the hair follicle to the epidermis in neonatal mice, which might play a role in the promotion of melanoma formation.

Induction of melanoma by neonatal UV exposure in the *Xiphophorus* hybrid Sp-*Couchianus* fish model compares with the early childhood exposures associated with truncal melanoma in humans. A retesting of early action spectrum studies in this model, on a larger scale, found that neonatal UVB but not UVA irradiation induced melanoma, in contrast to earlier results.

Many observations suggest that vitamin D can protect against cancer; however, the UVB required for vitamin D production induces skin cancer. Knock-out mice lacking the vitamin D receptor (VDR) were more susceptible to UV-induced skin carcinogenesis. Results suggested that the protection afforded by the VDR involves epidermal DNA repair, suppression of proliferation of keratinocytes and stimulation of differentiation. Further studies with the VDR ko mouse pointed to an involvement of impaired regulation of the hedgehog (Hh) and Wnt/β-catenin signalling pathways in UVB-induced tumorigenesis.

In an investigation of the origin of the stem cells from which melanocytes arise, it was demonstrated that isolated dermal cells could be maintained as dermal spheres that expressed stem cell markers not found in fibroblasts, keratinocytes or melanocytes. These dermal cells could develop into melanocytes localised to the basal membrane of the epidermis in three-dimensional skin constructs, thus mirroring melanoma cells in plasticity. The Notch signalling pathway was found to be activated in dermal stem cells. Gene expression profiling revealed up-regulation of the msx-1 transcription factor that is induced by Wnt, BMP and FGF signalling in dermal stem cells and melanoma cells. This transcription factor decreases melanocyte pigmentation and increases migration. The occurrence of similar processes in skin in response to carcinogens such as UV radiation has implications for melanoma initiation and progression.

In the hairless mouse model, chronic UV exposure results in *p53* mutant cell clones in the interfollicular (IF) epidermis that ultimately give rise to squamous cell carcinoma (SCC) with similar mutations. Ablation by acute UV overexposure of the IF basal layer, but not overlying layers, in mice carrying UV-induced *p53* clones delayed SCC formation in response to continuing chronic UV exposure by the same length of time as the prior UV exposure; thus *p53* mutant clones had been removed and carcinogenesis 'reset'. This resembles the human sunburn response in which underlying cells undergo apoptosis and cell death. Treatment of chronically UV-

irradiated hairless mice with the immunosuppressant drug rapamycin, which has anti-tumour effects, reduced the number of *p53* over-expressing clones. However, rapamycin-treated mice did not differ from controls in the onset of tumours up to 1 mm, but did show a decrease in tumours >4 mm. Thus the relationship between the *p53* clones and tumour development is ambiguous.

UVB-induced immunosuppression of the oxazolone-induced contact hypersensitivity (CHS) response in hairless mice could be significantly reduced by UVA irradiation, with HO implicated as a mediator. UVA also appears to be protective against immunosuppression occurring during solar-simulated UV (SSR) photocarcinogenesis in this model with a reduction in tumour multiplicity. 17-β-oestradiol also protected against SSR-induced immunosuppression and differences in response of male and female mice to the UVA-mediated protection against UVB immunosuppression of the CHS response were found.

1.3. Human studies of UV-induced disease

Accurately assessing the UV dose received by the skin is difficult and assays of CPDs in skin biopsies are not suitable for large populations or children. The alternative ^{32}P-postlabelling assay of urinary T=T dimers has been validated in a study of individuals exposed to UV in sunbeds. Chronically exposed individuals show steady-state dimer levels over a period of days. A study of the dimer levels in children and adults after sunbathing on Swedish beaches revealed that children did not excrete greater levels of T=T dimers than adults, indicating that they were not more sensitive to UV-induced DNA damage than adults.

Case–control studies of melanoma risk associated with exposure to sunlight give complex results: sunburn before age 15 is a significant risk factor; high sunbathing activities increase risk of truncal and limb melanoma; and total sun exposure is associated with risk for limb melanoma at low latitudes. Number of naevi is correlated with average holiday exposure and further data analysis also implicated sunburn. Paradoxically, the risk of melanoma associated with sunburn was greatest for those with fewest naevi. Regular weekend exposure in the summer at high latitudes might be protective; perhaps because of higher levels of serum vitamin D. Determinants of serum vitamin D levels include genetic factors, sun exposure, skin type and vitamin D supplementation. The data from an epidemiological study suggested that in real life attaining optimal level of 60 nmol/l at higher latitudes requires an average of 12 h outdoor exposure at weekends, rather more than the 15 min previously thought sufficient from laboratory studies. Population studies suggest that sun protection against both UVA and UVB is important and that fair-skinned people or those with large numbers of naevi might therefore need to take vitamin D supplements to ensure optimal serum levels.

The increased popularity of sunbeds, which emit largely UVA, although a small amount of UVB is unavoidable, has presented an opportunity for epidemiological studies of the role of UVA in melanomagenesis. First use of sunbeds before the age of 30 increases melanoma risk. An increase in melanoma incidence has paralleled the popularity of artificial tanning in the Nordic countries and a more recent increase in Iceland has been attributed to the later uptake of artificial tanning in this country. Subsequent decreases in the incidence of melanoma in Icelandic women are thought to result from health campaigns discouraging artificial tanning.

The early expectation that sunscreens that protect against sunburn, largely induced by UVB, would protect against melanoma was not met. Sunscreen use during intentional sun exposure led to increased melanoma risk. Use of high skin protection factor (SPF) sunscreen increased the time spent in the sun as burning was delayed but this might increase the UVA dose received depending on the sunscreen filters. The trunk would be expected to be particularly vulnerable as not regularly exposed in day-to-day life and this was borne out by observations in a Swedish population over recent decades.

Although melanoma incidence in young people in different populations has increased, mortality has not. The increase in melanomas was primarily due to thin melanomas of less than 2 mm, which might be less aggressive. Age-related 'thick' melanoma, associated with childhood rather than recent exposures, might reflect declining defence mechanisms with ageing.

Cohort studies of Swedish and Norwegian women showed an association between melanoma risk and sunburn, sunbathing holidays and solarium use during the age decades 10 to 39 years. The accumulating effect of intermittent sun exposure or solarium use over time suggested that reducing exposure in adult life could reduce risk. A study in the Swedish cohort suggested a reduced risk of breast cancer associated with sunbathing holidays between 10 and 29 years or solarium use between 10 and 39 years of age. This finding was not replicated in a study in the Norwegian cohort nor was an association found with vitamin D effective UV dose or high vitamin D intake. No association was found between UV exposure and non-Hodgkin lymphoma. Because of the contribution of solarium use to melanoma risk, knowledge of UV emissions of tanning equipment is crucial. Disturbingly, erythema-weighted UV exposure varied twofold for the same model in different facilities and up to threefold for different models in the same location, making the risk of sunburn greater. The higher proportion of UVA in newer sunbed models is also a cause for concern.

Genetic alterations in melanomas are of interest for the light that they might throw on etiology and mechanisms of initiation and progression. Analysis of cell lines from melanoma metastases by comparative genomic hybridization revealed gross but non-random copy number changes throughout the genome. Hotspots for loss of heterozygosity were observed and more amplifications than deletions were found. Patterns of changes were found, e.g. alterations in the tumour suppressor gene *CDKN2A* were more common in cell lines with *BRAF* or *NRAS* mutations; *PTEN* alterations were higher in cell lines carrying *BRAF* mutations. A recurrent deletion at the *CDKN2A* locus on chromosome 9 was found to result in loss of *CDKN2A* and formation of a novel fusion gene that was both transcribed and translated.
Gene expression profiling of advanced melanomas resulted in the identification of four tumour groups that predicted disease outcome: high immune response, proliferative, pigmentation and normal-like. The same subtypes were found in early stage melanomas. Further analysis allowed the development of two subtype signatures: high immune and normal-like (low-grade melanomas), or proliferative and pigmentation (high-grade melanomas), the latter subtype having a poorer prognosis. Gene expression profiling, DNA sequencing and *BRAF* mutational analyses of a clinically annotated cohort of melanoma patients is yielding interesting results, e.g. different metastases from the same patient can show gene expression profiles that fall into different subtypes.

The lack of a correlation between the minimal erythemal dose (MED) for UVB and UVA1 (340–400 nm) in a given individual indicates a different mechanism of action. For a given erythemal exposure the UVB induction of CPDs and 6-4PPs decreases with epidermal depth whereas UVA-induced CPDs increase with depth, suggesting that the basal layer might be vulnerable to UVA1-induced damage. Erythemally equivalent doses of UVB and UVA1 induced comparable expression of MMP-1 mRNA, a marker of photoageing, indicating that erythema and MMP-1 induction have common chromophores. Other experimental results suggest that CPDs trigger both MMP-1 and photoageing. Gene expression profiling of biopsies from UVA1- or UVB-exposed volunteers showed common changes in key pathways but also many differences. At 6 h after exposure the common pathways involved were immune response, inflammation, apoptosis and oxidative stress response genes. At 24 h, expression of genes in the extracellular matrix modelling pathway was enriched.

A novel hotspot mutation has been identified in the *BRM* gene, involved in chromatin remodelling, in human non-melanoma skin cancers. The G:C to T:A transversion is typical of oxidative stress, suggesting that UVA is responsible. *BRM* ko mice have an increased incidence of UV-induced skin tumours but are protected against UV-induced immunosuppression. Whether or not Brm inhibits UV-induced DNA damage is unclear. Data from a number of sources indicate that UVA immunosuppression, unlike that induced by UVB, has a bell-shaped dose response. Gene expression profiling in mice showed that up-regulation of genes in the alternative complement pathway correlated with UVA immunosuppression.
UVA exposure also results in reduced levels of ATP in keratinocytes. Nicotinamide has a critical role in ATP production and can prevent UV depletion of intracellular ATP. It can also reduce UV-induced CPDs and oxidative damage in skin explants. Topical application of nicotinamide protects against UVB- and UVA-induced immunosuppression and oral nicotinamide reduced the development of basal cell carcinomas (BCCs) and SCCs in a small trial.

Data from the Genes, Environment and Melanoma study (GEM), which compared cases with multiple primary melanomas with controls with a single primary melanoma for sun exposure, genotype and other lifestyle factors, did not confirm earlier observations that sun exposure prior to melanoma diagnosis is associated with survival from melanoma.

A high UV environment, such as might be found at lower latitudes, does not necessarily mean that serum vitamin D levels will be sufficient: levels were found to be deficient in 40% of adults in Brisbane, Australia at 27°S. Supplement intake makes an important contribution to adequate intake. Serum levels were higher overall in summer than in winter but, contrary to expectation, some individuals had lower serum vitamin D levels in summer than in winter.

Exposure of healthy adults to suberythemal UV doses of UVB, UVA or both together, for times designed to provide the same vitamin D effective dose, increased serum vitamin D levels, with UVA alone being less effective. Baseline levels were lower in skin type IV to VI than in I to III but no correlation was found for skin type and the change in levels after UV irradiation.

In a cross-sectional study of 7-year-old children in southern Sweden, changes in parental sun-protective regimes were found to result in a significant decrease in the

mean number of common melanocytic naevi in the children. This decrease occurred despite an increase in holidays at seaside resorts abroad over the years studied.

A study of sunbed use in a group of 16 to 17 year olds in Sweden showed that 40% had used a sunbed with more girls than boys having done so. Awareness of the risks was high. It is expected that sun burns would be as common in this group as in the youngest adults previously surveyed and prohibition of sunbed use, hire or sale to under 18 year olds is recommended.

1.4. Discussion

Results from recent experiments on the mechanism of induction of mutations in the skin by sunlight are consistent with a model in which extended stalling of the transcription complex at a photolesion in human and mouse cells increases the likelihood of deamination of CPDs. Translesion synthesis then induces transition mutations and intragenic deletions; the latter could be generated by the collapse of replication forks at a stalled transcription complex.

The role of different wavelengths of the UV spectrum in the induction of melanoma remains controversial. In *Xiphophorus*, the evidence suggests that only UVB is effective whereas in a mouse model, neonatal UVA has been shown to induce melanoma in black but not albino littermates while UVB is effective in both. In humans, the rise in melanoma with increasing use of UVA-rich sunbeds suggests a role for UVA. Children sunbathing on Swedish beaches do not excrete more urinary T=T dimers than adults but a greater understanding of the response of children's skin to UV radiation is desirable.

The greater susceptibility of fair-skinned individuals to melanoma suggests that melanin might be protective but the results in the mouse model indicate that it might also have a role in UVA-induced disease. The correlation of induction of CPDs by both UVB and UVA with MED in phototype II and IV human volunteers shows that the physical dose of light reaching the DNA and not photosensitiser content is the main risk factor.

Increased numbers of naevi are a potent melanoma risk factor and numbers are correlated with average holiday exposure more strongly than for sunburn. Paradoxically, the risk of melanoma associated with sunburn is greatest in those with fewest naevi, supportive perhaps of the theory of different routes for melanoma associated with intermittent sun exposure and naevi versus chronic sun exposure in individuals with normal naevus numbers.

The actively dividing cells and pigment-producing melanocytes in lower levels of the skin might be more vulnerable to damage, especially by the more highly penetrating UVA. Greater levels of 8-oxoGua found in the basal layer of the human epidermis in response to UVA support this idea. In addition, UVB induces many more CPDs in isolated keratinocytes than in skin, whereas the ratio for UVA is much less. Paradoxically, a bell-shaped UVA dose–response curve has been found for different measures of immunity in humans and mice. Also, in a hairless mouse model, SSR-induced tumour formation and immunosuppression of CHS can be abrogated by treatment with appropriate doses of UVA. This contrasts with the lack of reduction in cancer risk found with solarium (chiefly UVA) use.

Gender differences in response to UV irradiation have been found in the fish *Xiphophorus* and in the hairless mouse but the implications for humans are unclear as men and women have differing sun exposure and solarium use habits.

The search for melanoma risk factors has identified many germline genes such as *MC1R*, *TYR* and *ASIP* as well as single nucleotide polymorphisms in other low-risk genes that are implicated in susceptibility. Analysis of somatic mutations in melanomas is complicated by the difficulty in distinguishing among initiating mutations, mutations important to progression or metastasis and secondary mutations that contribute to cancer cell survival. Mutations in the *BRAF* or *NRAS* gene are universal in melanomas but those commonly found are not classic UV mutations. In contrast, a high percentage of non-melanoma skin cancers have *p53* mutations consistent with the induction of sunlight-induced CPDs.

Two categories of gene expression changes with diagnostic value have been revealed by expression profiling of melanomas. These include immune, proliferative and pigmentation genes and high-immune gene expression is associated with better survival. On-going work is relating these changes to mutations in the melanomas. Epidemiological studies demonstrate that at higher latitudes, 12 h of sun exposure at weekends is necessary to attain optimal serum vitamin D levels, not the 15 min previously considered sufficient. Even in a high UV environment, vitamin D supplementation might be necessary.

Increasing understanding of the mechanisms of photoageing of skin, and its parallels with normal ageing, are leading to the development of antagonists that can mitigate these effects.

Promising results in chemoprevention of UVA-induced CPDs and oxidative damage in skin with nicotinamide have been attained in volunteers but the need for care is illustrated by the finding of defects in skin function resulting from up-regulating a potentially protective gene in a mouse model. The introduction of multiple signalling pathway inhibitors for melanoma therapy offers hope.

The downturn in melanoma mortality in response to regulatory and public health initiatives in different countries is a source of optimism as is the reduction in naevi in Swedish children in response to changing parental sun-protective regimes.

To summarise, certain topics might merit further investigation:

- Differences between the skin of adults and children
- The role of melanin, naevi, phototype and ethnicity in the UV response
- Identification of further susceptibility genes by genome-wide association studies
- The role of somatic mutations and altered gene expression in melanoma prognosis and treatment
- The role of stem cells, environment and cellular interactions in melanoma initiation and progression
- Modulation of the immune response by UV irradiation
- Photoageing mechanisms and their relationship to photocarcinogenesis
- The effects of inhibiting multiple signalling pathways in melanoma
- Determinants of vitamin D levels

- Effective public health information and behavioural change; targeting susceptible groups

1.5. Recommendations

Discussion among participants resulted in the following recommendations:

- Broad-spectrum sunscreens to protect against both UVA and UVB
- Standardisation of sunscreens; quality control for effectiveness against UVA and UVB
- Raise awareness of high levels of UVA; discourage use of tanning parlours and sunbeds
- Vitamin D supplementation rather than tanning for those with fair skin or numerous naevi
- Individuals with skin types III and IV should also exercise caution in the sun
- Consider protective effect of clothing against sun-induced skin damage

2. Sammanfattning

Under 2007 anordnades vid Karolinska Institutet en internationell konferens som sponsrades av dåvarande svenska strålskyddsinstitutet (SSI) och Cancerfonden. Fokus för konferensen var presentation och diskussion av aktuella forskningsdata gällande biologisk påverkan av UV-strålning med betoning på effekter av UVA-och UVB-strålning och dess betydelse för sjukdom.

Kunskapsläget gällande sjukdomar inducerade av UV-strålning har under de senaste fem åren utvecklats snabbt. Bland annat har IARC klassificerat UV-strålning som cancerframkallande för människor, nya metoder för behandling av melanom har introducerats och framsteg har gjorts avseende riskfaktorer och biologiska mekanismer som är inblandade i utvecklingen av hudcancer. I maj 2012 anordnade forskare vid Karolinska Institutet med finansiellt stöd från Strålsäkerhetsmyndigheten en uppföljande konferens för att vidare presentera och diskutera kunskapsutvecklingen inom detta område.

Denna rapport bygger på resultat som lagts fram vid detta möte.

2.1. Cellulära effekter av UV-strålning

Den största delen av UV-strålning som når markytan består av UVA (320-400 nm) medan enbart ca 5 % består av UVB (280-320 nm). UVB-strålning inducerar DNA-skador, så kallade cyklobutan pyrimidin dimerer (CPDs), och då särskilt tymin dimerer (T = T), och även (6-4) fotoprodukter (6-4PPs) genom direkt fotokemisk reaktion med DNA. Dessa skador är inte jämnt fördelade längs DNA-strängen utan varierar efter hur DNA-strukturen ser ut (exempelvis beroende på metyleringsstatus och kromatinstruktur). Tidigare ansågs UVA-strålning orsaka främst oxidativa skador på DNA, exempelvis 8-oxo7,8-dihydroguanine (8-oxoGua), men det har även visat sig att CPDs kan induceras av UVA-strålning, även om mönstret varierar från det som orsakas av UVB. Förhållandet mellan CPDs till 8-oxoGua är ungefär 5:1 i de flesta typer av hudceller, men lägre i melanocyterna, vilket kan återspegla ökad oxidativ stress hos melanocyterna. Få 6-4PPs induceras av UVA-strålning.

Hos människa är den enda DNA reparationsvägen som kan avlägsna CPDs och 6-4PPs nukleotid-excision-reparation (NER). NER omfattar både global genomisk reparation (GGR) och transkriptions-kopplad reparation (TCR). Vid GGR har nya studier visat att även polyADP-ribos polymeras bidrar till att identifiera skadat DNA (”DNA recognition step”). Detta polymeras spelar en nyckelroll i stabiliseringen av DDB2 som är det första proteinet som anländer till UV-skadat kromatin. Rekrytering av kromatin-remodellerare gör sedan UV-skadat kromatin tillgängligt för reparation. Hos möss som saknar TCR är epidermis mer känsligt för UVB-inducerad solskada, med en ökning av apoptotiska celler. I embryonala stamcellslinjer från mus med en inducerbar *hprt* reportergen, var UV-inducerade nukleotidsubstitutioner vanligare när reportergenen transkriberades, men endast från UV-skador i den transkriberade strängen. Detta har man även sett vid p53-mutationer i solexponerad hud hos människa, vilket tros bero på en snabb transkriptions-beroende deaminering av cytosin vid CPDs som skulle kunna förhindras med TCR. Dessa experiment belyser

den betydande roll som transkription spelar vid solljus-inducerad mutagenes i huden och den avgörande funktionen som TCR har att motverka denna typ av mutagenes.

Hemoxygenas 1 (HO-1), som upprätthåller jämnvikten av hem och järn, induceras i fibroblaster vid oxidativ stress, inklusive vid UVA-bestrålning. Stabilisering av transkriptionsfaktorn Nrf2 genom UVA, bindning till DNA av transkriptions-suppressorproteinet Bach1 och därefter aktivering av HO-1 har visat sig medieras av hem. Långvarig uttryck av HO-1 hindras av UVA induktion av Bach1. Keratinocyter är mindre känsliga för UVA-inducerad membranskada, möjligen på grund av deras konstitutiva uttryck av hemoxygenas 2 (HO-2) som leder till låga hemnivåer som förhindrar aktivering av HO-1. En ökad förståelse av regleringen av det anti-inflammatoriska enzymet HO-1 kan leda till nya terapeutiska ingrepp.

Aktivering av Nrf2, som inducerar uttrycket av ett antal reaktiva syreradikal (ROS)-detoxifierande enzymer samt HO-1, kan ha terapeutiska fördelar vilket testades i en transgen musmodell som konstitutionellt uttrycker en aktiv mutant form av Nrf2 i keratinocyterna. Resultatet visade på reducerade nivåer av UVB-inducerad apoptos, ROS nivåer och p53-positiva keratinocyter, men utan effekt på bildandet av CPDs. Möss som uttrycker högre nivåer av Nrf2 visade epidermala avvikelser, liknande fenotypen hos patienter med iktyos. Microarray-expressions analyser visade förändringar i regleringen av gener som reglerar bildandet av den epidermala barriären. Man bör därför använda Nrf2-aktiverande föreningar med försiktighet vid försök med kemoprevention av UV-skador.

Förutom vid DNA-skada kan UVB effekter även förmedlas av arylhydrocarbon receptorn (AhR) och epidermal tillväxtfaktorreceptorn (EGFR). Nyligen genomförda experiment visade att 6-formyl-[3,2-b] karbazol (FICZ), bildad genom UVB bestrålning av tryptofan, är involverat i respons av AhR och EGFR. UVB-bestrålning av en keratinocytcellinje i närvaro av AhR antagonisten 3'-metoxi-4'-nitroflavone (KE) visade en minskning av CPDs, vilket kan tyda på att AhR är involverad i UVB-inducerad karcinogenes. MNF inhiberade också UVB induktion av metalloproteinaset MMP-1, som bryter ner kollagen, i keratinocyter vilket kan tyda på att AhR även är inblandad i fotoåldrande. Man har även utvecklat en AhR antagonist som effektivt minskar UVB-induktion av MMP-1 och andra enzymer i human hud med potentiella anti-åldrande effekter. Man har även sett att AhR uttrycks i melanocyter. AHR knock-out-möss uppvisade minskad UVB-inducerad solbränna och minskad förökning av melanocyter.

Rynkor i fotoåldrad hud tros bero på förändringar i syntes och nedbrytning av elastin. Extracellulära elastaser är välkända. I ett projekt med syfte att söka efter intracellulära enzymer, påvisades det lysosomala cysteinproteaset cathepsin K (catK) hos neonatala humana fibroblaster. Elastin inne i fibroblasterna degraderades genom catK. Bestrålning av hudtransplantatat med UVA, och i mindre utsträckning UVB, inducerade catK i fibroblaster i övre dermis. Fotoåldrande kan möjligtvis orsakas av en UV-inducerad acceleration av det inneboende åldrandet eftersom man observerat att progerin, trunkerat pre-lamin A, som förknippas med tidigt åldrande-syndrom, induceras i fibroblaster genom upprepad exponering av lågdos UVA. En enda UVA exponering framkallar progerin mRNA mer effektivt i fibroblaster som åldrats in vitro, samt i celler från äldre donatorer jämfört med i unga fibroblaster.

Man har analyserat om snabb pigmentering av mänsklig hud framkallad av UVA möjligtvis kan förmedlas genom ljustransduktion, vilket avslöjade en retinal-beroende Ca^{2+} mobilisering i epidermala melanocyter. Melanocyter befanns uttrycka

fullängds-rodopsin och en reducering av rhodopsin-uttrycket minskade även den UV-inducerade Ca^{2+} responsen. De ökade melaninnivåerna i UVA-bestrålade melanocyter i närvaro av retinal reducerades vid kalcium uttömning, vilket tyder på en ny väg för ljustransduktion att reglera snabb pigmentering.

2.2. Experimentalla modeller för UV-inducerad sjukdom

Utvecklingen av musmodeller av UV-inducerat melanom med "humaniserad" hud, t.ex. transgena HGF / SF möss, har inneburit ett viktigt framsteg för mekanistiska studier. Endast neonatal UVB-exponering initierar melanom i FVB-HGF albino möss, medan i svarta- och albinomöss från en korsning av C57BL/6 möss kunde man se att både UVA och UVB kunde inducera melanom hos de svarta mössen. Melanom inducerade av UVA skedde via en oxidativ process, medan UVB-inducerad sjukdom orsakades direkt från DNA-skador. Dessa resultat tyder på en komplex roll för melanin i UV-inducerad hudsjukdom.

UV-strålning främjar också tillväxt av UV-initierade melanom. UV-bestrålad neonatal hud ympad på HGF/SF vildtyp möss som också UV-bestrålats neonatalt producerar melanom snabbare än hos icke-bestrålade mottagare. UV-strålning ökar inte neonatal immuntolerans men leder till att melanocyter mobiliseras från hårsäcken till epidermis hos neonatala möss, vilket kan främja bildandet av melanom.

Induktion av melanom genom neonatal UV-exponering i fiskmodellen Xiphophorus hybrid Sp-Couchianus kan jämföras med UV-exponering under tidig barndom och melanom på bålen hos människa. Storskaliga studier av tidiga verkningsspektrum i denna fiskmodell visade att neonatal UVB-exponering, men inte UVA inducerade melanom i motsats till tidigare resultat.

Många observationer tyder på att D-vitamin kan skydda mot cancer, men UVB som krävs för att producera vitamin D kan även orsaka hudcancer. Knock-out (KO) möss som saknar vitamin D-receptorn (VDR) har visat sig vara mer känsliga för UV-inducerad hudcancer. Resultaten tyder på att det skydd som förmedlas av VDR inkluderar epidermal DNA-reparation, undertryckande av proliferation av keratinocyter och stimulering av mognad, differentiering av cellerna. Ytterligare studier med VDR KO musen pekar på en försämrad reglering av hedgehog (HH) och Wnt / Beta-catenin signalvägarna i UVB-inducerade tumörer.

Genom att analysera ursprunget för de stamceller som utvecklas till melanocyter, har man kunnat demonstrera att isolerade dermala celler kunde bibehållas som dermala sfärer som uttrycker stamcellsmarkörer som inte uttrycks av fibroblaster, keratinocyter eller melanocyter. Dessa dermala celler kan utvecklas till melanocyter lokaliserade till det basala membranet av epidermis i en tredimensionell hudmodell, vilket speglar melanomcellers anpassningsförmåga. Dermala stamceller var aktiverade genom Notch-signalvägen. Genuttrycks-profilering visade på en uppreglering av transkriptionsfaktorn MSX-1, som inducerades av Wnt, BMP och FGF signalering i dermala stamceller och melanomceller. Denna transkriptionsfaktor minskde melanocytpigmenteringen och ökade cellernas migration. Förekomsten av liknande processer i huden som svar på cancerframkallande ämnen såsom UV-strålning har visat sig leda till initiering och progression av melanom.

I en hårlös musmodell resulterade kronisk UV-exponering i p53 muterade cellkloner i interfollikulära (IF) epidermis, som i slutändan gav upphov till skivepitelcancer (SCC) med motsvarande mutationer. Ablation genom akut överexponering av UV i IF:s basala skikt, men inte i överliggande skikt, hos möss med UV-inducerade p53-kloner ledde till en försening av SCC utveckling som svar på fortsatt kronisk UV-exponering, vilket tyder på att de p53 muterade klonerna har eliminerats och att det skett en ”reset” av carcinogenesen. Detta kan liknas vid responsen hos människa vid solbränna, där underliggande celler genomgår apoptos och dör. Behandling av kroniskt UV-bestrålade hårlösa möss med den immunosuppressiva medicinen rapamycin, vilken har antitumöreffekter, minskar antalet kloner som överuttrycker p53. Rapamycin-behandlade möss skiljde sig inte från kontrollmössen när det gällde tumörer upp till 1 mm, men visar en minskning i antal tumörer > 4 mm. Således är förhållandet mellan p53-kloner och tumörutveckling komplex.

UVB-inducerad immunsuppression av oxazolon-inducerad kontaktöverkänslighet (CHS) hos hårlösa möss skulle kunna minskas avsevärt genom UVA-bestrålning, medierad av HO. UVA verkar också vara skyddande mot den immunosuppression som orsakas av sol-simulerad UV (SSR) fotokarcinogenes i denna musmodell, vilket visades genom en minskning av antalet tumörer. 17 - beta-östradiol verkar också skydda mot SSR-inducerad immunsuppression och man kunde se en skillnad i UVA-medierat skydd mot UVB immunosuppression vid CHS hos både manliga och kvinnliga möss.

2.3. Humana studier av UV-inducerade sjukdomar

Att noggrant utvärdera vilken dos av UV-strålning som huden exponerats för är svårt och estimering av CPDs i hudbiopsier är inte lämpligt för stora studier eller för studier på barn. En alternativ analys är att mäta radioaktivt märka in T = T dimerer i urinen. Denna analys har validerats hos individer som solat solarium där kroniskt exponerade individer visar förhöjda steady state-nivåer av T = T dimerer efter ett par dagar. I en studie av T = T-nivåer hos barn och vuxna efter solexponering vid svenska stränder visade på liknande nivåer hos barn och vuxna, vilket tyder på att barn inte är mer känsliga för uppkomst av UV-inducerade DNA-skador än vuxna.

Fall-kontrollstudier av melanomrisk i relation till solexponering ger ofta komplexa resultat: solbränna före 15 års ålder är en betydande riskfaktor, frekvent solbadande ökade risken för melanom på bål, armar och ben, och total solexponering är förenad med ökad risk för melanom på ben och armar. Antal nevi har kunnat korreleras med antal solsemestrar och även antal solbrännor verkar påverka risken för hudmelanom. Paradoxalt nog var risken för melanom förknippad med solbränna störst för de med minst antal nevi. Regelbunden solexponering på helgen under sommaren på höga breddgrader kan vara skyddande, möjligtvis på grund av att detta leder till högre nivåer av D- vitamin. Mängden D-vitamin hos en individ beror bland annat på genetiska faktorer, ålder, mängd solexponering och hudtyp. Data från en epidemiologisk studie visade att för att nå en optimal nivå av serum D-vitamin på 60 nmol/l krävde i genomsnitt 12 timmar utomhusvistelse på helgerna vid högre breddgrader, att jämföras med de 15 minuter man tidigare rapporterat med underlag från laboratoriestudier. Befolkningsstudier visar att det är viktigt att skydda sig mot både UVA-och UVB-strålning och att ljushyade personer eller personer med ett stort antal nevi kan behöva D-vitamintillskott för att garantera optimala serumnivåer.

Genom en ökad användning av solarier, som medierar stora mängder UVA-strålning, även om en liten mängd UVB är oundviklig, har öppnat möjligheter för epidemiologiska studier av UVA-strålningens roll i utveckling av melanom. Att nyttja solarium före 30 års ålder ökar risken att utveckla melanom. Man har sett en parallel ökning av melanomincidens tillsammans med ökad popularitet av solariienyttjande i de nordiska länderna. Den senare incidensökningen av melanom som setts på Island kan förklaras med att nyttjande av solarium kom senare till Island. Dock har man sett en minskning i incidens av melanom på senare år hos isländska kvinnor, vilket kan bero på minskad solarieanvändning till följd av hälsokampanjer mot solarieanvändning.

De tidiga förväntningarna på att solskyddsmedel som skyddar mot solbränna, som till stor del orsakas av UVB-strålning, också skulle skydda mot melanom har inte visat sig stämma. Användning av solskyddsmedel för att sedan avsiktligt kunna spendera mer tid i solen har visat sig öka melanomrisken. Detta skulle kunna förklaras med att användandet av solskyddsmedel ofta leder till ökad tid i solen eftersom man inte bränner sig lika fort, mendan man därigenom utsätts för en ökad mängd UVA-strålning (beroende på solskyddsmedlets filter). Hud som inte regelbundet utsätts för solexponering, såsom den på bålen, och som därför är extra känslig, blir särskilt utsatt för ökad melanomrisk.

Även om melanomincidensen hos unga människor har ökat i vissa populationer, verkar detta inte följas av en ökad dödlighet. Förklaringen till detta kan vara att ökningen främst består av tunna melanom på mindre än 2 mm, vilka normalt har en bättre prognos än tjockare melanom. Åldersrelaterade ”tjocka” melanom som orsakas av solexponering under barndomen snarare än senare exponeringar, kan återspegla försämrade försvarsmekanismer med ökad ålder.

Kohortstudier av svenska och norska kvinnor visade ett samband mellan melanomrisk och solbränna, antal solsemestrar och solarieanvändning under åldern 10-39 år. Den ackumulerande effekten av intermittent solexponering eller solarieanvändning över tid antyder att minskad solexponering senare under vuxenlivet kan minska risken för melanom. I en studie av den svenska kohorten föreslogs ett samband mellan minskad risk för bröstcancer och regelbundna solbad helgdagar under ålderperioden 10 och 29 år eller solarienyttjande mellan 10 och 39 års ålder. Detta fynd kunde inte replikeras i den norska kohorten och inte heller kunde man se ett samband med vitamin D och UV-exponering. Ingen association sågs mellan UV-exponering och non-Hodgkin lymfom. På grund av att solarienyttjande ökar risken för melanom, är kunskap om vilken UV-strålning som man utsätts för genom solarieanvändning viktig. Man har sett att den erytem-viktade UV-exponeringen varierar mellan solarier. Till exempel varierade strålningen dubbelt så mycket mellan två solarier av samma modell på olika anläggningar och upp till trefaldigt för olika modeller i samma lokal, vilket ökar risken att man bränner sig. Den högre andelen UVA i nyare modeller är också oroväckande.

Genetiska förändringar som uppkommer i melanomtumörer är av intresse eftersom de kan förklarara bakomliggande orsaker och mekanismer för initiering och progression av tumörer. Analys av cellinjer från melanommetastaser med teknologin ”comparative genomic hybridization” (CGH), har identifierat kromosomala regioner med icke slumpmässiga genetiska förändringar. Kromosomala regioner som ofta visade på förlust av den ena kromosomkopian, så kallad ”loss of heterozygosity” kunde identifierats och fler amplifikationer (ökat kopieantal av en viss kromosomal region) än deletioner (förlust av kromosomal region) hittades. Specifika mönster av

genetiska förändringar observerades, t.ex. var förändringar i tumörsuppressorgenen CDKN2A vanligare i cellinjer med mutationer i onkogenerna BRAF eller NRAS och förändringar i PTEN genlokuset var mer frekvent i cellinjer med BRAF mutationer. En återkommande deletion av CDKN2A lokuset på kromosom 9 befanns resultera dels i förlust av CDKN2A genen och dels i bildandet av en ny fusionsgen som både transkriberas och translateras.

Genuttrycks-profilering av avancerade melanom resulterade i identifiering av fyra olika grupper av tumörer som kan användas att prediktera prognosen vid melanomtumören: aktivt immunsvar, proliferation, pigmentering och normal-liknande. Samma subtyper kunde även detekteras hos primära melanom. Vidare analys kunde gruppera tumörerna till två huvudgrupper: aktivt immunsvar och normal-liknande (låggradigt melanom), gentemot hög proliferation och pigmentering (höggradigt melanom), där den senare gruppen har en sämre prognos. Genuttrycks-profiling, DNA-sekvensering och BRAF mutationsanalyser i en grupp av melanompatienter visade intressanta resultat, t.ex. att olika metastaser från samma patient kan visa genuttrycksprofiler från olika subtyper.

Avsaknaden av en korrelation mellan den minimala erytema-dosen (MED) för UVB och UVA1 (340-400 nm) hos en given individ tyder på att strålningstyperna har olika verkningsmekanismer. För en given erytemdos av UVB-strålning minskar bildandet av CPDs och 6-4PPs med epidermalt djup medan UVA-inducerade CPDs ökar med djupet, vilket tyder på att det basala hudlagret kan vara sårbar för UVA1-inducerad skada. Jämförbara erytemorsakande doser av UVB och UVA1-strålning har visat sig inducera ekvivalenta uttryck av MMP-1-mRNA, en markör för solskadad hud (photoageing), vilket tyder på att hudrodnad och MMP-1-uttryck har gemensamma kromoforer. Andra experimentella resultat tyder på att CPDs kan utlösa både MMP-1-uttryck och solskadad hud. Genutryck-profilering av biopsier från frivilliga försökspersoner som exponerats för UVA1-eller UVB-strålning visade liknande förändringar i viktiga signaleringsvägar men också många skillnader. 6 timmar efter exponering var vanligen signaleringsvägar inom immunsvar, inflammation, apoptos och oxidativ stressgener uppreglerade. Vid 24 timmar, var uttrycket av ett flertal gener som bygger upp och modellerar den extracellulära matrisen, vävnaden mellan kroppens celler, uppreglerade.

Man har nyligen identifierat en ny återkommande ”hotspot” mutation i genen BRM, som är involverad i kromatin-remodellering, i humana icke-melanom hudcancrar. Mutationen som är en G: C till T: A transversion är typisk för oxidativ stress, vilket tyder på att UVA är orsaken. BRM KO-möss har en ökad förekomst av UV-inducerade hudtumörer men skyddas mot UV-inducerad immunsuppression. Huruvida Brm inhiberar UV-inducerade DNA-skador är oklart. Data från ett antal källor tyder på att UVA immunsuppression, till skillnad från den som induceras av UVB, har en klockformad dosrespons. Genuttrycks-profilering på möss visade att uppreglering av gener i den alternativa komplementvägen korrelerade med UVA immunsuppression.

UVA-exponering resulterar även i minskade nivåer av ATP i keratinocyter. Nikotinamid har en avgörande roll för ATP-produktion och kan förhindra utfömning av intracellulärt ATP på grund av UV-exponering. Det kan också minska UV-inducerade CPDs och oxidativa skador i hudtransplantatat. Lokal applicering av nikotinamid skyddar mot UVB-och UVA-inducerad immunsuppression och peroral nikotinamid minskade utvecklingen av basaliom (BCC) och skivepitelcancer (SCC) i en liten studie.

I studien ”Genes, Environment and Melanoma study” (GEM) jämförde man melanompatienter med multipla primära melanom (fallgrupp) med patienter med ett enda primärt melanom (kontrollgrupp) för faktorer som solexponering, genotyp och andra livsstilsfaktorer. Resultat från studien kunde inte bekräfta tidigare data som visat att ökad solexponering före melanomdiagnos är associerad med bättre melanom-associerad överlevnad.

En miljö med hög UV-exponering, såsom länder vid lägre breddgrader, betyder inte nödvändigtvis att serum D-vitaminnivåer är tillräckliga hos dess invånare: D-vitaminnivåerna befanns vara bristfälliga hos 40% av de vuxna i Brisbane, Australien vid 27 °S. Intag av kosttillskott och berikade livsmedel gav ett viktigt bidrag till adekvata nivåer. De totala serumnivåerna var generellt högre på sommaren än på vintern, även om det fanns vissa individer som visade det motsatta, d.v.s lägre serum D-vitaminnivåer på sommaren än på vintern.

Exponering av friska vuxna för suberytemala UV-doser av UVB, UVA eller båda tillsammans, under den tid som ansågs ge samma effektiva D-vitamin dos, ökade serum D-vitaminnivåer för samtliga, men UVA ensamt gav minst ökning. Grundnivåerna var generellt lägre hos individer med hudtyp IV - VI än hos de med hudtyp I-III, men man kunde inte se en korrelation mellan hudtyp och D-vitaminnivå efter UV-bestrålning.

I en tvärsnittsstudie av 7-åriga barn från södra Sverige, visade att förändringar i hur föräldrar skyddar sina barn från solen har lett till en betydande minskning av medelantalet melanocytnevi hos barnen. Denna minskning inträffade trots att det skett en ökning av antal semestrar vid badorter utomlands under de studerande åren.
En studie av solarieanvändning gjord på en grupp 16 till 17-åringar i Sverige visade att 40% hade solat solarium och att det var fler flickor än pojkar som solade solarium. De flesta var medvetna om riskerna. Det förväntas att solbränna och solskador är lika vanliga i denna grupp som hos unga vuxna, som tidigare granskats, och därför är ett förbud mot solarieanvändning för personer under 18-år är att rekommendera.

2.4. Diskussion

Resultat från experiment på mekanismer bakom solljus-inducerade mutationer i huden överensstämmer med en modell där en förlängd blockering av transkriptionskomplexet vid en fotolesion i humana och musceller ökar sannolikheten för bildandet av CPDs. Translesionsyntes genererar då transitionsmutationer och intragena deletioner, där de senare skulle kunna genereras av kollaps av replikationsgaffeln vid ett avstannat transkriptionskomplex.

Den roll som olika UV-våglängder har vid uppkomst av melanom är fortfarande kontroversiell. I Xiphophorus, tyder mycket på att endast UVB är effektivt medan i en musmodell har det visat sig att neonatal UVA-exponering genererar melanom hos svarta men inte hos albinomöss från samma kull medan UVB orsakar melanom hos båda. Hos människan skulle en ökning av melanomincidensen kunna delvis bero på en ökad användning av solarier med UVA-berikad strålning, vilket talar för att UVA har en viktig roll. Barn som exponeras för solstrålning på svenska stränder visade sig inte utsöndra mer t = t-dimerer i urinen än vuxna, men en större förståelse för hur barns hud påverkas av UV-strålning är önskvärd.

Eftersom ljushyade individer har en större känslighet att insjukna i melanom har det föreslagits att pigmentet melanin kan vara skyddande men data från en musmodell visar att melaninet också kan ha en roll i UVA-inducerad sjukdom. Korrelationen mellan induktion av CPDs från både UVB-och UVA med MED hos frivilliga individer med hudtyp II och IV visar att det är den fysiska dosen av ljus som når DNA som är den viktigaste faktorn.

Ökat antal nevi är en potent riskfaktor för melanom och antal nevi har visat sig korrelera starkare med genomsnittlig semesterexponering än med solbränna. Paradoxalt nog är melanom starkast associerad med solbränna hos de individer med minst antal nevi, vilket skulle kunna stödja teorin om olika vägar för melanomuppkomst; intermittent solexponering och nevi kontra kronisk solexponering hos individer med normalt antal nevi.

Aktivt delande celler och pigment-producerande melanocyter i längre ner i huden kan vara mer sårbara för skador, särskilt orsakande av den mer genomträngande UVA-strålningen. Större mängd av 8-oxoGua som hittats i det basala lagret av humant epidermis som svar på UVA-strålning förstärker denna hypotes. Dessutom inducerar UVB många fler CPDs i isolerade keratinocyter än i huden, medan kvoten för UVA är betydligt mindre.

Förvånandsvärt nog har en klockformad UVA dosresponskurva funnits för olika mått på immunfunktion hos människor och möss. Också i en hårlös musmodell kan SSR-inducerad tumörbildning och immunosuppression av CHS upphävas genom behandling med lämpliga doser av UVA. Detta står i kontrast med att man inte ser en minskad cancerrisk vid solarium (främst UVA) användning.

Könsskillnader som svar på UV-strålning har påvisats hos fisken Xiphophorus och hos den hårlösa musen men hur det ser ut hos människan är oklart eftersom män och kvinnor har olika vanor avseende solexponering och solarieanvändning.

Sökandet efter faktorer som kan kopplas till ökad känslighet att insjukna i melanom har identifierat nedärvda genetiska förändringar i flera olika gener såsom MC1R, TYR och ASIP samt andra lågriskgener som är inblandade i ökad melanomkänslighet. Analys av somatiska mutationer i melanomtumörer kompliceras av svårigheten att skilja mellan initierande mutationer, mutationer viktiga för progression eller spridd sjukdom samt sekundära mutationer som bidrar till cancercellernas överlevnad. De vanligaste muterade generna i melanom är BRAF och NRAS, men de vanligt förekommande mutationerna i dessa gener är inte klassiska UV-mutationer. Däremot bär en hög andel icke-melanom hudcancrar på p53-mutationer som överensstämmer med mutationer orsakade av solljus-inducerade CPDs.

Två kategorier av genuttrycks-profiler med diagnostiskt värde har identifierats med hjälp av genuttrycksprofilering av melanomtumörer. Dessa inkluderar immun-, proliferativa- och pigmenteringsgener där överuttryck av immungener associeras med bättre överlevnad. Studier på hur dessa förändringar kan kopplas till mutationer i melanom pågår.

Epidemiologiska studier visar att 12 timmar i solen på helger vid högre breddgrader är nödvändigt för att uppnå optimala serum D-vitaminnivåer, inte 15 min som tidigare ansetts tillräckligt. Även i en hög UV-miljö, kan D-vitamintillskott vara nödvändigt.

Ökad förståelse för mekanismerna bakom solåldrande av huden, och dess paralleller med normalt åldrande, kan leda till utveckling av antagonister som kan mildra dessa effekter.

Hos frivilliga försökspersoner har man uppnått lovande resultat vid kemoprevention av UVA-inducerade CPDs och oxidativa skador i huden med nikotinamid men att det bör användas med försiktighet illustreras av upptäckten av defekter i hudens funktion genom upp-reglering av en potentiellt skyddande gen i en musmodell. Införandet av hämmare för olika signaleringsvägar har markant förbättrat möjligheterna att behandla melanom.

Nedgången i melanomdödlighet till följd av reglerings-och folkhälsoarbetet i olika länder är en källa till optimism, ett exempel är minskningen av nevi hos svenska barn som svar på förändrat solskyddsbeteende hos deras föräldrar.

Sammanfattning: Kunskapsluckor och områden av speciellt intresse som kom fram under mötet:
• Skillnader i huden mellan vuxna och barn
• Betydelse av melanin, nevi, fototyp och etnicitet avseende hur man reagerar mot UV-exponering
• Identifiering av ytterligare riskgener genom analyser av hela den humana arvsmassan
• Betydelse av somatiska mutationer och förändringar i genuttryck för prognos och behandling av melanom
• Betydelse av stamceller, miljö och cellulära interaktioner för initiering och progression av melanom
• Modulering av immunsvaret efter UV-exponering
• Mekanismer bakom solskadad hud ”fotoåldrande” och hur det påverkar fotokarcinogenes
• Effekterna av att nedreglera flera signaleringsvägar samtidigt i melanom
• Identifiering av för hälsan optimala D-vitaminnivåer
• Hur man kan effektivisera allmän hälsoinformation och åstadkomma beteendeförändring, till exempel genom målinriktad information mot känsliga grupper?

2.5. Rekommendationer

Diskussion bland deltagarna resulterade i följande rekommendationer:

- Att få bort solarieutnyttjande
- Att använda solskydd mot både UVB-och UVA
- Solskyddsmedel bör standardiseras, bred-spektrum bäst
- Att tillräckliga mängder av D-vitamin ska uppnås genom kost och kosttillskott och inte genom ökad UV-exponering, speciellt hos individer med ljus hy och många födelsemärken
- Öka medvetenheten om effekter av UVA
- Även personer med hudtyp III och IV bör varnas för riskerna med för hög UV-exponering
- Informera om skyddande effekter av kläder för att undvika sol-inducerade hudskador

3. Introduction

In 2007, an international conference sponsored by the former Swedish Radiation Protection Authority and the Swedish Cancer Society and held at Karolinska Institutet explored the state of knowledge concerning ultraviolet (UV) radiation-induced disease and the roles of UVA and UVB[1]. The following five years have seen the classification of the whole solar UV spectrum by IARC as carcinogenic to humans, the introduction of BRAF inhibitors for treatment of melanoma and advances in understanding of the mechanisms of skin carcinogenesis and the risk factors involved. In May 2012, a second, follow-up, conference was held at Karolinska Institutet, funded by the Swedish Radiation Safety Authority, to present and discuss progress in the field, again with emphasis on the differential effects of UVA and UVB and links to disease. This report is based on the evidence presented at that meeting.

[1] SSM Report number: **2009:24** ISSN: 2000-0456 - Available at www.stralsakerhetsmyndigheten.se UV radiation-induced disease - roles of UVA and UVB - Author: Jean Emeny

4. Role of UV radiation in disease

Exposure to sunlight has a variety of effects, including vitamin D synthesis, erythema, melanogenesis, immunomodulation, photoageing and skin cancer. Sunlight encompasses the electromagnetic spectrum and includes infrared, visible and ultraviolet (UV) radiation. Of the UV spectrum, the UVC component (wavelength 220–280 nm) and most of the UVB component (280–320 nm) are absorbed by the earth's atmosphere. Sunlight reaching the earth comprises 90–95% UVA (320–400 nm), depending on factors such as latitude and time of year, and 5–10% UVB.

4.1. Cellular effects of UV radiation

Understanding the mechanisms by which UV radiation induces deleterious effects in humans is crucial to the development of therapies and preventive measures. Much can be learned at the cellular level, including the contribution of different UV wavelengths to DNA damage and mutagenesis and the role of DNA repair. Cell systems are also being used to elucidate the mechanisms involved in maintenance of redox homeostasis, receptor-mediated changes in gene expression, photoageing, and phototransduction in response to UV irradiation.

4.1.1. Formation of UV-induced DNA damage

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UVB

UVB (280–320 nm) induces dimeric pyrimidine photoproducts, i.e. cyclobutane pyrimidine dimers (CPDs) and (6-4) photoproducts (6-4PPs) by direct photochemical reaction with DNA. Cyclobutane pyrimidine dimers, especially thymine dimer (T=T), are the most frequent whereas 6-4PPs represent about one-third of the lesions. The latter may be converted to Dewar isomers in the presence of UVA. Lesions are not induced evenly along the DNA; methylated cytosine is more reactive than cytosine; and chromatin structure can influence photochemistry. Telomeres are more photoreactive and formation of interstrand dimers is possible in the quadruplex region (Cadet et al. 2012 and references therein). Deamination of cytosine-containing CPDs leads to mutation at TC and CC dinucleotides. This deamination is enhanced during transcription and accelerated by adjacent G; however, binding of methyl CpG binding protein 2 (MeCP2) suppresses deamination (Cannistraro and Taylor 2010).

UVA

UVA irradiation of cells results in the formation of oxidised purines, oxidised pyrimidines and strand breaks in DNA, with the main product being 8-oxo-7,8-dihydroguanine (8-oxoGua). The mechanism is thought to be via the formation of singlet oxygen, mediated by intracellular photosensitisers, with hydroxyl radical formation playing a lesser part (Cadet et al. 2012 and references therein).

Although early evidence showed that UVA induces CPDs in bacteria (Tyrrell 1973), mammalian cells and human skin (see Mouret et al. 2006 for references), emphasis has been focused on oxidative damage to DNA. The distribution of photoproducts induced by UVA differs from that induced by UVB, with a preponderance of T=T

dimers and negligible production of 6-4PPs; the pattern is similar in fibroblasts, keratinocytes and skin (Mouret et al. 2006). The replication of this pattern in isolated DNA suggests that a photosensitiser is not involved (Mouret et al. 2010). Greater amounts of CPDs than 8-oxoGua are induced by UVA irradiation, with a fivefold ratio in fibroblasts, keratinocytes and skin; however, in melanocytes the ratio is 1.4-fold CPDs to 8-oxoGua, perhaps reflecting greater oxidative stress in this cell type (Mouret et al. 2012).

Photosensitivity of human skin to UVA and UVB

A comparison of UVB- and UVA-induced CPDs in skin and keratinocytes from the same donors showed that UVB induces 22-fold fewer lesions in skin than keratinocytes, whereas for UVA it was only 1.5-fold. The rate of removal of lesions was also lower for UVA damage in skin than for UVB (Mouret et al. 2006).

The role of phototype in sensitivity to CPD induction by UVA as compared with UVB was tested in skin biopsies from phototype II and IV volunteers (Mouret et al. 2011). Phototype and UVB-induced CPD formation and minimal erythemal dose (MED) were found to be correlated, with similar results found for UVA. UVB- and UVA-induced damage was also correlated for each volunteer, indicating that photosensitiser content, possibly higher in darker skin, was not a factor in lesion formation, the physical dose of light reaching the DNA being the main parameter.

4.1.2. Repair of DNA photolesions

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Nucleotide excision repair (NER), which in human is the only repair pathway capable of removing CPDs and 6-4PPs, involves several different steps. The first step is recognition of damage to DNA, followed by remodelling of chromatin, dual incision and removal of the damaged oligonucleotide, filling of the gap created and ligation. These processes involve 30 or more proteins. Nucleotide excision repair comprises transcription coupled repair (TCR), which removes lesions from the transcribed strand of active genes, and global genomic repair (GGR), which repairs damage at other sites.

Global genomic repair

Protein complexes involved in the pre-incision, DNA damage recognition step of GGR include UV-DDB, a heterodimer of DNA damage binding protein 1 (DDB1) and DDB2 (lacking in Xeroderma pigmentosum Group E (XPE) cells, which are deficient in CPD repair and have retarded 6-4PP repair). Recent studies by Pines et al. (2012) have shown that polyADP-ribose polymerase 1 (PARP1) is associated with DDB2 in UV-irradiated human cells. This polymerase plays a key role in the stabilisation of DDB2, the first protein that arrives at the UV-damaged chromatin, and the recruitment of chromatin remodellers to make the UV-damaged chromatin accessible for repair. PolyADP-ribosylation post-incision was also found that was independent of DDB2.

Chromatin remodelling in response to UV-induced DNA damage was investigated by Luijsterburg et al. (2012). Depletion of core histones was seen at DNA lesions in locally UV-damaged human cells; this depletion was reduced in the presence of a PARP inhibitor or siRNA, indicating that PARP is involved in the changes in chromatin structure. Luijsterburg et al. (2012) also demonstrated that polyADP-ribosylation stimulates the recruitment of XPC to UV-induced DNA lesions. In addition, Pines et al. (2012) showed that DDB2-dependent polyADP-ribosylation at the photolesion resulted in recruitment of the chromatin-remodelling enzyme ALC1.

Transcription-coupled repair

Transcription-coupled repair removes lesions from DNA strands transcribed by RNA polymerase II. In mice lacking TCR the epidermis is more sensitive to UVB-induced erythema with an increase in apoptotic cells (van Oosten et al. 2000). Analysis of the *p53* gene in mice lacking TCR showed a bias to mutations in the transcribed strand (Pines et al. 2010).

Mouse models of Cockayne syndrome, which lack TCR; XPC, which lack GGR; and XPA, lacking both pathways, were used to investigate the role of TCR in balancing mutagenesis and apoptosis in response to UVB-induced DNA damage. The effects of transcription on UV-induced mutation frequency were compared in repair-deficient ($XPA^{-/-}$) and -proficient mouse embryonic stem cell lines carrying an inducible *hprt* reporter gene. In the absence of transcription of the reporter gene, UV-induced mutations were only little increased in the repair-deficient lines relative to controls, indicating that GGR does not play a major role in repair of UV-induced damage in these cells. In the presence of transcription, a significant increase in nucleotide substitutions occurred in the repair-deficient cells, relative to controls, but only from damage in the transcribed strand (Hendriks et al. 2008, 2010). This strand bias, also seen in *p53* mutations in sun-exposed human skin, was due to a rapid transcription-dependent deamination of cytosine at CPDs that could be prevented by TCR. These experiments highlight the role of transcription in sunlight-induced mutagenesis in the skin and the crucial role of TCR in counteracting this type of mutagenesis.

The results are consistent with a model in which extended stalling of the transcription complex at a photolesion in repair-deficient cells increases the likelihood of deamination of cytosine dimers, which is increased in single-stranded DNA. Translesion synthesis then induces transitions (C>T or CC>TT). Intragenic deletions could be generated by the collapse of replication forks at a stalled transcription complex. Persistent stalling of the transcription complex also induces apoptosis. Transcription-coupled repair would prevent prolonged stalling of the transcription complexes and protect against DNA damage and apoptosis (Hendriks et al. 2010).

4.1.3. UVA-mediated regulation of the heme oxygenase 1 gene and potential therapeutic targets

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UVA contributes to the disruption of redox homeostasis in a number of ways, e.g. direct and indirect generation of reactive oxygen species (ROS), induction of the release of heme and iron and destruction of antioxidant enzymes such as catalase. Redox homeostasis can be restored by constitutive and UVA-induced antioxidant pathways. UVA-induced oxidative damage to DNA is greater in melanoma cell lines with higher levels of melanin.

The enzyme heme oxygenase 1 (HO-1) is induced by many forms of oxidative stress, including UVA, in skin fibroblasts but not keratinocytes. It breaks down heme and liberates iron and is important in the maintenance of heme and iron homeostasis. The enzyme has an anti-inflammatory action and may be protective in certain pathological states; hence, knowledge about its regulation may lead to therapeutic interventions.

Heme oxygenase 1 expression is activated by Nrf2, which forms a complex with MafK that binds to the Maf Recognition Element (MARE) upstream of the HO-1 gene. UVA irradiation of skin fibroblasts stabilises Nrf2 protein, enabling its migration to the nucleus. UVA-released heme binds to the inhibitor Bach1 and inhibits its

binding to DNA. Experimental reduction in heme levels prevents both Nrf2 stabilisation and UVA activation of HO-1. In addition, siRNA silencing of Nrf2 suppresses UVA-induced HO-1 activation and enhances membrane damage (Zhong et al. 2010). These results suggest that UVA-induced heme release mediates stabilisation of Nrf2 and activation of HO-1.
Inhibition of HO-1 expression is mediated by heme-dependent binding of the Bach1/MafK heterodimer to the MARE site. Transfection of skin fibroblasts with Bach1/MafK prevents UVA induction of HO-1. In contrast, siRNA knock out (ko) of Bach1 very much increases the expression of HO-1 in untreated skin fibroblasts and increases that in UVA-irradiated cells (Raval et al. 2012). The UVA induction of Bach1 prevents prolonged expression of HO-1 (Raval et al. 2012).
In contrast to skin fibroblasts, keratinocytes suffer less from UVA-induced membrane damage and HO-1 induction by UVA is much reduced. The latter is related to the observation that these cells express HO-2 constitutively, which will keep heme levels low. The role of HO-2 and Bach1 in the low basal and UVA-inducible levels of HO-1 in keratinocytes was investigated by Zhong et al. (2010). Silencing of HO-2 expression with siRNAs increased both basal and UVA-induced expression of HO-1 in keratinocytes. Silencing of Bach1 also substantially increased basal levels of HO-1 in these cells but neither additional silencing of HO-2 nor UVA treatment was additive. Bach1 silencing in keratinocytes also suppressed UVA-induced membrane damage (Zhong et al. 2010).

4.1.4. Cytoprotective signalling pathways in tissue repair and cancer

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The Nrf2 transcription factor has a central role in protection against oxidative damage to cells. It induces expression of a variety of ROS-detoxifying enzymes and other antioxidant proteins, including HO-1 (see Section 4.1.3). UVB induces a gradient of apoptotic ‘sunburn’ cells in mice from the basal to suprabasal layers of skin, but this is not seen in Nrf2 ko mice (Schäfer et al. 2010). This gradient enables upper layers of cells to survive and maintain skin integrity, whereas damaged basal cells, including stem cells, are eliminated, thus avoiding carcinogenesis.
To test whether activation of Nrf2 might have therapeutic benefits, Schäfer et al. (2010) analysed the UVB response of mice expressing a constitutively active Nrf2 mutant in keratinocytes. Apoptosis, ROS levels and p53-positive keratinocytes were reduced relative to controls; however, no change in the formation of CPDs was found. Transgenic mice expressing two- to three-fold higher levels of Nrf2 showed epidermal acanthosis and hyperkeratosis, impaired skin barrier function, inflammation and increased cell proliferation (Schäfer et al. 2012) and thus developed a phenotype resembling ichthyosis in humans.
Microarray analysis revealed changes in regulation of more than 100 genes in the high-level Nrf2-expressing transgenic mice. Of particular importance for the observed epidermal abnormalities are the genes encoding the secretory leukocyte peptidase inhibitor (Slpi), and small proline-rich proteins Sprr2d and Sprr2h, which were identified as direct targets of Nrf2. The known properties of these proteins suggest that up-regulation of Slpi could explain the hyperkeratosis observed and the Sprr proteins might contribute to the defects in barrier function and inflammation. Hence, therapeutic treatments that activate Nrf2 must be used carefully so as to avoid deleterious changes to the skin.

4.1.5. Role of the arylhydrocarbon receptor in the UVB response

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In addition to the DNA damage-mediated UVB stress response, cells also respond via mechanisms involving activation of membrane receptors. Fritsche et al. (2007) demonstrated that UVB, but not UVA, irradiation of a keratinocyte cell line induced translocation of the arylhydrocarbon receptor (AhR) from the cytoplasm to the nucleus and induction of expression via the xenobiotic response element (XRE) of the AhR-dependent *CYP1A* gene. In a second, non-genomic, signalling pathway, UVB induced internalisation of the epidermal growth factor receptor (EGFR) and phosphorylation of the EGFR downstream target ERK1/2. Both these responses were inhibited in AhR ko mice (Fritsche et al. 2007) as was UVB induction of *COX-2* expression, consistent with a role of the AhR in the EGFR-mediated UVB response. Further experiments revealed a role for 6-formyl[3,2-*b*]carbazole (FICZ), formed by UVB irradiation of tryptophan, in the AhR response. In tryptophan-starved cells, internalisation of EGFR was prevented and UVB induction of *CYPA1* and *COX-2* was abolished (Fritsche et al. 2007). FICZ, which has a high affinity for AhR, was demonstrated in irradiated cells by mass spectrometry. FICZ-treated cells internalised the EGFR receptor and showed *COX-2* mRNA expression but this was abolished in AhR ko cells, suggesting that FICZ is involved in the AhR-mediated UVB response.

A model has been described which links these observations (Krutmann et al. 2012). After UVB irradiation and FICZ formation, FICZ binds to the AhR complex. The FICZ-bound AhR then translocates to the nucleus, binds to XRE and activates genes such as *CYPA1*. In the membrane-mediated pathway, the c-src kinase disassociates from the AhR complex on FICZ binding, phosphorylates the EGFR and sets in motion a signalling pathway, including phosphorylation of pERK1/2, that results in activation of genes such as *COX-2*. These results also have relevance in vivo. UVB irradiation of shaved mice results in induction of *CYP1A1* and *COX-2* transcription in wild-type but not AhR ko mice, demonstrating AhR dependence.

An involvement of the AhR in UVB-induced carcinogenesis was revealed by experiments in which the effect of the AhR antagonist 3′-methoxy-4′-nitroflavone (MNF) was tested on the yield of CPDs formed in a keratinocyte cell line. Damage was found to be reduced by increasing doses of MNF. The use of signalling pathway inhibitors suggested that the non-genomic pathway was involved via phosphorylation of an unknown target protein and repression of dimer repair. On-going in vivo studies in ko mice are unravelling the contribution of the AhR in photocarcinogenesis.

There is also considerable interest in the contribution of the AhR to UVB-induced photoageing. Sunlight is thought to induce skin wrinkling by induction of matrix metalloproteinases (MMPs) which cause breakdown of collagen. In accord with this, UVB induction of MMP-1 in primary human keratinocytes was inhibited by MNF. An AhR antagonist, BIO-1031® (2-benzylidene-5,6-dimethoxy-3,3-dimethylindan-1-one) has been developed that inhibits UVB-induced *COX-2* transcription. It is also effective in human skin, reducing the UVB induction of *CYP1A1*, *COX-2* and *MMP-1* transcription (Krutmann J, unpublished results).

Evidence that AhR activation by toxins can cause changes in pigmentation suggested that it might be expressed in melanocytes and this was found to be the case (Jux et al. 2011). Induction of *CYP1A1* and *CYP1B1* in primary mouse melanocytes in response to the toxin 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), UVB-irradiated tryptophan and FICZ, was found, in accord with results in keratinocytes. UVB tan-

ning of mice, as measured by ear melanin content and tyrosinase activity, was also reduced in AhR ko mice. Microarray experiments and gene ontology analysis revealed a number of differences in expression of genes involved in cell differentiation, apoptosis, proliferation and pigmentation between wild-type and $AhR^{-/-}$ primary mouse melanocytes (Jux et al. 2011). The reduction in tanning in AhR ko mice was associated with reduced proliferation of melanocytes as determined by L-3,4-dihydroxyphenylalanine (DOPA) staining of cells. Primary wild-type but not $AhR^{-/-}$ melanocytes express stem cell factor in vitro and fluorescence activated cell sorting experiments show fewer $AhR^{-/-}$ melanocytes expressing c-kit on their surface (Jux et al. 2011). Thus, differences in expression of these proliferation and differentiation genes between wild-type and AhR ko mice may explain the difference in their response to UVB.

4.1.6. Ageing of the skin

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Both intrinsic ageing and photoageing (extrinsic ageing) result in detrimental changes to the skin. Photoageing and photocarcinogenesis often occur in parallel. It might be thought that UVA exposure has a greater photoageing effect because of its deeper penetration into the skin, although 'paracrine'-like effects have been demonstrated. Nevertheless, following are two examples in which UVA in particular, but not UVB, affects cell functions with relevance to ageing.

Cathepsin K

Accumulation of fibrillar basophilic material, mainly elastin and fibrillin, is seen in the dermis of photoaged skin and is termed actinic elastosis. Changes in elastin synthesis and degradation are thought to contribute to the accumulation of this fibrillar material. A number of extracellular elastases are known; however, intracellular elastases may also play a role. A possible candidate, cathepsin K (catK), is a lysosomal cysteine protease with elastolytic activity that is involved in bone resorption by osteoclasts and is found in fibroblasts in scar tissue, melanocytic naevi and melanomas but not in normal skin.

Cathepsin K was detected in cultured neonatal human fibroblasts by Rünger et al. (2007) and found to be inducible by UVA in vitro and in vivo (Codriansky et al. 2009). Fibroblasts were subsequently demonstrated to internalise elastin via the elastin-laminin receptor to lysosomes (Gan and Rünger 2011) where it was degraded by catK (Codriansky et al. 2009). These results indicate a role for catK in the regulation of elastin levels in fibroblasts and a possible involvement in photoageing. UVA irradiation of skin explants induced catK expression in fibroblasts in the upper dermis; UVB also induced this enzyme but to a lesser extent. Strikingly, catK was not induced by UVA in cultured fibroblasts from older donors, a lack that might result in changes in the balance of intracellular and extracellular elastin levels and contribute to actinic elastosis. Regulation of catK might represent an approach to reversing photoageing.

Progerin

Progerin, a truncated pre-lamin A, resulting from mutation of the lamin A gene, interferes with nuclear membrane function, and causes premature ageing in Hutchinson Gilford Progeria Syndrome. It also accumulates in aged cells and might be involved in photoageing. Repeated low dose exposure to UVA induces progerin in fibroblasts. Abnormal nuclear morphology is associated with this induction. Aged cells express a higher baseline level of progerin mRNA. In addition, a single exposure to UVA induces progerin mRNA more effectively in fibroblasts aged in vitro

and cells from older donors than in young fibroblasts. UVB was much less effective (Takeuchi and Rünger 2013). These results indicate that photoageing might represent an exacerbation of intrinsic ageing.

4.1.7. Ultraviolet light phototransduction in human skin

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Pigment darkening in human skin in response to UVB irradiation is mediated by DNA damage, activation of the melanocortin 1 receptor (MC1R) and melanin production. However, pigment darkening in response to UVA occurs within minutes and the mechanism is unknown.

Phototransduction in the eye is mediated by opsins in the presence of the chromophore retinal via G-protein activation and signal transduction. Wicks et al. (2011), using a microscope set up to allow UV exposure and live cell imaging, showed by fluorometric Ca^{2+} imaging that UV irradiation of human epidermal melanocytes resulted in a Ca^{2+} mobilisation response that was retinal dependent. Little response was seen with green or blue light and UVA was much more effective than UVB. Depletion of intracellular Ca^{2+} stores with Thapsigargin, an inhibitor of sarco/endoplasmic reticulum Ca^{2+} ATPases, reduced the response. The Ca^{2+} influx occurred via an ion channel and was reduced in the presence of a G protein or phospholipase C inhibitor, suggesting the involvement of a rhodopsin-like phototransduction pathway. A search for opsins expressed in melanocytes by RT-PCR revealed expression of full-length rhodopsin and western blot analysis with anti-rhodopsin antibodies showed that melanocytes could express rhodopsin. Reduction of rhodopsin expression with miRNA also reduced the UV radiation-induced Ca^{2+} response. However, the spectral profile of the light-induced Ca^{2+} response was atypical and this is unexplained (Wicks et al. 2011).

UV irradiation of human epidermal melanocytes, in the presence of retinal, resulted in an increase in melanin levels that was time and dose dependent but was significant within an hour (Wicks et al. 2011). UVA alone was sufficient for this melanin response. Calcium depletion abolished the Ca^{2+} response and reduced the increase in melanin levels. These results support the idea that the immediate pigment darkening seen in response to UVA is the result of a novel phototransduction pathway (Wicks et al. 2011).

4.2. Experimental models of UV-induced disease

The main animal models for sunlight-inducible melanoma are the opossum *Monodelphis domestica*, the *Xiphophorus* hybrid fish, and the transgenic HGF/SF mouse (Fernandez et al. 2012). The pathology of melanoma in the opossum model least resembles that of humans. *Xiphophorus* has the advantages of high fecundity, short breeding cycle and the availability of a collection of genetic models of melanoma (Patton et al. 2010). Mammalian models most resemble humans in terms of cell lineage and differentiation pathways. The development of human skin models such as dermal spheres complements these animal models.

4.2.1. Two UV pathways to melanoma

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The development of mouse models of UV-induced melanoma with 'humanized' skin represents an important step forward for mechanistic studies. The transgenic HGF/SF mouse model overexpresses the growth factor HGF/SF, which activates pathways relevant to human melanoma. A single UV dose given to 3 day old neonates results in melanoma development with the histopathological characteristics of human melanoma 6–12 months later (see Table 1, Noonan et al. 2012). UV irradiation of adults is without effect.

Normally, only UVB irradiation, and not UVA, initiates melanoma in FVB-HGF albino mice; however, when HGF mice were bred with a C57BL/6 background and black and albino littermates were compared, the black mice were found to be more susceptible to UV-induced melanoma (Noonan et al. 2012). They were also more susceptible to spontaneous melanoma. Irradiation of black mice with biologically relevant doses of UVB or UVA alone, showed that UVA was also effective in melanoma induction. Analysis of the data suggested that different mechanisms were responsible for UVB- and UVA-induced melanoma (Noonan et al. 2012).

Analysis of DNA damage by immunohistochemistry and high performance liquid chromatography-tandem mass spectrometry revealed comparable amounts of CPDs and 6-4PPs in UVB-irradiated black and albino mice. After UVA irradiation, only low levels of T=T dimers were detected but these were induced in both black and albino mouse strains. In contrast, 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo), representing oxidative damage to DNA, was found in melanocytes of neonatal skin and cultured melanocytes of black but not albino mice after UVA irradiation (Noonan et al. 2012). These results indicate that UVA induction of melanoma in pigmented mice occurs through an oxidative process that is melanin dependent, whereas UVB-induced disease results directly from DNA damage.

The results from the transgenic mouse model are relevant to human melanoma because they parallel the observations of increased melanoma risk from UVA-rich exposure in tanning salons and atypical naevi. The role of melanin in skin disease and protection from damage is paradoxical. Melanin can be protective in absorbing UV radiation, as a scavenger of free radicals and as an anti-oxidant. In dark-skinned people, melanin acts as an epidermal 'parasol' that protects against UV damage to melanocytes. In contrast to this, the melanin can be pro-oxidant in the presence of metals, or when partially polymerised, and melanin production occurs via a series of very reactive quinones.

Recent work (Zaidi et al. 2011) has profiled gene expression in isolated melanocytes from UVB-irradiated transgenic mice. An involvement of interferon was found as well as other immunoevasion genes and this could be blocked by antibody to interferon-γ (IFN-γ). Other observations suggested a role for IFN-γ in enhancing melanoma growth and survival, making this a possible target for therapeutic intervention. Using microarray analysis, it should also be possible to identify expression profiles for UVB- and UVA-induced melanomas in black and albino mice and to shed light on the mechanisms involved in melanomagenesis.

4.2.2. Neonatal UV irradiation promotes the outgrowth of UV-initiated melanomas: a role for mast cell-dependent neonatal immune tolerance

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In the transgenic HGF/SF mouse model, a single UV dose initiates melanoma, which appears at 6 to 12 months. Melanomas develop successfully on neonatally UV-irradiated transgenic HGF/SF skin which has been grafted onto wild-type

syngeneic mice. The appearance of melanomas in this grafted skin is significantly faster if the graft recipients have been UV irradiated as neonates, suggesting that UV irradiation facilitates outgrowth as well as initiation of melanoma. UV irradiation does not induce infiltration of $CD11b^+$ $Ly6G^+$ neutrophils in neonatal skin, unlike adult skin (Wolnicka-Glubisz et al. 2007), nor does it induce the inflammatory cytokines IL-1, Gro-α, IL-6 and tumour necrosis factor. Neonatal mice exposed to the contact sensitiser trinitrochlorobenzene (TNCB) showed a decreased contact hypersensitivity (CHS) response to the same antigen re-applied at 2 months, indicating neonatal immune tolerance (Wolnicka-Glubisz et al. 2007). This decreased CHS response to TNCB is ablated in the mast-cell-deficient Wsh mouse, suggesting a role for mast cells in neonatal immune tolerance.

Neonatal UV irradiation does not increase neonatal immune tolerance; however, UV irradiation does mobilise melanocytes from the hair follicle to the epidermis in neonates but not adults. To explain these and other results, Zaidi et al. (2011) have proposed a model in which neonatal UVB induces melanocytic expression of chemoattracting Ccr2 ligands that recruit $Ccr2^+$ macrophages into neonatal skin. In the neonatal tolerising environment, IFN-γ from these macrophages stimulates melanocyte proliferation and migration and the expression of genes involved in immunoevasion and survival.

4.2.3. Melanoma induction in a *Xiphophorus* hybrid fish model

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Early action spectrum studies in the *Xiphophorus* hybrid Sp-*Couchianus* implicated both UVB and UVA in melanoma formation and melanin photosensitisation (Setlow et al. 1993; Wood et al. 2006). Because the UVA effect was inconsistent with previous results from many other animal models (but see Section 2.2.1), the action spectrum for melanoma induction was re-examined in a large-scale experiment in *Xiphophorus* hybrid Sp-*Couchianus* (Mitchell et al. 2010). In contrast to the previous results, neonatal UVB but not UVA irradiation was found to induce melanomas in this experiment.

The fish melanoma model, using neonatal UV exposure, can be compared with the early childhood exposures associated with truncal melanoma in humans. Consideration of observations from the literature and of the structural differences between the most frequent UVB-induced DNA damage products CPDs and 6-4PPs, led to the development of a novel model of melanoma and non-melanoma skin cancer initiation (Mitchell and Fernandez 2011). In this model, an effect of UVB-induced 6-4PPs on gene expression via inhibition of DNA replication and transcription rather than mutagenesis is postulated to result in melanoma. In contrast, carcinoma induction is postulated to result from the mutagenic effects of UVB- and UVA-induced CPDs. Useful tools to test this hypothesis might be fish models with deficits in CPDs and 6-4PP photolyases, to test the effect of specific removal of lesions.

The greater susceptibility of males to UVB-induced but not spontaneous melanoma in this fish model is intriguing. UVB was found to reduce circulating hormone levels but conjugated testosterone increased in males later on. Hormone receptor changes were found in skin (Mitchell D et al., unpublished observations).

4.2.4. The vitamin D receptor as a tumour suppressor of UVB-induced skin cancer

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Many observations, including epidemiological and animal studies and human trials, suggest that vitamin D can protect against cancer. However, UVB radiation, required for vitamin D production, is a cancer inducer. To investigate the mechanisms involved, ko mice lacking the vitamin D receptor (VDR) were studied. These mice are more susceptible to chemically- and UV radiation-induced skin carcinogenesis (Ellison et al. 2008). Possible mechanisms for protection by vitamin D include promotion of DNA repair, or suppression of proliferation and stimulation of differentiation due to inhibition of signalling pathways.

In the epidermis of VDR ko mice, removal of UVB-induced CPDs was less than in the wild type, indicating a deficit in DNA repair. Keratinocytes lacking the VDR also showed decreased CPD clearance. Silencing of VDR by siRNA knockdown in cultured keratinocytes increased cell proliferation as determined by 5-bromo-2'-deoxyuridine staining and by the XTT assay and decreased apoptosis, as determined by the TUNEL assay. Silencing of the VDR also decreased early and late markers of cell differentiation. The epidermal hyperplasia and proliferation seen after UV irradiation was increased in VDR ko mice compared with the wild type (Teichert et al. 2011). Thus, the protection against UV irradiation by the VDR seems to involve DNA repair and also suppression of proliferation and stimulation of differentiation.

The nature of the tumours found in VDR ko mice suggested an involvement of the hedgehog (Hh) signalling pathway. Increased levels of proteins of the Hh pathway were found in epidermis and hair follicles in VDR ko mice (Teichert et al. 2011) and also in UVB-induced tumours. Given the interest in the use of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) as a potential anticancer agent, it was relevant that expression of the Hh pathway was repressed in $1,25(OH)_2D_3$-treated wild-type but not VDR ko mouse epidermis (Teichert et al. 2011).

Wnt/β-catenin signalling is also influenced by the VDR (Palmer et al. 2008). Silencing experiments showed that expression of target genes of the Wnt pathway is repressed by the presence of the VDR or $1,25(OH)_2D_3$. Localisation of E-cadherin/β-catenin to the cell membrane was also inhibited in the absence of the VDR, indicating a lack of control of this pathway. Thus the increased susceptibility of VDR null mice to UVB-induced tumours results from impaired signalling pathway regulation with consequent increased proliferation of damaged cells.

4.2.5. Stem cell differentiation and melanocyte de-differentiation

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Skin melanocytes are able to proliferate throughout life but the origin of the stem cells responsible has been unclear. To investigate this, Li et al. (2010) isolated dermal cells from foreskins, which lack hair follicles, and showed that they could be grown into ‘dermal spheres’ in vitro that expressed neural crest stem cell markers not found in fibroblasts, keratinocytes or melanocytes. In three-dimensional skin reconstructs, these dermal stem cells (DSCs) can develop into melanocytes localised to the basal membrane of the epidermis and anchor to keratinocytes through E-cadherin (Li et al. 2010). Thus, stem cells in the more protected dermis can give rise to epidermal melanocytes.

The proliferation of melanocytes in human skin is well regulated: keratinocytes and fibroblasts provide growth factors, keratinocytes control location, and anchorage to the basal membrane is essential. The processes of decoupling, division, reposition-

ing and re-coupling of melanocytes are controlled by a succession of signalling molecules. In melanoma, however, these processes become unregulated. Both DSCs and melanoma cells show plasticity and can give rise to other differentiated cell types such as osteoblasts or chondrocytes.
The Notch signalling pathway is activated in DSCs, which express Notch target genes such as *Hes1*. Differentiation and loss of viability of DSCs results from treatment with the Notch inhibitor DAPT. Interestingly, overexpression of the Notch intracellular domain in melanocytes results in the generation of induced neural crest stem cells (iNCSC) (Zabierowski et al. 2011).
Microarray gene expression profiling, confirmed by qRT-PCR, revealed up-regulation of msx1, a neural crest transcription factor induced by Wnt, BMP and FGF signalling, in DSCs, iNCSC and melanoma cells (Zabierowski et al. 2011). Msx1 decreases pigmentation in melanocytes and increases their migration. It also promotes formation of dermal spheres which express NGFRp75, a neural crest stem cell marker. If similar processes can occur in melanocytes in skin, perhaps as a result of exposure to carcinogens such as UV, this has implications for melanoma initiation and progression. The possibility of using fibroblasts to generate totipotent stem cells that can differentiate into melanocytes is being explored (Herlyn M, personal communication).

4.2.6. Ambiguity in the relationship between UV-induced *p53* mutant clones and skin carcinomas

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Chronic UV exposure of hairless mice results in cell clones overexpressing *p53* mutant in the interfollicular (IF) epidermis that appear to give rise to squamous cell carcinomas (SCCs) with similar mutations. Acute UV overexposure of dorsal skin of mice carrying such UV-induced *p53* clones, causing apoptosis of the IF basal layer but retention of overlying layers, delayed SCC formation by continuing low-dose chronic UV irradiation by the same length of time as prior UV exposure. Destruction of the IF layer had thus removed *p53* mutant clones and 'reset' carcinogenesis (Rebel et al. 2012). In humans, the sunburn response, with active apoptosis in underlying cells, cell death and peeling might prevent cell transformation, suggesting a target for therapy of SCCs (however, note that sunburns are a risk factor for malignant melanoma and basal cell carcinoma).
The immunosuppressive drugs used to suppress graft rejection are believed to cause the observed 65-fold increased incidence of SCCs in organ transplant recipients. Selecting an immunosuppressant with no or minimal pro-carcinogenic effects locally in the skin would minimise the risk. In a comparison of immunosuppressants admixed to the diet, rapamycin reduced foci with overexpressed mutant *p53* whereas cyclosporine and azathioprine increased the number of foci. Surprisingly, rapamycin did not reduce the onset of (small) tumours (<2 mm) whereas cyclosporine did (Voskamp et al. 2013). Dietary switching experiments ruled out an effect of rapamycin on mutant *p53* expression (Voskamp et al. 2012). Rapamycin did, however, decrease the yield of large tumours >4 mm (De Gruijl et al. 2010). Deep sequencing showed that none of the immunosuppressants affected the frequency of hotspot *p53* mutations around codon 270, and that cells carrying mutated *p53* are more numerous than the ones spotted in foci overexpressing mutant *p53* (Voskamp et al. 2013). The relationship between *p53*-overexpressing cell clones and tumour development is thus ambiguous.

4.2.7. UVA/UVB interaction for photoprotection

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In a hairless mouse strain, the CHS response to oxazolone is reduced by UVB irradiation or application of *cis*-urocanic acid (cis-UCA), a UV photoproduct. This immunosuppression can be significantly reduced by UVA irradiation (at a dose that is not immunosuppressive; see Section 4.3.8) before or after the UVB or cis-UCA exposure (Reeve and Tyrrell 1999). The HO inhibitor tin protoporphyrin (SnPP) abrogated this protective effect, implicating HO and its product CO in the response (Reeve and Tyrrell 1999).

The observation that topical application of the CO-releasing molecule tricarbonyldichlororuthenium (II) dimer reduced photocarcinogenesis in hairless mice chronically exposed to solar simulated radiation (SSR) suggested that UVA might also be protective against immunosuppression occurring during photocarcinogenesis (Allanson and Reeve 2007). A reduction in tumour multiplicity was seen with UVA treatment after SSR exposure; immunosuppression at 40 weeks as measured by CHS response was also abrogated by UVA treatment (Reeve V, manuscript in preparation).

Observations of the protective effects of phytoestrogens against UV damage to skin in mice led to the finding that 17-β-oestradiol could protect against SSR-induced immunosuppression. Treatment with the oestrogen receptor (ER) antagonist ICI 182780 inhibited the protective effect, implicating ER signalling in the response (Widyarini et al. 2006). These findings were extended to CHS suppression at 40 weeks and photocarcinogenesis in mice (Reeve V, manuscription in preparation). Because males are relatively deficient in ER signalling, the UV-induced immunosuppression of the oxazolone CHS response was compared in male and female mice. Male mice were significantly more sensitive (Reeve et al. 2012). Comparison of the protective effect of UVA irradiation against UVB-induced immunosuppression of the oxazolone CHS response in males and females again revealed that males were less responsive. Assay of HO-1 protein levels in male and female mice after UVA irradiation revealed that levels had increased by 24 h in male mice but did not reach the higher levels seen in females at 48 to 72 h post-UVA.

The importance of UVA source was demonstrated in the oxazolone/CHS model in the hairless mouse, with levels of protection against immunosuppression varying depending on the UVA source (Reeve V, unpublished observations).

4.3. Human studies of UV-induced disease

In vitro and animal models can provide important information about the initiation and progression of melanoma and other skin cancers but only studies in humans can establish whether these results represent a true picture of the human disease. Ethical and considerations rule out many kinds of human experiment but much can be learned from the molecular biology of naturally occurring skin disease and from population studies of cancer risk.

4.3.1. Analysis of thymine dimer in urine following exposure to sunlight

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The dose of UV radiation received by an individual while outdoors varies depending on factors such as latitude, season, and reflection from surrounding surfaces, e.g. water. Accurately assessing the dose received by the skin is difficult, despite the use of personal dosimeters, questionnaires and computer modelling. The production of CPDs, in particular T=T, is characteristic of UV-induced DNA damage. These can be assayed in skin biopsies, but this is not suitable for use in large populations or children. An alternative, ^{32}P-postlabelling analysis of T=T dimers excreted in urine, after NER of the DNA lesion, is non-invasive and widely applicable. In a validation study of urinary thymine dimers as a biomarker of UV exposure, Kotova et al. (2005) found that dimer excretion peaked at day 3 after a single sunbed session in most individuals, at >30-fold pre-exposure levels. Halving the exposure time or covering half of the solarium reduced dimer excretion proportionately, showing a dose–response effect.

Application of the method to chronically exposed individuals (lifeguards and farm workers) showed steady-state urinary T=T dimer levels over a period of days (Liljendahl et al. 2013). Because childhood exposure is a risk factor for skin cancer in adulthood, urinary dimer excretion was compared in children and adults after sunbathing on Swedish beaches (Liljendahl et al. 2012). The elevation in dimer excretion found after sunbathing correlated with dose as determined by a personal dosimeter for both adults and children. Children did not show greater urinary dimer excretion, indicating that they did not form more T=T in their skin than the adults. A comprehensive EC project, ICEPURE, looked at the impact of climatic and environmental factors on UV exposure. Elevated urinary dimer levels were found in children after two weeks summer exposure on a northern European beach and in adults holidaying in southern Europe in winter.

Understanding the relationship of urinary levels of T=T to levels in skin is important. Determining the effects of clothing, sunscreen use and health, e.g. vitamin D levels, immune function, will also be of practical value.

4.3.2. The genetic epidemiology of melanoma and what it suggest about melanoma risk, UVB, UVA and vitamin D

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Susceptibility to melanoma results from both genetically determined factors, such as skin colour, sun sensitivity and number of naevi, and exposure to sunlight. Genes that affect susceptibility include the melanocortin 1 receptor gene *MC1R*, which influences skin pigmentation, identified by a candidate gene approach, and genes such as *ASIP* and the tyrosinase gene *TYR*, and single nucleotide polymorphisms (SNPs) identified by genome-wide association studies (GWAS) (Amos et al. 2011 and references therein). Individuals carrying the phenotypes that predispose them to melanoma should receive targeted advice.

Melanoma risk associated with exposure to sunlight is very complex: sunburn is a significant risk factor; high sunbathing activities increase risk for melanoma on the trunk and limbs; however, large lifetime sun exposure is only associated with risk for melanoma on the limbs (and to a lesser extent head and neck) at low latitudes (Chang et al. 2009). Results from the Leeds Case–Control Study suggest that regular weekend sun exposure in the summer might be protective (Newton Bishop et al. 2011), perhaps because of changes in the skin such as photoadaption, or production of vitamin D.

In the Leeds Case–Control Study, higher levels of serum vitamin D were associated with greater weekend sun exposure. Analysis of vitamin D levels in a subset of cases

and sibling controls revealed significantly lower levels in cases (Newton Bishop et al. 2011). A protective role of vitamin D is supported by the observation that variants of the *VDR* gene can confer higher risk (Randerson-Moor et al. 2009). In addition, in a cohort study of melanoma patients, higher vitamin D levels were associated with thinner tumours and better survival, independent of Breslow thickness (Newton Bishop et al. 2009)

In the Leeds Case–Control Study, a strong correlation was found for number of naevi and average holiday exposure (Newton Bishop et al. 2010), but not sunburn. Further analysis of a large pooled data set (Chang et al. 2009) indicated an effect of sunburn on naevus count. Surprisingly, the risk of melanoma associated with sunburn was greatest in those with fewest naevi. In addition, stratification by photosensitivity and number of moles showed that those in the highest naevus count category with low photosensitivity had the lowest sunburn-associated melanoma risk.

Determinants of serum vitamin D levels include a SNP in the gene coding for the vitamin D binding protein, holiday sun exposure at low latitudes, weekend exposure in the summer, skin type, and, in particular, vitamin D supplementation (Davies et al. 2011 and references therein). The level generally considered optimal is 60 nmol/l; however, in the Leeds study this level was seen on average only in patients at this higher latitude spending an average of 12 h outside in warmer months at weekends, not the 15 min previously considered sufficient from laboratory studies.

The association of sunburn with melanoma risk suggests that UVB is important in the initiation or development of melanoma. Mutational patterns in naevi and melanoma in intermittently and chronically sun-exposed sites have been suggested to support a role for UVA in melanomagenesis but whole genome sequencing data do not support this. It might be hypothesised that UVA-induced *BRAF* mutations are involved in naevus development and that UVB acts later in development of melanoma.

Given that sunburn and sunny holidays are the major risk factors for melanoma, sun protection, against both UVB and UVA, is important. Fair-skinned people or those with large numbers of naevi, who need greater protection against sun exposure, might need to take vitamin D supplements in order to ensure optimal serum levels. Finally, consideration should be given to how risk is explained to those who are genetically predisposed to melanoma.

4.3.3. Epidemiological evidence that UVA is involved in the genesis of melanoma

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Because most UV sources comprise both UVB and UVA, disentangling their roles in skin disease in humans is difficult. The increased use of UV tanning beds, which emit 96–99% UVA, represents an opportunity for epidemiological studies of the role of UVA in melanomagenesis and naevus formation. Individuals who first used artificial tanning machines before the age of 30 had a 75% greater risk of melanoma than those who had no such exposure (for review see Gandini et al. 2011). ‘High’ exposure to artificial light also increases risk.

Artificial tanning has been popular in the Nordic countries and the incidence of melanoma has increased steadily over recent years. Until the 1990s, the incidence of melanoma in Iceland was lower than in Denmark and Sweden but by 2000 it had exceeded that of the other countries. The increase was mainly in melanoma of the trunk in younger women, suggesting that take up of artificial tanning was responsi-

ble (reviewed in Autier et al. 2011). Subsequent decreases in incidence in Icelandic women after 2001 are thought to result from health campaigns discouraging artificial tanning.

Early thinking regarding sunscreens was that prevention of sunburn, largely UVB induced, would protect against melanoma. Paradoxically, sunscreen use during intentional sun exposure led to an increased risk of melanoma or increased naevus numbers (see Autier et al. 2011 for references). Studies to investigate this revealed that use of high skin protection factor (SPF) sunscreen increased the time spent in the sun as sunburn was delayed. The extra exposure might increase the dose of UVA received, depending on the wavelengths filtered by the sunscreen. In addition, the trunk might be expected to be particularly vulnerable as this site is not regularly exposed in daily life. Studies of the distribution of melanoma by body site support this idea, e.g. Dal et al. (2007) observed a shift in melanomas from the head to the trunk in a Swedish population over recent decades, reflecting the changes in tanning habits.

Despite the increases in melanoma incidence in young people in different populations, *mortality* in these groups has not shown a concomitant increase. The rise in incidence in melanoma was primarily due to thin melanoma less than 2 mm (see Autier et al. 2011 for references). As an example, Montella et al. (2009) reported changing trends in melanoma incidence and mortality in Northern Ireland, with an increase in thin melanoma in men and women and a downward shift in mortality in women. Seasonal variations in thin melanoma, especially in women, were also observed in a study in Northern Ireland (Chaillol et al. 2011 and references therein) and it was suggested that this resulted from a short-term promotional effect of summer UV exposure.

To explain the changing patterns of exposure and melanoma incidence, Autier et al. (2011) have hypothesised that intermittent exposure to high UVA doses results in 'thin' rapidly developing but not aggressive melanomas. 'Thick', age-related melanoma may be associated with childhood, rather than recent, sun exposures and have a long latency. Age-related decline in defence mechanisms, coupled with possibly protective chronic sun exposure, could explain the patterns. In a study of migrants to Australia, migrating at a young age was a risk factor for both melanoma incidence and mortality (Khlat et al. 1992). Analysis of trends in melanoma mortality show differences between countries: downturns in mortality starting with the generation born in 1930–1940 were seen in Australia, the Nordic countries and the USA, and in later generations in the UK and Canada. Other countries showed no downturn (Severi et al. 2000). These patterns likely reflect the implementation of skin cancer prevention programmes.

4.3.4. Cohort study on sun and solarium exposure in relation to cancer risk

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Although exposure to UV from sunlight and artificial sources increases the risk of skin cancer it has an important role in maintaining levels of vitamin D in the body. In addition, studies suggestive of a protective effect of UV exposure against different cancer types have been published. To investigate further the relationships between the positive and negative effects of UV exposure, sunburn, sunbathing and solarium use data from over 100,000 women from the Norwegian Women and Cancer and the Swedish Women's Lifestyle and Health cohorts were analysed. Initial

questionnaires covered place of residence, pigmentation, sun and solarium exposure and vitamin D intake. Linkage to national registries allowed follow-up.
A trend was found between number of sunburns per year accumulated over the age decades 10–19, 20–29 and 30–39 years and risk of melanoma (Veierød et al. 2010a). A relative risk (RR) for melanoma of 1.69 was found for women who had had two or more sunburns per year during the decades from 10 to 39 relative to those having had none or only one per year. Similar results were found for sunbathing holidays: women who had taken ≥1 week sunbathing holiday per year over the decades 10 to 39 had an RR of melanoma of 1.54 compared with women who had had no such holidays. Solarium use once or more per month over two or three decades from 10 to 39 carried an RR of 2.37 relative to non-users of solariums. The observation of an accumulating effect of intermittent sun exposure or solarium use over time on melanoma risk suggests that reducing this exposure in adult life could reduce risk (Veierød et al. 2010a).
The association between non-Hodgkin lymphoma risk and sunburn, sunbathing holidays and solarium use was also studied in the two cohorts, but the results did not support an association between UV exposure and non-Hodgkin lymphoma (Veierød et al. 2010b).
A subsequent study of approximately 50,000 women from the Swedish Women's Lifestyle and Health cohort examined the effects of UV exposure from sunlight and solariums on overall cancer incidence and some cancers individually (Yang et al. 2011). Cumulative sunburn episodes, sunbathing holidays or solarium use up to age 39, had no effect on cancer incidence overall; however, ≥1 week/year sunbathing holidays between 10 and 29 years resulted in reduced risk. For individual cancers, the only correlations found were a reduced risk for breast cancer in those who had taken ≥1 week/year sunbathing holidays between 10 and 29 years or used solariums between 10 and 39 years of age or for lung cancer in those who had experienced ≥2 sunburns per year from 10 to 39 years of age (Yang et al. 2011).
Because breast cancer is one of the most frequent cancers and because of publicity highlighting solarium use and possible reduction in risk of this cancer, it is important to disentangle the factors contributing to risk. Edvardsen et al. (2011) looked at dietary vitamin D, vitamin D-effective UV dose at place of residence, sunburn, sunbathing holidays and solarium use among approximately 41,000 women from the Norwegian Women and Cancer cohort. No reduced risk of breast cancer was found for high vitamin D-effective UV dose or high vitamin D intake in this cohort (Edvardsen et al. 2011) nor was any effect of sunburn, sunbathing holidays or solarium use detected.
The contribution of solarium use to melanoma risk makes knowledge of the UV emissions of tanning equipment important. The UVA irradiance of tanning equipment from different regions of Norway was measured by Nilsen et al. (2011). Erythema weighted UV exposure varied twofold for the same model in different facilities and up to threefold for different models in the same location, making the risk of sunburn greater, especially without guidance. Modern sunbeds have a greater proportion of UVA than older models, which is disturbing because of the possible involvement of UVA in skin carcinogenesis.

4.3.5. Somatic alterations in melanoma genome: microarray based CGH study

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Sequencing of melanomas and comparison with peripheral blood cells from the same patients revealed elevated mutation rates in the melanomas and nucleotide substitutions consistent with exposure to UV radiation (Pleasance et al. 2010; Berger et al. 2012; Hodis et al. 2012). The *BRAF*^{V600E} mutation was found in 64% of the tumours and *NRAS* in 36%, mutually exclusively except in one case, where the mutation in *BRAF* did not involve the amino acid residue at position 600.
An analysis of the genome of 60 cell lines from metastasized melanomas by high-resolution array-based comparative genomic hybridization (CGH) showed large non-random copy number changes with more loci affected by amplification than by deletion (Gast et al. 2010). Large inter-individual differences were found in copy number changes between the cell lines, e.g. loss of heterozygosity (LOH) varied from 10% to 71%. Frequent LOH was found at 6q, 9p, 10, 11q, 17p. Amplification of the *BRAF* locus was found in 55% of cell lines and *NRAS* in 11%; other amplified oncogenes included *EGFR* (52%), *MITF* (40%) and *NOTCH2* (35%). Amplification of *BRAF*^{V600E} has been found in association with acquired resistance to BRAF inhibitors (Shi et al. 2012).
The *CDKN2A* tumour suppressor locus was the most frequently affected through homozygous or hemizygous deletions and often many other genes were found to be co-deleted within that complex locus. The *PTEN* locus was also often deleted (Gast et al. 2010).
Mutational analysis revealed that *CDKN2A* alterations were more frequent in melanoma cell lines carrying *BRAF* or *NRAS* mutations; *PTEN* alterations were significantly higher in cell lines carrying *BRAF* mutations. Interestingly, gene expression studies of melanocytic naevi carrying the *BRAF*^{V600E} mutation, revealed up-regulation of the cell cycle-regulating genes *CDKN2A* and *CDKN1C* and the melanocyte differentiation factor *MITF* (Bloethner et al. 2007), a process thought to be responsible for melanocytic senescence.
Overall, a higher frequency of amplifications was found in melanoma cell lines lacking *BRAF* or *NRAS* mutations than in the cell lines with mutations. In melanoma cell lines with *BRAF* or *NRAS* mutations, amplification of *BRAF* or *NRAS*, respectively, was increased. *PTEN* and *TP53* were reduced in lines carrying *BRAF* or *NRAS* mutations, respectively. *CDKN2A* was reduced in lines with *BRAF* or *NRAS* mutations and, to a lesser extent, in lines carrying neither mutation. In lines carrying neither *BRAF* nor *NRAS* mutations, the *CCND1* and *CDK4* loci were amplified but chromosomes 13q and 16q, carrying the *RB1* and *MC1R* loci, tended to be lost.
Analysis of a focal deletion at the *CDKN2A* locus revealed that a fusion gene formed by joining of remnants of *MTAP* and *ANRIL* was both transcribed as well as translated.
In summary, genetic alterations that are important in melanoma initiation, progression and metastasis include: the *BRAF* and *NRAS* oncogenes; the tumour suppressor loci *CDKN2A, PTEN* and *TP53*; the growth factor receptor *EGFR* and melanocyte differentiation factor *MITF*; the cell cycle regulators *CCND1* and *CDK4*; and tumour suppressor *RB1* and *MC1R*. Secondary genetic alterations contribute to survival of cancer cells and could be crucial in response to potential therapeutic inhibitors (Gast et al. 2010).

4.3.6. Molecular phenotypes with clinical implications in malignant melanoma

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The development of molecular markers for use in prognosis and prediction of the efficacy of treatment in melanoma patients would be of great value. In addition, such biomarkers might advance understanding of the mechanisms of melanomagenesis. To this end, Jönsson et al. (2010) used gene expression profiling of frozen samples from patients with stage IV melanoma. Four distinct gene expression patterns were identified: high-immune response; proliferative; pigmentation; and normal-like. The high-immune response group showed high expression of genes involved in immunological processes, although these could also be found in the pigmentation and normal-like groups. The proliferative group showed low expression of these genes and higher expression of cell cycle-associated genes and, in a prospective study, showed poor clinical outcome. The pigmentation group had higher expression of genes involved in melanin synthesis and melanocyte differentiation. *BRAF* and *NRAS* mutations were commonly found but were mutually exclusive. The gene expression subtypes were validated in a set of liver metastases and in three publically available datasets, which included stage III melanomas (Jönsson et al. 2010).

Gene expression analysis of 223 clinical samples of primary melanomas demonstrated that the four subgroups were also identifiable in early melanomas (Harbst et al. 2012). The high-immune and normal-like groups were again associated with better survival. Histological and survival similarities suggested that the high-immune and normal-like groups could be classified as 'low grade' and the proliferative and pigmentation as 'high grade', resulting in a 2-class prognostic grading system. This was established using the previously analysed stage IV melanomas. Significant differences in survival were found between the low- and high-grade primary melanomas.

High- and low-grade tumours could be distinguished within different clinical subtypes of melanoma and reflected survival. The grading system was validated in independent data sets. Compared with other prognostic factors, such as tumour thickness, mitotic rate and tumour infiltrating lymphocytes, molecular grading was a significant determinant of outcome. For melanomas ≥2 mm, only molecular grade was discriminatory. Taken together, the analyses showed that molecular tumour grade is an independent prognostic factor in primary melanoma (Harbst et al. 2012).

The potential of gene expression profiling and DNA sequencing to add value to prognosis is being studied in a clinically annotated, hospital-based cohort of melanoma patients. 1800 cancer/melanoma-related genes were selected for analysis in 274 melanomas. Results from the first 100 tumours showed a median number of 33 (1–344) mutations per tumour with *BRAF* and *NRAS* among the most frequent. Gene expression profiling revealed the four subgroups previously described, although few normal-like profiles were found. *BRAF* mutations were found in 55% of the proliferative subgroup tumours, with the high-immune and pigmentation subgroups having 39% and 40%, respectively. Analysis of multiple metastases from individual patients in some cases revealed that these differed in gene expression subgroup; differences in mutations and chromosomal rearrangements were also found. Correlation of mutations with molecular profiles has the potential to generate new therapies.

4.3.7. Molecular effects of erythemally equivalent doses of UVB and UVA1 on human skin in vivo

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The UV radiation in terrestrial sunlight is primarily UVA with only 5% UVB at most. Of the UVA, 75% is UVA1 (340–400 nm). The ratio of UVA to UVB varies

with latitude, season and time of day. Hence, although UVB is approximately 1,000 times more erythemogenic than UVA, changes in the ratio mean that UVA can contribute as much as 25% of the erythemally effective dose in mid-summer at higher latitudes (Tewari et al. 2013).

There is no correlation between the MED for UVB and UVA1 (340–400 nm) in a given individual, indicating that different chromophores are involved (Tewari et al. 2013). UVB irradiation of human skin induces both CPDs and 6-4PPs whereas UVA1 induces only CPDs, suggesting different mechanisms of photolesion formation (Tewari et al. 2012a). Quantification of immunostained skin biopsies showed that UVB-induced photolesions decreased with depth from the epidermis to the dermis; however, CPDs induced by UVA1 increased with depth (Tewari et al. 2012a, 2013). The mechanism of the 'reverse' gradient seen with UVA1 is unknown; however, this observation indicates that the basal layer of the epidermis, which contains melanocytes and proliferating keratinocytes, is especially vulnerable to UVA1-induced DNA damage.

The traditional view has been that UVB primarily induces skin burning (erythema) and cancer whereas UVA is responsible for photoageing. The extracellular matrix (ECM) composed mainly of collagen of the dermis is important for its structural integrity and function. UV-induced photoageing is thought to be mediated by degradation of collagen in the dermal ECM by MMP-1 (see Section 4.1.5). The action spectra for erythema and MMP-1 induction by UVB, UVA1 and SSR were tested in human skin in vivo. The dose–response curves for MMP-1 mRNA induction, using MED as the dose unit, were comparable for UVB and UVA1, suggesting that common chromophores mediate erythema and MMP-1 induction (Tewari et al. 2012b). In contrast, UVA1 was more effective than UVB in inducing expression of the MMP-1 inhibitor TIMP-1, perhaps protecting against photoageing. These results suggest that UVB might be more important in photoageing in the context of solar UV exposure.

Gene expression profiling of human skin exposed in vivo to comparable MEDs of UVB or UVA1 revealed more changes with UVA1 than UVB at 6 h post-irradiation. At 24 h UVB induced more changes. At 6 h, up-regulation of genes involved in inflammation, antioxidant activity, immunosuppression and apoptosis was seen; at 24 h ECM remodelling predominated. At 24 h, some MMPs were induced only by UVB or UVA1, e.g. MMP-12 was induced by UVA1, whereas MMP-10 and MMP-13 were induced by UVB. Validation of these results by RT-PCR is on-going.

4.3.8. Immunosuppression, inadequate DNA repair, and mutations in *Brm* may contribute to the role of UVA in human skin cancer

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Because it contains actively dividing cells, the basal layer of the epidermis is likely to be vulnerable to DNA damage and induction of mutations (see Section 2.3.7). UVA is able to penetrate deeper into the skin than UVB and has been associated with DNA damage resulting from the generation of ROS, which oxidise DNA bases forming primarily 8-oxoGua. At 1 h after UVA irradiation, 8-oxoGua predominates in the basal layer of the epidermis. Human 8-oxoguanine-DNA glycosylase, which is the main enzyme involved in repair of 8-oxoGua, is expressed more highly in the superficial than the basal layer of human epidermis, including foreskin (Javeri et al. 2008). After UVA irradiation of human skin, 8-oxoGua levels, which peaked by 30 min in the suprabasal epidermis, were completely repaired by 2 h post irradiation;

in the basal layer the levels did not show any indication of repair even at 2 h. This difference might lead to increased mutations occurring in the deeper layers.
The finding in human skin cancer of a hotspot mutation in an exon of the *Brm* gene, which encodes a subunit of the SW1/SNF chromatin-remodelling complex (Moloney et al. 2009), together with other evidence, suggested that this gene, which regulates multiple cellular processes, might be a tumour suppressor. *Brm*$^{-/-}$ ko mice showed an increased incidence of skin cancers (SCCs) after UV irradiation; however, unexpectedly, they were protected against UV-induced immunosuppression (Halliday et al. 2012).
Immunosuppression induced by UVA shows a bell-shaped dose–response in mice and humans for different measures of immunity. The action spectrum for immunosuppression shows a peak at 310 nm and a second, broader peak from 365 to 385 nm in the UVA range; however, high doses of UVA are not immunosuppressive. Gene expression analysis revealed that the alternative complement pathway was the only one significantly up-regulated in response to suppressive UVA doses in responsive mice (Halliday et al. 2011). Complement component 3, properdin and complement factor B were all up-regulated, suggesting that this pathway might act as a sensor of UVA-induced skin damage leading to immunosuppression. During normal daily activities, the UVA in sunlight is likely to make a larger contribution to immunosuppression; with the higher erythemal doses experienced during recreational activities, UVB-induced immunosuppression is likely to dominate (Halliday et al. 2011).
UVA has also been observed to inhibit oxidative phosphorylation and mitochondrial function in keratinocytes, resulting in reduced ATP levels (Svobodová et al. 2007). This inhibition can be prevented by nicotinamide, a precursor of nicotinamide adenine dinucleotide, which is important in ATP production (Park et al. 2010). The finding that nicotinamide can protect against UV radiation-induced CPDs and oxidative damage in skin explants (Surjana et al. 2013), and that topical (Sivapirabu et al. 2009) and oral (Yiasemides et al. 2009) nicotinamide protects against UV radiation-induced immunosuppression in human volunteers shows promise for its chemopreventive use.

4.3.9. Survival in melanoma – seven year follow-up in the Genes, Environment and Melanoma (GEM) study

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Although intermittent sun exposure is a risk factor for melanoma, some evidence has suggested that increased incidence of, and survival from, melanoma are associated with increased sun exposure. A case–control study based in Connecticut was conducted to test whether this might be related to increased early detection (Berwick et al. 2005). Factors associated with high levels of sun exposure, such as solar elastosis and, less so, sunburns and intermittent sun exposure were found to be inversely associated with death from melanoma. This association could not be explained by early detection or screening behaviour (Berwick et al. 2005). The involvement of sun exposure in synthesis in the skin of vitamin D and the anti-proliferative and pro-apoptotic effects of this vitamin might explain this apparent association.
Investigation of the role of serum vitamin D levels in melanoma risk and survival are complicated by the confusing standards for adequate levels, unreliable assay methods and differences in vitamin D status found among individuals even, e.g., with abundant sun exposure (Binkley et al. 2007). Germline and somatic mutations might also be expected to interact with sun exposure to modify disease risk and survival.

The contribution of sun exposure to the risk of more than one primary melanoma was investigated in the Genes, Environment and Melanoma (GEM) study. Participants with a single (controls) or multiple (cases) primary melanomas were recruited from countries at different latitudes with wide differences in ambient UV irradiance. The risk of multiple primary melanomas increased with ambient UV exposure at birth and 10 years, recreational exposure and sunburn; occupational sun exposure did not increase risk (Kricker et al. 2007). The risk of melanoma associated with sun exposure is modified by variants of genes such as *MC1R*, which affect melanin production, *XPD751*, a DNA repair gene and *CDKN2A*, the major familial melanoma gene (Berwick 2006, 2011; Kanetsky et al. 2006; Millikan et al. 2006; Kricker et al. 2010).

The GEM study evaluated the role of sun exposure prior to diagnosis of melanoma and found that high levels of sun exposure were not associated with survival with melanoma. This contrasts with the result from the Connecticut study and possible reasons for this are being explored.

4.3.10. Seasonal variations in serum 25-hydroxyvitamin D status due to the use of sun protection

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An understanding of the relationship between seasonal UV exposure, diet and sunscreen use and serum vitamin D levels is important in providing public health advice. In a high UV environment such as NE Australia, it might be expected that serum vitamin D levels would be sufficient; however, winter serum 25-hydroxyvitamin D (25(OH)D) levels in Brisbane (27°S) were found to be deficient or insufficient in approximately 40% of adults (Kimlin et al. 2007).

Only those taking supplements met the Adequate Intake for vitamin D with men having a greater intake from diet but less overall because of lower supplement usage. For those with high outdoor exposure (≥12 h per week), dietary intake of vitamin D has a negligible effect on serum 25(OH)D levels; however, with lower winter outdoor exposure (≤5 h per week), intake becomes important (Borradale and Kimlin 2010).

A study of healthy adults in Queensland, Australia (Kimlin 2010) showed that median serum 25(OH)D levels were higher in summer than winter with women having lower levels than men at both seasons. Contrary to the expectation that individuals maintain their relative ranking with season, it was found that 25% of the participants had lower levels in summer than in winter. Serum 25(OH)D levels showed a (non-significant) trend with time outdoors in the sun. Levels were positively correlated with sunscreen use and with the tanning response. It is unknown why serum 25(OH)D levels are lower in summer than winter for some individuals; possible reasons include changes in behaviour, little change in tanning ability, overexposure to UV causing 25(OH)D decay. It is clear that further knowledge is required about what determines vitamin D status and the dose of UV that is sufficient to generate enough vitamin D without unacceptable increases in skin cancer risk.

4.3.11. Serum 25-hydroxyvitamin D levels after whole body UV radiation with different wavelengths

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Exposure to sunlight is an important source of vitamin D but the amount that is adequate is uncertain. Groups of healthy adults recruited in Stockholm were exposed to suberythemal doses of UVB, UVA or UVA + UVB using medical UV treatment boxes for times designed to provide the same vitamin D effective dose. Serum 25(OH)D levels were increased by exposure to UVB or UVA + UVB and, significantly less so, by UVA. At the short exposure times used for UVA + UVB, no detrimental impact of the UVA was found on 25(OH)D levels although the short exposure time may have been insufficient to allow photodegradation. Baseline 25(OH)D levels were 27% lower in skin type IV to VI participants than in the group with skin types I–III but no correlation was found for skin type and the change in levels after UV irradiation.

4.3.12. Future reduction of cutaneous malignant melanoma due to improved sun tanning habits among Swedish children?

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Increased rates of cutaneous malignant melanoma have accompanied changes in lifestyles such as increased sunny holidays and changes in suntanning and clothing habits, especially in fair-skinned populations. Increased lifetime risk of melanoma is also associated with a high density of common melanocytic naevi. Cross-sectional studies of 7-year-old children in southern Sweden (57°N) have investigated the effects of changing trends in parental sun-protective regimes on the prevalence of common melanocytic naevi from 2002 to 2007 (Rodvall et al. 2007; Karlsson et al. 2011) and the results were confirmed in 2009. A significant increase in holidays at seaside resorts abroad was found. There were significant increases in factors such as never being naked in the sun, often staying in the shade or indoors and sunscreen use. In parallel, a significant decrease in the mean number of common melanocytic naevi was found in 7-year-old school children. Thus there is a trend for increased parental awareness of the risks of sun exposure and adoption of measures to protect children. In addition, the results support the use of density of common melanocytic naevi as an indicator of sun exposure in children (Karlsson et al. 2011).

4.3.13. Adolescents' sunbed use in Sweden (Poster)

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An SSM survey in 2010 of 500 Swedish 16 to 17 year olds revealed that 40% had used a sunbed. A higher percentage of girls had done so and they tended to start earlier than boys. In the previous 12 months, 30% had used sunbeds more than six times, 40% two to five times and 30% once at most. Awareness of the risks of sunbed use was high. This usage was similar to that of the youngest adult group previously surveyed and it would be expected that skin burns would be equally common. Prohibition of the use, sale or hire of sunbeds to those under 18 years of age is recommended.

5. Discussion

This section summarise issues that arose from the presentations and the discussions immediately after them. It also raises points considered in a discussion led by Dan Segerbäck, Rune Toftgård and Johan Hansson on the final day of the meeting. Recent experiments have shed light on the mechanism of induction of mutations in the skin by sunlight. The results are consistent with a model in which extended stalling of the transcription complex at a photolesion in human and mouse cells increases the likelihood of deamination of cyclobutane pyrimidine dimers (CPDs). Translesion synthesis then induces transition mutations and intragenic deletions; the latter could be generated by the collapse of replication forks at a stalled transcription complex.

The role of ultraviolet (UV) radiation of different wavelengths in the induction of melanoma has long been a subject of controversy. Results in mouse models have led to the assumption that only UVB is involved, in contrast to an early action spectrum in *Xiphophorus* that implicated both UVB and UVA. Recent results have turned this debate on its head: a large-scale study in *Xiphophorus* found that only UVB induced melanoma in this model; however, the observation that neonatal UVA can induce melanoma in black but not albino littermates in the HGF mouse model throws open the whole question. (De Fabo, 4.2.1, Mitchell, 4.2.3) In humans, a role for UVA is indicated by the increase in melanoma incidence that has accompanied the increase in use of sunbeds (emitting largely UVA) and decreasing incidence after public health campaigns (Autier, 4.3.3). Given the importance of early sun exposure to melanoma risk, little is known about skin in childhood. However, children sunbathing on Swedish beaches did not show a greater level of urinary excretion of thymine dimers (T=T) than adults (Segerbäck, 4.3.1).

Fair skin in humans predisposes to melanoma susceptibility (Newton Bishop, 4.3.2), suggesting that melanin in individuals with greater levels of skin pigmentation might offer protection by, for example, absorbing UV radiation, scavenging free radicals or as an antioxidant. However, melanin production occurs via a series of very reactive quinones and melanin can be pro-oxidant in the presence of metals (De Fabo, 4.2.1). The results with albino and black mice (De Fabo, 4.2.1) hint at a role of melanin in facilitating melanoma induction by UVA. Further studies indicated an oxidative process in the induction by UVA in black mice. The induction of CPDs in skin biopsies from phototype II and IV human volunteers by both UVB and UVA (Douki, 4.1.1) was found to be correlated with minimal erythemal dose (MED), implicating physical dose of light reaching DNA, and not photosensitiser content, as the main risk factor. The discovery of a novel phototransduction pathway in melanocytes and its possible involvement in UVA-induced immediate pigment darkening (Oancea, 4.1.7) is very interesting. Clearly, the role of melanin and phototype in protection against melanoma and in photocarcinogenesis needs further investigation.

Increased numbers of melanocytic naevi, benign proliferations of melanocytes, are also a potent risk factor for melanoma. The number of naevi has been found to be correlated with average holiday exposure (Newton Bishop, 4.3.2). Paradoxically, the risk of melanoma associated with sunburn was greatest in those with fewest naevi, supportive perhaps of the theory of different routes for melanoma associated with intermittent sun exposure and naevi versus chronic sun exposure in individuals with normal naevus numbers. Little work has been done on ethnicity, naevi and the risk

of melanoma. In a Chinese study, approximately 60% of melanocytic naevi carried *BRAF* mutation T1799A but this was found in only 15% of melanoma cases, suggesting that the mutation is not necessarily associated with development of disease (Qi et al. 2011). The roles of phototype, number of naevi and ethnicity in melanoma susceptibility merit further study and might add to understanding of the mechanisms of UV photocarcinogenesis.

Because UVA penetrates more deeply into the skin than UVB, UVA effects would be expected to predominate at lower levels, not only in the dermis, but also in the lower levels of the epidermis, as compared with the upper levels of the epidermis. The basal layer of the epidermis might be more vulnerable to damage and mutation because of the presence of actively dividing cells and pigment-producing melanocytes. In support of this idea, UVA-induced 8-oxo-7,8-dihydroguanine (8-oxoGua) levels were found to predominate in the basal layer of the human epidermis and remain unrepaired after levels in the suprabasal epidermis had returned to control unirradiated levels. In addition, human 8-oxoguanine-DNA glycosylase, involved in the repair of 8-oxoGua lesions was expressed more highly in superficial layers (Halliday, 4.3.8). Regarding CPDs, UVB induces 22-fold more CPDs in isolated keratinocytes than in skin, whereas UVA induces only 1.5-fold, perhaps reflecting its greater ability to penetrate skin (Douki, 4.1.1).

In humans and mice, a bell-shaped UVA dose–response curve has been found for different measures of immunity (Halliday, 4.3.8). In a hairless mouse model, solar-simulated radiation (SSR)-induced tumour formation (and reduction in oxazolone-induced contact hypersensitivity (CHS) at 40 weeks) can be abrogated by UVA treatment at a dose that is not immunosuppressive (Reeve, 4.2.7). However, in an epidemiological study, no reduced risk of cancer associated with solarium use (predominantly UVA) was detected (Veierød, 4.3.4).

Some examples of gender-related effects have been noted. In the fish model *Xiphophorus*, males are more susceptible to UVB-induced but not spontaneous melanoma. UVB was found to induce both hormonal and hormone receptor changes (Mitchell, 4.2.3). In the hairless mouse model, 17-β-oestradiol protected against SSR-induced immunosuppression. Male mice were more sensitive to UV-induced suppression of the CHS response to oxazolone and the protective effect of UVA was less (Reeve, 4.2.7). The implications of these observations for humans are unclear because of differing sun exposure habits and solarium use by men and women (see Wester, 4.3.13).

Although factors identified several years ago to identify high-risk patients are still valid, the search for new parameters continues. Genome-wide association studies (GWAS) have identified many single nucleotide polymorphisms that affect susceptibility to melanoma and non-melanoma skin cancer in addition to the well-known genes such as the melanocortin 1 receptor gene (*MC1R*), which influences skin pigmentation, *TYR*, involved in the conversion of tyrosine to melanin, and *ASIP*, involved in regulation of melanogenesis (Newton Bishop, 4.3.2).

Analysis of somatic mutations in melanomas has revealed that most base substitutions are C>T transitions, consistent with the UV (UVB and/or UVA) induction of CPDs (Kumar, 4.3.5). *BRAF* or *NRAS* mutations are found in all tumours, almost mutually exclusively; however, the common mutations in these genes are not classic UV mutations (Pfeifer and Besaratinia 2012) and what causes them is unknown. Genetic alterations found to be important to melanoma development include *BRAF*,

NRAS, *CDKN2A*, *PTEN* and others (see Kumar, 4.3.5). Distinguishing among initiating mutations, mutations that enable progression or metastasis and mutations that are merely passengers is difficult but identifying secondary genetic alterations that contribute to cancer cell survival might be important in the search for therapeutic inhibitors.

Non-melanoma skin cancers, which originate from keratinocytes, represent the majority of skin cancers. A high percentage of these have mutations in the *p53* tumour suppressor gene (Pfeifer and Besaratinia 2012 and references therein). The evidence is consistent with mutations resulting from sunlight-induced CPDs.

Gene expression profiling of melanomas has the potential to identify molecular markers for clinical use and also the pathways involved in melanomagenesis and metastasis (Jönsson, 4.3.6). Analysis of stage IV melanomas and primary melanomas enabled identification of two categories of gene expression changes that represented an independent prognostic factor in primary melanoma. Immune, proliferative and pigmentation genes were represented in the classification. High immune gene expression is associated with better survival. A comprehensive gene expression profiling and DNA sequencing study of a clinically annotated hospital-based cohort of melanoma patients seeks to correlate mutations with molecular profiles and enable the development of new therapies (Jönsson, 4.3.6).

Many observations have suggested that vitamin D is protective against cancer. Mice lacking the vitamin D receptor are more susceptible to chemically and UV-induced skin carcinogenesis (Bikle, 4.2.4). In humans lower serum vitamin D levels are found in cancer cases than controls and variant vitamin D binding genes convey a higher risk of melanoma (Newton Bishop, 4.3.2). Because UV irradiation is necessary for production of vitamin D but also induces skin cancer, a balance between sufficient sun exposure to maintain serum vitamin D levels but minimise risk of skin cancer is clearly needed. Epidemiological studies suggest that to attain the optimal level of serum vitamin D (60 nmol/l) at higher latitudes requires 12 h of sun at weekends, not the 15 min previously considered adequate (Newton Bishop, 4.3.2). Even in a high UV environment, winter serum vitamin D levels levels can be less than optimal in many adults and supplement intake may be necessary to attain adequate levels (Kimlin, 4.3.10). In addition, in a study of the effects of exposure of volunteers to UV, baseline serum vitamin D levels were found to be lower in individuals with skin types IV to VI than in I–III, although changes in level after UV irradiation were not correlated with skin type (Sallander, 4.3.11). Clearly, much remains to be understood about the determinants of serum vitamin D level and how to avoid unacceptable increases in skin cancer risk.

Exposure to sunlight results in detrimental changes to the skin which resemble those of ageing, including the accumulation of fibrillar material in the dermis. The finding that the intracellular elastase cathepsin K is inducible by UVA in dermal fibroblasts from younger but not older donors suggests that regulation of elastin levels may be less effective in the latter. The UVA induction of progerin, a truncated pre-lamin A that causes certain premature ageing syndromes, in dermal fibroblasts may result in ageing because of interference with membrane function (Rünger, 4.1.6). Breakdown of collagen via the induction of matrix metalloproteinases (MMPs) may also play a part in photoageing. Both UVB and UVA1 induce MMP-1 in human skin in vivo but UVA1, however, more effectively induced the MMP-1 inhibitor TIMP-1, indicating that UVB might be more important in photoageing (Young, 4.3.7). UVB induction of MMPs is inhibited by 3'-methoxy-4'-nitroflavone, an arylhydrocarbon

receptor (AhR) antagonist, implicating this receptor in UV-induced photoageing. A novel AhR antagonist, BIO-1031® has been developed that is effective in reducing UVB-induced MMP-1 transcription in human skin (Krutmann, 4.1.5).

Therapeutic advances are being made against UV-induced damage and disease. Nicotinamide, important in ATP production, protects against UV-induced CPDs and oxidative damage in skin explants and in topical and oral form protects against UV-induced immunosuppression in human volunteers, which is promising for chemo-preventive use (Halliday, 4.3.8). However, care must be taken to rule out unintended effects when implementing new therapeutic approaches. Testing of the effects of constitutive Nrf2 expression in a mouse model, to determine whether this would afford protection against oxidative damage, revealed hyperkeratosis, defects in skin barrier function and inflammation (Werner, 4.1.4). Although a comprehensive model of the pathway to melanoma cannot yet be constructed, and many signalling pathways seem to be involved, experience in the clinic with inhibitors targeting a single molecule has shown short-term inhibitory effects. For long-term effects, combinations seem to be more effective, e.g. *BRAF* and *MEC* and recently *PAK* inhibitors have also been added (Ong et al. 2013).

There is encouraging evidence that regulatory and public health initiatives can be effective in altering behaviour and reducing risk of adverse effects of UV and sunlight exposure. Downturns in melanoma mortality in different countries parallel the implementation of skin cancer preventive programmes (Autier, 4.2.3) and the finding that changing parental behaviours have resulted in a decrease in common melanocytic naevi in 7-year-old Swedish schoolchildren is very promising (Rodvall, 4.3.12).

To summarise, certain topics might merit further investigation:

- Differences between the skin of adults and children
- The role of melanin, naevi, phototype and ethnicity in the UV response
- Identification of further susceptibility genes (by GWAS)
- The role of somatic mutations and altered gene expression in melanoma prognosis and treatment
- The role of stem cells, environment and cellular interactions in melanoma initiation and progression
- Modulation of the immune response by UV irradiation
- Photoageing mechanisms and their relationship to photocarcinogenesis
- The effects of inhibiting multiple signalling pathways in melanoma
- Determinants of vitamin D levels
- Effective public health information and behavioural change; targeting susceptible groups

6. Recommendations

After discussion of the results presented at the meeting, participants were asked what recommendations they would make to protect the public against ultraviolet radiation (UV)-induced skin disease. The following conclusions were reached:

- Broad-spectrum sunscreens to protect against both UVA and UVB
- Standardisation of sunscreens; quality control for effectiveness against UVA and UVB
- Raise awareness of high levels of UVA; discourage use of tanning parlours and sunbeds
- Vitamin D supplementation rather than tanning for those with fair skin or numerous naevi
- Individuals with skin types III and IV should also exercise caution in the sun
- Consider protective effect of clothing against sun-induced skin damage

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8. Acronyms

$1,25(OH)_2D_3$	1,25-dihydroxyvitamin D_3
25(OH)D	25-hydroxyvitamin D
6-4PP	(6-4) photoproduct
8-oxodGuo	8-oxo-7,8-dihydro-2'-deoxyguanosine
8-oxoGua	8-oxo-7,8-dihydroguanine
AhR	arylhydrocarbon receptor
BCC	basal cell carcinoma
catK	cathepsin K
CGH	comparative genomic hybridization
CHS	contact hypersensitivity
CPD	cyclobutane pyrimidine dimer
DDB1	DNA damage binding protein 1
DSC	dermal stem cell
ECM	extracellular matrix
EGFR	epidermal growth factor receptor
ER	oestrogen receptor
FICZ	6-formyl[3,2-*b*]carbazole
GGR	global genomic repair
HGF	hepatocyte growth factor
HO-1	heme oxygenase 1
hOGG1	human 8-oxoguanine-DNA glycosylase
IF	interfollicular
IFN-γ	interferon γ
ko	knock out
LOH	loss of heterozygosity
MARE	Maf recognition element
MC1R	melanocortin 1 receptor
MED	minimal erythemal dose
miRNA	microRNA
MMP	matrix metalloproteinase
MNF	3'-methoxy-4'-nitroflavone
NER	nucleotide excision repair
NMSC	non-melanoma skin cancer
PARP1	polyADP-ribose polymerase 1
qRT-PCR	quantitative real-time polymerase chain reaction
ROS	reactive oxygen species
RR	relative risk
RT-PCR	reverse transcription polymerase chain reaction
SCC	squamous cell carcinoma
siRNA	short interfering RNA
SNP	single nucleotide polymorphism
SPF	skin protection factor
SSR	solar simulated radiation
T=T	thymine dimer
TCCD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TCR	transcription coupled repair
TNCB	2,4,6-trinitrochlorobenzene
UV	ultraviolet radiation
VDR	vitamin D receptor

9. Annex I. Programme

UV radiation-induced disease – roles of UVA and UVB
Thursday–Saturday 24–26 May 2012
Nobel Forum, Wallenbergsalen | Karolinska Institutet, Solna

THURSDAY MAY 24 OPENING DAY

09:00–11:15 Registration & Coffee

11:15–11:30 Welcome Address - Johan Hansson (Chairman of the Organizing Committee)

SESSION 1: CELLULAR EFFECTS OF UV-RADIATION

Chairpersons: Dan Segerbäck & Thierry Douki

11:30–12:00 *Recent advances in the formation of UV-induced DNA damage*. Thierry Douki (France)

12:00–12:30 "*Here comes the sun": from UV-photolesion recognition and processing to biological effects.* Leon Mullenders (The Netherlands)

12:30–13:30 Lunch

13:30–14:00 *Cellular effects of UVA and UVB: New insights into the mechanisms of photoaging.* Thomas Rünger (USA)

14:00–14:30 *UVA mediated regulation of the heme oxygenase 1 gene - potential therapeutic targets.* Rex Tyrrell (UK)

14:30–15:00 Coffee break

15:00–15:30 *Cytoprotective signalling pathways in UV response and skin cancer*. Sabine Werner (Switzerland)

15:30–16:00 *Role of the Ahr in the UVB response*. Jean Krutmann (Germany)

16:00–16:30 *UVA signal transduction in human skin*. Elena Oancea (USA)

FRIDAY MAY 25

SESSION 2: EXPERIMENTAL MODELS OF UV-INDUCED DISEASE

Chairpersons: Rune Toftgård & Meenhard Herlyn

08:30–09:00 *Two UV pathways to melanoma*. Edward De Fabo (USA)

09:00–09:30 *The role of UVA and UVB in melanoma: studies using the Xiphophorus hybrid fish model.* David Mitchell (USA)

09:30–10:00 *The vitamin D receptor as a tumor suppressor of UVB induced skin cancer*. Daniel Bikle (USA)

10:00–10:30 Coffee break

10:30–11:00 *Which cell in human skin transforms to melanoma?* Meenhard Herlyn (USA)

11:00–11:30 *Ambiguity in the relationship between UV-induced p53 mutant clones and skin carcinomas.* Frank de Gruijl (The Netherlands)

SESSION 3: HUMAN STUDIES OF UV-INDUCED DISEASE

Chairpersons: Veronica Höiom & Göran Jönsson

11:30–12:00 *Analysis of thymine dimer in urine following exposure to sunlight*. Dan Segerbäck (Sweden)

12:00–13:00 Lunch

13:00–13:30 *The epidemiology of melanoma and what it suggests about melanoma risk, UVB, UVA and vitamin D*. Julia Newton Bishop (UK)

13:30–14:00 *Epidemiological evidence that UVA is involved in the genesis of melanoma.* Philippe Autier (France)

14:00–14:30 *Cohort study on sun and solarium exposure in relation to cancer risk.* Marit B. Veierød (Norway)

14:30–15:00 Coffee break

15:00–16:30 SHORT TALKS
Vivienne Reeve (Australia), Frances Noonan (USA), Michael Kimlin (Australia), Ellinor Sallander (Sweden), Ylva Rodvall (Sweden), Ting Xiao (China)
19:00 Conference Dinner
SATURDAY MAY 26
SESSION 4: HUMAN STUDIES OF UV-INDUCED DISEASE
Chairpersons: Johan Hansson & Marianne Berwick
09:00-09:30 *Somatic alterations in melanoma genome: microarray based CGH study*. Rajiv Kumar (Germany)
09:30-10:00 *Molecular phenotypes with clinical implications in malignant melanoma*. Göran Jönsson (Sweden)
10:00-10:30 *Molecular effects of erythemally equivalent doses of UVB and UVA1 on human skin in vivo.* Antony Young (UK)
10:30-11:00 Coffee break
11:00-11:30 *Immunosuppression, inadequate DNA repair, and mutations in Brm may contribute to role of UVA in human skin cancer*. Gary Halliday (Australia)
11:00-11:30 *Survival in melanoma – seven-year follow-up in GEM*. Marianne Berwick (USA)
11:30-12:15 GENERAL DISCUSSION AND RECOMMENDATIONS
12:15-12:30 CLOSING OF THE MEETING

10. Annex II. Participants

Name	Institution	Country
Aleksandra Lesiak	Medical University of Łódź	Poland
Alireza Azimi	Karolinska Institutet	Sweden
Antony Young	King's College London	UK
Bernt Lindelöf	Karolinska University Hospital	Sweden
Bobby Li	Karolinska Institutet	Sweden
Carolina Johansson	Karolinska Institutet	Sweden
Dan Segerbäck	Karolinska Institutet	Sweden
Daniel Bikle	VA Medical Center and University of California	USA
David Mitchell	The University of Texas MD Anderson Cancer Center	USA
Desiree Wiegleb Edström	Karolinska Institutet	Sweden
Diana Lindén	Karolinska Institutet	Sweden
Diane Forsberg	Karolinska Institutet	Sweden
Edward De Fabo	The George Washington University Medical Center	USA
Elena Oancea	Brown University	USA
Ellinor Sallander	Karolinska Institutet	Sweden
Emil Bengtsson	Swedish Radiation Safety Authority (SSM)	Sweden
Esther Azizi	Tel-Aviv University	Israel
Frances Noonan	The George Washington University Medical Center	USA
Frank de Gruijl	Leiden University Medical Center	The Neth-erlands
Gary Halliday	University of Sydney	Australia
Göran Jönsson	Lund University	Sweden
Hanna Eriksson	Karolinska Institutet	Sweden
Helene Asp	Swedish Radiation Safety Authority (SSM)	Sweden
Jean Emeny	University of Leicester	UK
Jean Krutmann	Leibniz Research Institute for Environmental Medicine	Germany
Joanna Narbutt	Medical University of Łódź	Poland
Johan Gulliksson	Swedish Radiation Safety Authority (SSM)	Sweden
Johan Hansson	Karolinska Institutet	Sweden
Julia Newton Bishop	Leeds Institute of Molecular Medicine	UK
Leon Mullenders	Leiden University Medical Center	The Neth-erlands
Maria Wolodarski	Karolinska University Hospital	Sweden
Marianne Berwick	University of New Mexico	USA
Marit B. Veierød	University of Oslo	Norway
Martin Meier	Swiss Federal Office of Public Health	Switzer-

Name	Institution	Country
		land
Meenhard Herlyn	The Wistar Institute	USA
Michael Kimlin	Queensland University of Technology	Australia
Michal Rogowski-Tylman	Medical University of Łódź	Poland
Philippe Autier	International Prevention Research Institute	France
Rainer Tuominen	Karolinska Institutet	Sweden
Rajiv Kumar	German Cancer Research Center	Germany
Rex Tyrrell	University of Bath	UK
Robert Biggar	Queensland University of Technology	Australia
Rune Toftgård	Karolinska Institutet	Sweden
Sabine Werner	ETH Zürich	Switzer-land
Sattar Zebary	Karolinska Institutet	Sweden
Suzanne Egyhazi	Karolinska Institutet	Sweden
Thierry Douki	Université Joseph Fourier	France
Thomas Rünger	Boston University School of Medicine	USA
Tove Sandberg	Karolinska Institutet	Sweden
Ulf Wester	Swedish Radiation Safety Authority (SSM)	Sweden
Veronica Höiom	Karolinska Institutet	Sweden
Vivianne Reeve	University of Sydney	Australia
Yeshiwas Ayalneh Belew	Karolinska Institutet	Sweden
Ylva Rodvall	Karolinska Institutet	Sweden

11. Annex III. Abstracts[2]

11.1. Recent advances in the formation of UV-induced DNA damage

Thierry Douki
Laboratoire « Lésions des Acides Nucléiques », Université Joseph Fourier – Grenoble 1 / CEA / Institut Nanoscience et Cryogénie / SCIB, UMR-E3, Grenoble France
Solar UV radiation is the main etiologic agent involved in the induction of skin cancer. This deleterious effect arises from its ability to induce DNA damage that, in spite of efficient repair mechanisms, may result in mutations in key genes and trigger tumorogenesis. The chemical structure of the produced DNA damage greatly depends on the wavelength range considered. UVB (280-320 nm) is efficiently absorbed by DNA and leads to dimerization reactions between adjacent pyrimidine bases (thymine and cytosine). Two main types of photoproducts are produced, namely the cyclobutane pyrimidine dimers (CPD) and the (6-4) photoproducts (64PP). These photoproducts are well known but recent spectroscopic and theoretical studies have provided interesting insights into their formation mechanisms. In addition, evidence is accumulating for a major role of UVA (320-400 nm) rather than UVB as long believed in the conversion of 64PP in their Dewar valence isomers. It may be added that, although all are handled by the nucleotide excision repair pathway, pyrimidine dimeric photoproducts are not removed with the same efficiency.
Not only UVB but also UVA exhibit genotoxic properties. Formation of oxidative damage such as 8-oxo-7,8-dihydroguanine (8-oxoGua) and DNA strand breaks is well documented in cells exposed to UVA radiation. These lesions are mostly produced through the photosensitized production of singlet oxygen and to a lesser extent by the metal-catalyzed formation of hydroxyl radicals. Mechanisms involving type I photosensitization has also been recently proposed to account for the formation of DNA-protein crosslinks. In addition to these oxidative damage, UVA irradiation leads to the formation of CPDs, but not 64PP, both in cells and skin. A number of recent studies have shown that the frequency of CPDs was higher than that of 8-oxoGua. Interestingly, the ratio between the yields of these lesions is lower in melanocytes which seem more sensitive to oxidative stress than other cell types. Rather than a photosensitized mechanism, UVA-induced formation of CPDs is most likely triggered by the weak but significant absorption of DNA in this wavelengths range, favored in the double helix compared to the free monomeric bases.
In summary, although discovered more than half a century ago, UV-induced DNA damage is still the focus of intense research. Development of new analytical techniques makes possible a better description of the distribution of photoproducts in cells and of the formation mechanisms.

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

11.2. “Here comes the sun”: from UV-photolesion recognition and processing to biological effects.

Leon Mullenders
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Repair of the two major UV-induced dimeric photolesions (CPD, 6-4PP) in human is solely dependent on nucleotide excision repair (NER). Two mechanistically distinct NER subpathways have been identified: Global genome NER (GG-NER) and transcription-coupled repair (TC-NER). In the currently prevailing model, NER factors are sequentially assembled into pre- and post-incision complexes; however, the regulation of NER in vivo is poorly understood (Overmeer et al. 2011). Non-cycling nucleotide excision repair (GG-NER) proficient cells initiate a rapid but transient activation of the damage response. Surprisingly, repair deficient cells display persistent signaling and inhibition of cell cycle progression upon release from G0 phase due to checkpoint activation and formation of DNA breaks. These data reveal the existence of a novel NER independent, but APE1 dependent mechanism of UV-photolesion processing in human cells preventing entrance of unrepaired cells into S-phase (Vrouwe et al. 2011).

TC-NER removes UV-photolesions that arrest RNA polymerase and that elicit a strong signal for cell cycle arrest and apoptosis. Deficiency in TCR is associated with the rare human disorder Cockayne syndrome (CS) clinically associated with dwarfism, mental retardation, and photosensitivity. In mouse models TC-NER is a critical survival pathway that protects against acute toxic and long-term effects (cancer) due to UVB exposure. Moreover, TC-NER counteracts UV-induced mutagenesis associated with the transcription process itself (Hendriks et al. 2010). Hence, the lack of cancer susceptibility of CS patients is remarkable and emphasizes the role of GG-NER in preventing UV-induced carcinogenesis in the absence of TC-NER.

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11.3. Cellular effects of UVA and UVB: new insights into the mechanisms of photoaging

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Photoaging of the skin results from cumulative detrimental effects of prolonged and repetitive exposures to ultraviolet light. This extrinsic aging is superimposed on the age-associated degenerative changes of the skin (intrinsic aging). The relative contribution of different UV-wavelengths (e.g. UVA vs. UVB) to photoaging remains a matter of debate.

In contrast to only intrinsically aged skin, photoaged skin typically exhibits large deposits of fibrillary basophilic material in the upper and mid dermis, composed mainly of elastin and fibrillin (solar elastosis). UV-induced inflammation is well known to induce elastin synthesis and to recruit elastase-expressing cells into the skin, but the exact mechanism of how solar elastosis forms is still unknown. We recently described that skin fibroblasts internalize extracellular matrix proteins, including elastin into lysosomes for degradation by the protease cathepsin K. The finding that UVA (but not UVB) induces cathepsin K in young fibroblasts, but fails to do so in fibroblasts from older donors, suggests that solar elastosis is the consequence of failed clearing of elastin fragments from the extracellular matrix. This scenario is very reminiscent of the general view that considers cellular aging the consequence of accumulated abnormal, misfolded, toxic, or damaged proteins in the intracellular space due to failing lysosomal clearing by chaperone-mediated autophagy.

Human genetic diseases with premature aging (progerias) are intriguing models to study aging. Hutchinson Gilford progeria syndrome (HGPS) is characterized by premature aging of many organs, including the skin. It is caused by a mutation of the LMNA gene that activates a cryptic splice site and results in the expression of a truncated form of Lamin A, called progerin. Accumulation of progerin in the nuclei of HGPS cells impairs nuclear functions and causes abnormal nuclear morphology with blebbing and invaginations of the nuclear membrane. Accumulation of progerin has been described not only in HGPS, but also during normal intrinsic aging. We recently found that UVA (but not UVB) induces expression and accumulation of progerin in normal skin fibroblasts and that this is accompanied by nuclei with HGPS-like abnormal morphology. This effect of UVA is more prominent in cells from aged donors, as compared to younger donors. These data indicate that an HGPS-like cellular phenotype not only occurs during intrinsic aging, but is also induced by UV, in particular by UVA. This suggests that at least some aspects of photoaging should be regarded as a process of damage-accelerated intrinsic aging.

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11.4. UVA mediated regulation of the heme oxygenase 1 gene–potential therapeutic targets

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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. Biologically relevant doses of UVA radiation induce the heme catabolic enzyme, heme oxygenase 1 (HO-1, Keyse and Tyrrell 1989). This is now known to be a general response to oxidative stress in mammalian cells. In the case of UVA, the response is mediated via singlet oxygen (Basu-Modak and Tyrrell 1993).HO-1 was soon shown to be a key protective enzyme in cells (Vile et al. 1994) and is now known to be a major anti-inflammatory mechanism in mammals and implicated in many disease states (Otterburn and Zuckerbraun 2005) Understanding the underlying mechanisms will be key to developing new therapies including those potentially reversing endothelial dysfunction.

Using UVA as a model oxidant in skin cells provides a powerful experimental system to understand the regulatory pathways underlying activation of HO-1.The negative regulation of this anti-inflammatory oxidant stress protein will be crucial to maintaining cellular homeostasis under stress-free conditions. 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. Changes in heme status and Nrf2 activity are clearly involved in up-regulation of HO-1 by UVA and both heme and Bach1 are involved in maintaining low expression. Under acute UVA stress, Bach1 binds to released heme, loses its DNA binding and is exported from the nucleus allowing transcriptional up-regulation of HO-1. Human keratinocytes are constitutively refractory to induction of the HO-1 response by UVA (and are UVA radiation resistant) and we hypothesised that this is due to the high basal levels of heme oxygenase 2 (HO-2) which will maintain heme levels low and lead to strong binding of Bach1 to the HO-1 promoter. Using SiRNA knockdown technology we have now clearly demonstrated the crucial role of HO-2 in the lack of UVA upregulated HO-1 expression in keratinocytes and shown that HO-1 promoter binding by Bach1 is the dominant factor in the strong suppression of HO-1 up-regulation in these skin cells (Zhong et al. 2010). More recently we have further defined the role of Bach1 in negative regulation of HO-1 expression following UVA radiation (Raval et al. 2012). Such studies contribute to the understanding of the crucial role played by this stress pathway in maintaining cellular homeostasis, protecting organs and preventing disease. They also define a key protein that can be targeted to modulate pathophysiological responses.

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11.5. Cytoprotective signalling pathways in UV response and skin cancer

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The Nrf2 transcription factor is a key regulator of the cellular antioxidant response, since it induces the expression of various genes encoding enzymes that detoxify reactive oxygen species (ROS) as well as other antioxidant proteins. To study the role of Nrf2 in the UV response and in skin carcinogenesis we analyzed mice with a gain- and a loss-of function of Nrf2 in keratinocytes. We found that endogenous Nrf2 establishes a gradient of cytoprotection within the epidermis that allows selective UVB-induced apoptosis of basal cells while preserving the integrity of the suprabasal layers. Loss of Nrf2 in the epidermis affected the UVB response of the skin. In particular, it strongly increased the susceptibility to skin carcinogenesis in two different models. On the other hand, further activation of Nrf2 in keratinocytes using pharmaceutical and genetic approaches protected keratinocytes from UVB-induced apoptosis. However, long-term Nrf2 activation disrupted the epidermal barrier and resulted in the development of an ichthyosis-like phenotype in mice. These data demonstrate that Nrf2 links epidermal barrier function with ROS protection. Furthermore, they show that the extent of Nrf2 activation in the skin needs to be tightly controlled and that overactivation of this transcription factor can be detrimental.

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11.6. Role of the AHR in the UVB response

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Within the past 2 decades strong efforts have been made to elucidate the molecular basis of the UVB stress response in mammalian cells in general and human skin cells in particular. As DNA is the major chromophore for UVB, it was thought that the UVB response is initiated in the cell's nucleus as a consequence of UVB-induced DNA damage. This concept is supported by numerous studies which show enhanced UVB responses in cells deficient in nucleotide excision repair and diminished UVB responses if irradiated cells were treated with exogenously added DNA repair enzymes. It was however challenged by the observation that UVB stress responses can occur in enucleated cells. Subsequent work established that part of the UVB response is indeed independent of DNA damage and instead involves changes at the level of the cell membrane, such as clustering and internalization of cell membrane-bound growth factor receptors as initiating events. For many years, however, the nature of the responsible chromophore and its localization within the cells remained enigmatic. In 2007 we answered this question by showing that the arylhydrocarbon receptor (AhR) is an integral part of the UVB stress response in human skin cells, and that its activation in human epidermal keratinocytes caused the DNA-damage independent part of the UVB stress response. The AhR is a member of the basic helix-loop-helix protein family and functionally serves as a transcription factor which in its inactivated state is part of an intracytoplasmic complex containing the AhR, a c-src kinase and heat shock protein 90. We showed that the AhR is activated in human epidermal keratinocytes upon exposure to UVB, but not UVA. The chromophore for UVB-induced AhR activation is the amino free acid tryptophan, which is present in the cytoplasm and upon UVB irradiation forms a number of photoproducts including formyl-indolo-3,2-carbazole (FICZ) serving as physiological AhR ligands. We showed that exposure of human keratinocytes to physiologically relevant doses of UVB leads to the intracellular formation of FICZ, the subsequent activation of the AhR signaling pathway and - via two different (genomic and non-genomic) signaling pathways – to increased expression of genes. We also showed that the non-genomic pathway elicited upon UVB-induced AhR activation mediates the DNA damage-independent activation of cell membrane associated growth factor receptors and subsequent downstream signaling events of the UVB response. UVB-induced AhR activation is of obvious clinical relevance because it causes increased expression of numerous genes including cytochrome P450 1A1 and 1B1, but also COX-2, MMP-1 and thus most likely contributes to photocarcinogenesis and – aging. In collaboration with an industry partner we have therefore developed a topical AhR antagonist for use in sunscreen products. In recent studies we have discovered that the AhR is a negative regulator of nucleotide excision repair in human epidermal keratinocytes and that it plays a critical role in the regulation of UVB-induced apoptosis. We have also shown that UVB-induced AhR activation does not exclusively occur in keratinocytes. For example, melanocytes express functionally active AhRs and UVB-induced activation of AhR signaling in melanocytes leads to melanocyte proliferation and / or melanin synthesis.

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11.7. UVA signal transduction in human skin

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Exposure of human skin to solar ultraviolet radiation, a powerful environmental carcinogen comprising ~95% UVA and ~5% UVB at the Earth's surface, promotes melanin synthesis in epidermal melanocytes and leads to increased pigmentation (Gilchrest et al. 1996; Lin and Fisher 2007; Miyamura et al. 2007). To initiate signaling pathways that lead to pigment synthesis, UV must be absorbed by specific chromophores in skin. DNA is a chromophore for UVB (Young 1997) and the UVB-DNA damage pathway (reviewed in (Lin and Fisher 2007) results in delayed pigmentation that occurs within 1 - 2 days of UV exposure. In contrast, UVA exposure causes immediate pigment darkening (IPD) of the skin within minutes via an unknown mechanism (Pathak et al. 1962; Kaidbey and Kligman 1979; Beitner 1988; Routaboul et al. 1999). At the cellular level, UVA can cause DNA lesions indirectly through oxidative damage (Besaratinia et al. 2004; Besaratinia et al. 2004; Marrot et al. 2005; Marrot and Meunier 2008), but no receptor directly mediating UVA phototransduction has been identified.

We have recently demonstrated that exposure to physiological UVA doses activates opsin receptors expressed in human epidermal melanocytes (HEMs). We found that among opsins, light-sensitive G protein-coupled receptors (GPCRs) that use a retinal chromophore to mediate both visual and non-visual photoreception (Baylor 1996; Terakita 2005; Nickle and Robinson 2007), rhodopsin is expressed in human melanocytes and contributes to UVA phototransduction. UVA exposure evoked retinal-dependent, rapid intracellular calcium transients via G-protein and phospholipase C (PLC) activation, and resulted in augmentation of cellular melanin concentration. Significant melanin synthesis was measured as early as one hour after UV exposure and continued to increase up to 5-fold over 24 hours. Our results reveal a novel UVA-sensitive phototransduction mechanism in human melanocytes that involves rhodopsin activation, calcium mobilization and melanin synthesis. These findings have broad implications for understanding UV-induced signal transduction and its physiological consequences on human skin.

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11.8. Two UV pathways to melanoma

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The role(s) of UVB (280-320nm) and UVA (320-400nm) in melanoma are unclear but can be addressed in experimental animal models. The hepatocyte growth factor transgenic (HGF/SF) mouse has extrafollicular ectopic melanocytes which are absent in wild-type mice and develops melanomas recapitulating human disease after neonatal UV exposure. We used specialized optical sources to deliver precisely defined UVA or UVB radiation to albino and pigmented HGF/SF transgenic mice and compared melanoma formation. Unexpectedly, the presence of melanin pigment exacerbated melanoma. In pigmented HGF/SF transgenics, melanin was largely confined to melanocytes and protective epidermal melanin was sparse, enabling direct exposure of melanocytes to UV radiation. UVB initiated melanoma and produced cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (6-4PPs) quantified by HPLC-MS/MS in neonatal skin independent of pigment status. UVA initiation of CMM, however, was completely dependent on the presence of melanin pigment. UVA irradiation produced only TT-CPDs but at 10-fold lower levels than UVB and melanin did not increase their formation. UVA-induced oxidative DNA damage, however, quantified as nuclear 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo), was found at significantly higher levels in melanocytes in the presence of melanin, both in vivo and in vitro, supporting a photooxidative role for melanin and/or its precursors in UVA melanomagenesis. Our findings have identified two wavelength-dependent pathways to UV-induced melanoma and demonstrate a novel and important role for melanin/melanin precursors in melanoma. These studies also provide experimental support for the epidemiologic associations between solar UV exposure, or use of UVA-emitting tanning lamps and increased melanoma risk.

11.9. The role of UVA and UVB in melanoma: studies using the *Xiphophorus* hybrid fish model

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11.10. The vitamin D receptor as a tumor suppressor of UVB-induced skin cancer

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Epidemiologic evidence supporting the role of vitamin D in the prevention of a wide variety of tumors is strong. Likewise the use of the active metabolite of vitamin D, $1,25(OH)_2D_3$, and its analogs to prevent and/or treat a wide variety of malignancies in animals is well established. The evidence has been less compelling for epidermal carcinogenesis perhaps because the same agent that produces vitamin D in the skin, UVB radiation (UVR), is also the same agent that results in most epidermal malignancies. However, recent studies indicate that the role of vitamin D and its receptor (VDR) in protecting against the development of epidermal tumors deserves a closer look. In the first of these studies mice lacking the VDR, unlike their normal littermates, developed epidermal tumors following the oral administration of 7,12 dimethylbenzanthracene (DMBA). More recently we and others have demonstrated increased susceptibility of the skin of VDR null mice to tumor formation following UVR. Acute UVB exposure increases the proliferation while decreasing CPD clearance in the epidermis of VDR null mice. Our in vitro studies confirm that keratinocytes lacking VDR are hyperproliferative with decreased apoptosis. Two interacting pathways critical for normal hair follicle cycling, beta-catenin and hedgehog (Hh), when activated abnormally also result in epidermal tumors. Thus, we considered the possibility that VDR suppresses either or both beta-catenin and Hh signaling such that loss of VDR predisposes to epidermal tumor formation due to their uncontrolled activation. We determined that all elements of the Hh signaling pathway were upregulated in the epidermis and utricles of the adult VDR null mouse including sonic hedgehog (Shh), its receptor patched 1 (Ptch1), smoothened (Smoh) and the gli family (Gli1, Gli2) of transcription factors, whereas $1,25(OH)_2D_3$ could suppress their expression in normal epidermal explants. Although no striking changes in beta-catenin expression were observed in the VDR null keratinocyte, the transcriptional activity of beta-catenin was increased. Knockdown of VDR in keratinocytes inhibited the expression and membrane localization of E-cadherin (the major binding protein for beta-catenin in the plasma membrane) with increased expression of several beta-catenin target genes and promoter constructs. Furthermore, $1,25(OH)_2D_3$ administration and overexpression of VDR with or without $1,25(OH)_2D_3$ inhibited beta-catenin transcriptional activity (TOPGlow) in keratinocytes. Thus we attribute the increased predisposition of VDR null mouse skin to UVB induced tumor formation to the failure to regulate both Hh and beta-catenin pathways leading to increased proliferation in cells with UVB induced DNA damage.

11.11. Which cell in human skin transforms to melanoma?

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Normal human melanocytes are spread singly among basal layer epidermal keratinocytes, which tightly control their proliferation rates and expression of cell surface molecules. Melanocytes' dendrites reach into the upper layers of the epidermis to distribute pigment-containing melanosomes to the keratinocytes. We have dissected the biological events of the homeostatic balance between epidermal melanocytes and keratinocytes including cell-cell adhesion through E-cadherin, formation of gap junctions through connexin 43 or matrix adhesion receptors such as a6ß1 laminin receptor or DDR1 collagen receptor, and roles of growth factor receptors such as c-kit, endothelin receptor B, or fibroblast growth factor receptor 1. These studies helped us to understand the autonomy of melanoma cells from keratinocytes and their close association with fibroblasts and endothelial cells by switching from E-cadherin to N-cadherin expression. Melanoma cells no longer secrete the matricellular protein CCN3 but instead secrete tenascin, osteopontin or SPARC, they produce autocrine growth factors such as bFGF or HGF, switch signaling from the canonical to the non-canonical Wnt signaling pathway, and constitutively activate Notch signaling. Our discovery of potential stem cell reservoirs for melanocytes in the dermis and hair follicle changes the criteria defining normal and malignant melanocytes: Dermal multi-potent stem cells, in contrast to epidermal melanocytes, share many properties with melanoma cells such as high migration and invasion rates, close association with mesenchymal-type cells, activation of non-canonical Wnt signaling, and expression of Notch receptors and their ligands suggesting that melanoma cells have a stem cell-like phenotype. Many attributes of melanoma cells that the melanoma research community had labeled as 'tumor-specific' or 'tumor-associated' are based on comparison to the differentiated phenotype of melanocytes, and not that of melanocyte stem cells. Understanding the biology of normal stem cell self-renewal and differentiation will help us to better define truly tumor-specific changes. We then need to develop new models to proof the concept that melanomas can arise also from stem cell populations and not only from the mature cells. As a fourth cell for melanomas may serve dedifferentiated neural crest-like stem cells that are derived from melanocytes by Notch activation. These NIC (Notch intracellular domain)-activated stem cells have similar characteristics to the dermal stem cells suggesting that also in skin reprogramming from a mature to a multi-potent stem cell can occur.

11.12. Ambiguity in the relationship between UV-induced *p53* mutant clones and skin carcinomas

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A majority of human skin carcinomas were shown to carry mutations in the *p53* tumor suppressor gene, and these mutations were characteristic of UV radiation (C to T at dipyrimidinic sites). Experimentally UVB-induced skin carcinomas in (hairless) mice showed predominantly similar mutations in *p53*, and were found to be preceded by microscopic clusters of epidermal cell overexpressing mutant p53. Such putative microscopic precursor lesions were also found in regularly sun-exposed human skin. We have recently shown that severe sunburn ablated the complete interfollicular epidermal basal layer in mouse skin by massive caspase-3-driven apoptosis while leaving the overlying epidermis with sunburn cells (dyskeratotic, eosinophilic cytoplasm and compacted nuclei) intact. This eradicated the p53-mutant overexpressing cell clusters and nullified the carcinogenic effect of prior chronic UV exposure, demonstrating that UV-induced p53-mutant clusters and squamous cell carcinomas are of interfollicular origin. In testing immunosuppressants on effects on early UV carcinogenesis, we subsequently and unexpectedly found disconcordancies between effects on p53-mutant cell clusters and onset of the tumors with (overexpressed) mutant-p53. Deep sequencing revealed that far more epidermal cells were mutated than those overexpressing mutant-p53. Immunosuppressants could affect numbers of overexpressing cell cluster, but had no discernible effect on the pool of p53-mutated cells. This experiment showed that p53-mutant overexpressing cell clusters may be associated with skin carcinogenesis, but are not truly precursors of squamous cell carcinomas.

These results pertain to UVB-driven carcinogenesis, but the effects of UVA1 radiation tend to be different. Although we found UVA1 radiation to induce skin carcinomas, only a small minority of these tumors bore the p53 mutations characteristic of UV (B) radiation. Furthermore, UVA1 irradiation at sunburn dosages (after hours of exposure) induces p53 overexpression in hairless mouse skin, but without any sign of apoptotic cells, very much in contrast with UVB radiation. Hence, p53 mutations and apoptosis appear to be of less importance in UVA1 carcinogenesis.

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11.13. Analysis of thymine dimer in urine following exposure to sunlight

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Ultraviolet radiation (UVR) causes damage and mutations in cellular DNA and exposure, mainly in the form of sunlight, is considered to be the predominant cause of skin cancers. Biological monitoring of DNA damage due to UVR may provide a valuable complement to assessment with personal dosimeters. Ideally, such monitoring could take into account protection of certain areas of the skin by clothing or sunscreen, as well as individual sensitivity due to pigmentation and other factors. Thymine dimer (T=T), the most common UVR-induced DNA lesion, is excreted in the urine after repair. A ^{32}P-postlabelling assay for analysis of T=T in human urine has been validated using a sunbed and it was shown that there is a dose-response relationship for levels of T=T (1). In other more recent studies it has been shown that some kind of steady-state level is built up after chronic exposure (2) and our results have not indicated that children would have higher levels of urinary T=T than adults (3). The aim of the present investigations was to evaluate the feasibility of employing urinary T=T as a biomarker of exposure to UVR in various field investigations within the EU-funded project ICEPURE (www.icepure.eu). Several different studies were carried out and so far urine samples from Polish children who went to a summer camp during 2 weeks, Danes and Spaniards who went to Tenerife for one week in February and Danes who went skiing in the Alps in March have been analysed. The samples were collected before travelling and after returning home. Levels of urinary T=T before travelling were generally below detection limit or in a low range and dependent upon season and country. After being exposed to sunlight, T=T could be detected in all urine samples of the different groups and levels increased up to 20-fold. These investigations show that urinary levels of T=T increase dramatically after being exposed to sunlight. T=T is thus a very sensitive biomarker of exposure to UVR and can be applied to studies of recreational sunlight exposure.

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11.14. The epidemiology of melanoma and what it suggests about melanoma risk, UVB, UVA and vitamin D

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11.15. Epidemiological evidence that UVA is involved in the genesis of melanoma

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Nearly all UV sources are a mixture of both UVA and UVB and human exposure to pure UVA or UVB sources is rare. However, epidemiological studies can inform on the type of wavelength possibly involved in nevus and melanoma occurrence. First, the association between artificial "UVA-tanning" and melanoma provides evidence that exposure of sun-susceptible individuals to high UVA fluxes can trigger melanoma. Indeed, some UVB is always present in the UV spectrum of sun-tanning lamps but the genuine characteristics of the majority of modern canopy-like UV-tanning units is to deliver UVA dosages that are much higher than what is delivered, for example, by the summer midday sun on a Mediterranean beach. Second, the raised melanoma risk associated with increased sun exposure duration induced by sunscreen use would be due to greater exposure to the UVA radiation. Third, high SPF sunscreens enable subjects to withstand high UVB fluxes, which in turn probably leads to greater exposure to high UVA fluxes. Fourth, one randomised trial during which individual UVA and UVB dosimeters were used by subjects using a SPF 10 or a SPF 30 sunscreen, indicate that amounts of UVB that reached the skin were similar in both groups, whilst amounts of UVA that reached the skin were higher in the SPF 30 group. Fifth, sunburn history and lower latitude holidays are associated with large nevi (≥5mm) in children but not with small nevi (2 to 4.9 mm). In contrast, quantities and durations of holidays are associated with numbers of small but not of large nevi. These results suggest that the UVB would be the main trigger of the radial growth phase of nevi, leading to their enlargement and probably also to acquisition of clinical features of "atypia". UV wavelength other than the UVB, i.e., the UVA, would be involved in the initial steps triggering nevus formation. Lastly, studies on sunbed use have shown that the thicker the melanoma, the weaker the association with past sunbed use. The two epidemic episodes of melanoma described in Australia and in Icelandic had no influence on melanoma mortality rates. The Iceland epidemic was largely due to artificial UVA tanning. These observations indicate that melanoma associated with "UVA" exposure during adolescence and adulthood are generally thin, of low lethal potential and would constitute the majority of the melanoma epidemic observed in light-skinned populations.

11.16. Cohort study on sun and solarium exposure in relation to cancer risk

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Background Sun exposure is an established and modifiable risk factor for skin cancer. Use of indoor tanning equipment (solariums) has also been associated with increased skin cancer risk, but less consistently than sun exposure. Moreover, the importance of ultraviolet (UV) exposure in different periods of life, and possible effect modification by an individual's sensitivity to UV exposure is not completely understood. Sun and solarium exposure are sources of vitamin D which is essential to prevent bone diseases. Ecological and observational epidemiological studies have suggested beneficial effects of sun and solarium exposure for several diseases including cancer. This complicated stage of knowledge, namely the negative effect of UV exposure on skin cancer risk but preventive effect on bone health, and possibly (although not yet proven) on other diseases – makes the public health communication particularly challenging. We will present results on individual characteristics and sun and solarium exposure in relation to risk of cutaneous malignant melanoma (CMM), breast, ovary, lung, colon-rectum, and brain cancers, non-Hodgkin lymphoma (NHL), and preliminary results regarding squamous cell carcinoma (SCC).
Material and methods The Norwegian Women and Cancer Study (NOWAC) (Edvardsen et al. 2011), the Swedish Women's Lifestyle and Health (WLH) Study (Yang et al. 2011), and the combined cohort, the Norwegian-Swedish Women's Lifestyle and Health Cohort Study (Veierød et al. 2010a, b) , were used. The latter included more than 100,000 women aged 30-50 years at recruitment in 1991/92. Information on sun exposure, solarium use, and individuals' characteristics at cohort enrolment was collected through a self-administered questionnaire. Frequency of sunburns, sunbathing vacations, and solarium use were recorded for five age decades (0-49 years). We combined the exposure across each of the three decades of life that were recorded for all women (10-30 years). Vitamin D effective solar radiation (VD dose; Edvardsen et al. 2011) was calculated in NOWAC. Complete follow-up was achieved by linkage of the study database to national registries in Norway and Sweden. Relative risks (RRs) and 95% confidence intervals (CIs) were estimated by Poisson and Cox regression.
Results In the combined cohort, CMM risk increased significantly with increasing sunburns and bathing vacations in the first three age decades ($P_{trend} \leq 0.04$), and with solarium use at ages 30 to 39, and 40 to 49 years, with RRs (95% CIs) of 1.49 (1.11, 2.00) and 1.61 (1.10,2.35), respectively, for solarium use of one time and more per month versus "never" use. No significant associations were found between any cumulative measure of UV exposure for cancer of the breast, ovary, lung, colon-rectum, and brain in the WLH study, or for NHL in the combined cohort. VD dose was studied in relation to breast cancer risk in NOWAC, and no significant associa-

tion was found, RR=1.17 (95% CI (0.95, 1.44); highest vs lowest category). Results for SCC are in progress.

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11.17. Somatic alterations in the melanoma genome: a high-resolution array based comparative genomic hybridization study - a detailed investigation of deletions at the *CDKN2A* locus

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Melanoma accounts for the highest number of fatalities for any skin cancer. It originates from transformation of melanocytes through a series of morphological alterations and innumerable genetic alterations. Out data from high-resolution array based comparative genome hybridization on DNA from melanoma cell lines and corresponding normal tissues showed gross but non-random somatic changes specific to the tumor genome. While the *CDKN2A* (78%) and *PTEN* (70%) loci were the major targets of mono-allelic and bi-allelic deletions; amplifications affected loci with *BRAF* (53%) and *NRAS* (12%) as well as *EGFR* (52%), *MITF* (40%), *NOTCH2* (35%), *CCND1* (18%), *MDM2* (18%), *CCNE1* (10%) and *CDK4* (8%). The amplified loci also carried additional genes, many of which could potentially play a role in melanoma. Distinct patterns of copy number changes showed that alterations in *CDKN2A* tended to be more clustered in cell lines with mutations in the *BRAF* and *NRAS* genes; the *PTEN* locus was targeted mainly in conjunction with *BRAF* mutations. Amplification of *CCND1*, *CDK4* and other loci was significantly increased in cell lines without *BRAF-NRAS* mutations and so was the loss of chromosome arms 13q and 16q. Our data suggest involvement of distinct genetic pathways that are driven either through oncogenic BRAF and NRAS mutations complemented by aberrations in the *CDKN2A* and *PTEN* genes or involve amplification of oncogenic genomic loci and loss of 13q and 16q. Further focus on deletions at the *CDKN2A* revealed that in many cases the deletions encompassed a large affecting several genes. However, we also discovered a recurrent focal deletion that besides loss of *CDKN2A* resulted in creation of a fusion gene from parts of the *MTAP* and non-coding *ANRIL* gene, which is both transcribed and translated. In conclusion, it, thus, emerges that each tumor besides being affected by major and most common somatic genetic alterations also acquires additional genetic alterations that could be crucial in determining response to small molecular inhibitors that are being currently pursued.

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11.18. Molecular phenotypes with clinical implications in malignant melanoma

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Malignant melanoma is a disease that demonstrates a wide range of clinical behavior from relatively indolent to aggressive metastatic disease. Our own studies of metastatic melanoma reveal a division into four gene expression entities that are significantly correlated to outcome. We have repeated this observation in stage III/IV and primary melanomas suggesting that these gene signatures clearly reflect the aggressive behavior of certain melanomas. Our studies on melanoma progression reveal extensive genetic tumor heterogeneity, which challenges the current approach to treatment of metastatic disease. Our aim is to address this issue by searching for novel molecularly targetable molecules by using a large-scale genetic screen that includes deep-sequencing of 1,800 cancer- and melanoma-relevant genes in combination with molecular tumor phenotype information. One such molecular target, the BRAF oncogene, was identified almost a decade ago through a systematic mutation screen in genes of the MAPK signaling pathway. Now, Vemurafenib, an FDA approved BRAF-inhibitor has revolutionized treatment of melanoma patients illustrating the success of large-scale genetic screening approaches and the potential to define new therapeutic targets in melanoma. Thus, we believe that genetic and molecular information on melanoma tumors will ultimately lead to improved knowledge in biology, diagnosis, prognosis and treatment prediction.

11.19. Molecular effects of erythemally equivalent doses of UVB and UVA1 on human skin in vivo

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The sun is primarily a UVA source but most human skin photobiology research has been done with UVB, because it is much more effective at inducing erythema. Thus, we lack data on the acute and long-term effects of UVA, especially UVA1 (340 – 400nm), which is the main spectral region in solar UVR. Apart from solar exposure, people are increasingly treated with UVA1 phototherapy.

We have compared erythemally equivalent doses of UVB (monochromatic 300nm) and UVA1 in human skin types I/II *in vivo*. In all cases, the minimal erythema dose (MED) was determined. As expected, there was a distribution of MED with each spectrum but there was no correlation between individual UVB and UVA1 MED which suggests different independent mechanisms.

UVB and UVA1 readily induced epidermal and dermal cyclobutane pyrimidine dimers (CPD) detected using an antibody for thymine dimers (TT). For a given erythemal exposure, there were approximately 3-4 times more TT with UVB compared with UVA1. 6-4 photoproducts were also observed with UVB but not with UVA1. The level of UVB-induced TT decreased with epidermal depth and this trend continued into the dermis. In contrast, UVA1 showed the opposite trend in the epidermis with no trend in the dermis. This suggests there is scattering of UVA1 in the dermis. Irrespective of mechanism, our data suggest that the basal layer DNA is especially susceptible to UVA1 damage.

We have also compared the effects of UVB and UVA1 on expression of a wide range of genes using micro-array and RT-PCR. It is often stated that UVA is the main cause of photoageing which is believed to be mediated via matrix metalloproteinases (MMP) especially MMP-1. We tested this by comparing erythemally equivalent doses of UVB and UVA1, and showed that they had comparable effects on MMP-1 mRNA expression. However, UVA-1 was more effective at inducing expression of tissue inhibitor of MMP-1 (TIMP-1) which, unlike MMP-1, is only expressed in the dermis. Thus, we conclude that solar UVB is the main cause of photoageing, as is the case with erythema.

Data will also be presented other skin genes expressed by UVB and UVA1.

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11.20. Immunosuppression, inadequate DNA repair, and mutations in *Brm* may contribute to the role of UVA in human skin cancer

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UVB and UVA are most likely co-carcinogens for human skin cancer. Exposure of engineered human skin showed that UVA but not UVB induced mutations predominated in basal epidermis that contain dividing keratinocytes thought to give rise to skin tumours (Huang et al. 2009). Reactive oxygen species (ROS) oxidize guanine to 8-oxo-7,8-dihydroguanine (8-oxo-dG), which is thought to be the predominant DNA damage formed in response to UVA. This is repaired by 8-oxoguanine-DNA glycosylase 1 (OGG1). Anti-OGG1 staining of human skin showed the highest expression in the superficial epidermal layer. UVA-induced 8-oxo-dG was repaired more rapidly in the upper layer of human skin compared to the lower layers. This indicates that weaker expression of the OGG1 enzyme in the basal cells of human epidermis may result in a lack of DNA repair in these cells and therefore accumulation of UVA-induced oxidative DNA mutations (Javeri et al. 2008).

We have recently discovered a novel hotspot mutation in the chromatin remodelling gene *Brm* in human skin cancers. This G:C to T:A transversion is typical of mutations occurring following oxidation of guanine to 8-oxo-dG, which is consistent with being caused by UVA in the absence of repair from OGG1 (Moloney et al. 2009). The Brm protein is expressed at very low levels in human skin cancers, and photocarcinogenesis studies in mice have confirmed that knockdown of Brm increases sensitivity to photocarcinogenesis. UV suppression of immunity is another critical biological mechanism responsible for skin cancer. Both UVB and UVA are potently immunosuppressive in humans, enabling mutated cells to grow unchecked. Our recent action spectrum shows two immunosuppressive peaks, one in the UVB range at 300 nm and a second in the high wavelength UVA at 370 nm. The action spectra for UVA-induced immunosuppression and ROS production are similar, suggesting that ROS production may be responsible for UVA immunosuppression in humans (Halliday et al. 2011). There is now accumulating evidence that the role of UVA in causing skin cancer in humans has been underestimated, and ROS may be critically involved.

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11.21. Survival in melanoma – seven-year follow-up in GEM

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In the international population-based GEM study, data were available for 3,579 melanoma patients. Of the clinical characteristics Breslow thickness was, as anticipated, the main determinant of survival. Of the host characteristics, low socioeconomic status, measured by education level achieved, male gender and older age at diagnosis were associated with poorer survival. Of the behavioral characteristics, contrary to our prior hypothesis that high levels of solar exposure would be inversely associated with mortality, there was little association in either direction with measures of sun exposure prior to diagnosis. These results support the need to investigate further genetic associations with melanoma progression and mortality.

12. Annex IV. Short talks

12.1. The protective role of UVA radiation against UVB-induced immunosuppression and skin carcinogenesis involves inducible haem oxygenase-1 (HO-1) and oestrogen receptor-β (ER-β) signalling – studies in mice.

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A normal endogenous photoprotective role for ER-β signalling has been identified in the skin of the mouse. Topical application of phytoestrogens or the natural ligand 17β-oestradiol dose-dependently protected mice from photoimmunosuppression, whereas a deficiency of ER signalling produced by the antiestrogen ICI 182780, or by a null mutation of the ER-β gene (ER-β -/- mice), increased the severity of photoimmunosuppression and exacerbated the UVB-induced epidermal cytokine dysregulation. The mechanism involved the inducibility of the photoprotective stress enzyme, (HO-1). In mice injected with the specific HO inhibitor Sn protoporphyrin-IX (SnPP), topical oestradiol was no longer protective against SSUV-induced immunosuppression, and ER blockade by ICI 182780 prevented the protective effect of the HO enzyme product CO, against *cis*-urocanic acid-induced immunosuppression.

It is the UVA waveband that is responsible for the induction of cutaneous HO-1. However, environmentally relevant doses of UVA radiation can also protect against UVB-induced immunosuppression. Therefore it appears that there is an interdependence of HO-1 and ER-β signalling that regulates UVA photoimmune protection. We found that ER-β -/- mice are refractory to UVA-induction of HO-1 and to UVA immunoprotection against UVB or *cis*-urocanic acid. Further, comparisons between male and female hairless mice reveal that normal but relatively ER-deficient male mice display increased photoimmune suppression by SSUV, refractoriness to UVA photoimmune protection, and a reduced UVA-induction of HO-1 in the skin.

This pathway appears also to afford endogenous protection against skin carcinogenesis. In separate models of transplanted murine skin cancer cells, tumour growth of B16 melanoma cells was significantly enhanced in the ER-β -/- mouse compared with normal wild type mice, and growth of a SSUV-induced squamous cell carcinoma was enhanced in hairless mice by ICI 182,780 (ER blockade) treatment. Photocarcinogenesis induction in hairless mice was inhibited by topical oestradiol treatments at doses that also reduced the severity of photoimmunosuppression. We also found that photocarcinogenesis is abrogated in hairless mice by continuing daily UVA irradiations following an otherwise cumulative carcinogenic regime of 50 daily SSUV exposures. Therefore, in mouse skin, successful signalling by the ER-β, which is associated with UVA exposure and HO-1 responsiveness, appears to provide a form of endogenous protection not only against photoimmune suppression but also against photocarcinogenesis. These findings are supported by epidemiological evidence of greater skin cancer susceptibility in men than in women.

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12.2. Mast cell-dependent neonatal immune tolerance and UV melanoma

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Childhood sunlight exposure is associated with increased melanoma risk. We used a transgenic mouse model to investigate the mechanisms responsible. Melanoma was initiated by UV irradiation of neonatal hepatocyte growth factor/scatter factor transgenic pups. UV irradiated skin from these animals was transplanted onto wild-type recipients. Melanomas were observed on grafted skin after 6-12 months and appeared significantly faster if graft recipients had been UV irradiated as neonates. Thus, neonatal UV irradiation not only initiated melanoma but also facilitated subsequent melanoma outgrowth, suggesting initiation of an immunosuppressive environment. We identified several factors contributing to an immune tolerizing environment in neonatal skin. Neonates did not respond to UV with an increase in the inflammatory cytokines IL-1□, IL-6, Gro-□□or TNF-□ in skin, in contrast to adults. Application of a contact sensitizer to neonatal skin resulted in immune tolerance. Genetic deficiency of the immunosuppressive cytokine IL-10 did not restore the production of Gro-□□or the influx of inflammatory neutrophils in response to UV or alter neonatal immune tolerance. Mast cell deficiency, in contrast, ablated neonatal tolerance but without restoring the classic inflammatory parameters. UV irradiation prior to application of the contact sensitizer did not increase neonatal tolerance. Importantly, however, melanocytes were mobilized from the hair follicle into neonatal but not into adult epidermis in response to UV. We propose that, following UV, antigens from mobilized extra-follicular melanocytes interact with the tolerizing environment we have described in neonatal skin to generate an immune tolerance which facilitates subsequent melanoma outgrowth.

12.3. Variations in serum 25(OH)D status due to the use of sun protection

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Background Low serum 25(OH)D status has been associated with a range of health outcomes, the most strongest evidence for playing a role in optimum bone health. This study aimed to evaluate the impact of sun protection and changes in sun protection with season on serum 25(OH)D status.

Methods This research was undertaken as a observational cohort study to test this assumption among healthy free-living adults aged 18 to 87 years, in south-east Queensland, Australia (27°S), at the end of winter and summer. This research was approved by Queensland University of Technology Human Research Ethics Committee and conducted under the guidelines of the Declaration of Helsinki. Vitamin D (25OHD) was used as a the dependant variable, whilst independent sun protection variables [Having worn a hat in the past month (0=never, 1=rare/sometimes; 2=usually/almost always; Having worn sunscreen in the past month (0=never, 1=rare/sometimes; 2=usually/almost always); Having worn a long sleeve shirt in the past month (0=never, 1=rare/sometimes; 2=usually/almost always; Having worn a long pants in the past month (0=never, 1=rare/sometimes; 2=usually/almost always] were analysed.

Results Unsurprisingly, sun protection varied between the seasons (Table 1) where in winter, participants were less likely to wear a hat or use sunscreen, but more likely to wear a long sleeve shirt or long pants. Mean Vitamin D also increased between the seasons.

Table 1. Mean scores (SDs) of study variables in winter and summer samples

	Winter sample (N=125)	*Summer sample (N=114)*	*t-test*
Worn a hat	1.06 (0.75)	1.34 (0.68)	.002
Worn sunscreen	0.93 (0.76)	1.27 (0.73)	<.001
Worn a long sleeve shirt	1.28 (0.66)	0.73 (0.69)	<.001
Worn a long pants	1.53 (0.64)	0.96 (0.71)	<.001
Vitamin D Status	61.64 (30.60)	80.91 (28.09)	<.001

Wearing a hat was positively associated with Vitamin D status (Table 2), while wearing a long sleeve shirt or long pants was negatively correlated to vitamin D level in the winter sample. In summer sample, however, wearing sunscreen was positively, but wearing long pants was negatively related to vitamin D level.

Table 2. Pearson correlations coefficients between clothing and sunscreen scores and vitamin D level in winter and summer, respectively

	Winter sample (N=125)	*Summer sample (N=114)*
Worn a hat	.21 *	.07
Worn sunscreen	.16	.25 *
Worn a long sleeve shirt	-.18 *	.02
Worn a long pants	-.19 *	-.31 **

When all these sun protection factors were taken into consideration spontaneously (Table 3), in winter sample, none of these factors were significantly associated with vitamin D level. However, in summer sample, wearing sunscreen was positively

associated with Vitamin D, while wearing long pants was negatively associated with the vitamin D concentration.

Table 3. Multiple regressions of vitamin D level on clothing and sunscreen scores in winter and summer samples

	Winter sample (N=125)	*Summer sample (N=114)*
Worn a hat	.16	-.11
Worn sunscreen	.13	.29 **
Worn a long sleeve shirt	-.16	.19
Worn a long pants	-.12	-.41 **

Conclusions: These results, show in real life situations that the use of sun protection does not impede with the production of Vitamin D in human skin. However, in the winter months, there was a greater proportion of the skin not exposed, leading to a negative association with Vitamin D level. Further research is needed within this area, particularly the role of local climate on sun protection measures.

12.4. Serum 25-hydroxyvitamin D levels after the same vitamin-D effective UV-exposures without and with high UVA-doses

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Background Vitamin D status is crucial for bone structure, and may play a broader health role. What´s adequate and how to get it is controversial (Wolpowitz et al. 2006).

Exposure to sunlight is a main source of vitamin D. Mainly UVB radiation, but not UVA, contributes to form previtamin D3 in the skin.

The photosynthesis of vit-D3 is known, its wavelength dependency and action spectrum (CIE 2006). However there also is a photodegradation process reported (IARC 2008).

Aim To examine how the same vitamin-D effective UV radiation dose affects 25(OH) D if there is very little UVA or if there also are high UVA-doses.

Material and methods Three comparable groups of healthy subjects were given suberythemal UV-doses (< 1 SED) with different spectral compositions in the clinic´s medical UV-boxes: "UVA" (UVA tube lamps, 9 minutes, n=10), "UVB" (UVB lamps, 1 minute, n=23) and "UVAB" (UVB+UVA-lamps, 1 minute, n=23). Spectral distributions 250-400 nm of the UV-boxes were measured with a spectroradiometer, and exposure times were chosen to give the same calculated vitamin D effective dose. The radiation was given for two consecutive weeks, three times a week. Blood samples were collected before the first irradiation, after the 5th irradiation and 2 days after the last irradiation and compared to a control group (n=20).

Results UVB radiation increased serum 25(OH) D significantly as expected. Increase in the UVAB group was almost identical. A considerably smaller increase was seen in the UVA-group. There was a small decrease of 25(OH) D in the control group.

Conclusion The same vitamin D weighted irradiation gave less serum 25(OH) vitamin D levels after a relatively long irradiation of weak UVB and strong UVA compared to short irradiations from strong UVB with or without UVA - possibly due to photodegradation effects.

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12.5. Future reduction of cutaneous malignant melanoma due to improved sun tanning habits among Swedish children?

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We have previously reported increased parental sun protection regimen and a decrease of common melanocytic nevi among 7-year old Swedish children. The purpose of this study was to investigate whether the trend persists and whether common melanocytic nevi density per se could be an indicator of UV exposure.

In 2009 a third, cross-sectional study was performed among 7-year-old children in two municipalities at latitude 57° in the south of Sweden (n=480). The two previous were conducted in 2001–2002 and in 2007. The parents answered a questionnaire about their child's tanning habits at different ages since birth, and nevi >2 mm were counted by the same research nurse.

The significantly increased improvement in sun-protective regimens, such as often use of sunscreen (+27%), staying in shade (+49 %), or indoors (+127 %) between 2002 and 2009 confirmed the results from 2007. Number of sunburns had decreased at all ages compared to 2007 and 2002. A significant decrease in mean nevus density from 9.7 (95 % CI: 8.6-10.9) in 2002 to 7.3 (95 % CI: 6.4-8.3) in 2009 was seen. These results confirm a current trend in Sweden regarding increased parental awareness of the risks of sun overexposure and a decrease in number of nevi among 7-year-olds. If this persists, a future reduction of cutaneous malignant melanoma incidence in Sweden might be anticipated. The results also indicate that nevi count *per se* could be used as an indicator of UV exposure.

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2015:16

The Swedish Radiation Safety Authority has a comprehensive responsibility to ensure that society is safe from the effects of radiation. The Authority works to achieve radiation safety in a number of areas: nuclear power, medical care as well as commercial products and services. The Authority also works to achieve protection from natural radiation and to increase the level of radiation safety internationally.

The Swedish Radiation Safety Authority works proactively and preventively to protect people and the environment from the harmful effects of radiation, now and in the future. The Authority issues regulations and supervises compliance, while also supporting research, providing training and information, and issuing advice. Often, activities involving radiation require licences issued by the Authority. The Swedish Radiation Safety Authority maintains emergency preparedness around the clock with the aim of limiting the aftermath of radiation accidents and the unintentional spreading of radioactive substances. The Authority participates in international co-operation in order to promote radiation safety and finances projects aiming to raise the level of radiation safety in certain Eastern European countries.

The Authority reports to the Ministry of the Environment and has around 300 employees with competencies in the fields of engineering, natural and behavioural sciences, law, economics and communications. We have received quality, environmental and working environment certification.

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