

Ki-67 and p53 Expression in Cutaneous Bowen's Disease: An Immunohistochemical Study of Fixed-embedded Tissue Sections

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The monoclonal Ki-67-specific MIB-1 and p53 protein-specific DO-1 antibodies were used to identify proliferating cell fractions on microwave-pretreated paraffin sections of 7 cutaneous Bowen's disease specimens. A high Ki-67 score was characteristic of all cases examined, and significant p53-positivity was seen in 4 cases. In the peritumoral, histologically normal epidermis, Ki-67- and p53-positive cells were frequently present, in one case with very high scores (89% as well as 82%, respectively). These findings indicate an increase in the proliferative activity of the Bowen cells (high Ki-67 score) and that p53 mutations are frequently found in this disease. The p53 expression was not related to the Ki-67 score. The expression of Ki-67 and p53 in morphologically normal epidermal cells is also discussed. The histologically normal epidermal cells expressing p53 and Ki-67 antigens may correspond to pre-malignant clones.

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The Ki-67 antigen is a cell cycle marker, which can be readily detected using immunohistochemical methods, and is valuable for assessing the growth fractions of tumours, since it occurs only in the nuclei of proliferating cells (1, 2). The recently described MIB-1 monoclonal antibody reacts with a formalin-resistant epitope of the Ki-67 antigen (3–6).

The p53 phosphoprotein is a tumour suppressor gene product and a negative regulator of the cell cycle (7–9). A mutation of the p53 gene may lead to cancer (10). The DO-1 monoclonal antibody recognizes both the wild-type and the mutant forms of the p53 protein (11). The normal protein has a very short half-life, and thus the mutant form with its extended half-life can be detected immunohistochemically (12, 13). The epitope of the DO-1 antibody is also formalin-resistant (11).

We studied the patterns of expression of these two proliferating cell markers on Bouin's-fixed and paraffin-embedded tissue sections from cases of Bowen's disease. Our objective was to compare the distribution of Ki-67- and p53-positive cells in tumorous areas as well as in peritumoral, histologically normal epidermis.

MATERIALS AND METHODS

The tissue sections tested were prepared from routine Bouin's-fixed, paraffin-embedded samples of 7 cases of cutaneous Bowen's disease and were obtained from the Laboratory of Pathology at La Rochelle. Routine histological examinations were of haematoxylin-eosin-stained sections.

Immunohistochemical staining was performed on 4- μ m serial tissue sections mounted on chrome-alum-gelatin-coated slides and dried at

either 37°C overnight or at room temperature for 48 h. For good immunostaining we used a household microwave oven (Toshiba F21MA) to pretreat the de-waxed and trypsinized sections at 750 W for three times 5 min (14). For incubation in the microwave oven, we used a citrate buffer (6) as a substitute for widely used toxic, lead-containing solutions. We used the MIB-1 and DO-1 monoclonal antibodies (IMMUNOTECH, Marseille, France) and the streptavidin-biotin-peroxidase technique employing the Universal kit (IMMUNOTECH). Immunostaining with ascites fluid from non-immunized mice served as control.

The counting of positive nuclei was performed in the tumorous, as well as in histologically normal epidermal areas without atypical cells. At least 400 cells were counted per section and per area. The ratio of positive over total number of nuclei was expressed as percent Ki-67 or p53 index.

RESULTS

The immunohistological results are summarized in Table I. Immunostaining was nuclear with both MIB-1 and DO-1 antibodies and showed very heterogeneous distribution in the same specimen.

Tumorous areas

The Ki-67 score (% of positively stained cells) was high (20–58%) in the tumorous areas with heterogeneous distribution and staining patterns. Both atypical and histologically normal cells were positive (Fig. 1).

Increased expression of the p53 protein was found in 4 cases of Bowen's disease (29–57%).

Peritumoral, histologically normal epidermal areas

In the epidermis surrounding the tumour, the parabasal layer cells and some rare cells dispersed in the upper epidermal layers expressed Ki-67. In one case (Case 4) the Ki-67 score in the histologically normal epidermis was higher than in the tumour (89% and 58%, respectively).

In 3 cases, weak nuclear p53 positivity was seen in the histologically normal epidermis surrounding the tumour

Table I. Ki-67 and p53 immunostaining in Bowen's disease (% of cells showing nuclear immunostaining)

Case	Tumour		Peritumoral epidermis	
	p53	Ki-67	p53	Ki-67
1	<1	37	0	<1
2	29	28	0	<1
3	54	20	<1	<1
4	49	58	82	89
5	<1	26	0	<1
6	<1	57	<1	<1
7	57	45	<1	<1

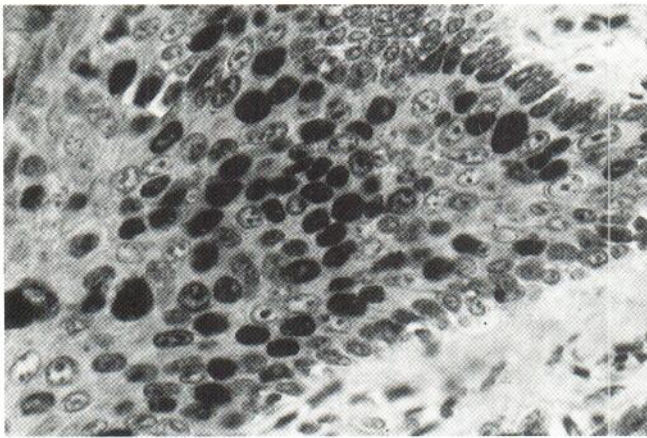


Fig. 1. Nuclear immunoreactivity for Ki-67 with the MIB-1 monoclonal antibody in Bowen's disease showing strong nuclear immunostaining of tumour cells.

(Fig. 2). However, in one case (Case 4), very strong p53 immunostaining was observed (82% of epidermal cells were stained) with a high Ki-67 index in the histologically normal epidermis (Fig. 3).

DISCUSSION

The high Ki-67 score in the cases studied corresponds to the high intra-epithelial proliferative activity of Bowen cells and of keratinocytes in the tumorous areas. In the surrounding epidermis, the Ki-67 positivity of suprabasal layer cells and of a few epidermal cells, located in the upper layers, was a characteristic finding. However, in one case (Case 4), we found a very high Ki-67 index (89%) with a high number of p53-positive cells (82%).

Using the p53-specific DO-1 antibody, the specimens were classed into two groups: 4 p53⁺ and 3 p53⁻ cases. The strong immunohistochemical staining may correspond to an overexpression of mutant-type p53 protein. Mutations of the p53 gene are frequently found in squamous cell carcinomas of the skin (15, 16). Molès et al. (16), using a combination of the polymerase chain reaction and single-stranded conformation polymorphism techniques, did not find p53 gene mutations in the 6 cases of Bowen's disease examined. In view of our immunohistochemical findings, a need for additional molecular biological analysis seems to be indicated.

The strong overexpression of the Ki-67 and p53 proteins in the peritumoral epidermis in one case (Case 4) of Bowen's disease and the presence of weakly staining Ki-67- and p53-positive cells in the histologically normal epithelium in the other specimens studied could be interpreted as positivity of pre-malignant clones (17). The finding of Barbareschi et al. (13) that the occurrence of p53-positive cells in skin and skin appendages, not associated with malignant lesions, suggests that p53 immunoreactivity may not be related to gene mutation, and that the positivity of normal cells might reflect a very high expression of wild-type p53 protein (13). The accumulation of p53 protein is a consequence of its stabilization. The frequently

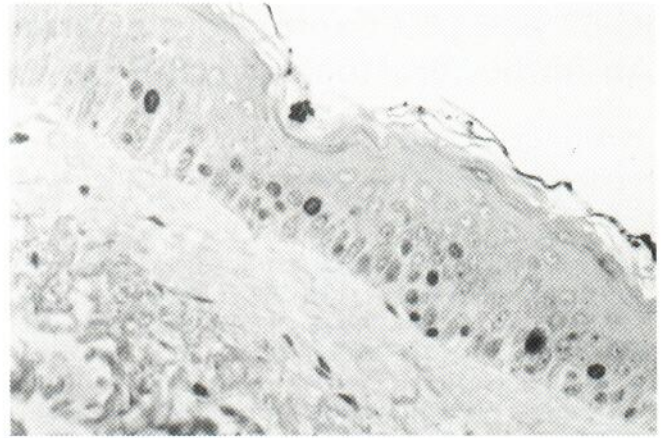


Fig. 2. The peritumoral, histologically non-neoplastic epidermis contains p53-positive nuclei.

observed point mutations of the p53 gene in human cancer are involved in the modification of its conformation and stability (9, 10). Its accumulation seems to be a common step in the development of most cancers; however, immunohistological identification of p53 may occasionally occur in non-malignant tissue (12, 18, 19). The observation of a large amount of p53 and Ki-67 in tumour cells and peritumoral, histologically normal epidermis in one case of our series is therefore in favour of a mutated p53 gene in the non-neoplastic cells and may reflect a failure in the control of cell proliferation. Further molecular biological studies will be necessary to clarify the significance of the weak positive immunostaining. Other antibodies which bind to different epitopes of the p53 protein may prove useful in identifying mutations in the p53 gene (9).

Cattoretti et al. (20) and Barbareschi et al. (21) reported positive correlation of p53- and Ki-67-immunostaining in breast cancer. Sasano et al. (22) suggested that p53 expression was strongly associated with proliferation of tumour cells detected in esophageal carcinoma, using Ki-67- and proliferating cell nuclear antigen (PCNA)-specific antibodies. Barbareschi et al. (23) in central nervous system tumours, as well as Monden et al. (24) in colorectal carcinomas reported the lack of association of p53 and PCNA expression. We did not find association of p53 expression with proliferative activity as detected by Ki-67 im-

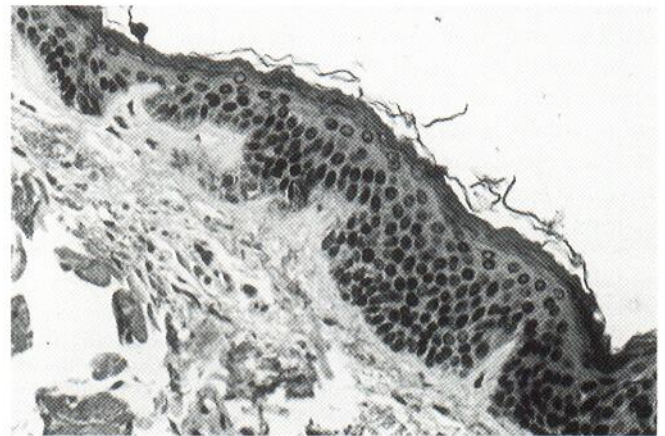


Fig. 3. Case 4, peritumoral epidermis. High p53 expression of the epidermal cells.

munostaining. Both p53⁺ and p53⁻ tumours exhibited high levels of Ki-67.

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