Original Paper

Immunoexpression of Transforming Growth Factor Beta 3 (TGFβ3) and Its Receptor Type III (TGFβRIII) in Basal Cell Carcinomas

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ABSTRACT: Basal cell carcinomas (BCCs) are malignant tumors with particular biological prognosis and behavior, and the biomolecular investigation of these lesions can provide important therapeutic targets for epithelial neoplasia. In this study we analyzed the immunoexpression of transforming growth factor beta 3 (TGFβ3) and its receptor type III (TGFβRIII) for 53 cases of BCC in relation to the main histopathological prognostic parameters. The results indicated statistically significant differences of TGFβ3 and TGFβRIII expression related to histological type and lesion stage, the both proteins being higher expressed in adenoid and morpheaform advanced stage tumors. In this study, TGFβ3 and TGFβRIII immunoexpression analysis indicated their utility for identifying aggressive BCCs with potential for tumor progression.

KEYWORDS: *transforming growth factor, basal cell carcinoma*

Introduction

Basal cell carcinomas are frequent tumors with a particular biological behavior dependent on the histopathological type, which is generally characterized by local invasiveness and low metastatic rate [1,2]. Understanding the biomolecular mechanisms underlying BCC progression may lead to the identification of therapeutic targets for epithelial neoplasia.

Transforming growth factor beta 3 (TGFβ3) and its receptor type III (TGFβRIII) represent a multifunctional cytokine and one of its receptors, which interact directly or indirectly within complex biomolecular pathways [3,4]. The effect of expression of TGFβ and its receptors was described for different carcinoma localizations, the results being contradictory and ultimately suggesting the existence of a dual effect in the progression of malignant tumors, respectively biological effects of suppression or tumor stimulation depending on the progression stage of the lesions [3-5].

The literature data for TGFβ3 and TGFβRIII are rare, mostly performed on experimental models and quantification by genetic amplification methods. In this study we analyzed the TGFβ3 and TGFβRIII immunoexpression in relation to the main histopathological prognostic parameters of BCCs.

Material and methods

The study included 53 basal cell carcinomas (BCC) diagnosed for the first time in pacients admitted, investigated and operated in Dermatology and Plastic Surgery Clinics of Emergency County Hospital of Craiova during 2013-2015. The lesions were histopathological assessed in accordance with the criteria elaborated by the AJCC (American Joint Committee on Cancer) for non-melanocytic skin tumors [2] by two specialists (CS and AS) of the Pathology Department of the same hospital.

After the tissues fixation (10% neutral buffered formalin) the paraffin embedding and Hematoxylin-Eosin (HE) staining were done within the classic histopathological technique. In this study we analyzed the main prognostic parameters of BCC represented by histopathological type and tumor stage (including the site) depending on the immunoexpression of TGFβ3 and its receptor TGFβRIII.

The immunohistochemical analysis was made on serial sections using a ready to use polymeric amplification detection system (Histofine polymer-Horseradish Peroxidase, Nichirei, Japan, code 414151F). We work with

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rabbit antihuman polyclonal antibodies TGFβ3 and TGFβRIII, both in dilution of 1:50, using an antigen retrieval represented by microwaving in citrate buffer pH6. The chromogen 3,3'-diaminobenzidine tetrahydrochloride (DAB, Redox, Bucharest, code 3467) was used for signal visualization and external negative (by omitting the primary antibody) and positive (placenta) controls validated the reactions.

For the quantification of immunohistochemical reactions a positive composite score was obtained by multiplying the reaction intensity score (1-mild, 2-moderate, 3-strong) with the percentage of labelled cells score (1-40%, 2-40-60%, 3-over 60%). For the statistical analysis, the score levels were considered low for values 1-4 and high for values of 6-9.

For the statistical analysis the Statistical Package for the Social Sciences (SPSS) 10 software (ANOVA comparison tests) was used, the p-values <0.05 being considered significant. The Nikon Eclipse E600 microscope and Lucia 5 software were used for the image acquisition process. The study was approved by the local ethical committee and the written informed consent was obtained from all the patients.

Results

Basal cell carcinomas investigated in this study were diagnosed in a group of patients with a mean age of 61.2 years, the lesions being located mainly in the head (71.6%), with sizes under 2cm (52.8%). In the group of 53 BBCs analyzed the nodular type was the most common (52.8%), followed by adenoid and morpheaform types (47.2%). Most lesions were diagnosed in stage I and II tumors (56.6% vs 35.8%), and the least in stages III and IV (5.6% vs. 2%) (Table 1).

Table.1. BCCs distribution depending on histopathological types and tumor stages

BCCs (No.) Stage/Type	**Nodular**	**Adenoid**	**Morpheaform**
I	18	9	3
II	10	7	2
III	-		3
IV	-		1

The immunohistochemical analysis indicated the presence of TGFβ3 immunoreactions in 47 cases, which accounted 88.7% of the analyzed BCCs.

The immunostaining have been observed in the cytoplasm of tumor cells as well as stromal elements represented by fibroblasts, lymphocytes, plasma cells and endothelial cells.

For the entire group, the reactions present variable intensity, a mean value of labelled cells of 57.2±14.5 and a mean composite score of 3.9.

TGFβ3 immunoreaction had differences in relation to the type and stage of BCCs.

Thus, in relation to the type of tumor in the case of nodular type, the number of labeled cells was between 30-75% with a mean value of 54.5±12.5, the intensity of the reactions was reduced, and the average composite score was 2.2.

By comparison, for adenoid and morpheaform types, the number of marked cells was between 30-85%, with mean value of 59.3±16/ 5.1, respectively 60.5±17/ 6.2.

In these cases, TGFβ3 reactions were variable in intensity, predominantly moderate and intense, and the average composite score values were 5.1 for adenoid type and 6.2 for morpheaform type (Fig. 1A-C, Table 2).

The analysis of TGFβ3 expression in relation to the tumor stage revealed the highest values in stage III/IV tumors, where the number of marked cells was between 65-85%, with an average value of 70±10.8/7.5, with moderate/increased intensity of reactions and a composite average score of 7.5.

By comparison, for stages I and II, the mean values of marked cells were 53.7±12.8 and 58.9±15.8, the intensity of the reactions was variable, and the average composite scores were 3.2 and respectively 4 (Table 2).

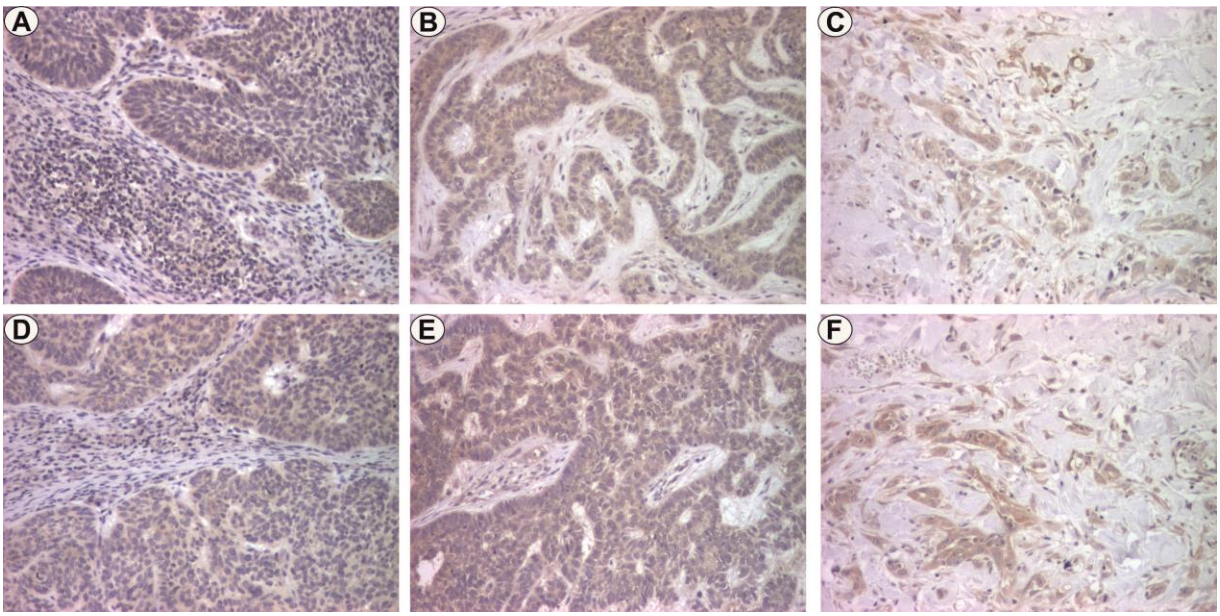

Fig. 1. BCC, x100. A. Nodular type, TGFβ3 immunostaining; B. Adenoid type, TGFβ3 immunostaining; C. Morpheaform type, TGFβ3 immunostaining; D. Nodular type, TGFβRIII immunostaining; E. Adenoid type, TGFβRIII immunostaining; F. Morpheaform type, TGFβRIII immunostaining

Table 2. TGFβ3 and TGFβRIII immunoexpression depending on BCCs histopathological parameters

Labelled cells (%)/ composite score (average values)	TGFβ3	p value (χ2 test)	TGFβRIII	p value (χ2 test)
Nodular	54.5±12.5/ 2.2	<0.001	48.6±13.2/ 2.2	0.006
Adenoid	59.3±16/ 5.1		50±17.9/ 3.2	
Morpheaform	60.5±17/ 6.2		59.7±15.6/ 5.7	
Stage I	53.7±12.8/ 3.2	0.008	48.4±17.5/ 2.8	0.073
Stage II	58.9±15.8/ 4.0		51.9±12.8/ 3.0	
Stage III/IV	70±10.8/ 7.5		64.5±7.5/ 4.0	

The immunohistochemical analysis indicated the presence of TGFβRIII reactions in 45 cases, representing 84.9% of the analyzed BCCs. Immunostainings have been observed in the cytoplasm of tumor cells, as well as stromal elements mainly represented by fibroblasts, lymphocytes and endothelial cells. For the entire BCCs analyzed group the number of labelled cells was between 25-85%, with an average value of 51.2±15.5 and an average composite score of 3.2.

TGFβRIII reactions were higher in the case of morpheaform type, the number of labeled cells was between 35-85% with a mean value of 59.7±15.6, high intensity/moderate intensity and an average composite score of 5.7. The values were lower for the nodular and adenoid types, the number of labeled cells was between 25-85% with mean values of 48.6±13.2 and 50±17.9, low/moderate reactions intensity and average composite scores of 2.2 and 3.2 (Fig. 1D-F).

Analysis of TGFβRIII immunoreactions in relation with BCCs stage indicated mean values of 48.4±17.5 marked cells for stage I lesions, 51.9±12.8 for stage II, and 64.5±7.5 for stage III, the average composite scores being 2.8, 3.0 and respectively 4.0.

The statistical analysis of the results indicated significant differences in TGFβ3 expression related to the tumor type ($p<0.001$, χ 2 test) and tumor stage ($p=0.008$, χ 2 test), as well as significant differences in TGFβRIII expression related to the tumor type ($p=0.006$, χ 2 test) (Table 2, Fig. 2A-C). Although the values of TGFβRIII scores were higher in advanced stages lesions, the aspect was not statistically significant ($p=0.073$, χ 2 test). The analysis of TGFβ3 and TGFβRIII reactions percentage values indicated a positive linear correlation of the two markers immunoexpression ($p<0.001$, Pearson test) (figure 2D).

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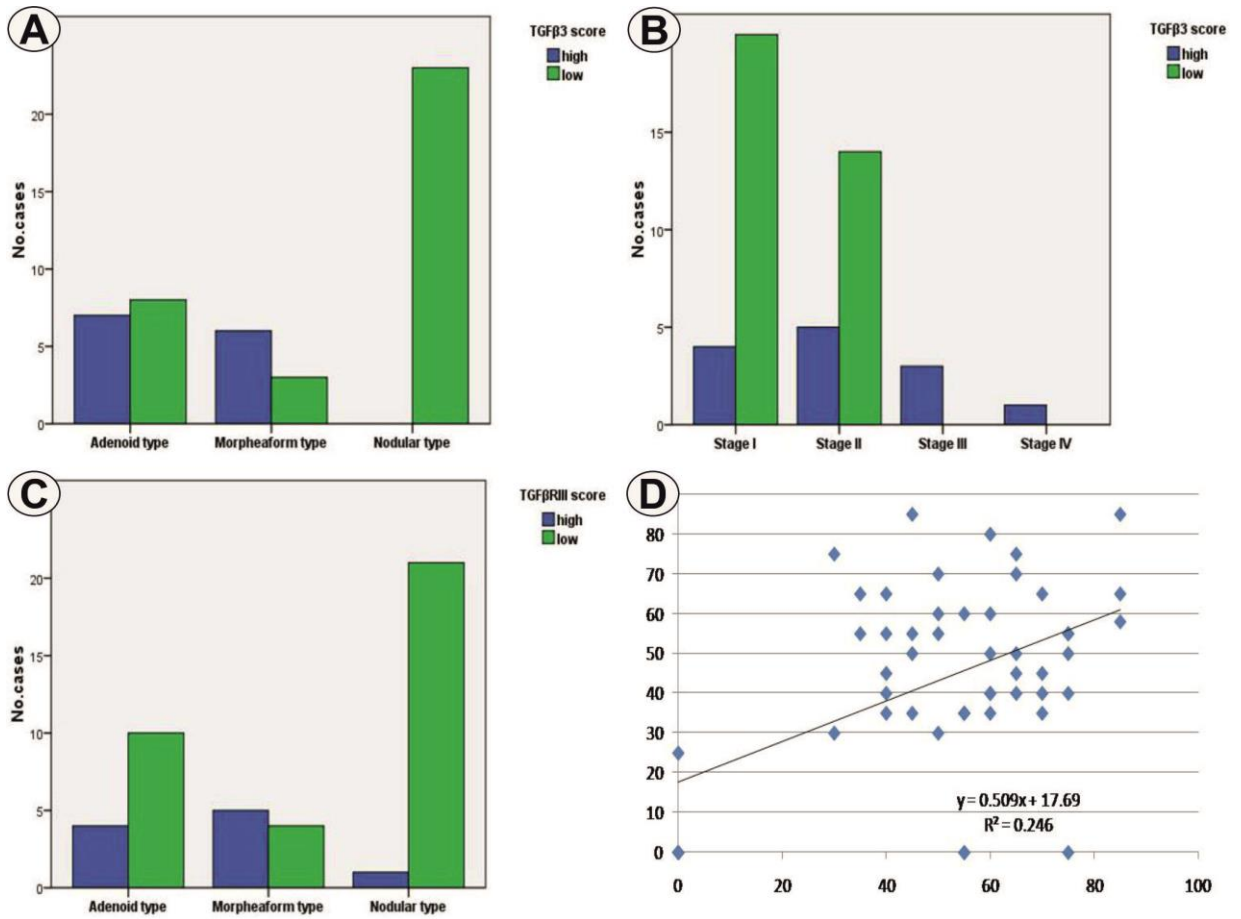

Fig. 2. A. TGFβ3 immunoexpression depending on BCC types; B. TGFβ3 immunoexpression depending on BCC stages; C. TGFβRIII immunoexpression depending on BCC types; D. TGFβ3 and TGFβRIII values distribution

Discussions

Data from the literature on TGFβ3 and TGFβRIII immunoexpression in basal cell carcinomas are rare or absent. Thus, assaying TGFβ3 expression by in situ hybridization indicated protein diminution in stromal cells and tumor cells from basal cell carcinomas compared to normal tissues, although were reported BCCs in which the expression was superior [6]. By the same techniques, there was also an overexpression of TGFβ1 and SMAD3 in basal cell carcinomas, with involvement in paracrine stimulation of BCC [6,7].

In this study we found high expression of TGFβ3 and TGFβRIII in andenoid and morpheaform types compared to the nodular type, as well as a higher expression in advanced lesions.

TGFβ is a cytokine with four isoforms (TGFβ1-4) belonging to transforming growth factor superfamily, a group that also includes growth/differentiation factors, inhibins, activins and bone morphogenetic proteins, mullerian inhibitory substance [3,8].

TGFβ family members play a role in regulating immune and hormonal response, cell growth, apoptosis, tissue repair, and remodeling of the extracellular matrix [3]. Data from the literature indicates a dual role of TGFβ in cancers. Thus, in the case of normal tissues and early carcinomas, TGFβ has a suppressive effect with inhibitory effects of processes involved in local tumor development such as inhibition of cellular proliferation, induction of apoptosis, inhibition of cellular immortalization [3]. On the contrary, in the case of aggressive and invasive tumors, by activating complex biomolecular mechanisms, such as epithelio-mesenchymal transition and angiogenesis, the migration, invasion and metastasis of cancer cells are promoted [3].

Published data indicate overexpression of TGFβ in most mammary cancers, but also in those with pulmonary, pancreatic, esophageal, gastrocolic or prostatic localization [3]. Also, overexpression of TGFβ is generally associated

with high grade and advanced stage carcinomas [9,10].

TGFβ3 is involved in embryogenesis, cell differentiation and extracellular matrix formation, the principal receptor with which it interacts being represented by TGFβRII, so that, as well as the TGFβ1 and TGFβ2 isomorphs, activates the pathogenic pathway of TGFβ [3].

Data from the literature indicates the existence of three receptors for TGFβ, respectively types I (TGFβR1), II (TGFβRII) and III (TGFβRIII) [4,11]. The pathogenic canonical pathway of TGFβ involves ligand binding of the activated TGFβRII, which results in phosphorylation and subsequent activation of TGFβR1, with subsequent phosphorylation and SMAD activating effects involved in cellular transcription [4,11].

TGFβRIII (betaglican) is a transmembrane proteoglycan involved in the regulation of TGFβ-activated TGFβRII binding and also appears to be involved in prolonging the activity of the TGFβRII-TGFβR1 receptor complex [4,11-13]. This receptor does not have intrinsic signaling activity but has a high affinity for all TGFβ isomorphs [13].

The published results support a dual role of TGFβRIII in the sense of stimulating on the one hand of the TGFβ basal expression and implicitly promoting progression and metastasis and on the other hand decreasing dependent ligand signaling by preventing ligand blockade within the TGFβRII-TGFβRI complex [4].

Overexpression of TGFβRIII was observed in seminomas and loss of expression is associated with increased risk of metastasis in prostatic, pulmonary and pancreatic carcinomas [4,5,14]. Also for triple-negative mammary tumors, TGFβRIII appears to be necessary for the migration and invasion of tumor cells, including the growth of xenografts in vivo [15].

In our study, we found expression of TGFβ3 and stromal elements. Data from the literature also indicates this aspect, especially in stromal fibroblasts, endothelial cells and immune cells [3]. A particular relationship is described in the literature between TGFβ and the extracellular matrix. Recent data indicates that the activated ligand precursor is deposited at the matrix and activation of tumor cell migration is followed by stimulation of matrix-metalloproteinase secretion, matrix degradation/remodeling and precursor protein release, thereby providing autocrine stimulation of TGFβ [3,16].

Conclusions

In this study we found significant differences in TGFβ3 and TGFβRIII immunoexpression related to the BCCs tumor type. We found a positive linear correlation of the analyzed markers and their immunoexpression was superior in the case of advanced BCCs. The obtained results indicate the involvement of TGFβ3 and TGFβRIII in the aggressiveness of BCCs, which supports the inclusion of the analyzed markers in the group of potential therapeutic targets for these tumors.

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