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Immunohistochemical Detection of p53 in Epidermal Proliferations Overlying Dermatofibromas

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Basaloid proliferations of epidermis overlying dermatofibromas and morphologically resembling superficial basal cell carcinomas might possess a malignant potential. In order to elucidate whether these basaloid proliferations share phenotypic characteristics with malignancy, we examined immunohistochemically 19 cases of dermatofibroma with overlying epidermal basaloid proliferations, 10 dermatofibromas with overlying simple epidermal proliferations, and 10 invasive basal cell carcinomas for expression of p53.

Simple and basaloid proliferations showed sparse positive immunostaining for p53, as seen in normal epidermis. No differences in staining pattern or number of positive keratinocytes could be demonstrated between these conditions. The dermatofibromas were negative. The invasive basal cell carcinomas showed abundant p53 positivity.

The lack of p53 immunoreactivity in the epidermal basaloid proliferations overlying dermatofibromas indicates that these lesions have not acquired a phenotype as seen in malignant conditions.

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Epithelium overlying dermatofibromas often shows hyperplasia or acanthosis, and epidermal proliferations which morphologically resemble superficial basal cell carcinomas appear in 2–5% of the lesions (1). These epidermal proliferations have an aberrant expression pattern of β-2-microglobulin that is similar to the expression pattern in basal cell carcinomas (2). In rare cases invasive basal cell carcinoma with ulceration has been associated with dermatofibromas (3).

Mutation of the p53 gene is one of the most frequent genetic abnormalities in human cancers, and accumulation of the p53 protein has been found in premalignant conditions as well as in a wide variety of human cancers (4–7), including basal cell carcinoma (8). Studies have shown that cells with inactivation of the p53 possess growth advantage over their neighbours (9). The p53 gene, which was initially considered to be an oncogene, has tumour suppressor properties (10–12). The abrogation of the tumour suppressor activity of the gene most often occurs through point mutations and expression of conformationally altered and functionally defective proteins. The wild-type p53 protein is virtually undetectable immunohistochemically, because of its short half-life and low amount present (13), whereas the majority of mutated proteins have a longer half-life permitting their detection by immunohistochemistry.

In this study the immunohistochemical expression of p53 protein in epidermal basaloid proliferations overlying dermatofibromas was examined, to elucidate whether these epidermal changes might be premalignant lesions.

Nineteen dermatofibromas with overlying epidermal basaloid proliferations, 10 dermatofibromas with overlying simple hyperplasia and/or acanthosis, 10 basal cell carcinomas, and 4 cases with normal epidermis were included in this study.

Five-μm sections were cut, dewaxed, rehydrated, and pretreated in a microwave oven twice for 5 min in citrate buffer. Sections incubated with anti-p53 were incubated overnight at 4°C. Anti-p53 (DO-7, DAKO, Glostrup, Denmark), an IgG_2 monoclonal mouse antibody directed against both the wildtype and the mutated p53 protein, was used in a 1:100 dilution. The antibody was diluted in TRIS-buffered saline (TBS), pH 7.6, with azide/bovine albumin. Since the avidin-biotin-complex (ABC) technique was used, the second layer was biotinylated rabbit-anti-mouse immunoglobulin (DAKO, Copenhagen, DK) used in a 1:400 dilution. The sections were then incubated with avidin-biotin peroxidase (ABC)-complex (DAKO, Glostrup, Denmark) for 30 min. Between incubations the sections were washed 3 times in TBS, pH 7.6, for 5 min. The colour was developed using 3-amino-9-ethylcarbazole (AEC). Sections were counterstained with Mayer's haematoxylin. Sections from a primary colon carcinoma and a primary breast carcinoma, known to show strong positive immunoreactivity for p53, were used as positive controls in each run. First and second level negative controls were included. These sections were negative.

Only cells with distinct nuclear staining for p53 were recorded as positive.

In normal epidermals p53 positivity was located primarily in the basal and the parabasal keratinocytes. Positive cells were scattered sparsely throughout the epidermis, but less than 1% of the keratinocytes were p53-positive. In the simple and the basaloid hyperproliferations, p53-positive cells were scattered sparsely through the lesions (Fig. 1). No differences in the staining pattern and in the number of positive keratinocytes could be demonstrated between the lesions. The underlying dermatofibromas were all negative.

Nine out of 10 basal cell carcinomas showed strong p53 positivity, in particular at the periphery of the tumour islets (Fig. 2). In these tumours more than 50% of the tumour cells were positive. In one basal cell carcinoma positive tumour cells occurred sparsely scattered throughout the tumour islets. Less than 1% of the tumour cells were positive. In all cases with basal cell carcinomas p53 immunoreactivity in the normal adjacent epidermis appeared in the keratinocytes, as described above.

Most reports in the literature analysing the p53 gene sequence in parallel with immunohistochemistry conclude that most mutant p53 proteins can be detected by immunohistochemistry. However, false negative cases may be caused by total deletion

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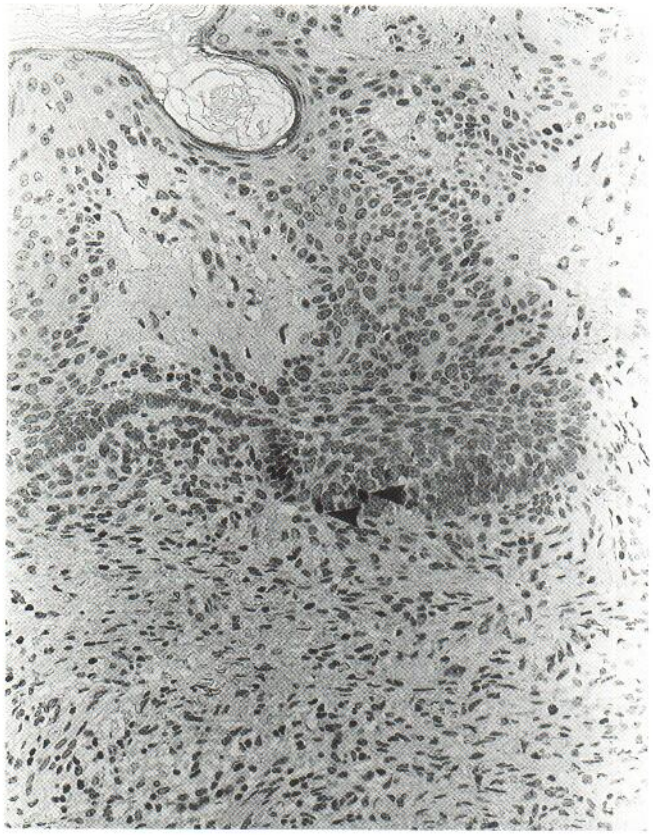

Fig. 1. Epidermal basaloid proliferation overlying a dermatofibroma, showing positive immunoreactivity for p53 in a few cells in the periphery of the lesion. *Arrows* point at p53-positive keratinocytes (ABC-technique, ×200).

of the gene, or the tumour may contain a point mutation which may not sufficiently stabilise the protein to reach detectable levels by immunohistochemistry (14).

In this study the keratinocytes in normal epidermis showed sparse positive p53 immunoreactivity primarily in the basal and parabasal layers. This finding is in accordance with the finding of Hall et al. (15) who showed that normal skin can accumulate wild-type p53 protein as a physiological response to UV-irradiation. The immunodetection level for p53 is lowered by the use of highly sensitive immunohistochemical techniques and antigen retrieval methods such as microwave irradiation (16, 17). These factors can explain the presence of p53 positivity in normal epidermis found in this study.

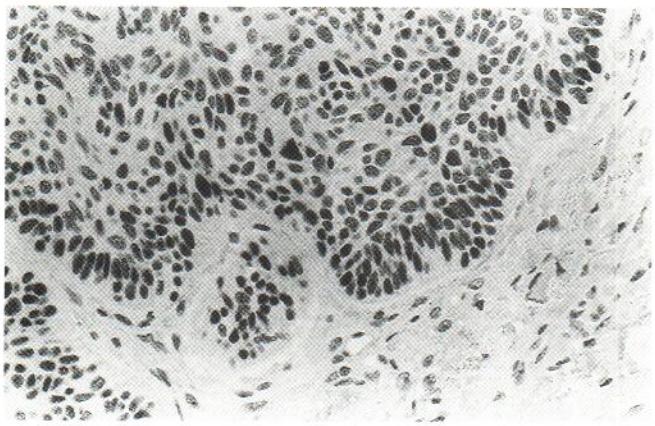

Fig. 2. An invasive basal cell carcinoma showing p53 overexpression, mainly at the periphery of the tumour islets (ABC-technique, ×200).

The presence of p53 immunoreactivity in the simple and the basaloid proliferations demonstrated in this study may reflect an association with the proliferative capacity of these conditions, since accumulation of p53 protein occurs in non-neoplastic rapidly proliferating conditions (14). The underlying dermatofibromas did not, as expected, show any positive p53 immunostaining.

Over-expression of p53 immunoreactivity appeared in 9 out of 10 basal cell carcinomas. This is in accordance with a study by Shea et al. (8) who examined 41 basal cell carcinomas, out of which 31 (83%) were p53-positive, and the p53 immunoreactivity also appeared at the periphery of the tumour islets.

Basal cell carcinomas more often develop in sun-exposed areas of the skin and in elderly persons contrary to dermatofibromas. Certain mutagens can produce characteristic patterns of DNA alterations, and recently it has been shown that certain types of DNA alterations characteristic of UV-irradiation are present in basal cell and squamous cell carcinoma of the skin. Thus the strong p53 positivity found in the basal cell carcinomas may reflect this phenomenon. In contrast, the sparse p53 positivity demonstrated in the basaloid proliferations is probably a result of the immunohistochemical technique used and/or the proliferative rate of the lesion. Previous studies have demonstrated that microwave processing before incubation with the primary antibody lowers the threshold for detection of the protein. It has also been demonstrated that accumulation of the wild-type p53 protein can occur in proliferative conditions. Thus the positivity demonstrated in the basaloid proliferations is considered as the presence of the wild-type p53 protein.

It is also noteworthy that the adjacent normal epidermis in the lesions with basal cell carcinomas or basaloid proliferation showed the same pattern of immunostaining and the same number of p53-positive keratinocytes. Thus, although we did not have information on the age of the patients or location of the lesions, it is less likely that the difference in p53 expression found in basal cell carcinomas and epidermal basaloid proliferation overlying dermatofibromas is biased or misinterpreted by differences in these factors.

In conclusion, the lack of p53 immunoreactivity in the epidermal basaloid proliferations overlying dermatofibromas, although morphologically resembling basal cell carcinomas, indicates that these lesions have not acquired a phenotype as seen in malignant lesions.

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