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 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 (Leat 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;
Biomarkers in Cancer Chemoprevention

Table 1. Inherited diseases with increased skin cancer susceptibility

<table>
<thead>
<tr>
<th>Disease</th>
<th>Gene defect or gene locus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oculocutaneous albinism</td>
<td>TYR, P gene</td>
</tr>
<tr>
<td>Xeroderma pigmentosum</td>
<td>XP genes</td>
</tr>
<tr>
<td>Xeroderma pigmentosum variant</td>
<td>DNA polymerase eta</td>
</tr>
<tr>
<td>Nevoid basal-cell carcinoma syndrome</td>
<td>PTCH</td>
</tr>
<tr>
<td>Cutaneous malignant melanoma</td>
<td>CDKN2A, CDK</td>
</tr>
<tr>
<td>Inherited retinoblastoma</td>
<td>pRB</td>
</tr>
<tr>
<td>Li–Fraumeni syndrome</td>
<td>p53</td>
</tr>
<tr>
<td>Rombo syndrome</td>
<td>?</td>
</tr>
<tr>
<td>Bazex syndrome</td>
<td>Xq</td>
</tr>
<tr>
<td>Multiple self-healing epithelioma</td>
<td>9q31</td>
</tr>
<tr>
<td>Muir–Torre syndrome</td>
<td>MSH2, MLH</td>
</tr>
<tr>
<td>Cowden’s syndrome</td>
<td>PTEN</td>
</tr>
</tbody>
</table>

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 \( \varepsilon \)ta (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-
Genetically determined susceptibility markers in skin cancer

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.

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

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Tumour number; accrual (Lear et al., 1996, 1997)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTM1 null</td>
<td>Tumour number; accrual (Lear et al., 1996)</td>
</tr>
<tr>
<td>GSTT1 null</td>
<td>Accrual (Lear et al., 1996)</td>
</tr>
<tr>
<td>CYP2D6 extensive metabolizer</td>
<td>Tumour number, accrual (Lear et al., 1996)</td>
</tr>
<tr>
<td>CYP1A1 M11</td>
<td>Tumour number (Lear et al., 1996)</td>
</tr>
<tr>
<td>CYP1A1 Val/Val and CYP1A1 Val/Val</td>
<td>Tumour number (Lear et al., 1996)</td>
</tr>
<tr>
<td>NQO1 null</td>
<td>Tumour number (Clairmont et al., 1999)</td>
</tr>
</tbody>
</table>

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)F1 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, p21Wafl 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.

References


Genetically determined susceptibility markers in skin cancer


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