Bcl-2 overexpression in basaloid proliferations
overlying dermatofibromas and basal cell carcinomas

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Basaloid proliferations overlying dermatofibromas resembling superficial basal cell carcinomas have been interpreted both as reactive/regressive and frankly malignant. Basal cell carcinoma is a slow-growing tumour, which so far has been regarded as an actively proliferating lesion with a high apoptotic activity. We examined immunohistochemically 6 dermatofibromas with overlying simple hyperplasia, 12 dermatofibromas with overlying basaloid proliferations, and 24 basal cell carcinomas for expression of Ki-67 protein and bcl-2 protein. The Ki-67 labelling index represents an estimate of proliferative activity. Bcl-2 protein suppresses apoptosis. The Ki-67 labelling indexes of basaloid proliferations, basal cell carcinomas, and normal epidermis were similar (11–15%, p<0.05, Mann-Whitney test). Bcl-2 protein was expressed in all cells of basaloid proliferations, similar to the expression pattern in basal cell carcinomas. We suggest that basaloid proliferations overlying dermatofibromas might have achieved a phenotype that equals an early stage of BCC carcinogenesis.

Key words: Epidermal proliferation; dermatofibroma; basal cell carcinoma; bcl-2; immunohistochemistry.

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The epidermis overlying 2–8% of dermatofibromas shows basaloid proliferations that are morphologically indistinguishable from superficial basal cell carcinoma (4). These basaloid proliferations have an aberrant expression pattern of β2-microglobulin (18) and metallothionein (19) similar to that in basal cell carcinoma, but regarding p53 expression, they have not acquired the phenotype seen in basal cell carcinoma (6).

Basal cell carcinoma (BCC) is the most common form of cutaneous malignancy, and represents a slow growing type of neoplasm (12). Different methods of estimating proliferation fractions and cell cycling duration indicate that BCCs are actively proliferating lesions, whose proliferative activity equals or exceeds that of normal epidermis (7, 14, 20, 24), and the slow growth of BCCs has been explained by prominent cell death (9, 12). BCCs normally respond to most forms of local therapy, but one subgroup, the infiltrative and the morphea-like type, is more prone to recurrence (12).

The Ki67 protein is a nuclear proliferation associated protein that is expressed in cycling cells of late G1, S, G2, and M-phase. Even though little is known about the nature of the Ki67 protein, the Ki67 labelling index has for several years been used as a marker of proliferative activity, and numerous studies indicate that this

Received July 29, 1996.
Accepted October 10, 1996.
index is of prognostic relevance when studying human tumours (1). Most studies on Ki67 protein expression have been done on frozen materials. With the MIB1 antibody, it has become possible to examine formalin-fixed, paraffin-embedded tissue. Regarding the influence of antibody concentration on the immunohistochemical quantification of cell proliferation, the MIB1 (Ki67) labelling index has been shown not to be falsely elevated by high antibody concentrations (11). Furthermore, the MIB1 labelling index correlates well with the assessment of proliferation by BrdU incorporation (20).

Apoptosis is a physiological single cell death that differs from pathological cell death (necrosis) in its well-defined ultrastructural and biochemical features. In several human tissues, including epidermis, apoptosis represents an important homeostatic mechanism (5). The bcl-2 gene is a protooncogene that is involved in growth regulation by suppressing apoptosis (10, 16). The bcl-2 gene was first described in follicular non-Hodgkin’s lymphomas, where the neoplastic cells often possess a chromosomal translocation t (14, 18) which juxtaposes the bcl-2 gene to the immunoglobulin heavy chain region, resulting in overexpression of bcl-2 protein (10, 16). The bcl-2 gene product is an intracellular protein that is associated with mitochondrial membrane, nuclear envelope, and endoplasmic reticulum. It normally occurs in tissues characterized by apoptotic activity (10, 16). Overexpression of bcl-2 protein is reported in several malignant neoplasms, including BCC of the skin (2, 13, 15, 17, 22, 25).

In this study, we investigated the immunohistochemical expression of Ki-67 protein and bcl-2 protein in normal epidermis and bcl-2 protein in normal epidermis, simple hyperplasia overlying dermatofibromas, basaloid proliferations overlying dermatofibromas, and different types of BCC.

MATERIALS AND METHODS

The study comprised 6 cases of normal epidermis, 6 dermatofibromas with overlying simple hyperplasia, 12 dermatofibromas with overlying basaloid proliferations, and 24 BCCs (6 superficial, 6 nodular, 6 infiltrating and 6 morphea-like). The tissue was formalin-fixed and paraffin-embedded.

The primary antibodies were anti-Ki67-protein mouse monoclonal antibody MIB1 (Immunotech, Marseille, France) and anti bcl-2 protein mouse monoclonal antibody (clone 124, DAKO, Denmark). Sections (5µm) were deparaffinized, rehydrated, and pretreated in a microwave oven (650 Watts for 25 min) in Tris-HCl 0.05 M, pH 9.0. After cooling in buffer to room temperature, blocking of endogenous peroxidase, and preincubation (1% bovine albumin, A4503 Sigma, in TBS pH 7.6 for 10 min), sections were incubated with the primary antibody diluted in Tris-buffered saline (TBS) pH 7.6 with azide/bovine albumin for 30 min at room temperature. MIB1 was diluted 1:50, anti bcl-2 1:10. As the immunological reaction was visualized using a peroxidase-conjugated avidin-biotin-complex technique, the second layer was biotinylated rabbit anti-mouse immunoglobulin (E354 DAKO, Denmark), and the third layer was avidin-biotin-peroxidase complex (K 355, DAKO, Denmark). Between each step the sections were washed in TBS, pH 7.6. The colour was developed using 3-amino-9-ethylcarbazole, and the sections were counterstained with Mayer’s haematoxylin. A positive control was achieved by running a normal lymph node concurrently, and as a negative control the primary antibody was omitted.

The Ki67 labelling index was estimated by counting 10 randomly selected ocular counting frame-defined fields, or 20 in cases of normal epidermis and simple hyperplasia. The counting was performed using a ×100 objective, in each case with 200 to 500 cells. When counting normal epidermis and simple hyperplasia, the terminal differentiated non-cycling cells of the superficial layers were not included. Statistical analysis was carried out using the Mann-Whitney test.

RESULTS

Ki67

In normal epidermis and simple hyperplasia overlying dermatofibroma cells with Ki67 positive nuclei were only located in the basal and parabasal layers, which is in accordance with the fact that the more superficially located and terminal differentiated cells are non-cycling (8). For that reason, the Ki67 labelling indexes of normal epidermis and simple hyperplasia overlying dermatofibromas were estimated for the proliferating pool of cells, i.e. the basal and parabasal cells. In most cases of basaloid proliferations overlying dermatofibromas and BCC, the cells with Ki67 positive nuclei were randomly located, but sometimes they were located mainly at the periphery of the tumour islands. In
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TABLE 1. Ki-67 labelling index, i.e. percentage of tumour cell nuclei immunoreactive with MIB1 antibody, in normal epidermis, simple hyperplasia overlying dermatofibromas, basaloid proliferations overlying dermatofibromas, superficial basal cell carcinoma (BCC), nodular BCC, infiltrating BCC, and morphea-like BCC.

<table>
<thead>
<tr>
<th>Type of lesion</th>
<th>Number of cases</th>
<th>Ki-67 labelling index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mean</td>
</tr>
<tr>
<td>Normal epidermis</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>Simple hyperplasia</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>Basaloid proliferations</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Superficial BCC</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>Nodular BCC</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Infiltrating BCC</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>Morphea-like BCC</td>
<td>6</td>
<td>15</td>
</tr>
</tbody>
</table>

Differences do not reach statistical significance (p<0.05, Mann-Whitney test).

A balance between cell proliferation, terminal differentiation and cell death is essential to homeostasis in many tissues (5). Tumour growth has been described as a function of the rate of cell proliferation minus the rate of cell death (16).

Epidermis is known to be a quickly renewing tissue compartment, and our study of the Ki-67 labelling index indicates that BCC cells proliferate at the same rate as the proliferating pool of cells in the normal epidermis. This is in accordance with other studies on BCC proliferative activity (7, 14, 20, 24), using different methods such as tritiated thymidine incorporation (24), Ki-67 labelling index (7, 20), BrdU incorporation (14, 20), and in situ detection of histone mRNA (20). Furthermore, the Ki67 labelling index of malignant neoplasms such as metastatic malignant melanoma, 14.0% (21), and invasive breast carcinomas, 15.3–16.6% (1), does not in essence differ from that of BCC. Thus, we do not think that the slow clinical growth of BCC can be plausibly explained by a low proliferative activity.

Normal epidermis showed a weak bcl-2 protein immunoreactivity in the basal keratinocytes, or no reaction at all. This is in accordance with some earlier studies (15, 25), but not with others (2, 17, 22), which have described a consistent immunoreactivity in the basal keratinocytes. Since the same antibody (clone 124, DAKO, Denmark) is used in most of the studies, the discrepancy may be due to differences in fixation and/or staining procedures.

In agreement with other studies (2, 13, 15, 17,
we showed that BCC consistently had overexpression of bcl-2 protein. This finding has given rise to a hypothesis that explains the slow clinical growth rate of BCC by a low apoptotic activity, and suggests that deregulation of the bcl-2 gene is a crucial event in the development of BCC (2, 13, 15, 17, 22, 25). There is a disparity between this hypothesis and former assumptions that prominent cell death is one of the main causes of the slow growth rate of BCC (9, 12). Furthermore, the hypothesis indirectly assumes that BCC has a relatively low proliferative activity: a concept that is challenged by the present results and other studies on BCC.

Fig. 1. Bcl-2 immunoreactivity in simple hyperplasia overlying dermatofibroma. Note: Immunoreactivity in lymphocytes and melanocytes, and no reaction in keratinocytes. (Original magnification: ×400).

Fig. 2. Bcl-2 immunoreactivity in nodular BCC. Note: Prominent cytoplasmic and perinuclear staining in all tumour cells and no reaction in overlying epidermis. (Original magnification: ×400.)

Fig. 3. Bcl-2 immunoreactivity in basaloid proliferation overlying dermatofibroma. Note: Cytoplasmic and perinuclear staining as seen in BCC. (Original magnification: ×400.)
proliferative activity (7, 14, 20, 24). As a consequence of these reservations, we propose that the high bcl-2 protein expression in BCC represents a homeostatic feedback mechanism that is initiated to suppress a high apoptotic activity controlled by other means. Thus, in BCC, the bcl-2 protein is presumably ineffective in preventing apoptosis. This ineffectiveness can be explained by inhibition of the bcl-2 protein function or by the presence of a bcl-2-independent pathway to apoptosis (16). Inhibition of the bcl-2 protein function possibly occurs as a consequence of heterodimerization of the bcl-2 protein with gene products of other members of the bcl-2 gene family (10, 16). Bcl-2-independent pathways to cell death are, among others, complement-mediated lysis, cytotoxic T cells, amyloid-β peptide, tumour necrosis factor (TNF), and withdrawal of lymphokines (16). Examples of physiological regulation of bcl-2 protein expression are given in studies on lymphocytic differentiation indicating that both T- and B lymphocytes at some stages of development are able to regulate bcl-2 protein expression, presumably by translational and post-translational control mechanisms (3, 23).

The apparently less intensive bcl-2 protein expression in the more aggressive infiltrative and morphea-like BCCs as compared to the superficial and nodular BCCs, which was also found by Rodriguez-Villanueva et al. (17), possibly reflects either a less active apoptosis suppression or a partial loss of homeostatic feedback mechanism in these tumours.

Basaloid proliferations overlying dermatofibromas showed the same overexpression of bcl-2 protein as is seen in BCC. Regardless of whether the bcl-2 overexpression reflects a primary oncogenic event in the development of the BCC or a homeostatic response to apoptosis, it is interesting that an epithelial lesion, presumably induced by a paracrine signal from the underlying dermatofibroma, shares this phenotypical change with BCC. Previously, we have shown that basaloid proliferations are differentiated in the same way as BCC concerning β2-microglobulin (18), the invariant chain of the HLA-class-I molecule, and metallothionein (19), a possible marker of early carcinogenesis. Furthermore, after reporting one series of basaloid proliferations which did not have increased p53 protein expression (6), we have had one single case of basaloid proliferations with increased expression of p53 protein. The latter case might represent a coincidence of a true superficial BCC with a dermatofibroma, but it might also represent basaloid proliferations with the potential to develop into true BCC.

In conclusion, the present and previous immunohistochemical findings (6, 18, 19) show that basaloid proliferations share several pheno-typical characteristics with BCC. On the basis of these observations, we propose that basaloid proliferations might have achieved a phenotype that equals an early stage of BCC carcinogenesis.

This study was supported by a grant from Aage Bang’s Foundation to K.R. We thank Ms LaTease Copeland-Toft for checking the English of the manuscript.

REFERENCES


