ORIGINAL ARTICLE

EXPRESSION OF ANTIAPOPTOTIC PROTEIN B-CELL LYMPHOMA-2 IN CUTANEOUS BASAL CELL CARCINOMA

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Abstract

Purpose: Overexpression of antiapoptotic B-cell lymphoma-2 (Bcl-2) protein is one of the major contributors to oncogenesis and high levels have been identified in a variety of tumour types. We investigated an immunohistochemical expression of Bcl-2 protein in cutaneous basal cell carcinomas (BCCs) to elucidate whether there are differences in the expression pattern related to tumour growth phenotype.

Materials and Methods: The study group consisted of 45 cutaneous BCCs, which were categorised into the non-aggressive (NA-BCCs; 31 cases) and aggressive histologic variants (A-BCCs; 14 cases).

Results: There were 3 tumours (6.6%) with negative staining and 42 tumours (93.4%) with positive staining for Bcl-2 protein, 10 of which (23.8%) displayed low and remaining 32 cases (76.2%) exhibited high expression. All three "Bcl-2 negative" BCCs showed aggressive-growth features (infiltrative subtypes). When Bcl-2 values were evaluated as negative/low versus high expression, there was significantly lower Bcl-2 protein expression in the A-BCCs compared to the NA-BCCs. Even an intensity of immunostaining showed a tendency of being weaker in the A-BCCs. In spite of that, three infiltrative BCCs showed a diffuse strong immunoreactivity.

Conclusion: An immunohistochemical positivity of Bcl-2 protein in the neoplastic cells of cutaneous BCC was nearly constant feature, and its decreased staining was associated with an infiltrative growth pattern. It suggests that a low Bcl-2 protein expression in tumor tissue might be considered an unfavorable prognostic indicator.

Key words: Basal cell carcinoma, B-cell lymphoma-2 protein, biological behavior

Introduction

Continuous programmed cell death (apoptosis) is crucial for maintaining tissue homeostasis.[1] An important component of the apoptotic pathway is a diverse group of proteins commonly known as the B-cell lymphoma-2 (Bcl-2) protein family. Some of them have pro-apoptotic effects (e.g., Bax, Bak, and Bok), while another (e.g., Bcl-2, Bcl-xL, and Bcl-w) possess antiapoptotic (pro-survival) properties.[1] Since the Bcl-2 family is a key regulator of apoptosis, the abnormalities in its function have been implicated in many human diseases including the cancer development.[1] The antiapoptotic protein Bcl-2 is an important member of the Bcl-2 family, which controls the release of pro-apoptotic factors responsible for the activation of caspases by stabilising the mitochondrial outer membrane.[1] It is located in the endoplasmic reticulum, mitochondria, and nuclear envelope membranes. Overexpression of Bcl-2 protein is one of the major contributors to oncogenesis.[1] Until now, the Bcl-2 expression status detected by immunohistochemistry has been reported as a valuable prognostic indicator in many tumours including hematological malignancies, prostate cancer, lung cancer, ovary cancer, or breast cancer.[2] Due to the prominent role in inhibiting apoptosis, Bcl-2 protein has been recognised as promising target for the development of novel anticancer drugs, for example, Obatoclax, Gossypol, and its derivatives.[2]

Basal cell carcinoma (BCC) is the most common skin cancer in humans, which typically grows slowly, expands

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only locally and virtually never metastasizes.[3] One of the explanations for such indolent and prognostically favorable behavior of a majority of BCCs is a high apoptotic rate of tumour cells.[4] A number of papers addressing an immunohistochemical expression of Bcl-2 protein in cutaneous BCC have been published so far.[5-16] Although this field has been quite well documented in literature, some authors have reported contradictory results. For example, the frequency of Bcl-2 protein staining in BCC has been widely varied from 39% to 100% in published series. Further, while the majority of papers have revealed, the Bcl-2 immunoreactivity was significantly stronger and more diffuse in non-aggressive (NA) BCC variants compared with aggressive counterparts,[6,8,10,12,15] some authors did not confirm such conclusions.[5,11,13] Herein, we investigated an immunohistochemical expression of Bcl-2 protein in a set of cutaneous BCCs to elucidate, how often does it occur and whether there are differences in the expression pattern related to tumour growth phenotype.

Materials and Methods

Biopsy samples from 45 cases of cutaneous BCCs from 41 patients were enrolled into the study. All specimens were histopathologically investigated at the Department of Pathology in Faculty Hospital in Žilina (Slovakia). We selected the specimens of the BCC of various histological subtypes. As reported previously,[10] they were divided into two subgroups. The first one comprised 31 NA BCC subtypes (NA-BCCs; i.e., superficial, superficial-nodular, and nodular), while the second one included 14 aggressive BCC subtypes (A-BCCs; i.e., infiltrative and nodular-infiltrative) having an infiltrative growth pattern.

Biopsy specimens were routinely processed and immunohistochemically stained for Bcl-2 protein according to manufacturer's instructions and finally evaluated in the light microscope. Specific monoclonal mouse antibody against the Bcl-2 protein (clone 124, DAKO, ready to use) was used for staining. Positive reaction on stromal lymphocytes served as internal control. After including a total percentage of immunolabeled tumour cells, according to previous papers,[8,16] we adopted following scoring system: Negative score 0 (≤5% positive cells), score 1+ (6–25% positive cells), score 2+ (26–50% positive cells), score 3+ (51–75% positive cells), and score 4+ (≥76% positive cells). We considered a tumour to be Bcl-2 positive if it had a score at least 1+. If the proportion of stained cells did not exceed 5% (score 0), the result was classified as negative. Low expression encompassed scores 1+ and 2+ and high expression referred to scores 3+ and 4+. For statistical analysis, the cases were merged into two separate categories: (a) Negative/low expression and (b) high expression. In addition, an intensity of immunostaining was categorised as weak (when a majority of immunolabeled tumour cells was apparently weaker than stromal lymphocytes) and strong (when a majority of neoplastic cells stained with the same intensity like stromal lymphocytes).

Data were collected in a databank, using software SPSS Statistics. For the statistical analysis, Chi-square test was employed and $P < 0.05$ was considered to indicate statistical significance.

Results

In our series, there were 3 tumours (6.6%) with negative staining (score 0) and 42 tumours (93.4%) with positive staining for Bcl-2 protein, 10 of which (23.8%) displayed low and remaining 32 cases (76.2%) high expression [Figure 1]. Within the"Bcl-2 positive" subset of cancers, a cytoplasmic immunoreactivity varied in the range of 10–100% of total tumour tissue (mean value 70%). It was weak in 19 lesions (45.2%) and strong in 23 lesions (57.5%).

All three "Bcl-2 negative" BCCs showed aggressive-growth features (infiltrative subtypes) [Figure 2]. In some

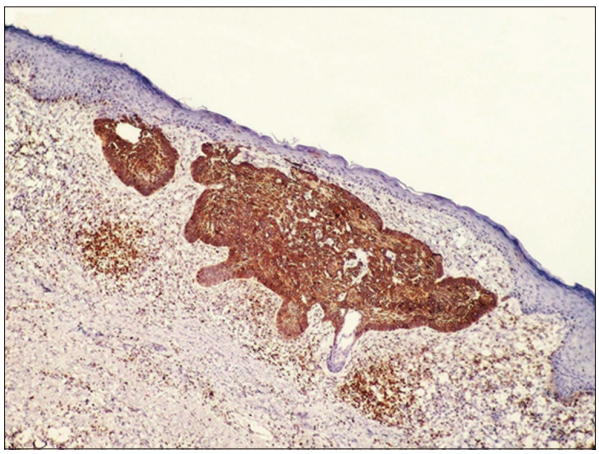

Figure 1: Diffuse strong expression (score 4+) of Bcl-2 protein in superficial basal cell carcinoma (magnification ×40)

BCCs with mixed nodular-infiltrative growth pattern, it seemed to be stronger and more expressive Bcl-2 protein staining in nodular component, compared with deeper infiltrative neoplastic formations [Figure 3].

When we statistically assessed two categories of Bcl-2 protein status in tumour tissue, i.e., negative/low expression versus high expression, we have confirmed a correlation between them and both, the NA-BCC and A-BCC variants. Bcl-2 protein expression was significantly lower in the A-BCCs compared to the NA-BCCs ($P < 0.001$). Even an intensity of immunostaining showed a tendency of being weaker in the A-BCCs (strong intensity found in 28.5% of the cases) than in the NA-BCCs (strong intensity seen in 61.3% of the cases). In spite of that, three infiltrative BCCs showed a diffuse strong immunoreactivity [Figure 4].

In the adjacent non-neoplastic surface epidermis and epidermis of the hair follicles, Bcl-2 protein immunoreactivity was confined to basal keratinocytes, while the suprabasal and upper layers were consistently negative [Figure 5]. A summary of the immunohistochemical findings in our set of BCCs is presented in Table 1.

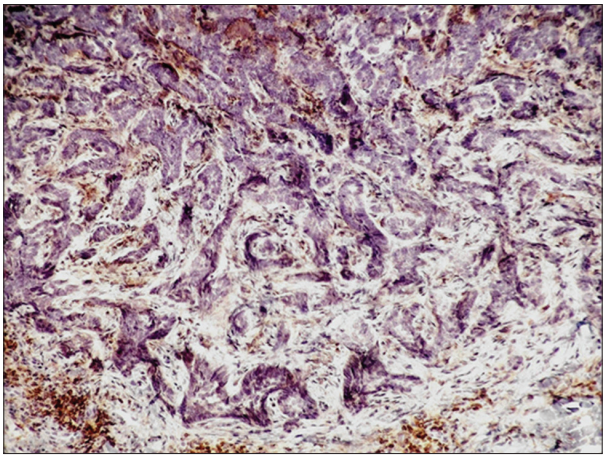

Figure 2: Negative staining (score 0) for B-cell lymphoma-2 protein in infiltrative BCC (magnification ×100)

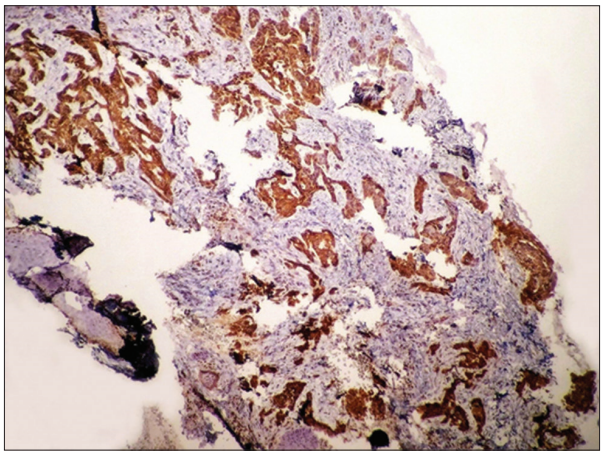

Figure 4: Diffuse strong expression (score 4+) of B-cell lymphoma-2 protein in infiltrative basal cell carcinoma (magnification ×40)

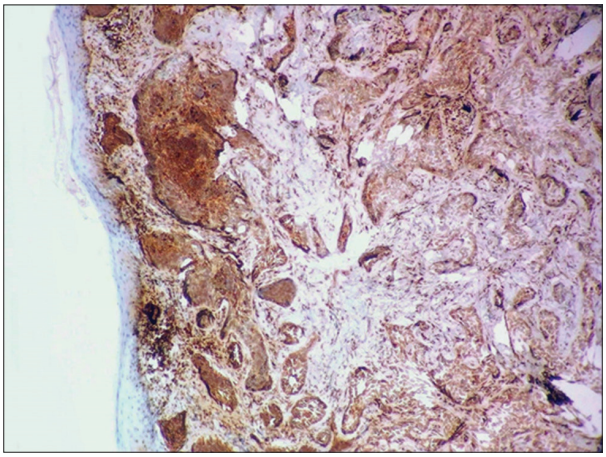

Figure 3: Stronger and more expressive immunoreactivity for B-cell lymphoma-2 protein in the nodular tumor component beneath the epidermis (left) compared to more deeply situated infiltrative tumor component (right) (magnification ×40)

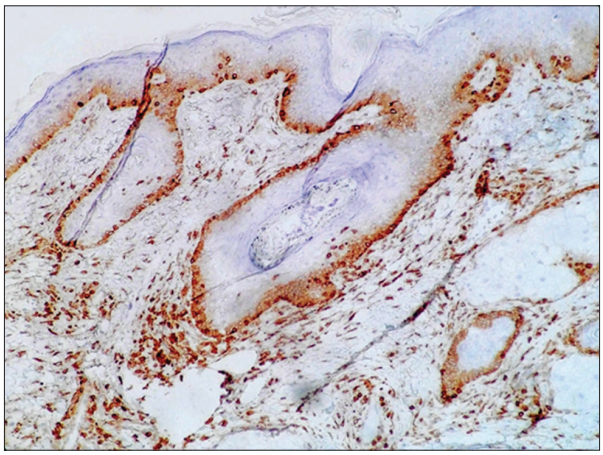

Figure 5: In non-neoplastic skin, the surface epidermis and the epidermis of the hair follicles express B-cell lymphoma-2 protein only in the basal layer. Stromal lymphocytes are also positive (magnification ×120)

Table 1: A summary of the immunohistochemical findings in our set of 45 BCCs we investigated

BCC phenotype	Negative Bcl-2 expression	Low Bcl-2 expression	High Bcl-2 expression
Non-aggressive (31 cases)	0 (0%)	4 (12.9%) - weak (4 cases) - strong (no case)	27 (87.1%) - weak (8 cases) - strong (19 cases)
Aggressive (14 cases)	3 (21.4%)	6 (42.9%) - weak (6 cases) - strong (no case)	5 (35.7%) - weak (1 case) - strong (4 cases)

BCCs: Basal cell carcinomas

Discussion

The Bcl-2 proto-oncogene is a key inhibitor of apoptosis, playing a crucial role in the maintenance of physiological balance between cell survival and loss. It has been reported in literature[5-8,10-16] that overexpression of antiapoptotic Bcl-2 protein is very frequent or even constant histopathological finding in cutaneous BCCs. The current study describes

immunohistochemical expression status of Bcl-2 protein in a panel of 45 human cutaneous BCCs. We have found an immunoreactivity (exceeding 5% of entire tumour tissue) in 93.4% of the cases. It is in accordance with many previous studies which have revealed, this feature was present in 68–100% of all BCCs investigated.[5-8,10-13] The only exception is the work published by Cho *et al*.,[9] who showed just 39% of "Bcl-2 positive" BCCs in a collection of 33 cases. In general, common Bcl-2 protein immunoreactivity in cutaneous BCC probably reflects its histogenetic origin. In normal skin, "Bcl-2 positive" keratinocytes are found in the basal layer of the epidermis and hair follicles, which represent the major proliferative cellular compartment, from which the squamous epithelium continuously renews itself. Permanent cytoplasmic production of Bcl-2 protein serves as a protector of the basal cells from death after apoptotic stimuli. As BCC of the skin is currently thought to originate from pluripotential cells in the basal layer of the epidermis and hair follicles,[3] Bcl-2 protein expression has been constantly detected in the vast majority of the cases. This is in contrast with cutaneous squamous cell carcinoma (SCC), which is presumed to originate from the suprabasal (more differentiated) keratinocytes.[5,12] This cell population, as well as cutaneous SCC, do not express Bcl-2 protein.[5,7,11]

Biologic and prognostic impact of Bcl-2 protein production in the neoplastic cells of cutaneous BCC is not entirely elucidated. The majority of papers have confirmed[6,8,10,12,15] that an immunohistochemical expression was higher in the NA-BCCs and lower in BCCs with aggressive histomorphologic phenotype. For example, in a series of 50 excisional biopsy samples, Bozdogan *et al*.[10] detected Bcl-2 protein expression in 83.9% of the NA-BCCs but only in 42.1% of the A-BCC specimens. Ramdial *et al*.[8] investigated a set of 50 clinically NA and 25 clinically aggressive BCCs (including tumour recurrences). They showed that low Bcl-2 protein labelling was a statistically significant feature of the A-BCCs. Puizina-Ivić *et al*.[12] revealed that morphoeic BCCs showed reduced amount of Bcl-2 protein, compared with solid, adenoid, and cystic BCC variants. Similar data have been published by Crowson *et al*.[6] and Sivrikoz and Kandiloğlu.[15] However, another researchers did not confirm such conclusions. For instance, Zheng *et al*.[11] investigated 47 BCCs and they did not found significant differences in Bcl-2 protein expression among the histologic subtypes. Even Verhaegh *et al*.[5] examined 20 BCCs and they observed no staining differences between solid and morphoeic growth patterns. In addition, Corrêa Mde *et al*.[13] seen the most expressive immunostaining of Bcl-2 protein in sclerosing BCC subtype, a lower in nodular and the weakest in superficial BCC subtype. Therefore, this relationship seems to be far from being clearly understood. Discordant literature data may be the result of different number of tumour lesions investigated, different sample processing methodology, or disunity in the interpretation of Bcl-2 protein immunopositivity. Consistently with the first group of researchers,[6,8,10,12,15] we detected significantly decreased Bcl-2 protein labelling in the A-BCCs, while the NA-BCCs overexpressed it. Although there was only small number of tumours belonging to the A-BCC category, we suppose that decreasing in Bcl-2 protein

production in cutaneous BCC is associated with more aggressive biological behavior of tumour. On the other hand, a few cases of infiltrative BCCs with diffuse strong immunostaining for Bcl-2 protein suggest that a certain subset of BCC behave aggressively without any loss of Bcl-2 protein production. Selection bias of biopsy samples is most likely the reason, why some studies did not see differences in Bcl-2 protein expression among the histologic subtypes of cutaneous BCC. From a practical point of view, it should be interesting to observe, how does Bcl-2 protein expression change after a local application of drugs containing substances with pro-apoptotic effects. Vidal *et al*.[17] evaluated an effect of imiquimod administration on the expression of selected biomarkers in BCC tissue. They found that an application of this remedy was significantly associated with decreasing of Bcl-2 protein immunoreactivity and increasing of apoptotic index. In a similar study, Urosevic *et al*.[18] compared biopsies from six patients with BCC before and after treatment with imiquimod. At the baseline level, BCC demonstrated strong Bcl-2 protein immunoreactivity. After treatment, Bcl-2 protein staining in cancer decreased substantially or become negative in four of six subjects. One case demonstrated no change, but interestingly, another one showed an increase in tumour Bcl-2 protein immunoreactivity. Otsuka *et al*.[19] assessed biopsy samples from five individuals with BCC before and after 4 weeks of therapy with Hedgehog pathway inhibitor. In all cases, they found dramatic reduction of Bcl-2 protein in cancer. A decreased production of Bcl-2 protein after local administration of that drugs indicates that they cause the tumour to be less resistant (i.e., more susceptible) to pro-apoptotic signals by altering programmed cell death/survival homeostasis.

Conclusion

In the current paper, a decreased immunohistochemical staining of Bcl-2 protein in cutaneous BCC was associated with an infiltrative growth pattern. It suggests that a low Bcl-2 protein expression in tumour tissue might be considered an unfavorable prognostic indicator. Although the main limitation of our study was that the aggressive and NA-BCC subsets were segregated only on the basis of histomorphology with lack of clinical correlations, we suppose, an assessment of Bcl-2 protein can be of use in biopsy practice. For example, it might be useful in selecting those BCC patients, who could benefit from a potential Bcl-2 family targeting therapeutical strategy. Further, incompletely excised BCCs with a markedly reduced Bcl-2 expression would preferably be the subject for a subsequent surgical reexcision due to their greater probability of local recurrence.

Conflict of Interest

The authors declare that they have no conflict of interest.

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