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Genetically determined susceptibility markers in skin cancer and their application to chemoprevention

H. Hahn

Development of skin cancer is a result of interactions between genetic and environmental factors. Exposure to sunlight is an established cause of non-melanoma skin cancer as well as of melanoma. Other additional factors such as exposure to environmental chemicals (e.g., chimney soot, arsenic compounds), chronic irritation of the skin, viral infections and the immune status of the host may predispose to skin cancer. The high incidence of skin cancer highlights the need for development of more effective chemopreventive agents. This requires a better understanding of genetically determined host susceptibility, which is increasingly acknowledged as a major factor in the causation of skin tumours.

Introduction

Skin cancer accounts for approximately one third of all newly diagnosed cancers in the United States (Boring *et al.*, 1994). Non-melanoma skin cancers are the most common cancers in the white population, with 900 000 to 1 200 000 new cases diagnosed annually in the United States (Miller & Weinstock, 1994). The most frequently diagnosed cancer in this group is basal-cell carcinoma (BCC), followed by squamous-cell carcinoma (SCC). The incidence of non-melanoma skin cancers is rising worldwide and it has been estimated that 28% to 33% of Caucasians born after 1994 will develop a basal-cell carcinoma in their lifetime (Miller & Weinstock, 1994; Hughes *et al.*, 1995; Gloster & Brodland, 1996). In 1997, more than 40 000 cases of cutaneous melanoma were diagnosed in the United States (Gilchrest *et al.*, 1999). The incidence of no other cancer is increasing so fast, and mortality from cutaneous melanoma has doubled in the last 35–40 years (Balch *et al.*, 1997).

The reason for the increasing incidence of skin cancer is unclear. Increased recreational exposure to sunlight may play a major role, as may an increase in the ultraviolet (UV) radiation that reaches the Earth's surface (Balch *et al.*, 1997). Another factor is probably the increased use of sunscreens which induce individuals to spend more time outdoors. Sunscreens are very effective in preventing sunburn. However, there is no unequivocal evidence that they protect against melanoma formation (Ley & Reeve, 1997).

The identification of genes underlying several rare hereditary syndromes associated with increased skin cancer incidence has had an enormous impact on the understanding of cutaneous malignancies at the molecular level (Halpern & Altman, 1999). Additionally, evidence is emerging that allelic variants of genes involved in detoxification of a variety of exogenous and endogenous substrates may also contribute to the increased number of skin tumours (Lear *et al.*, 2000). Furthermore, it is likely that a number of still unknown genetic modifiers influencing individual susceptibility to skin cancer segregate within the population and their identification should allow better assessment of individual skin cancer risk (Nagase *et al.*, 1999).

High-penetrance susceptibility genes

Inherited conditions entailing increased predisposition to skin cancer and the underlying genes are listed in Table 1.

Albinism

Albinism comprises a group of genetic disorders characterized by deficient synthesis of the pigment melanin. Oculocutaneous albinism Type 1 (OCA1;

Table 1. Inherited diseases with increased skin cancer susceptibility

Disease	Gene defect or gene locus
Oculocutaneous albinism	*TYR, P* gene
Xeroderma pigmentosum	*XP* genes
Xeroderma pigmentosum variant	DNA polymerase eta
Naevoid basal-cell carcinoma syndrome	*PTCH*
Cutaneous malignant melanoma	*CDKN2A, CDK*
Inherited retinoblastoma	*pRB*
Li–Fraumeni syndrome	*p53*
Rombo syndrome	?
Bazex syndrome	Xq
Multiple self-healing epithelioma	9q31
Muir–Torre syndrome	*MSH2, MLH*
Cowden's syndrome	*PTEN*

autosomal recessive inheritance) is caused by mutations in the tyrosinase gene, which encodes the enzyme that catalyses at least three steps in melanin biosynthesis (Spritz, 1994). Oculocutaneous albinism Type 2 (OCA2; autosomal recessive inheritance) results from mutations in the P gene, which is probably involved in the transport of the melanin-precursor tyrosine within the melanocyte (Spritz, 1994). OCA2 is the most prevalent type of albinism worldwide. In the United States, only 1 in 36 000 inhabitants is affected with this disease (Lee *et al.*, 1994). However, in Nigeria it is one of the most common recessive genetic disorders, with a prevalence of about 1 in 1100 (Okoro, 1975). The most frequent type of skin cancer associated with albinism in African albinos is squamous-cell carcinoma, in contrast to Caucasians, in whom basal-cell carcinoma is most frequent (Yakubu & Mabogunje, 1993).

Melanin has photoprotective functions in the skin and directly absorbs both UV photons and reactive oxygen species generated by the interaction of UV radiation with membrane lipids or other cellular components (Riley, 1997). Besides mutations in the tyrosinase gene and in the P gene, mutations or polymorphisms in other genes involved in the synthesis and transport of melanin may play critical roles in susceptibility to skin cancer in the general population (Valverde *et al.*, 1995, 1996).

Xeroderma pigmentosum and xeroderma pigmentosum variant

Exposure to UVB radiation induces the formation of photoproducts which may lead to accumulation of skin cancer-inducing mutations (Brash, 1997). The main pathway by which mammalian cells remove DNA damage caused by UV radiation and other mutagens is nucleotide excision repair (NER). Genes which are defective in the inherited disorder xeroderma pigmentosum (XP) are the best characterized components of the human NER process (Araujo & Wood, 1999). XP shows autosomal recessive inheritance and predisposes the affected individual to cutaneous malignancies. The risk of developing skin cancer on sun-exposed areas is more than 1000-fold increased in XP patients and most frequently the patients develop basal-cell carcinomas and squamous-cell carcinomas (Kraemer *et al.*, 1994). An increase in skin cancer predisposition has also been described in XP heterozygotes (Swift *et al.*, 1979). In this context, it is of great interest that reduced repair of UVB-induced DNA damage in patients with basal-cell carcinoma has been described (Wei *et al.*, 1995).

The xeroderma pigmentosum variant (XPV) is an inherited disease which is associated with increased incidence of sunlight-induced skin cancer. Unlike other XP-cells, XPV cells carry out normal nucleotide excision repair but are deficient in replication of UV-damaged DNA (Cordonnier & Fuchs, 1999). Recently XPV has been shown to

arise from mutations in the DNA polymerase *eta* (Masutani *et al.*, 1999; Johnson *et al.*, 1999).

Naevoid basal-cell carcinoma syndrome (NBCCS)

It is well known that inherited defects in oncogenes and tumour-suppressor genes influence skin cancer susceptibility. For example, a large proportion of sporadic basal-cell carcinomas show mutations in the tumour-suppressor gene *patched* (*PTCH*). *PTCH* was cloned from the locus for NBCCS, which is a rare autosomal dominant disorder that predisposes the affected individuals to basal-cell carcinoma, several other cancers and developmental defects (Johnson *et al.*, 1996; Hahn *et al.*, 1996). More than 50% of basal-cell carcinomas show loss of heterozygosity (LOH) at the *PTCH* locus on 9q22–31. Mutation screening revealed that 30% of basal-cell carcinomas with LOH have mutations in the remaining allele of *PTCH* (Gailani *et al.*, 1996a, b). Interestingly, missense mutations in the ligand of PTCH, *sonic hedgehog* (*SHH*) as well as in its signalling partner, *smoothened* (*SMO*) in basal-cell carcinomas have been reported (Oro *et al.*, 1997; Xie *et al.*, 1998). Overexpression of *SHH* (Oro *et al.*, 1997), *SMO* (Xie *et al.*, 1998) and of the *PTCH* pathway targets GLI1 (Nilsson *et al.*, 2000) and GLI2 (Grachtchouk *et al.*, 2000) in murine skin leads to abnormalities resembling human basal-cell carcinomas. Therefore, it is possible that carcinogenesis can be initiated by mutations in all known components of the pathway. Essentially all basal-cell carcinomas overexpress targets of an activated SHH/PTCH/SMO pathway, which include PTCH itself, GLI1 and SMO (Dahmane *et al.*, 1997; Reifenberger *et al.*, 1998; Tojo *et al.*, 1999) (Figure 1). Expression of these genes seems to be very specific for basal-cell carcinomas in comparison with other skin tumours and the level of expression correlates with progression of basal-cell carcinomas (Tojo *et al.*, 1999; Unden *et al.*, 1997; Kallassy *et al.*, 1997). Overall, these data suggest a major role for the SHH/PTCH/SMO signalling pathway in the development of basal-cell carcinoma.

Approximately 40% of *PTCH* mutations in basal-cell carcinomas are C→T or CC→TT transitions at dipyrimidine sites which are typical of UVB-induced DNA damage, thus implicating UVB in mutagenesis of *PTCH* (Aszterbaum *et al.*, 1999). A small proportion of basal-cell carcinomas have point mutations in the *Ras*-homologues H-*ras* and K-*ras* (van der Schroeff *et al.*, 1990; Ananthaswamy & Pierceall, 1990; Lieu *et al.*, 1991). *p53* mutations have been identified in 50% of basal-cell carcinomas and 65% of these are of the UVB-type (Rady *et al.*, 1992; Ziegler *et al.*, 1993; van der Riet *et al.*, 1994; Urano *et al.*, 1995). This suggests a cooperation between either *Ras* or *p53* and *PTCH* in basal-cell carcinoma formation, although no experimental evidence for a direct interaction between these genes has yet been reported.

Melanoma-prone families

Melanoma is the most aggressive of skin cancers; it affects young individuals and much effort has been made to elucidate its development. Atypical or dysplastic naevi are major risk factors in both high-risk families and in the general population. Another significant risk factor is a positive family history of melanoma. Family cases, however, constitute only a small proportion (1–2%) of all cutaneous melanomas (Kefford *et al.*, 1999). In these families, melanoma susceptibility is enhanced by mutations in the cyclin-dependent kinase inhibitor *CDKN2A* and in the cyclin-dependent kinase *CDK4*. These genes have been found to confer elevated risk in 20–40% of melanoma-prone families. The genetic basis for melanoma-predisposition in the remaining 60–80% of families is not known (Kefford *et al.*, 1999). *CDK4* mutations are assumed to generate dominant oncogenes that are resistant to normal inhibition of the cell cycle by p16 (Zuo *et al.*, 1996). The *CDKN2A* gene encodes two distinct proteins which arise through alternative splicing. The p16INK4A protein regulates G1 phase exit by inhibiting the cyclin/CDK-mediated phosphorylation of the pRB protein. The other protein, p14ARF, acts via the p53 pathway to induce cell cycle arrest or apoptosis in response to hyperproliferative signals. Consequently, mutations in *CDKN2A* impair the function of both the p53 and the pRB pathways (Chin *et al.*, 1998). The involvement of these pathways in the pathogenesis of melanoma is underlined by the fact that cutaneous melanoma is the most common second cancer in individuals with inherited retinoblastoma and occurs also in families with Li–Fraumeni syndrome (Eeles, 1995; Moll *et al.*, 1997). The penetrance of *CDKN2A*

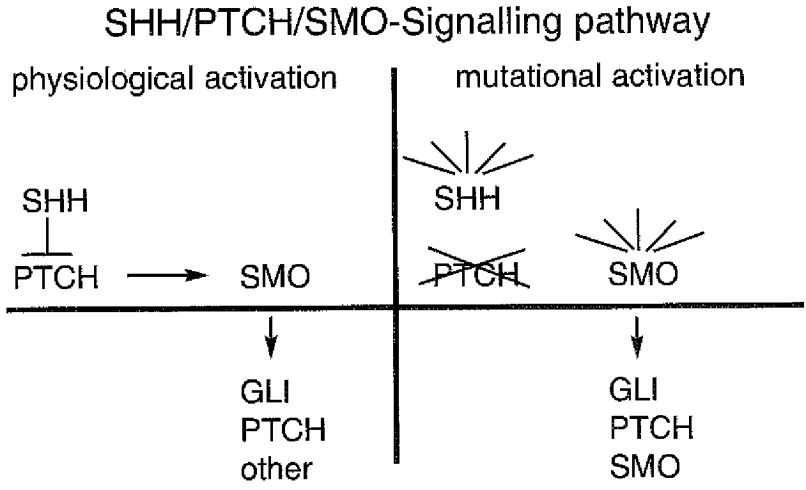

Figure 1. The SHH/PTCH/SMO signalling pathway plays a critical role in the development of basal-cell carcinoma.
Left panel: Physiological activation of the pathway occurs during embryogenesis and is triggered by the ligand of PTCH, SHH. Binding of SHH blocks PTCH, thereby activating SMO, which leads to expression of target genes. Right panel: Mutational activation of the pathway is accomplished by inactivating mutations in PTCH or activating mutations in either SHH or SMO. In basal-cell carcinomas, mutational activation of the pathway results in overexpression of GLI1, SMO and PTCH transcripts.

mutations is strongly influenced by the level of sun exposure and possibly by the action of unknown modifier genes (Goldstein *et al.*, 1998) (see also below, under *Allelic variants of tumour modifiers*). At the present time, routine clinical testing for CDKN2A mutations is not recommended (Haluska & Hodi, 1998) because of uncertainties regarding the penetrance and the correlation between *CDKN2A/CDK4* mutations and the clinical phenotype.

Low-penetrance susceptibility genes

Only 1% of cancer patients have a clearly identifiable inherited component (familial cases) (Fearon, 1997). The remaining 99% of cases are called 'sporadic'. However, there is increasing evidence that sporadic cancers also have heritable determinants that segregate within the general population and thus contribute to individual tumour susceptibility (Ponder, 1990). This appears to be true for skin cancers.

Allelic variants of effect-modulators of carcinogen exposure

UVB radiation (280–320 nm) directly damages DNA, whereas UVA radiation (320–400 nm) acts on DNA via an oxidative stress mechanism which results in the formation of reactive oxygen species in the skin. Reactive oxygen species damage DNA (as well as lipids and amino acids), leading to elevated mutation rates and tumorigenesis (Lear *et al.*, 2000). Detoxification of products of UV-induced stress is the task of numerous cellular proteins, which are also involved in the detoxification of exogenous chemicals. Many of these proteins demonstrate polymorphisms, some of which may result in reduced ability to remove potential carcinogens (Lear *et al.*, 2000). Much interest has been focused on polymorphisms in the *GSTM1* and *GSTT1* genes, which are both expressed in the skin. Homozygotes for the respective null alleles of both the *GSTM1* and *GSTT1* genes express no protein and exhibit greater skin cancer risk (Lear *et al.*, 2000) (Table 2). Further-

Table 2. Polymorphisms in detoxifying enzymes associated with increased basal-cell carcinoma number and accrual

GSTM1 null (in combination with skin type, male gender or tumour site)	Tumour number, accrual (Lear *et al.*, 1996, 1997)
GSTT1 null	Accrual (Lear *et al.*, 1996)
CYP2D6 extensive metabolizer	Tumour number, accrual (Lear *et al.*, 1996)
*CYP1A1*m1m1	Tumour number (Lear *et al.*, 1996)
*CYP1A1*Ile/Val and *CYP1A1*Val/Val	Accrual (Lear *et al.*, 1996)
NQO1 null	Tumour number (Clairmont *et al.*, 1999)

more, *GSTP*, another member of the glutathione *S*-transferase (GST) family of genes, may play an important role in skin cancer development, since homozygous GSTP knock-out mice develop up to 10 times more skin tumours (papillomas) than wild-type-mice (Henderson *et al.*, 1998).

Genes of the cytochrome P450 (*CYP*) family encode enzymes with mono-oxygenase activity. They metabolize a wide range of structurally diverse substrates and participate in the defence against oxidative stress. Many of the *CYP* genes are polymorphic and some of the polymorphisms have significant phenotypic consequences. For example, mutations in *CYP2D6* lead to extensive, intermediate or poor metabolizer phenotypes (Smith *et al.*, 1995). In patients with basal-cell carcinomas, the CYP2D6 extensive metabolizer phenotype has been associated with increased tumour number and accrual (Lear *et al.*, 1996) (Table 2). The latter phenotypic features have also been associated with specific polymorphisms in *CYP1A1* (Table 2). In addition, either *CYP2D6* extensive metabolizer phenotype, *GSTM1* null allele or male gender have been shown to significantly reduce time of presentation of further tumours in patients with truncal basal-cell carcinomas (Lear *et al.*, 1997).

Another antioxidant enzyme which protects cells against reactive oxygen species is NAD(P)H:quinone oxidoreductase (NQO1). A significant association between *NQO1* null allele and basal-cell carcinoma number has been described (Clairmont *et al.*, 1999). The elucidation of the specific roles of detoxifying enzymes and the determination of their contribution to overall susceptibility to skin cancer is becoming a hot topic in the field of skin cancer predisposition.

Allelic variants of tumour modifiers

Tumour modifiers are thought to segregate within a population and to play major functions in determination of individual tumour susceptibility. Most of the information about tumour modifiers of skin cancer has been obtained from studies using laboratory animals. In contrast to *Mus musculus*, *Mus spretus* mice are resistant to chemically induced skin cancer (Nagase *et al.*, 1995). Using a large (*NIH/Ola* × *Mus spretus*)F_1 backcross, it was possible to identify several quantitative trait loci involved in the regulation of skin cancer incidence or multiplicity. Three of these map to loci harbouring members of the family of cyclin-dependent kinase inhibitors p57Kip2, p21Waf1 and p27Kip2 (Nagase *et al.*, 1999).

Mapping of additional loci that modulate skin cancer susceptibility should be possible using the carcinogenesis-resistant (Car-R) and carcinogenesis-susceptible (Car-S) mice which have been obtained applying bi-directional selective breeding. In an initiation (7,12-dimethylbenz[*a*]anthracene)/progression (12-*O*-tetradecanoylphorbol 13-acetate) protocol for tumour induction, skin papillomas occurred in 100% of Car-S mice compared with 3.3% of Car-R mice (Saran *et al.*, 1999).

Conclusions

The agents used today for prevention of skin cancer are either sunscreens or antioxidants. Sunscreens reduce the formation of pyrimidine dimers and are undoubtedly effective in preventing sunburn.

However, epidemiological and laboratory studies indicate that sunscreens may not prevent melanoma formation (Ley *et al.*, 1997).

Only in a minority of skin cancers does the family history show a clear inherited component. For the majority of cases, the identification of the underlying genetic risk factors might be of tremendous value for preventive therapy. The assessment of the individual genetic risk factors for skin cancer requires:

(*a*) association studies using polymorphisms in genes which confer risk for skin tumours (e.g., *GST, CYP*);

(*b*) identification of predisposing polymorphisms in genes which are known to be defective in heritable diseases (e.g., *PTCH, CDKN2A*);

(*c*) identification of new skin cancer susceptibility genes using human and murine genetic studies.

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Corresponding author:

H. Hahn
Institute of Pathology,
Technical University Munich/GSF-Research Center for Environment and Health,
Ingolstädter Landstrasse 1,
85764 Neuherberg,
Germany