High risk groups in cutaneous oncology
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SUMMARY AND CONCLUSIONS

Aim of the study

Cutaneous neoplasms represent 10-50% of all malignancies occurring in Caucasians. About 90% of these cutaneous neoplasms are non-melanoma skin cancers: basal cell carcinoma (BCC) or squamous cell carcinoma (SCC). Several factors are known to increase the risk of development of cutaneous carcinomas. In general the most important factor in Caucasians is the degree of sunlight exposure, especially UV-B irradiation, which acts as an initiator and promoter of carcinogenesis. Dysfunction of immune surveillance is thought to be another, possibly important, factor, as a deficient immune response can facilitate tumor growth (pseudo-promotor effect). Evidence in support of this concept, derived from animal and clinical studies, has been presented indicating that the risk of development of tumors induced by oncogenic viruses or physical - and possibly also chemical - carcinogens is increased by a deficient immune response (Introduction). In patients with multiple carcinomas, in whom an impaired immune response might enhance tumor growth, oral retinoid treatment may be effective in the prevention of tumor growth (Introduction).

In the studies collected in this thesis three hereditary afflictions were investigated, in which the number of cutaneous carcinomas frequently ranges from >10 to >100: the Basal cell nevus syndrome, Xeroderma pigmentosum and Epi- dermodysplasia verruciformis. The principal characteristics of these disorders are listed in Table 1.

The Basal cell nevus syndrome (BCNS) is a rare autosomal dominant hereditary disorder, which is relatively frequently encountered in the Northern provinces of the Netherlands. Its main features are multiple BCCs in about half of the patients with BCNS (sometimes already present in large numbers in the first decade of life), milia, palmoplantar pits, jaw cysts (keratocysts), skeletal abnormalities and ectopic calcification. These features suggest that BCNS can be partly defined as a genetically determined epidermal cell differentiation disorder. Agressive growth of the BCCs generally does not occur until after puberty and is mostly restricted to sunlight or ionizing radiation exposed skin areas. No other studies are known concerning the immune response of patients with BCNS, except for a Russian study which is difficult to interpret. Systemic treatment with the retinoids isotretinoin and etretinate appeared to be effective in the prevention of tumor growth in some BCNS patients, as suggested by the literature.
Xeroderma pigmentosum (XP) is a rare autosomal recessive hereditary disorder, characterized clinically by cutaneous and ocular photosensitivity, cutaneous pigmentary changes and a propensity for the development of oculo-cutaneous malignancies, particularly SCCs and BCCs in sun-exposed areas of the body. The basic anomaly of XP is defective UV light induced DNA excision repair. The increased frequency of UV light induced mutations, probably a result of the DNA repair defect, is thought to be responsible for the development of neoplasms in XP. The results of the few immunologic studies performed in XP are conflicting. Normal and abnormal results have been found using DNBC sensitization and lymphocyte proliferation tests. In several XP patients reported in the literature a dose-dependent preventive effect on tumor growth of systemic retinoid treatment with etretinate has been observed.

Epidermodysplasia verruciformis (EV) is caused by a persistent cutaneous infection with certain, EV-specific, human papillomaviruses (HPVs), generally in association with an impaired immune response. This rare disease is thought to be an autosomal recessive or possibly X-linked hereditary disorder. In about 30% of the EV patients reported in the literature multiple cutaneous SCCs developed, mostly on sun-exposed skin areas. DNA of EV-specific HPVs (especially HPV5 and 8) has been detected in SCCs of some EV patients. Thus, circumstantial evidence suggests that certain HPVs are potentially oncogenic. Other oncogenic factors appear to be sunlight-exposure and probably the impaired immune response. Two studies reported in the literature suggested that systemic retinoid treatment with etretinate has a positive effect on the immune response of EV patients.

The investigations reported in this thesis were centred around two themes: a) impairment of immune surveillance, possibly implicating enhancement of tumor growth; b) treatment and prevention of tumor growth by systemic retinoid medication.

a) First we investigated whether the immune response was impaired in 14 patients with BCNS (chapter 1) and 1 patient with XP (chapter 2.1), assessing 1) the in vivo cellular immune response by a semiquantitative DNBC sensitization test, 2) the in vivo humoral immune response by measuring the antigen-specific antibody response after immunization with the primary immunogen Helix pomatia Haemocyanin (HPH), 3) the in vitro lymphoproliferative response induced by mitogens, irradiated allogeneic lymphocytes and HPH, 4) phytohaemagglutinin-induced T-cell cytotoxicity in 2 BCNS patients with multiple BCCs (chapter 1). In addition, a quantitative and morphologic in situ analysis of epidermal Langerhans cells was performed in patients with BCNS (chapter 1) and XP (chapter 2.1), as these cells appear to be involved in the immunologic response against cutaneous carcinomas.

Next we investigated 2 patients with EV and an impaired immune response.
The quantity and morphology of epidermal Langerhans cells was studied in situ in one EV patient (chapter 3.1), as these cells might be involved in the antigen-specific immune response to the persistent HPV infection. Viral analysis was performed of an invasive SCC of the second EV patient (chapter 3.2), in order to provide further circumstantial evidence, that certain HPVs can be oncogenic in EV. Finally a genetic study was performed of an EV patient (chapter 3.1), in order to investigate whether EV is a chromosomal instability disease. Chromosomal instability, present in XP and ataxia telangiectasia, probably increases the risk of cancer and can be associated with an impaired immune response.

b) The value of systemic retinoid medication for the therapy and prevention of multiple cutaneous carcinomas was investigated in 3 patients with BCNS (chapter 1) and in 1 patient with XP (chapter 2.1). The influence of systemic retinoid medication on the immune response was also investigated in these patients (chapter 1 and chapter 2.1) and in 1 EV patient (chapter 3.1). Furthermore, a study was performed in order to determine whether retinoids affect the basic anomaly of XP, i.e. defective UV light induced DNA excision repair (chapter 2.2).

Finally, (potential) hepatologic side effects of systemic retinoid treatment, particularly of long-term treatment, were evaluated (chapter 4). The occurrence of severe liver test disturbances associated with short-term retinoid treatment with etretinate was analysed in two patients (chapter 4.1). Hepatologic side effects of long-term retinoid treatment were analysed in 20 patients without liver test abnormalities (chapter 4.2 and chapter 4.3), using 1) light microscopy of liver tissue, also stained to visualize Ito cells, 2) measurement of splenic pressure in order to detect portal hypertension and 3) quantitative analysis of retinoids in serum, liver tissue, subcutaneous fat and epidermis-dermis samples of patients treated with etretinate, retinoic acid and isotretinoin.

Summary of the results

The results of the studies reported in chapter 1, 2.1 and 3.1 are summarized in Table 1. These studies focused on the immune response and the clinical and immunologic effect of systemic retinoid treatment of patients with BCNS, XP and EV.

Chapter 1 deals with the results of the studies concerning BCNS. 14 BCNS patients were screened for the presence of decreased immune responsiveness, which might enhance the development of the multiple BCCs occurring in 8 of
Table 1. Characteristics of three hereditary disorders with a propensity for the development of multiple cutaneous carcinomas. This table also represents the results of investigations related to these disorders and reported in this thesis.

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Basic abnormality</th>
<th>Type of skin carcinoma</th>
<th>Suggested role of UV light</th>
<th>CMI type</th>
<th>Retinoid treatment in vivo/in vitro</th>
<th>Cutaneous carcinomas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal cell nevus syndrome</td>
<td>cell differentiation disorder</td>
<td>basal cell carcinoma</td>
<td>promoter/promo-promptor</td>
<td>N⁺ (\uparrow) N⁻ (\downarrow)</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Xeroderma pigmentosum</td>
<td>UV light induced DNA repair disorder</td>
<td>squamous cell carcinoma, basal cell carcinoma</td>
<td>initiator/promo-promptor</td>
<td>1 (\uparrow) N⁻ (\downarrow)</td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td>Epidermodysplasia verruciformis</td>
<td>persistent infection with oncogenic viruses (HPVs) (^*)</td>
<td>squamous cell carcinoma</td>
<td>co-initiator/co-promo-promptor</td>
<td>1** (\uparrow) 1** (\downarrow)</td>
<td>±</td>
<td>?</td>
</tr>
</tbody>
</table>

Encircled data are data, which were confirmed by investigations reported in this thesis.

\(^*\) CMI: Cell Mediated Immunity (non-HPV-specific).

\(^\dagger\) N: Normal.

\(^\ast\) HPV: Human Papilloma Virus.

\(^\ast\ast\) In 2 patients from the literature, one with and one without cutaneous carcinomas, the CMI response in vivo and in vitro was normal.
these patients. In addition the clinical and immunologic effect of retinoid treatment with etretinate was assessed in 3 BCNS patients with multiple BCCs. Immunologic evaluation showed: 1) a normal DNCB skin test score in vivo of all 6 BCNS patients without multiple BCCs, but a decreased score of 4 of the 8 BCNS patients with multiple BCCs; 2) a normal antigen-specific humoral immune response in vivo after immunization with HPH of 3 patients with multiple BCCs, except for a decreased IgG-class antibody response of 2/3 patients; 3) a normal in vitro lymphoproliferative response after stimulation with mitogens, allogeneic lymphocytes and HPH of 5 patients with multiple BCCs; 4) normal PHA-induced lymphocyte cytotoxicity of 2 patients with multiple BCCs; 5) a normal number of T6+ and HLA-DR+ epidermal Langerhans cells in clinically normal skin from the forehead and back of 6 patients with multiple BCCs; 6) epidermal buds of BCC tissue in clinically normal skin from the forehead of 2 patients with multiple BCCs.

Retinoid treatment with etretinate of 3 patients with multiple BCCs (dosage 0.3-1.0 mg/kg/day) supported the notion of a preventive effect on the development of BCCs, which lasted until about 9 months after discontinuation of treatment. The therapeutic effect of etretinate on clinically apparent BCCs was limited, recurrences occurring in all patients. Hepatitis, probably etretinate-induced, occurred in one patient and was associated with massive recurrences of BCCs. Etretinate treatment did not markedly affect the immunologic parameters followed, i.e. the lymphoproliferative response in vitro and phytohaemagglutinin-induced T-cell cytotoxicity.

In conclusion: a) the immune response of the BCNS patients tested was normal, except for a possible defect of the cellular immune response in vivo in some patients with multiple BCCs, which might be due to the epidermal cell differentiation disturbance of BCNS, b) systemic retinoid medication with etretinate was of preventive value in the treatment of the multiple BCCs, which frequently occur in BCNS patients and did not affect the immune response.

Chapter 2.1 describes the immunologic and clinical investigations of a patient with XP, belonging to complementation group C and treated with the retinoid etretinate. During a Northern winter (in order to minimize the effect of sunlight-exposure) immunologic evaluation showed: 1) a decreased number of peripheral blood lymphocytes with relatively normal T-cell subset percentages; 2) a decreased antigen-specific humoral and cellular immune response in vitro as measured by the class-specific antibody response after immunization with HPH and the DNCB sensitization test; 3) an in vitro lymphoproliferative response after stimulation with mitogens and allogeneic lymphocytes, which was in the lower range of normal; 4) a decreased number of T6+ and HLA-
DR+ Langerhans cells per mm² of epidermis on the forehead compared to
the back, supposedly due to sunlight exposure, which might impair the local
immune response to tumor growth; 5) HLA-DR expression of epidermal ker-
ratinocytes in association with the presence of Leu-3a+ and OKT-8+ T-cells
in the papillary dermis, which is a common reaction pattern of unknown signi-
ficance.8

Clinical observation during 5 years suggested that treatment with the reti-
noid etretinate, 0.9 mg/kg/day, prevented the growth of cutaneous carcino-
mas. Retinoid treatment did not markedly affect the immunologic param-
ters, except for a transient stimulation of the mitogen induced in vitro lymphoproliferative response. Spontaneous corneal perforation, the finding of a keratoconus and the beneficial influence of corneal transplantation stressed
the need for ophthalmologic follow-up.

In conclusion: a) immunologic investigations of this XP patient during a
Northern winter indicated a suboptimal immune response, which might en-
hance the growth of the oculocutaneous UV light induced neoplasms; b) sys-
temic retinoid treatment with etretinate appeared to be effective in the pre-
vention of growth of cutaneous carcinomas of this XP patient and exerted a
transient effect on the immune response.

Chapter 2.2 shows the results of a study concerning the possible effect of reti-
noid treatment on the basic anomaly of XP, i.e. defective UV light induced
DNA excision repair. The rate of unscheduled DNA synthesis after in vitro
UV light exposure of fibroblasts from a XP patient and a control was not in-
fluenced by the addition of different concentrations of retinoic acid to the me-
dium in which the fibroblasts were cultured. This suggests that the prophylac-
tic effect of retinoid treatment on tumor growth in XP patients is not likely to
be due to modulation of DNA repair capacity.

Chapter 3.1 describes an immunohistologic, immunologic and cytogenetic
study of a negroid EV patient with cutaneous SCCs and an impaired immune
response, as measured by the in vitro lymphoproliferative response and the
dNCB sensitization test in vivo, who was treated with the retinoid etretinate.
The results showed: 1) the presence of microscopic EV lesions in clinically
normal skin from the forehead; 2) the virtual absence of T6+ and HLA-DR+
Langerhans cells in the koilocytic areas of epidermis histologically involved
with EV, koilocytosis being a HPV-induced specific cytopathic effect; 3) a
change in the in situ pattern of T6+ and HLA-DR+ epidermal Langerhans
cells in epidermis histologically involved with EV, i.e. the presence of these
cells in koilocytic areas, after 8 days of consecutive retinoid treatment; 4) an
increase of the in vitro phytohaemagglutinin- and concanavalin A-induced
lymphoproliferative response after 10 consecutive days of retinoid treatment; 5) no abnormalities in the frequency of sister chromatid exchanges, the number of spontaneous and UV light induced chromosomal breaks and the rate of UV light induced unscheduled DNA synthesis.

These results suggest: a) a deficient role of epidermal Langerhans cells in the HPV-specific cellular immune response in EV; b) a modulatory effect of the retinoid etretinate on the immune response; c) that increased chromosomal instability is unlikely to be the cause of EV, while the absence of cell hypersensitivity, already shown by other investigators, was confirmed.

Chapter 3.2 describes a virologic study of a Caucasian EV patient with multiple SCCs and an impaired immune response, who also developed a primary hepatocellular carcinoma associated with a chronic hepatitis B virus infection. Five different HPV5 were detected in the benign disseminated skin lesions of EV, i.e. HPV5, 8, 17, 19 and 24, the latter three being newly characterized HPVs. HPV5 DNA sequences were demonstrated in the central part of an invasive cutaneous SCC. This case provides an example of the circumstantial evidence which suggests that certain types of HPV are potentially oncogenic and stresses the importance of immune surveillance in the protection against virus-associated tumors.

Chapter 4.1 deals with hepatologic side effects of short-term systemic retinoid treatment with etretinate. Two patients are described in whom an histologically confirmed, clinically inapparent and reversible hepatitis occurred within the first months after introduction of etretinate therapy. Causes of hepatitis other than etretinate were not found. Reintroduction of etretinate resulted in reactivation and/or persistence of the hepatitis. These data strongly suggest that the hepatitis in both patients was caused by etretinate. No liver test disturbances were observed during treatment of one of these patients with 13-cis-retinoic acid.

Chapter 4.2 deals with hepatologic side effects of long-term systemic retinoid treatment of 13 patients without liver test abnormalities treated with etretinate (n=6), all-trans-retinoic acid (RA; n=6) and 13-cis-retinoic acid (13-cis-RA; n=1). In routine histological sections of liver tissue no abnormalities were observed apart from slight steatosis in some cases. Perisinusoidal fibrosis was notably absent. The number of Ito cells, i.e. perisinusoidal hepatic cells with fat-storing and fibrogenetic capacities, which can be visualized by light microscopy using accumulation of lipid droplets within these cells as a marker, was increased in 6/6 etretinate and in 5/6 RA patients. Lipid droplets causing protrusion of Ito cells into the sinusoidal lumen were observed in 3/6
etretinate and 3/6 RA patients. Thin-needle splenic pressure measurements in 2 etretinate patients with such protruding Ito cells showed slightly elevated values, indicative of portal hypertension. This finding is unlikely to have serious consequences, as the risk of complications of portal hypertension is very low at this pressure level. These results indicate that the risk of serious hepatologic side effects of long-term systemic retinoid treatment, such as have been seen to occur in hypervitaminosis A, i.e. fibrosis and portal hypertension, are minimal.

In chapter 4.3 the results are reported of a quantitative analysis of serum, liver tissue, subcutaneous fat and epidermis-dermis samples of 20 patients treated with etretinate (n=9), RA (n=9) and 13-cis-RA (n=2). In liver tissue of etretinate treated patients accumulation of presumably etretinate derived metabolites including the main metabolite Ro 10-1670 was found, whereas subcutaneous fat showed accumulation of etretinate. Similar findings were made in tissue samples from RA treated patients, also showing the presence of metabolites in liver tissue and the presence of RA itself in subcutaneous fat. Tissue samples did not show detectable amounts of 13-cis-RA. These quantitative data appear to correlate with the histological findings presented in chapter 4.2, both favouring accumulation of metabolites of etretinate and probably also of RA in liver tissue, possibly mainly in Ito cells.

Conclusions

The investigations reported in this thesis were centred around two themes: a) impairment of immune surveillance, possibly implicating enhancement of tumor growth; b) treatment and prevention of tumor growth by systemic retinoid medication. The conclusions drawn from the results of the investigations are presented for each theme separately.

Regarding the first theme the following is concluded:

1) The immune response of BCNS patients is not impaired except for a possible defect in the delayed type hypersensitivity response of some BCNS patients with multiple BCCs. One could speculate whether such a defect can be explained by immunologic dysfunction of the keratinocyte, as the cause of the cutaneous symptoms of BCNS appears to be an epidermal cell differentiation disorder.

2) The immune response of XP patients can be impaired and the number of T6+ and HLA-DR+ epidermal Langerhans cells in sunlight exposed skin of XP patients can be decreased, even in the winter season with a relatively low
Ire measurements were slightly elevated and unlikely to have serious hypertension is very likely to have severe hepatic impairment, such as have portal hypertension. Analysis of serum, liver biopsy was performed in 20 patients treated with etretinate. Liver tissue of etretinate derived metabolism was found, whereas subcutaneous fat. Tissue rings were made in presence of metamereous fat. Tissue and these quantitative data are presented in chapter 9 and probably
and two themes: a) enhancement of tumor growth by systemic retinoid treatment, b) the investigations

1) except for a possible defect of some BCNS patients, such a defect can be ascribed to the degree of cell differentiation and the number of light exposed skin of patients with a relatively low amount of sunlight exposure. Both findings might be associated with enhancement of tumor growth. The immune response is not impaired in all XP patients, however. The presence or absence of an impaired immune response in XP may be linked with the severity of the clinical picture, i.e. to the degree of sensitivity to UV-induced cell damage.

3) Data from the literature indicating that the non-HPV-specific immune response is impaired in most EV patients, were confirmed. An immunohistologic study suggested a deficient role of the epidermal Langerhans cell in the HPV-specific cellular immune response in EV. A cytogenetic and cell biological study appeared to exclude chromosomal instability and cell hypersensitivity as the cause of EV. A virologic study demonstrated the presence of DNA of an EV-specific HPV (HPV5) in an invasive SCC of an EV patient, who also developed a primary hepatocellular carcinoma associated with a persistent hepatitis B virus infection. These data provide further circumstantial evidence for the potential oncogenicity of certain HPVs and stress the importance of immune surveillance in the protection against virus-associated tumors.

Regarding the second theme the following is concluded:
1) Systemic retinoid treatment with etretinate appeared to prevent growth of cutaneous carcinomas in patients with BCNS and XP. The limited therapeutic effect of etretinate treatment on clinically detectable BCCs and the risk of recurrence does not justify treatment of these carcinomas with systemic retinoid medication.
2) Systemic retinoid treatment with etretinate did not have any consistent effect on the immune response of BCNS patients. The suboptimal in vitro lymphoproliferative response of a XP and an EV patient appeared only transiently stimulated by etretinate treatment.
3) Retinoic acid did not affect defective UV-light induced DNA excision repair in vitro, the basic disorder of XP. Enhancement of normal cell differentiation may be the main factor responsible for the tumor prophylactic effect of retinoid treatment.
4) The retinoid etretinate can induce hepatitis without the first few months after starting treatment, which appears to occur in about 1% of the patients. In patients with normal liver tests the risk of serious hepatologic side effects of long-term systemic retinoid treatment appears to be minimal.

Future investigations may answer some of the many questions left regarding the role of immune surveillance in cutaneous oncology, particularly questions concerning the immune response of patients with BCCs and questions in the field of photoimmunology. Dynamic studies may shed light on the interaction
of the immune system in man and sunlight exposure. The role of human papillomaviruses in oncogenesis is actively investigated in many centres, also in the Netherlands, especially as regards the role of these viruses in the aetiology of carcinoma of the cervix uteri. The Basal cell nevus syndrome is an interesting clinical model, in which the possible role of oncogenes can be studied.

REFERENCES