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Original Article

Assessment of BCL-2 expression in actinic keratosis, keratoacanthoma and seborrheic keratosis cases

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Abstract

Aim: One of the main functions of apoptosis is the elimination of cells with damaged DNA, including premalignant cells in skin. Apoptosis is regulated by the balance between antiapoptotic and proapoptotic proteins. The bcl–2 protein has been shown antiapoptotic effect. It protects cell against apoptosis induced by different death-inducing signals.

Material and Methods: In this study the authors have analyzed imunohistochemically the expression of bcl-2 protein in the histopathological variants of the precancerous lesion [actinic keratosis (AK) (28 cases) and keratoacanthomas (KA) (12 cases) and seborrheic keratosis (SK) (22 cases) in skin.

Results: In cases of AK, bcl-2 expression was confined to basal cell layer, as well as in two cases of thorough epidermis. SK expression of bcl–2 protein was in areas of basaloid proliferation.KA expression of bcl-2 protein was not dome-shaped keratin-filled crater areas but bcl-2 expression was confined of non crater areas.

Conclusion: In conclusion, bcl-2 expression supports the observation that tumor cells are derived from basal keratinocytes in AK and SK and not support the observation that tumor cells are derived from basal keratinocytes in KA.

Keywords: bcl-2; Actinic Keratosis; Keratoacanthoma; Seborrheic Keratosis.

INTRODUCTION

Skin is a multilayered organ that protects the body from the external environment. The outer layer of the skin comprises the epidermis. The epidermis contains epidermal keratinocytes and low numbers of melanocytes distributed towards the basal layer (1).

Seborrheic keratosis (SK) is a benign skin tumor that may be observed in all body regions. It is more commonly observed in areas exposed to the sun. It is more commonly described in males and increases with age (2). Actinic keratosis (AK) and keratoacanthoma (KA) are tumors associated with sun exposure (3-5).

Apoptosis is regulated by the balance between antiapoptotic and proapoptotic proteins. Bcl-2 is an antiapoptotic protein preventing cell death. It protects the cell against different death-inducing signals. The Bcl-2 gene family (bcl-2, bcl-xL, bax, bak, bad) have a determinative role in apoptosis regulation (3-5). Bcl-2 is expressed in tissue during physiologic and pathologic events in the intrauterine and extrauterine period, and is an apoptosis inhibitor ensuring cell viability without causing cell proliferation (5,6). It prevents or clearly reduces cell death begun by activation of intrinsic and extrinsic pathways by various stimuli like radiation, ultraviolet (UV), p53, chemotherapeutic medication and reactive oxygen radicals (1,6).

This study aimed to analyze the staining features in actinic keratosis, keratoacanthoma and seborrheic keratosis of immunohistochemical expression of Bcl-2.

MATERIAL and METHODS

This study assessed a total of 62 cases including 28 actinic keratosis (AK) cases, 12 keratoacanthoma (KA) cases and 22 seborrheic keratosis (SK) cases. Ethics committee approval was obtained from the Clinical Studies Ethics Committee of Ordu University, Faculty of Medicine on 16.04.2015 and numbered 2015/4. Tissue samples were reviewed in hematoxylin-eosin slides and appropriate paraffin blocks were chosen. From the selected blocks,

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3-micron thickness sections were taken on polylysine slides. Tissues had Bcl-2 monoclonal antibody (dilution rate: 1/400) applied. Immunohistochemical staining was applied by deparaffinization, dehydration and incubation in buffered citrate. An Ultra Vision Polyvalent, HRP-AEC kit (Neomarkers-Biogen, Lab Vision Corp. USA) was used for antigen staining with Bcl-2 (mouse) protein. The obtained slides were assessed with a light microscope. Lymph node was stained for positive control (Figure 1c). Slides were graded according to the staining level in the epidermis (Figures 1-3).

The assessment of distribution of Bcl-2 protein in the skin, including the epidermis and adnexal structures may be evaluating the potential contribution of Bcl-2 expression to the development of cutaneous lesion. Bcl-2 is expressed in basal keratinocytes, melanocytes, outer root sheath and dermal papillae of hair follicles. Interestingly, it is expressed epithelial cells of the secretory coils of eccrine sweat glands and in cells located in the excretory ducts of sebaceous glands. Bcl-2 protein is expressed moderate or high levels of basal cell proliferation (7).

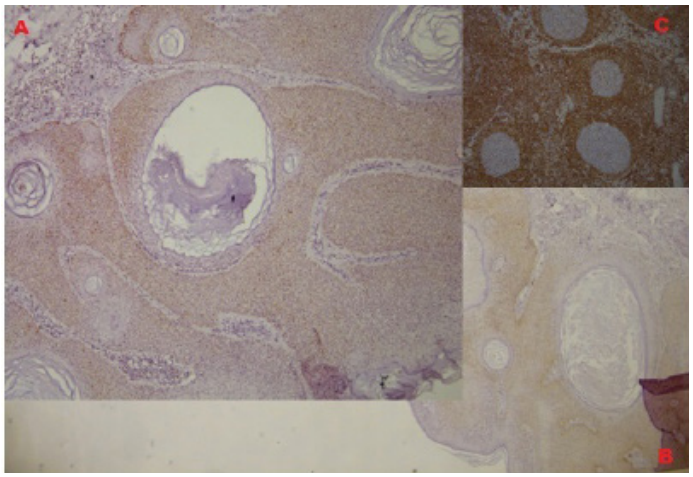

Figure 1. Nearly full-layer Bcl-2 expression in basaloid cells in seborrheic keratosis (A-large picture x40, B-small picture x100, C-right top corner, control lymph node)

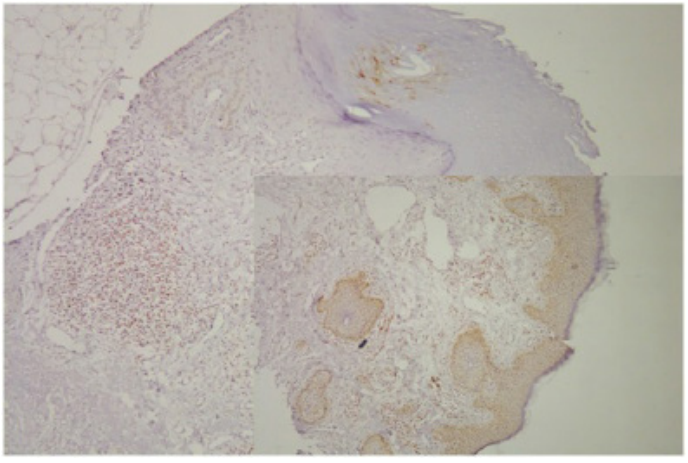

Figure 2. Bcl-2 expression in basal cells in actinic keratosis (large picture x100, small picture x100)

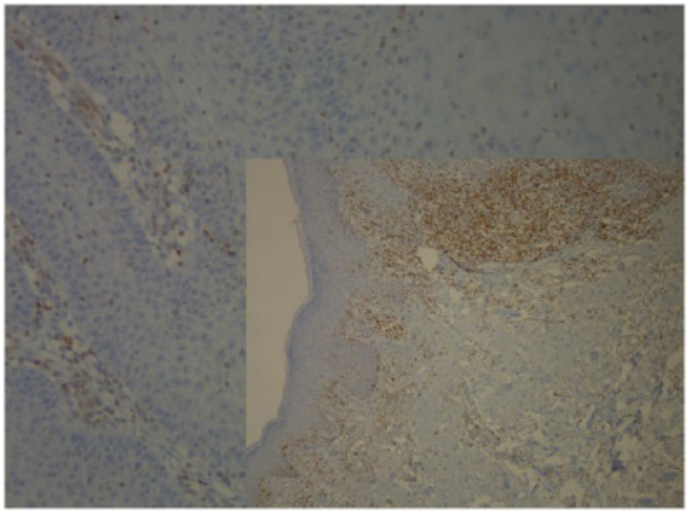

Figure 3. Negative staining in keratoacanthoma crater (x200), positive staining of basal cells in areas outside the crater (small picture x40)

RESULTS

For AK cases, in two cases all layers of the epidermis were stained while for other cases only the basal layer was stained. For SK cases, ten cases had lower (1/3) staining, seven cases had middle layer (2/3) staining and five cases had full layer (3/3) staining observed. For KA cases, the keratin-filled crater area was not observed to have bcl-2 staining, while staining was observed in the areas outside the crater (Table 1). Of all cases 61.2% (n:38) were males. 38.8% (n:24) were females. There were 40 cases (64.5%) with head and neck localization, and 22 cases (35.5%) with localization other than the head and neck.

Table 1. Distribution of Bcl-2 staining in keratoacanthoma, seborrheic keratosis and actinic keratosis

	KA	SK	AK
Lower (1/3)	0	10	26
Middle (2/3)	0	7	0
Full layer (3/3)	0	5	2

DISCUSSION

Apoptosis is one of the mechanisms playing a basic role in homeostasis of the epidermis. Apoptotic cells are identified in normal and diseased skin, while apoptosis inhibiting mechanisms have not been fully explained at skin level (8).

Bcl-2 is a member of a gene family continuing proto-oncogenic cell death suppressors and cell death promotors (9). Bcl-2 was discovered by chromosomal translocation analysis related to proto-oncogenic human follicular B-cell lymphoma. The Bcl-2 gene codes proteins localized to the endoplasmic reticulum and the nuclear membrane on the internal surface of mitochondria (10). These proteins regulate apoptosis making cell survival easier and acting as a cell death suppressor (9,10). Functions occur by regulating mitochondrial release of cytochrome c and interacting with apoptotic activating

factors (11,12). Expression of Bcl-2 in embryonic and fetal skin and presence in hair follicle bulge cells leads to the consideration that Bcl-2 may be a stem cell marker (13). In normal adult human epidermis, it was previously reported that Bcl-2 was expressed in keratinocytes in the basal layer (13). As the epidermis matures towards the surface, Bcl-2 expression is lost (6). A study by Bircan et al. showed that Bcl-2 was expressed in cells in the basal cell layer and around mature hair follicles (14). In our study, in accordance with the literature, Bcl-2 expression was identified to intensify in the basal layer.

AK is a cutaneous intraepithelial neoplasm observed in areas with solar damage, especially in elderly individuals (15-17). For cases without treatment, there is the potential to transform to squamous cell carcinoma (SCC). The clear cause is not known; however, the main risk factor is chronic ultraviolet (UV) exposure (18-21). UV causes variations in a variety of genes in many apoptosis cascades (11,22). There are many studies showing Bcl-2 expression among AK cases (23,24). A study by Thomas et al. showed that typically AK cases had 35-41% weak positive Bcl-2 expression (25). Contrary to this, Hussein et al. showed high Bcl-2 expression in 73% of AK cases. This situation showed that high expression values prevent apoptotic cell death of dysplastic cells and ensure their transformation to SCC (12,23). One study showed that Bcl-2 expression was limited to the basal layer in hypertrophic and atrophic AK. One case was observed to have staining of suprabasal cells. Among SK cases, Bcl-2 expression is observed in basaloid proliferation areas. Bcl-2 expression was not observed in squamous differentiation areas (8). In our study, in accordance with the literature, 26 out of 28 AK cases were observed to have staining in the basal layer. However, in 2 cases staining was observed in all layers of the epidermis.

KA and SCC are cutaneous neoplasms occurring mainly in fair-skinned people in regions with exposure to the sun. KA rapidly grows for 4-6 weeks and then spontaneously regresses in 4-6 weeks and heals leaving an annular scar with slight depression (26). Both tumors have similar cytological characteristics which may make both clinical and histological differentiation of KA from SCC difficult (27,28). In order to make this differentiation, molecular biological markers for both lesions have been broadly studied and developed (29-32).

A study by Ribeiro et al. accepted that Bcl-2 expression among KA and well differentiated SCC cases was generally negative. They showed that Bcl-2 did not play an important role in SCC and KA differentiation (10). A study by Tamara et al. Found KA and SCC cases were Bcl-2 immunonegative. Some previous studies have shown there is Bcl-2 overexpression in BCC and there is no Bcl-2 expression in KA and SCC (7,33,34). In our study, in accordance with the literature, there was no Bcl-2 expression in the epidermis of all KA cases. The reason for this may be the suprabasal origin of the tumor cells.

When SK is compared with normal skin, the apoptosis rates in all pathologic sequelae appear to be lower (35). In a study comparing with SCC, the apoptosis rate was shown to reduce in SK while Bcl-2 expression increased. Additionally, very rapidly growing lesions were identified to have higher rates of apoptosis compared to slow-growing lesions (36). The Bcl-2 increase in SK was stated to possibly be associated with slipping of the cell cycle linked to infection or trauma (35,36). In SK lesions, keratinocytes and dendritic cells positive for Bcl-2 were identified in some studies. In these studies, SCC cases were assessed and typically Bcl-2 expression was not observed. These studies showed that contrary to suprabasal Bcl-2 negative keratinocytes causing SCC, SK was shown to be due to Bcl-2 positive basal keratinocytes. This situation explains the different staining patterns (8,36-38). In our study staining was observed to be greater in SK with more basaloid proliferation.

CONCLUSION

In conclusion, Bcl-2 expression was observed in tumor cells derived from basal cells in AK and SK supporting the possible association with UV effect. Additionally, Bcl-2 expression was not present in KA which may be associated with KA not being derived from basal cells. There is a need for multi-center studies with higher numbers to obtain more detailed and clearer data.

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