ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

Expression of CXCR4, MMP-13 and β-catenin in different histological subtypes of facial basal cell carcinoma

M. E. CIUREA[1)], DANIELA CERNEA[2)], C. C. GEORGESCU[3)], O. S. COTOI[4)], V. PĂTRAŞCU[5)], H. PÂRVĂNESCU[1)], D. POPA[1)], V. PÂRVĂNESCU[1)], RALUCA NICULINA CIUREA[5)], R. MERCUŢ[6)]

[1)]*Department of Plastic Surgery, University of Medicine and Pharmacy of Craiova*
[2)]*Clinic of Anesthesia and Intensive Care, Emergency County Hospital, Craiova*
[3)]*Department of Pharmacology, University of Medicine and Pharmacy of Craiova*
[4)]*Department of Cell and Molecular Biology, University of Medicine and Pharmacy of Tirgu Mures*
[5)]*Department of Dermatology*
[6)]*PhD student*
University of Medicine and Pharmacy of Craiova

Abstract

Basal cell carcinoma (BCC) is one of the most common skin neoplasms in humans, accounting for almost 80% of all non-melanoma skin cancers worldwide. The nodular and infiltrative-morpheaform are the most common BCC types in the head and neck region and together with the micronodular subtypes are the most aggressive tumors, because of their tendency to infiltrate the deep subcutis, muscles and even bones. To explain the local aggressive behavior and their metastatic potential, many studies have been performed to identify the molecular determinants implicated in BCC tumor progression. For this reason, we investigated the immunohistochemical expression of CXCR4, MMP-13 and β-catenin expression in six metatypical, eight infiltrative-morpheaform, six micronodular and five superficial facial BCCs. For all three markers, the tumor reactivity varied with the histological type. The highest reactivity was observed in metatypical subtype, especially at the level of areas with squamous cells differentiation. The lowest reactivity was recorded in micronodular and superficial BCC subtypes. Regardless histological subtype, the tumor reactivity was higher at the advancing edge and additional a strong stromal reaction was noticed for all investigated markers peculiar in fibroblasts, inflammatory cells and endothelial cells. All these data proved the utility of CXCR4, MMP-13 and β-catenin immunohistochemical investigation in BCCs both for identification of high-aggressive tumors and to develop novel more efficient therapeutic strategy for these patients by targeting these biomarkers.

Keywords: basal cell carcinoma, CXCR4, MMP-13, β-catenin, immunohistochemistry, histological subtypes.

Introduction

Basal cell carcinoma (BCC) is one of the most common skin neoplasm in humans, accounting for almost 80% of all no melanoma skin cancers worldwide [1, 2]. It is slowly progressive and poorly metastasizing skin cancer with propensity to be locally destructive [3]. Although these patients have lower mortality, the tumors may behave aggressively with deep invasion, recurrence, and regional and distant metastasis [4]. These authors proposed as prognostic markers of aggressiveness: tumor size, duration of development, histology and perineural spread. About 85% of metastatic BCC arise from primary lesions in the head and neck region [5] and histological types such as micronodular, infiltrative-morpheaform and mixed type have been classified as aggressive because they more frequently exhibit deep invasion [4, 6].

The invasion of tumor cells is a complex, multistage process, which starts with the degradation of the extracellular matrix (ECM) and the basement membranes that surround the primary tumor by proteases, such as matrix metalloproteinases (MMP). Further, to facilitate the cell motility, invading cells need to change the cell–cell adhesion properties, rearrange the ECM environment, suppress anoikis and reorganize their cytoskeletons [7]. These processes are governed by complex interactions between various biomarkers, especially MMPs (MMP-9, MMP-13), cell–cell adhesion molecules (β-catenin) and chemokine receptor-ligand complexes (peculiar the SDF-1/CXCR4). In this regard, it seems that β-catenin is a promising key factor of SDF-1/CXCR4 signaling to regulate the metastasis, mainly in pancreatic cancer [8]. Moreover, several MMPs, including MMP-2, MMP-9, and MMP-13, directly or indirectly interact with CXCR4, which could alter MMP-mediated pericellular proteolysis [9–11].

CXCR4 (C-X-C chemokine receptor type 4 or fusin or CD184) is an alpha-chemokine receptor specific for

stromal-derived-factor-1 (SDF-1, also called CXCL12), being expressed in normal condition by hematopoietic cells [12], vascular endothelial cells [13], neurons, microglia and astrocytes [14] and functionally is linked to human immunodeficiency virus-1 (HIV-1) infection, hematopoiesis, embryogenesis, organogenesis and angiogenesis, along with its important roles in lymphocyte trafficking and recruitment at sites of inflammation [15, 16]. Also, its interaction with CXCL12 has been found to play an important role in tumorigenicity, proliferation, metastasis, and angiogenesis in many cancers, such as lung cancer, melanoma, esophageal cancer, ovarian cancer, glioblastoma, pancreatic cancer, cholangiocarcinoma, and basal cell carcinoma cells [17–24].

Given the limited information on BCC tumor progression biomarkers, we were interested here in the immunohistochemical investigation of CXCR4, MMP-13 and β-catenin expression in the aggressive type (infiltrative-morpheaform, micronodular and metatypical) *versus* superficial facial BCCs.

Materials and Methods

We reviewed the medical records from the Laboratory of Pathology, Emergency County Hospital, Craiova, Romania, and identified 25 patients who had been diagnosed with BCCs. The histopathological diagnosis was made according to the *WHO* histological classification of keratinocytic skin tumors [25]. The BCC growth pattern, assigned as described [26] comprised six metatypical, eight infiltrative-morpheaform, six micronodular and five superficial tumors. The study cohort included 15 women and 10 men aged 46 to 81 years (mean age 64 years). In relation to the site of tumor origin, were selected only the cases that developed in the facial skin and they belonged to the following anatomic regions: nasal (nine cases), lip (six cases), orbit (five cases) and frontal (five cases). The study was carried out after approval by the local ethics committee.

Immunohistochemistry was performed on 4 μm-thick sections from one selected block for each case. The sections were deparaffinized in xylene, dehydrated in ethanol, and immersed in distillated water containing 3% hydrogen peroxide for 30 minutes to block endogenous peroxidase activity. Then, we performed an antigen-unmasking step of 20 minutes heat-induced epitope retrieval in DakoCytomation Target Retrieval solution, code S1700. Subsequently, the unspecific binding sites were blocked with a 5% Bovine Serum Albumin (BSA) in PBS for one hour. Briefly, the primary antibodies were used at a dilution of 1:1000 for CXCR4 (polyclonal rabbit anti-human, SDIX, Cheminpress, Romania, code CAB 011447), 1:50 for MMP-13 (MM0019-12E10, mouse anti-human, monoclonal, Santa Cruz, Redox, Romania, Code sc-101564) and 1:200 for β-catenin (β-Catenin-1, mouse anti-human, monoclonal, Dako, Redox, Romania, code M3539). The primary antibodies were amplified with biotinylated species-specific secondaries and a LSAB2 (Dako, Redox, Romania, code K0675) system. Visualization was done with 3,3'-Diaminobenzidine (DAB) (Dako, Redox, Romania, code K3468). For counterstaining, we used Mayer's Hematoxylin. Negative-control stainings were done by omitting the primary antibodies.

For the immunostaining assessment, we used the immunoreactive score (IRS) of the Remmele W and Stegner HE (1987) [27]. According to this, the intensity of marker expression was quantified using the following scores: 0 = negative, 1 = weakly positive, 2 = moderately positive, 3 = strongly positive. The extent of marker expression was quantified by evaluating the percentage of the positive staining areas in relation to the whole cancer areas in the core. A score of 0 point was given for 1–10% reactivity, 1 point was assigned for 10–25% reactivity, 2 points were assigned for 25–50% reactivity, 3 points were assigned for 50–75% reactivity, and 4 points were assigned for 75–100% reactivity. The final immunoreactive score was determined by multiplying the positive intensity and the positive area extent scores, yielding a range from 0 to 12. β-Catenin reactivity was assessed in tumor cell taking into account all cellular localizations of this marker (membranous, cytoplasmic and nuclear). The stromal reactivity was assed qualitatively noting the presence or absence of immunoreactivity, noting the reactive cellular type.

The images were acquired by utilizing a Nikon Eclipse 55i microscope (Nikon, Apidrag, Bucharest, Romania) equipped with a 5-megapixel cooled CCD camera and the Image ProPlus AMS7 software (Media Cybernetics Inc., Buckinghamshire, UK). All recorded values were exported and analyzed in Excel (Microsoft Corporation). Data were expressed as average ± standard deviation for each tumor subtype, and all for subtypes were compared utilizing ANOVA testing. Correlations between the expression levels were thought utilizing Pearson's correlation coefficients. Statistical significance was deemed for p-values <0.05.

Results

Immunohistochemical expression of CXCR4

In the normal epidermis adjacent to the tumor lesions, we observed an increased cytoplasmic CXCR4 expression in the differentiated keratinocytes, the reactivity decreasing form parabasal to the upper layer of the epidermis, a low reactivity in the basal and parabasal layers, and it was absent in the granulosum layer (Figure 1A). At the level of hair follicles, the CXCR4 reactivity was present especially in the bulb, and at the root sheaths, the expression was more obvious in inner sheath (Figure 1B). Also, the CXCR4 expression was noticed in the sebaceous and sweats glands (Figure 1C), in endothelial cells and in the inflammatory cell infiltrate (Figure 1D). The most intense reaction was observed in the hyperplastic and dysplastic lesions that were associated with BCCs (Figure 1, E and F).

At the tumoral level, the highest reactivity was noticed in metatypical subtype of BCCs especially at the level of areas with squamous cells differentiation (Figure 2A). All six investigated cases were positive with IRS scores varying from 4 to 8 and score 6 as most frequently found. On the second place as CXCR4 reactivity, we noticed

the infiltrative-morpheaform subtype with both tumor cells and adjacent fibroblasts positive to this marker (Figure 2, B and C). In all eight investigated cases was noticed this reactivity, with score 3 as the lowest IRS score recorded and score 8 as the highest one (in half of the cases being recorded score 6). These were followed by superficial and micronodular subtype with the central tumoral cells being more reactive than palisading peripheral cells (Figure 2, D and E). Two micronodular and one superficial BCCs were CXCR4 negative. The lowest IRS scores were recorded in the micronodular subtype. Regardless of histological subtype, we also noticed CXCR4 reactivity at the level of endothelial cells and of the associated inflammatory cell infiltrate (Figure 2F).

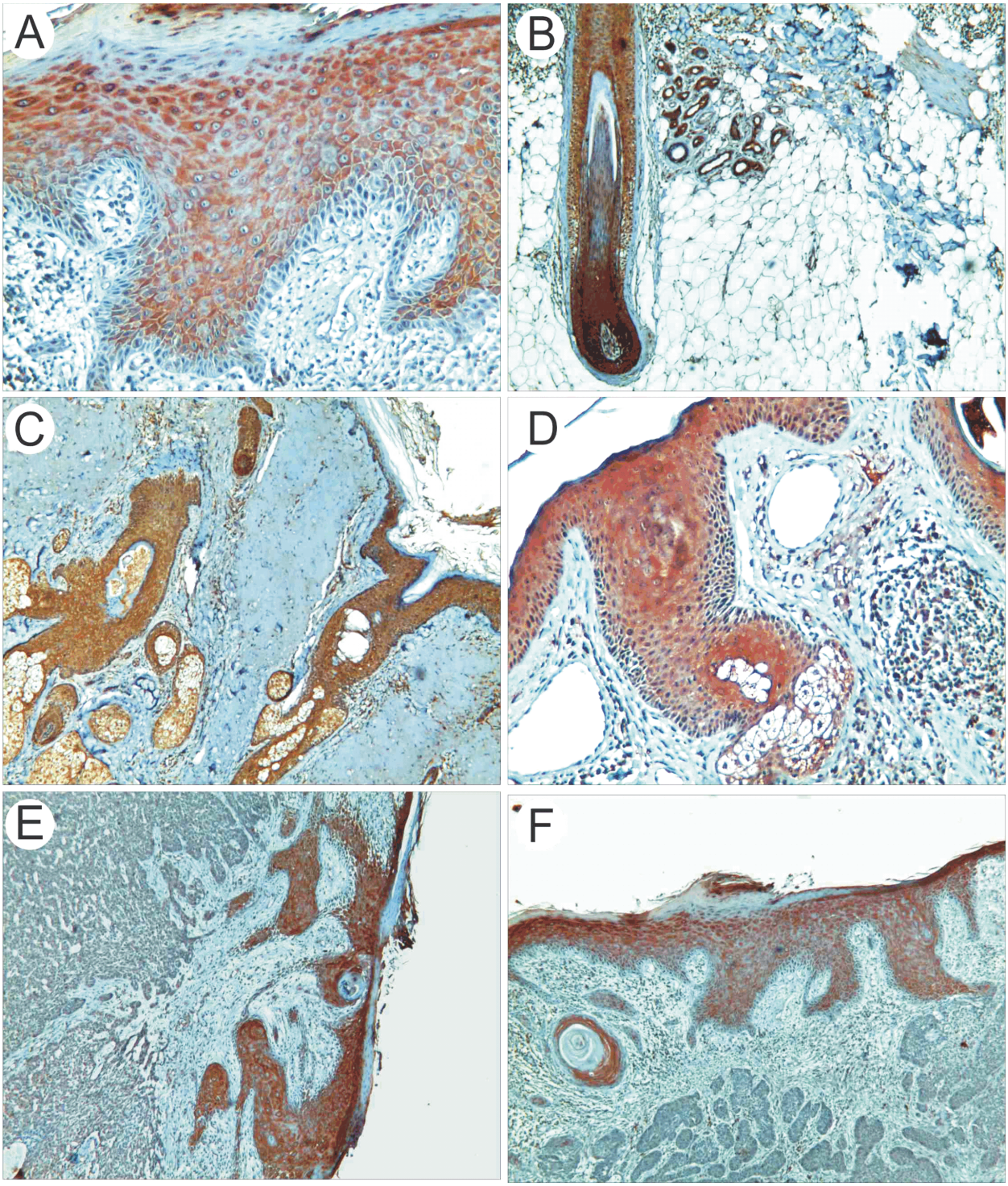

Figure 1 – ***Immunohistochemical reactivity to CXCR4 in normal skin adjacent to facial BCCs: (A) Positive reaction in differentiated keratinocytes along skin spinosum stratum. DAB (brown), ×100; (B) Positive reaction in follicle hair structures and sweat glands. DAB (brown), ×40; (C) Positive reaction in pilosebaceous follicle structures. DAB (brown), ×100; (D) Positive reaction in endothelial cells and in associated inflammatory cell infiltrate. DAB (brown), ×100; (E) Positive reaction in hyperplastic-associated lesions. DAB (brown), ×40; (F) Positive reaction in dysplastic-associated lesions. DAB (brown), ×40.***

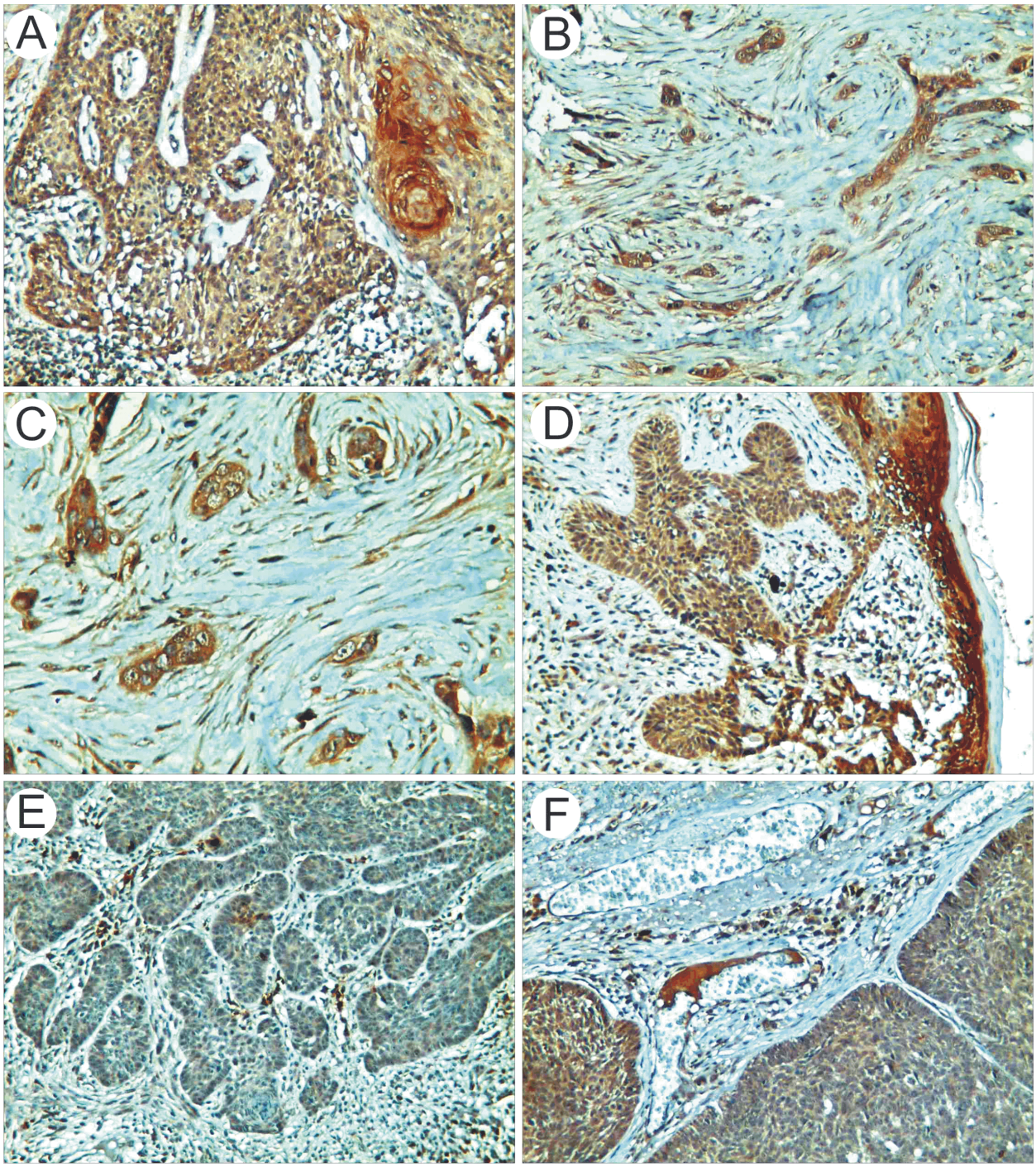

Figure 2 – ***Immunohistochemical reactivity to CXCR4 in facial BCCs: (A) Positive reaction in tumor cells of metatypical BCC subtype, with more intense reaction in areas with squamous cells differentiation. DAB (brown), ×100; (B and C) Positive reaction in tumor cells and adjacent fibroblasts of infiltrative-morpheaform BCC subtype. DAB (brown), ×100/×200; (D) Positive reaction in tumor cells of superficial BCC subtype. DAB (brown), ×100; (E) Positive reaction in tumor cells of micronodular BCC subtype. DAB (brown), ×100; (F) Positive reaction in endothelial cells and associated inflammatory cell infiltrate. DAB (brown), ×100.***

Immunohistochemical expression of MMP-13

Into the normal epidermis, the MMP-13 reactivity was restricted to the basal, parabasal and spinous layers with the latter showing the highest reactivity (Figure 3A). Related to the cutaneous appendages, MMP-13 was observed in the hair root sheaths (more intense in the outer sheath) and sebaceous gland (more accurate in the intact sebum-containing secretory cells) (Figure 3B). Also, some reactivity was noticed in to the dermis, especially in fibroblast and endothelial cells (Figure 3C). A more extensive reactivity was observed in hyperplastic and dysplastic lesions associated to the investigated BCCs (Figure 3D).

All investigated BCCs specimens were positive for MMP-13, but the cytoplasmic reaction varied from case to case depending on the histological subtype. The most reactive was the metatypical BCC subtype with the most cases (50%) scored IRS as 8. The MMP-13 reactivity predominates inside the tumor proliferations, especially in the areas with squamous cells differentiation (Figure 4A).

The second most reactive BCC subtype was the infiltrative-morpheaform tumors mostly with IRS score 6 and 8 (Figure 4B). The least reactive was the micronodular subtype with half of the cases scored as 1 and in which MMP-13 was limited to few central tumor cells from inside micronodular proliferations (Figure 4C). In all these three types of facial BCCs, the MMP-13 reactivity was more prominent at the tumor advancing edge. The superficial BCC cases had a heterogeneous MMP-13 reactivity with cases scored from 1 to 6. The MMP-13 staining was also observed in the cytoplasm of palisading peripheral cells (Figure 4D). Regardless of histological subtype in all investigated cases, we noticed a stromal MMP-13 expression, peculiar in fibroblasts, inflammatory cells and endothelial cells (Figure 4, E and F). Overall, the stromal MMP-13 reaction exceeds tumor cell reactivity.

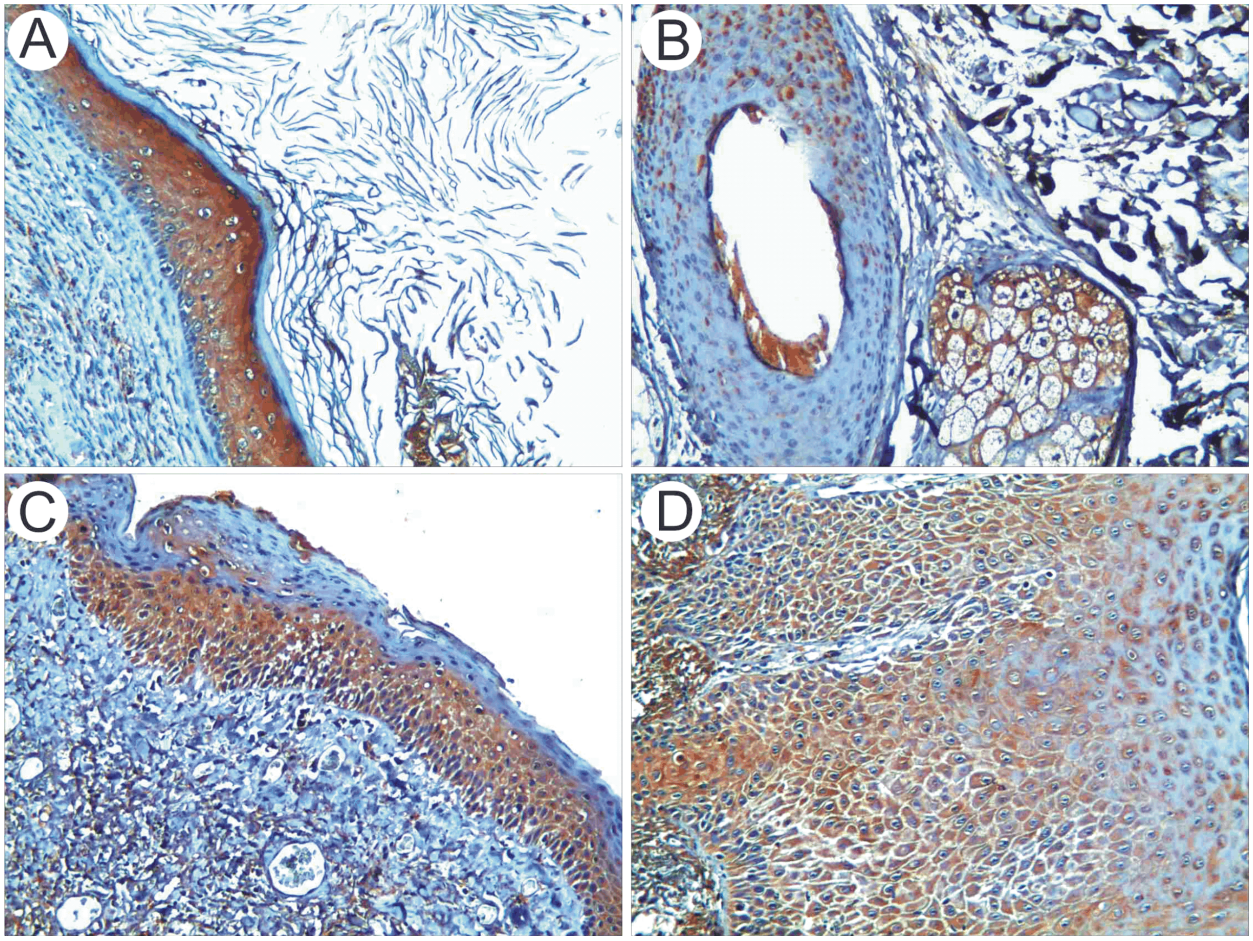

Figure 3 – ***Immunohistochemical reactivity to MMP-13 in normal skin adjacent to facial BCCs: (A) Positive reaction along basal, parabasal and spinous layers with the latter showing the highest reactivity. DAB (brown), ×100; (B) Positive reaction in pilosebaceous follicle structures. DAB (brown), ×100; (C) Positive reaction in fibroblast and endothelial cells from dermis. DAB (brown), ×100; (D) Positive reaction in dysplastic-associated lesions. DAB (brown), ×100.***

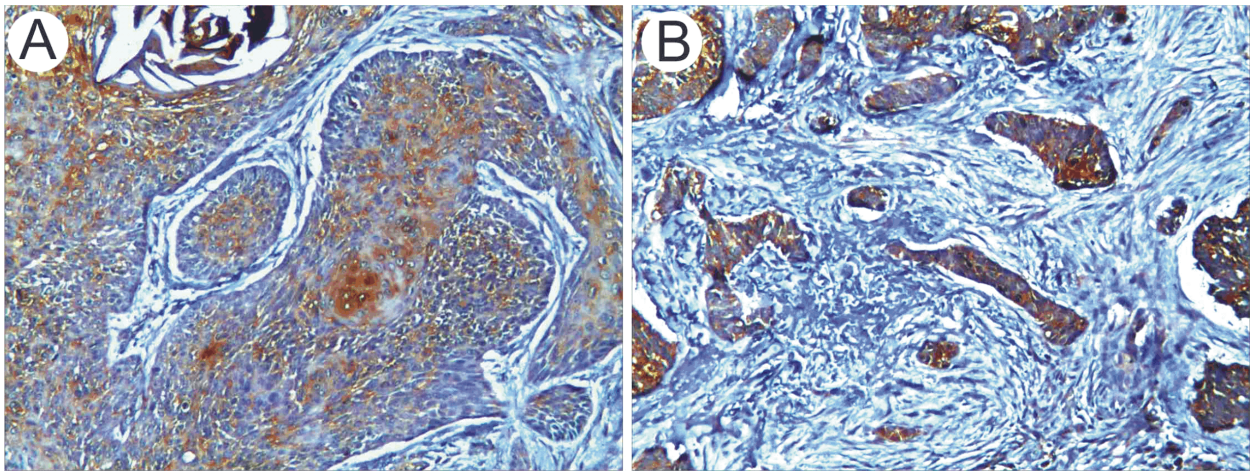

Figure 4 – ***Immunohistochemical reactivity to MMP-13 in facial BCCs: (A) Positive reaction in tumor cells of meta-typical BCC subtype, with more intense reaction in areas with squamous cells differentiation. DAB (brown), ×100; (B) Positive reaction in tumor cells and adjacent fibroblasts of infiltrative-morpheaform BCC subtype. DAB (brown), ×100.***

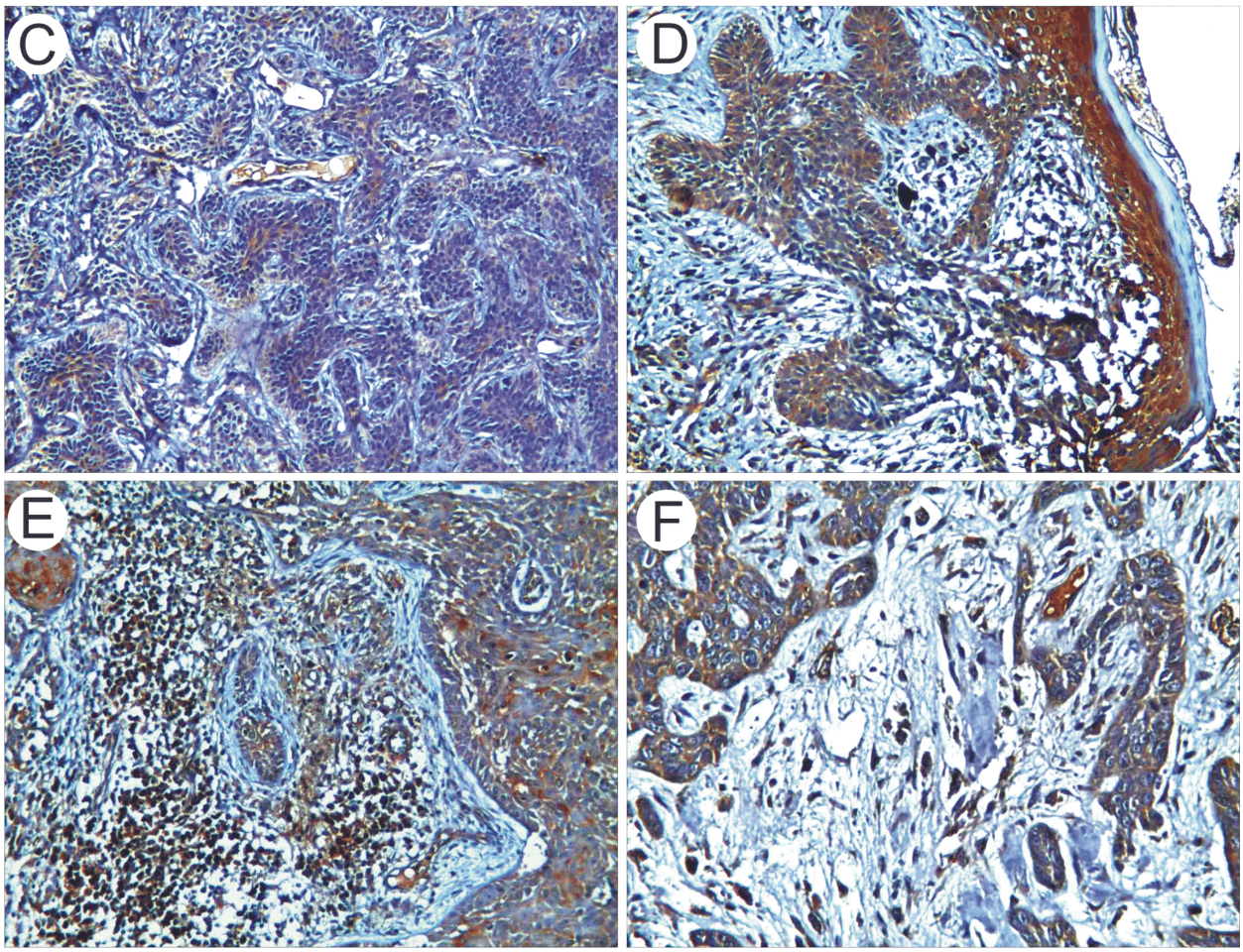

Figure 4 (continued) – ***Immunohistochemical reactivity to MMP-13 in facial BCCs: (C) Positive reaction in tumor cells from inside islands of micronodular BCC subtype. DAB (brown), ×100; (D) Positive reaction in tumor cells of superficial BCC subtype. DAB (brown), ×100; (E and F) Positive reaction in endothelial cells and associated inflammatory cell infiltrate. DAB (brown), ×100/×200.***

Immunohistochemical expression of β-catenin

In the normal epidermis, β-catenin reactivity was predominant membranous and was observed throughout basal, parabasal and spinous layers (Figure 5A). The reactivity decrease along superficial spinous layers and disappears in the granulosum stratum. Occasionally we noticed a nucleo-cytoplasmic staining in the basal layer (Figure 5B). Also, β-catenin was expressed in the cell membranes of the outer and inner root sheaths and in matrix cells located at the base and periphery of the hair follicle bulb (Figure 5C). The same cell membrane pattern reaction was observed in sebaceous and sweat glands (Figure 5D).

All the investigated BCC cases were reactive to β-catenin but with variable intensity from case to case and even in the same tumor from one area to another. The tumor β-catenin staining was influenced both by histological grade and lesion topography (upper part *versus* advancing edge). Thus, the highest IRS scores were recorded in metatypical BCC subtype, most cases being scored as 9. The reaction was more obvious in the areas with squamous cells differentiation and inside tumor proliferation compared with the more peripheral tumor cells. The predominant pattern was the membranous one especially in the areas with squamous cells differentiation, while in the proliferative areas with basal cells differentiation the staining was cytoplasmic and membranous (Figure 6A). The cytoplasmic reaction was increased in the peripheral palisading portion of proliferative islands (Figure 6B). Few nuclear β-catenin reactive tumoral cells were observed at the advancing edge. A moderate β-catenin staining was noticed in infiltrative-morpheaform BCC subtype with IRS scores ranging from 2 to 6 and score 3 as most encountered (37.5% cases). In this type of BCC, we observed a decrease in the membrane cells reactivity and an increased cytoplasmic β-catenin expression (Figure 6C). The nuclear staining was rarer than in metatypical BCC subtype and was also observed at the invasion front. A situation somewhat comparable to that of infiltrative-morpheaform BCC subtype was also recorded in the micronodular tumors. The IRS scores varied between 2 and 6 with score 2 and 3 as most encountered (each one with 33.33%). The prevalent β-catenin staining pattern was membranous, the cytoplasmic reactivity being seen in few peripheral cells from the advancing edge (Figure 6D). We did found any nuclear reactivity in the investigated cases. The lowest reactivity we recorded in superficial BCC subtype with only one cases scored as 3. In these tumors, the β-catenin reactivity was predominant in the cytoplasm and also some scattered peripheral cells from the advancing edge had nuclear reactivity (Figure 6E).

Excluding the superficial subtype, all other tumors regardless of histological subtype presented high β-catenin reactivity at the advancing edge. In addition, we observed especially for the infiltrative-morpheaform BCC cases an increase number of stromal cells with β-catenin immunoreactivity adjacent to tumor cells (Figure 6F). Also, a weak β-catenin reactivity was recorded in the endothelial cells of tumor vessels and in the associated inflammatory cell infiltrates.

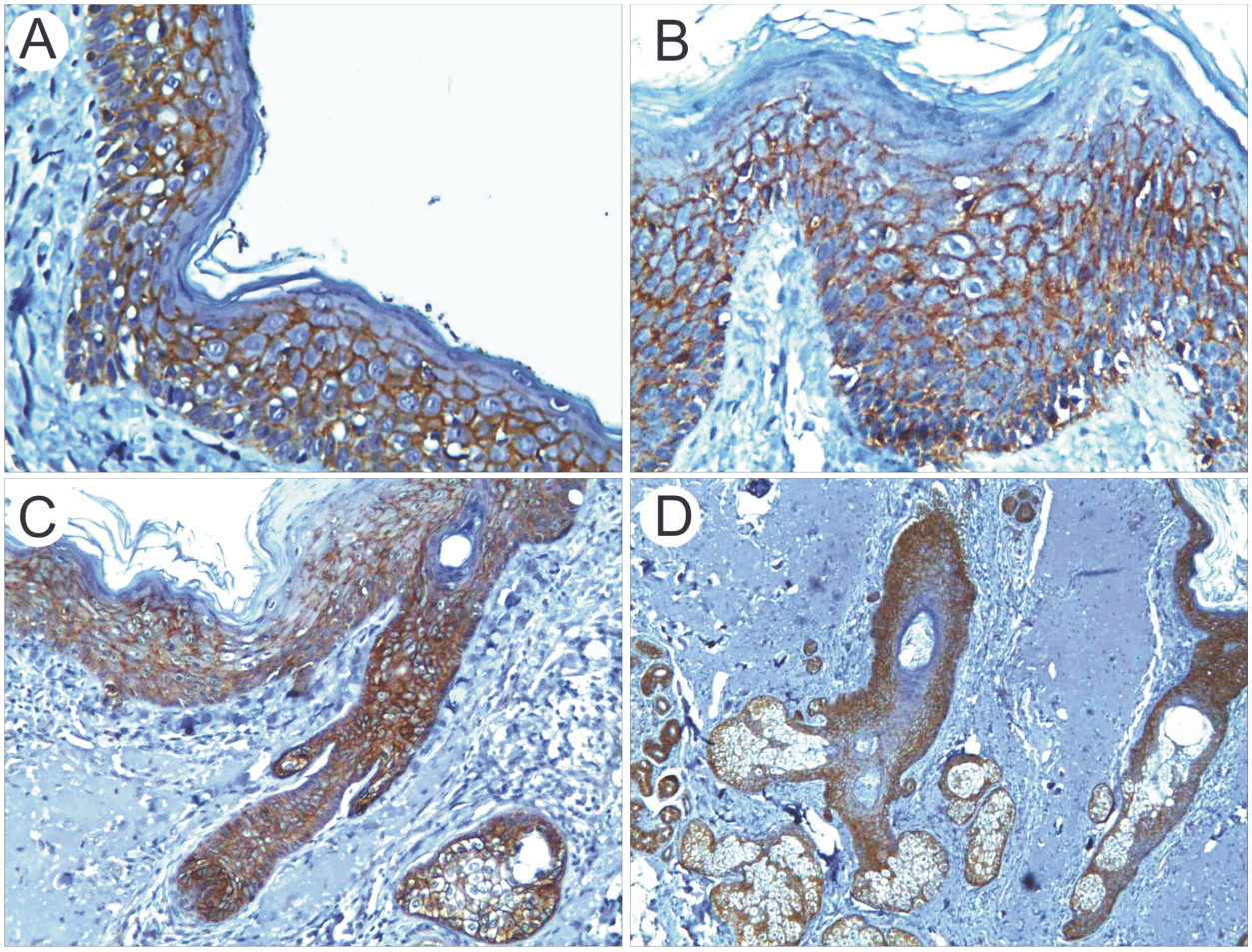

Figure 5 – ***Immunohistochemical reactivity to β-catenin in normal skin adjacent to facial BCCs: (A) Positive reaction along basal, parabasal and spinous layers with decreasing in the upper spinous layers. DAB (brown), ×200; (B) Nucleo-cytoplasmic reactivity in cells of epidermis basal layer. DAB (brown), ×200; (C and D) Positive reaction in pilosebaceous follicle structures and sweat glands. DAB (brown), ×100.***

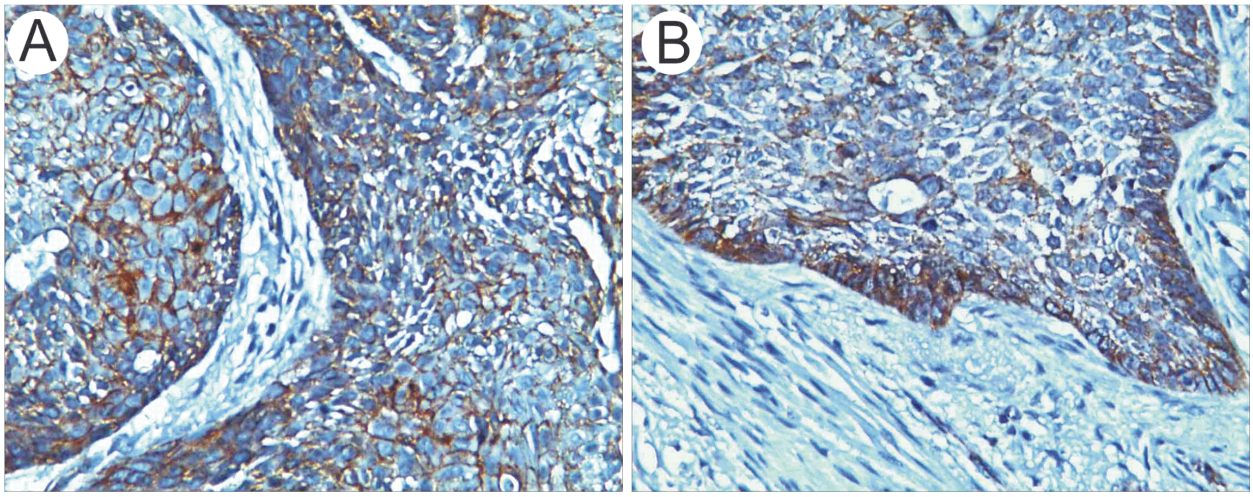

Figure 6 – ***Immunohistochemical reactivity to β-catenin in facial BCCs: (A) Positive reaction with prevalent membranous pattern in tumor cells of metatypical BCC subtype, with more intense reaction in areas with squamous cells differentiation. DAB (brown), ×200; (B) The cytoplasmic reaction was increased in the peripheral palisading portion of proliferative islands from metatypical BCC subtype. DAB (brown), ×200.***

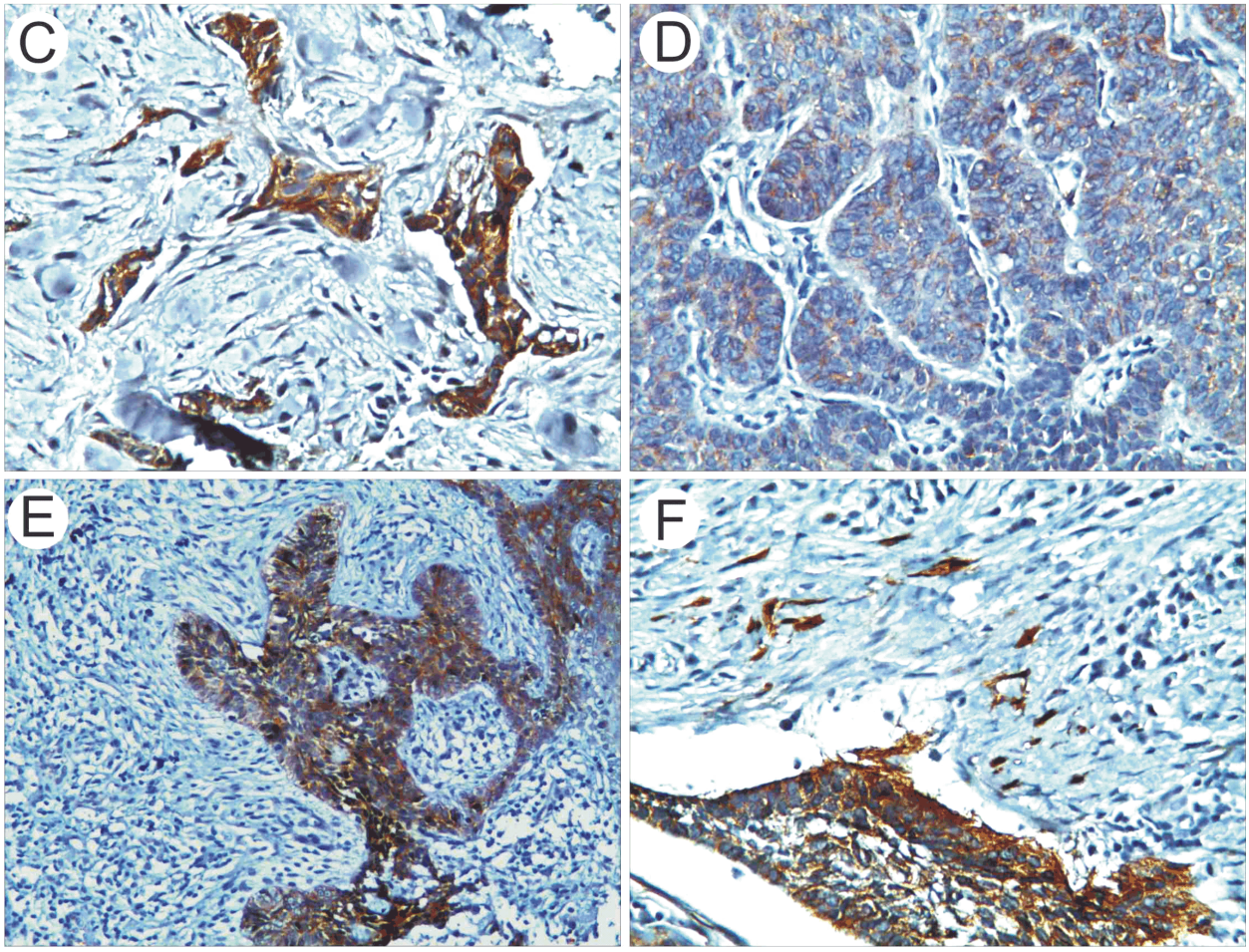

Figure 6 (continued) – ***Immunohistochemical reactivity to β-catenin in facial BCCs: (C) Positive reaction with membranous and cytoplasmic pattern in tumor cells of infiltrative-morpheaform BCC subtype. DAB (brown), ×200; (D) Positive reaction with prevalent membranous pattern in tumor cells of micronodular BCC subtype. DAB (brown), ×200; (E) Positive reaction with membranous and cytoplasmic pattern in tumor cells of superficial BCC subtype. DAB (brown), ×100; (F) Positive reaction in stromal cells adjacent to tumor cells of infiltrative-morpheaform BCC subtype. DAB (brown), ×200.***

Statistically, we noticed significant differences between IRS scores of different BCC histological subtypes for all three investigated markers with the metatypical as the highest reactive form for all markers and micronodular as the lowest reactive subtype for CXCR4 and MMP-13 while for β-catenin the superficial form was the less reactive (Figure 7).

The Pearson tests proved a very strong correlation between CXCR4–MMP-13 IRS scores [$r(21)=0.973$, $p<0.01$] and a strong correlation between IRS pairs scores CXCR4–β-catenin [$r(21)=0.785$, $p<0.01$] and MMP-13–β-catenin [$r(21)=0.737$, $p<0.01$] (Figure 8).

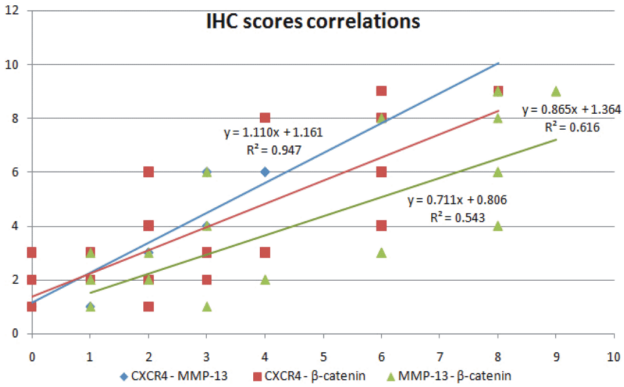

Figure 7 – ***Comparative statistics of the averages of all expression levels. ANOVA testing showed very significant variations between all three markers for the pathological subtypes. P<0.001 for all tests.***

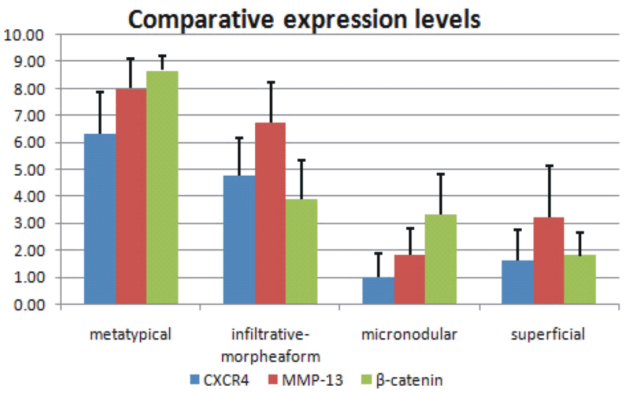

Figure 8 – ***Correlation analysis showed strong dependences between all three tested pairs on Pearson testing (r≥0.73, p≤0.05).***

Discussion

BCC is a non-melanotic skin cancer locally invasive, with slowly spreading which rarely metastasize, arising in the epidermis or hair follicles and in which, in particular, the peripheral cells usually simulate the basal cells of the epidermis [28]. BCCs occurs most frequently at sun-exposed sites, with the head and neck being the common areas and more precisely develops on the face with the nose and lip most commonly affected [29, 30]. According to the type of growth pattern, BCC can be classified into: nodular, superficial, infiltrative-morphoeic, micronodular and others [6]. The nodular and infiltrative-morphoeic are the most common BCC type in head and neck region and together with micronodular subtypes are the most aggressive tumors, because of their tendency to infiltrate the deep subcutis, muscles and even bones [31, 32]. Also, about 85% of metastatic BCC arise from primary lesions in the head and neck region [5].

To explain the local aggressive behavior and their metastatic potential many studies have been performed to identify the molecular determinants implicated in BCC tumor progression. For this reason we investigated the immunohistochemical expression of CXCR4, MMP-13 and β-catenin expression in the aggressive type (infiltrative-morpheaform, micronodular and metatypical) *versus* superficial facial BCC.

In our study, we noticed CXCR4 reactivity in normal epidermis at the level of stratum spinosum especially in the lower layers, in the bulb and at the root sheaths of hair follicles, in sebaceous and sweats glands, in endothelial cells and in the inflammatory cell infiltrate form the dermis. Following skin burns, Avniel S *et al.* observed an increased CXCR4 expression in proliferating epithelial cells as well as in eosinophils and mononuclear cells infiltrate [33]. The authors found that CXCR4 inhibition increase the rate of re-epithelialization following burn injury, suggesting a regulatory role for CXCR4 in skin repair or re-epithelialization. Moreover, Takekoshi T *et al.* noticed a CXCR4 upregulation in the junctional region at the border of human skin psoriatic plaques inhibiting keratinocytes proliferation and mitigating the effects of proliferative T-helper type 17 cytokines [34]. In the same direction, given that stromal reactions surrounding BCC proliferations mimic a wound healing process, it was suspected that the CXCL12 secretion of surrounding fibroblasts might contribute in a paracrine manner to the tumor invasion [35].

In our BCCs specimens, the CXCR4 reactivity varied by histological type. Thus, the highest reactivity was observed in metatypical subtype especially at the level of areas with squamous cells differentiation. The lowest IRS scores were recorded in the micronodular subtype, two cases being negative. In the superficial and micronodular subtypes, the central tumoral cells were more reactive than palisading peripheral cells. Also, we noticed CXCR4 reactivity regardless of histological subtype at the level of endothelial cells and of the associated inflammatory cell infiltrate. Chen GS *et al.* proved a high significantly CXCR4 immunoreactivity in nodulo-ulcerative and sclerosing type *versus* superficial type BCC, suggesting that CXCR4 expression may vary with different subtypes of BCC [18]. Also, the authors established that increased CXCR4 expression enhances proliferation, resistance to apoptosis, migration and angiogenesis of BCC cells *in vitro* and, more importantly, BCC tumorigenesis *in vivo*. Thus, they concluded that specific blockade of CXCR4 leads to tumor regression of BCC *in vivo* and may serve as a potential therapeutic strategy for more aggressive BCC types.

Chu CY *et al.* observed no CXCR4 immunoreactivity in seborrheic keratosis lesions but the tumoral lesions were reactive, the recurrent BCC cases being the most reactive (89.5%), followed by the invasive BCC cases (72.7%) and non-invasive BCC cases (38.7%) [9]. Statistically, the authors found that CXCR4 expression was significantly higher in invasive histological types (micronodular, infiltrative and mixed) of BCC compared to noninvasive types, indicating that CXCR4 may be involved in BCC invasiveness.

Other studies had proved that CXCR4/CXCL12 play an important role in BCC angiogenesis, the paracrine effects of CXCL12 on human BCCs may induce the expression and secretion of several angiogenesis-associated factors and lead to higher MVD [36], which has been found to correlate with the aggressive phenotype of human BCC [37, 38].

Our study proved that MMP-13 reactivity in normal epidermis was restricted to the basal, parabasal and spinous layers with the latter showing the highest reactivity. Also, the cutaneous appendages were reactive and some MMP-13 staining was noticed in dermis, especially in fibroblast and endothelial cells. Vaalamo M *et al.* proved that MMP-13 was not expressed in normal epidermal keratinocytes or in the acute skin wounds, but this collagenase-3 was highly expressed by fibroblasts deep in the chronic ulcer bed suggesting its involvement in the remodeling of collagenous matrix in chronic wounds [39]. In contrast, the expression of human MMP-13 by fibroblasts has been noted in normal human gingival and fetal skin wounds characterized by scarless wound healing [40, 41]. Also, MMP-13 has been shown to enhance the remodeling of three-dimensional (3D) collagen matrix, cell morphology and cell viability of dermal fibroblasts *in vitro* [42]. Moreover, Toriseva M *et al.* had showed the importance of MMP-13 in wound healing by coordinating cellular activities important in the growth and maturation of granulation tissue, including myofibroblast function, inflammation, angiogenesis, and proteolysis [43].

Matrix metalloproteinases (MMPs) have important roles in the tumor invasion especially by their proteolytic activities assisting in degradation of extracellular matrix (ECM) and basement membrane [44, 45]. Since 1997, Airola K *et al.* proved collagenase-3 mRNA expression in focal areas of keratinized cells from BCCs suggesting that its expression is associated with terminal differentiation of epithelial cells in this type of skin neoplasia [46].

All BCCs specimens investigated by us were positive to MMP-13, but the cytoplasmic reaction varied from case to case depending on the histological subtype. The most reactive was the metatypical BCC in which the MMP-13 reactivity predominates inside the tumor proliferations, especially in the areas with squamous cells differentiation. The least reactive was the micronodular subtype in which

the MMP-13 was limited to few central tumor cells from inside micronodular proliferations. The tumor reactivity was more prominent at the tumor invading edge and regardless of histological subtype, it was exceed by stromal MMP-13 expression, recorded peculiar in fibroblasts, inflammatory cells and endothelial cells.

Balbín M *et al.* noticed the production of MMP-13 by a variety of malignant tumors including BCCs of the skin and its expression was associated with aggressive tumors [47]. Also, the authors found that MMP-13 expression was not restricted to tumor cells, this enzyme being produce also in stromal cells surrounding epithelial tumor cells. The same aspect was observed by Alvarez Suárez ML *et al.* in 65% of the eyelids BCCs, which also noticed an MMP-13 up-regulation in the epithelial tumoral cells located at the advancing edge, which could explain the aggressive behavior of this kind of tumors [48]. In the same location of BCCs, Zlatarova ZI *et al.* found an MMP-13 overexpression both in tumor cells (57% of cases) and surrounding stroma (86%) but with various intensity in all subtypes of investigated tumors [49]. Tumor MMP-13 reactivity was more prominent at the invading edge of the BCC, but it was exceeded by the stroma where the MMP-13 expression was noticed in fibroblasts, inflammatory cells and endothelial cells. In other study, MMP-13 was detected in the vasculature from 17 of 20 human BCCs of unknown origin as well in the capillaries of normal human skin taken from the wound margin [50]. The authors conclude that endothelial cells in the skin are a source of MMP-13 and that enzyme expression is upregulated under conditions that promote endothelial cell growth and vascular differentiation. In BCCs, as in other solid cancers, matrix metalloproteinases enable tumor angiogenesis as they allow endothelial cells to invade through basement membrane to form new blood vessels and also by regulation of endothelial cell attachment, proliferation, migration and growth, either directly or by the release of growth factors [51, 52]. In the study of Zlatarova ZI *et al.*, inflammatory cells form connective tissue surrounding the BCCs were also positive for MMP-13 together with MMP-1, MMP-9, and TIMP-1, indicating an important role of inflammation in the regulation of tumor progression [49]. Related to the aggressiveness of some BCCs, Chu CY *et al.* found that the CXCR4 ligand (CXCL12), directed BCC invasion and that this was mediated by time-dependent upregulation of mRNA expression and gelatinase activity of MMP-13 [9].

β-Catenin is a multifunctional protein that controls a number of cell activities, both at the membrane and the nuclear level [53]. Bridging between cytoskeleton and cadherins β-catenin regulate cell-cell interactions [54]. In the nucleus, β-catenin mediates the Wnt/TCF signaling [55–57], regulating a number of gene transcription, such as cyclin D1 [56, 58], metalloproteinase matrilysin [59], survivin [60], MITF [61], TCF-1 [62, 63] and AXIN2 [64], that are involved in the control of cell proliferation and differentiation.

In the normal epidermis, we noticed predominant membranous β-catenin reactivity throughout basal, parabasal and spinous layers. Occasionally, we noticed a nucleo-cytoplasmic staining in the basal layer. Also, β-catenin was expressed in all the skin appendages. Doglioni C *et al.* proved a β-catenin membrane immunoreactivity of epithelial cells of epidermis, eccrine, apocrine, and sebaceous glands of normal human skin [65]. Overall, the differentiated structures of hair follicles displayed a membrane reaction pattern, but in hair matrix cells were also observed an intense nucleo-cytoplasmic staining. Occasionally, β-catenin nucleo-cytoplasmic reactivity was noticed in basal cell of epidermis, in scattered differentiated keratinocytes of the upper layer of the epidermis and in luminal cells of sebaceous glands. In dermis, mainly the fibroblasts and endothelial cells showed membranous reaction pattern with some nuclear reactivity, especially in mesenchymal cells of the dermal papilla [65]. Fukumaru K *et al.* observed that β-catenin expression in normal epidermis was restricted on the keratinocyte cell membrane facing adjacent keratinocytes and as the keratinocytes differentiated, the expression became significantly weaker, suggesting that β-catenin is associated with keratinocyte differentiation as well as cell adhesion in normal keratinocytes [66].

In our study, we noticed β-catenin reactivity in all tumor specimens but with variable intensity from case to case and even in the same tumor from one area to another. The tumor β-catenin staining was influenced both by histological grade and lesion topography (upper part versus advancing edge). The highest reactivity was observed in metatypical BCC subtype, especially in the areas with squamous cells differentiation and inside tumor proliferation. The predominant pattern was membranous and the cytoplasm reactivity was noticed in the proliferative areas with basal cells differentiation, more obvious in the peripheral palisading tumor cells. In infiltrative-morpheaform BCC subtype was observed an increased cytoplasmic β-catenin expression. The nuclear staining was rare and was recorded at the invasion front of metatypical and infiltrative-morpheaform BCC subtypes. The lowest reactivity was recorded in superficial BCC subtype where the β-catenin staining was predominant and in some scattered peripheral cells from the advancing edge that had nuclear reactivity. In addition, we observed peculiar for the infiltrative-morpheaform BCC cases an increase number of stromal cells with β-catenin immunoreactivity adjacent to tumor cells and also regardless histological subtype's weak β-catenin reactivity was recorded in the endothelial cells of tumor vessels and in the associated inflammatory cell infiltrates.

In literature, data regarding the β-catenin reactivity in BCCs are controversial. Thus, while El-Bahrawy M *et al.* observed nuclear staining in 55% (n=56) of their BCC casuistry [67], Saldanha G *et al.* noticed such immunolocalization in only 23% of the 86 BCC examined [68], but Fukumaru K *et al.* (33 cases) and Boonchai W *et al.* (195 cases) showed only membrane expression [66, 69]. The same ambiguity was observed regarding the correlation between β-catenin localization and histological subtype. Thus, El-Bahrawy M *et al.* showed that the immunohistological localization of β-catenin gives a degree of credence to the histological classification of BCCs currently used by pathologists with all subtypes having their own characteristic pattern of membranous, cytoplasmic and nuclear staining [67]. The nuclear localization was most

notable in the infiltrative and morphoeic variants, followed by the superficial variant, and seen least in nodular BCC. Also, the authors showed that micronodular BCC emerged as a distinct subtype, with strong membranous staining, weak cytoplasmic and no nuclear β-catenin staining [67]. Contrariwise, Brinkhuizen T *et al.*, Saldanha G *et al.* and Fukumaru K *et al.* could not find any correlation between β-catenin localization and histological subtype [66, 68, 70]. Also, contrary to El-Bahrawy M *et al.*, Oh ST *et al.* observed an increased β-catenin expression in the tumor cells of micronodular BCC, and the reaction localization was also seen in the nucleus [71]. It seems that the absence of nuclear β-catenin in many cases may be due to high E-cadherin levels, which would also be consistent with the general inability of BCC to metastasize [72]. Nuclear localization of β-catenin was most notably seen at the advancing edge of the infiltrative BCCs and also at the periphery of the nodules in the more indolent variants, strongly supports the hypothesis that β-catenin plays a role in tumor invasion [67, 71]. Nuclear β-catenin translocation directly mediate the downstream Wnt signaling pathway events through transactivation of transcription factors of the lymphocyte enhancer factor (Lef)/T-cell factor (Tcf) family, increasing the MMP production, especially the membrane type-1 matrix metalloproteinase (MT1-MMP) in high-risk BCC [68, 71]. On the other hand, it seems that the CXCR4/SDF-1 axis is correlated with E-cadherin/β-catenin complex expression in invasion and metastasis at least in colorectal cancers [73, 74].

Conclusions

The expression of CXCR4, MMP-13 and β-catenin varied between different types of investigated BCCs. The highest reactivity was observed in metatypical subtype especially at the level of areas with squamous cells differentiation. The lowest reactivity was recorded in micronodular and superficial BCC subtypes. Regardless histological subtype the tumor reactivity was higher at advancing edge and additional a strong stromal reaction was noticed for all investigated markers peculiar in fibroblasts, inflammatory cells and endothelial cells. Thus, we suggest that immunohistochemical investigation in BCCs of all these three markers it can be useful both for identification of high aggressive tumors and to develop novel more efficient therapeutic strategy for these patients by targeting these biomarkers.

Contribution Note

All authors contributed equally to the manuscript.

References

[1] Katalinic A, Kunze U, Schäfer T, *Epidemiology of cutaneous melanoma and non-melanoma skin cancer in Schleswig–Holstein, Germany: incidence, clinical subtypes, tumour stages and localization (epidemiology of skin cancer)*, Br J Dermatol, 2003, 149(6):1200–1206.

[2] Raasch BA, Buettner PG, Garbe C, *Basal cell carcinoma: histological classification and body-site distribution*, Br J Dermatol, 2006, 155(2):401–407.

[3] Lear JT, Harvey I, de Berker D, Strange RC, Fryer AA, *Basal cell carcinoma*, J R Soc Med, 1998, 91(11):585–588.

[4] Walling HW, Fosko SW, Geraminejad PA, Whitaker DC, Arpey CJ, *Aggressive basal cell carcinoma: presentation, pathogenesis, and management*, Cancer Metastasis Rev, 2004, 23(3–4):389–402.

[5] Malone JP, Fedok FG, Belchis DA, Maloney ME, *Basal cell carcinoma metastatic to the parotid: report of a new case and review of the literature*, Ear Nose Throat J, 2000, 79(7): 511–515, 518–519.

[6] Rippey JJ, *Why classify basal cell carcinomas?* Histopathology, 1998, 32(5):393–398.

[7] Woodhouse EC, Chuaqui RF, Liotta LA, *General mechanisms of metastasis*, Cancer, 1997, 80(8 Suppl):1529–1537.

[8] Wang Z, Ma Q, *Beta-catenin is a promising key factor in the SDF-1/CXCR4 axis on metastasis of pancreatic cancer*, Med Hypotheses, 2007, 69(4):816–820.

[9] Chu CY, Cha ST, Chang CC, Hsiao CH, Tan CT, Lu YC, Jee SH, Kuo ML, *Involvement of matrix metalloproteinase-13 in stromal-cell-derived factor 1 alpha-directed invasion of human basal cell carcinoma cells*, Oncogene, 2007, 26(17): 2491–2501.

[10] Son BR, Marquez-Curtis LA, Kucia M, Wysoczynski M, Turner AR, Ratajczak J, Ratajczak MZ, Janowska-Wieczorek A, *Migration of bone marrow and cord blood mesenchymal stem cells in vitro is regulated by stromal-derived factor-1-CXCR4 and hepatocyte growth factor-c-met axes and involves matrix metalloproteinases*, Stem Cells, 2006, 24(5):1254–1264.

[11] Tang CH, Tan TW, Fu WM, Yang RS, *Involvement of matrix metalloproteinase-9 in stromal cell-derived factor-1/CXCR4 pathway of lung cancer metastasis*, Carcinogenesis, 2008, 29(1):35–43.

[12] Bleul CC, Wu L, Hoxie JA, Springer TA, Mackay CR, *The HIV coreceptors CXCR4 and CCR5 are differentially expressed and regulated on human T lymphocytes*, Proc Natl Acad Sci U S A, 1997, 94(5):1925–1930.

[13] Gupta SK, Lysko PG, Pillarisetti K, Ohlstein E, Stadel JM, *Chemokine receptors in human endothelial cells. Functional expression of CXCR4 and its transcriptional regulation by inflammatory cytokines*, J Biol Chem, 1998, 273(7):4282–4287.

[14] Hesselgesser J, Halks-Miller M, DelVecchio V, Peiper SC, Hoxie J, Kolson DL, Taub D, Horuk R, *CD4-independent association between HIV-1 gp120 and CXCR4: functional chemokine receptors are expressed in human neurons*, Curr Biol, 1997, 7(2):112–121.

[15] Horuk R, *Chemokine receptors*, Cytokine Growth Factor Rev, 2001, 12(4):313–335.

[16] Murdoch C, *CXCR4: chemokine receptor extraordinaire*, Immunol Rev, 2000, 177:175–184.

[17] Barbero S, Bonavia R, Bajetto A, Porcile C, Pirani P, Ravetti JL, Zona GL, Spaziante R, Florio T, Schettini G, *Stromal cell-derived factor 1alpha stimulates human glioblastoma cell growth through the activation of both extracellular signal-regulated kinases 1/2 and Akt*, Cancer Res, 2003, 63(8):1969–1974.

[18] Chen GS, Yu HS, Lan CC, Chow KC, Lin TY, Kok LF, Lu MP, Liu CH, Wu MT, *CXC chemokine receptor CXCR4 expression enhances tumorigenesis and angiogenesis of basal cell carcinoma*, Br J Dermatol, 2006, 154(5):910–918.

[19] Jiang YP, Wu XH, Shi B, Wu WX, Yin GR, *Expression of chemokine CXCL12 and its receptor CXCR4 in human epithelial ovarian cancer: an independent prognostic factor for tumor progression*, Gynecol Oncol, 2006, 103(1):226–233.

[20] Koishi K, Yoshikawa R, Tsujimura T, Hashimoto-Tamaoki T, Kojima S, Yanagi H, Yamamura T, Fujiwara Y, *Persistent CXCR4 expression after preoperative chemoradiotherapy predicts early recurrence and poor prognosis in esophageal cancer*, World J Gastroenterol, 2006, 12(47):7585–7590.

[21] Ohira S, Sasaki M, Harada K, Sato Y, Zen Y, Isse K, Kozaka K, Ishikawa A, Oda K, Nimura Y, Nakanuma Y, *Possible regulation of migration of intrahepatic cholangiocarcinoma cells by interaction of CXCR4 expressed in carcinoma cells with tumor necrosis factor-alpha and stromal-derived factor-1 released in stroma*, Am J Pathol, 2006, 168(4):1155–1168.

[22] Spano JP, Andre F, Morat L, Sabatier L, Besse B, Combadiere C, Deterre P, Martin A, Azorin J, Valeyre D, Khayat D, Le Chevalier T, Soria JC, *Chemokine receptor CXCR4 and early-stage non-small cell lung cancer: pattern of expression and correlation with outcome*, Ann Oncol, 2004, 15(4):613–617.

[23] Scala S, Ottaiano A, Ascierto PA, Cavalli M, Simeone E, Giuliano P, Napolitano M, Franco R, Botti G, Castello G, *Expression of CXCR4 predicts poor prognosis in patients with malignant melanoma*, Clin Cancer Res, 2005, 11(5):1835–1841.

[24] Saur D, Seidler B, Schneider G, Algül H, Beck R, Senekowitsch-Schmidtke R, Schwaiger M, Schmid RM, *CXCR4 expression increases liver and lung metastasis in a mouse model of pancreatic cancer*, Gastroenterology, 2005, 129(4):1237–1250.

[25] Kossard S, Epstein Jr. EH, Cerio R, Yu LL, Weedon D, Basal cell carcinoma. In: LeBoit PE, Burg G, Weedon D, Sarasin A (eds), *Pathology and genetics of skin tumours*, World Health Organization Classification of Tumors, IARC Press, Lyon, 2006, 13–19.

[26] Saldanha G, Fletcher A, Slater DN, *Basal cell carcinoma: a dermatopathological and molecular biological update*, Br J Dermatol, 2003, 148(2):195–202.

[27] Remmele W, Stegner HE, *Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue*, Pathologe, 1987, 8(3):138–140.

[28] Jacobs GH, Rippey JJ, Altini M, *Prediction of aggressive behavior in basal cell carcinoma*, Cancer, 1982, 49(3):533–537.

[29] Choi JH, Kim YJ, Kim H, Nam SH, Choi YW, *Distribution of basal cell carcinoma and squamous cell carcinoma by facial esthetic unit*, Arch Plast Surg, 2013, 40(4):387–391.

[30] Narayanan DL, Saladi RN, Fox JL, *Ultraviolet radiation and skin cancer*, Int J Dermatol, 2010, 49(9):978–986.

[31] Bastiaens MT, Hoefnagel JJ, Bruijn JA, Westendorp RG, Vermeer BJ, Bouwes Bavinck JN, *Differences in age, site distribution, and sex between nodular and superficial basal cell carcinoma indicate different types of tumors*, J Invest Dermatol, 1998, 110(6):880–884.

[32] McCormack CJ, Kelly JW, Dorevitch AP, *Differences in age and body site distribution of the histological subtypes of basal cell carcinoma. A possible indicator of differing causes*, Arch Dermatol, 1997, 133(5):593–596.

[33] Avniel S, Arik Z, Maly A, Sagie A, Basst HB, Yahana MD, Weiss ID, Pal B, Wald O, Ad-El D, Fujii N, Arenzana-Seisdedos F, Jung S, Galun E, Gur E, Peled A, *Involvement of the CXCL12/CXCR4 pathway in the recovery of skin following burns*, J Invest Dermatol, 2006, 126(2):468–476.

[34] Takekoshi T, Wu X, Mitsui H, Tada Y, Kao MC, Sato S, Dwinell MB, Hwang ST, *CXCR4 negatively regulates keratinocyte proliferation in IL-23-mediated psoriasiform dermatitis*, J Invest Dermatol, 2013, 133(11):2530–2537.

[35] Mueller MM, Fusenig NE, *Tumor–stroma interactions directing phenotype and progression of epithelial skin tumor cells*, Differentiation, 2002, 70(9–10):486–497.

[36] Chu CY, Cha ST, Lin WC, Lu PH, Tan CT, Chang CC, Lin BR, Jee SH, Kuo ML, *Stromal cell-derived factor-1alpha (SDF-1alpha/CXCL12)-enhanced angiogenesis of human basal cell carcinoma cells involves ERK1/2-NF-kappaB/interleukin-6 pathway*, Carcinogenesis, 2009, 30(2):205–213.

[37] Oh CK, Kwon YW, Kim YS, Jang HS, Kwon KS, *Expression of basic fibroblast growth factor, vascular endothelial growth factor, and thrombospondin-1 related to microvessel density in nonaggressive and aggressive basal cell carcinomas*, J Dermatol, 2003, 30(4):306–313.

[38] Staibano S, Boscaino A, Salvatore G, Orabona P, Palombini L, De Rosa G, *The prognostic significance of tumor angiogenesis in nonaggressive and aggressive basal cell carcinoma of the human skin*, Hum Pathol, 1996, 27(7):695–700.

[39] Vaalamo M, Mattila L, Johansson N, Kariniemi AL, Karjalainen-Lindsberg ML, Kähäri VM, Saarialho-Kere U, *Distinct populations of stromal cells express collagenase-3 (MMP-13) and collagenase-1 (MMP-1) in chronic ulcers but not in normally healing wounds*, J Invest Dermatol, 1997, 109(1):96–101.

[40] Ravanti L, Häkkinen L, Larjava H, Saarialho-Kere U, Foschi M, Han J, Kähäri VM, *Transforming growth factor-beta induces collagenase-3 expression by human gingival fibroblasts via p38 mitogen-activated protein kinase*, J Biol Chem, 1999, 274(52):37292–37300.

[41] Ravanti L, Toriseva M, Penttinen R, Crombleholme T, Foschi M, Han J, Kähäri VM, *Expression of human collagenase-3 (MMP-13) by fetal skin fibroblasts is induced by transforming growth factor beta via p38 mitogen-activated protein kinase*, FASEB J, 2001, 15(6):1098–1100.

[42] Toriseva MJ, Ala-aho R, Karvinen J, Baker AH, Marjomäki VS, Heino J, Kähäri VM, *Collagenase-3 (MMP-13) enhances remodeling of three-dimensional collagen and promotes survival of human skin fibroblasts*, J Invest Dermatol, 2007, 127(1):49–59.

[43] Toriseva M, Laato M, Carpén O, Ruohonen ST, Savontaus E, Inada M, Krane SM, Kähäri VM, *MMP-13 regulates growth of wound granulation tissue and modulates gene expression signatures involved in inflammation, proteolysis, and cell viability*, PLoS One, 2012, 7(8):e42596.

[44] Egeblad M, Werb Z, *New functions for the matrix metalloproteinases in cancer progression*, Nat Rev Cancer, 2002, 2(3):161–174.

[45] Kerkelä E, Saarialho-Kere U, *Matrix metalloproteinases in tumor progression: focus on basal and squamous cell skin cancer*, Exp Dermatol, 2003, 12(2):109–125.

[46] Airola K, Johansson N, Kariniemi AL, Kähäri VM, Saarialho-Kere UK, *Human collagenase-3 is expressed in malignant squamous epithelium of the skin*, J Invest Dermatol, 1997, 109(2):225–231.

[47] Balbín M, Pendás AM, Uría JA, Jiménez MG, Freije JP, López-Otín C, *Expression and regulation of collagenase-3 (MMP-13) in human malignant tumors*, APMIS, 1999, 107(1):45–53.

[48] Alvarez Suárez ML, González Vázquez LO, Barbón García JJ, Vázquez Rojo J, Lamelas Suárez-Pola ML, Vizoso Piñeiro FJ, *Collagenase-3 (MMP-13) expression in epithelial cancers of the eyelids*, Arch Soc Esp Oftalmol, 2004, 79(6):281–288.

[49] Zlatarova ZI, Softova EB, Dokova KG, Messmer EM, *Expression of matrix metalloproteinase-1, -9, -13, and tissue inhibitor of metalloproteinases-1 in basal cell carcinomas of the eyelid*, Graefes Arch Clin Exp Ophthalmol, 2012, 250(3):425–431.

[50] Hattori Y, Nerusu KC, Bhagavathula N, Brennan M, Hattori N, Murphy HS, Su LD, Wang TS, Johnson TM, Varani J, *Vascular expression of matrix metalloproteinase-13 (collagenase-3) in basal cell carcinoma*, Exp Mol Pathol, 2003, 74(3):230–237.

[51] Foda HD, Zucker S, *Matrix metalloproteinases in cancer invasion, metastasis and angiogenesis*, Drug Discov Today, 2001, 6(9):478–482.

[52] Hadler-Olsen E, Winberg JO, Uhlin-Hansen L, *Matrix metalloproteinases in cancer: their value as diagnostic and prognostic markers and therapeutic targets*, Tumour Biol, 2013, 34(4):2041–2051.

[53] Peifer M, Polakis P, *Wnt signaling in oncogenesis and embryogenesis – a look outside the nucleus*, Science, 2000, 287(5458):1606–1609.

[54] Ozawa M, Baribault H, Kemler R, *The cytoplasmic domain of the cell adhesion molecule uvomorulin associates with three independent proteins structurally related in different species*, EMBO J, 1989, 8(6):1711–1717.

[55] Behrens J, von Kries JP, Kühl M, Bruhn L, Wedlich D, Grosschedl R, Birchmeier W, *Functional interaction of beta-catenin with the transcription factor LEF-1*, Nature, 1996, 382(6592):638–642.

[56] Shtutman M, Zhurinsky J, Simcha I, Albanese C, D'Amico M, Pestell R, Ben-Ze'ev A, *The cyclin D1 gene is a target of the beta-catenin/LEF-1 pathway*, Proc Natl Acad Sci U S A, 1999, 96(10):5522–5527.

[57] van Noort M, Clevers H, *TCF transcription factors, mediators of Wnt-signaling in development and cancers*, Dev Biol, 2002, 244(1):1–8.

[58] Tetsu O, McCormick F, *Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells*, Nature, 1999, 398(6726):422–426.

[59] Crawford HC, Fingleton BM, Rudolph-Owen LA, Goss KJ, Rubinfeld B, Polakis P, Matrisian LM, *The metalloproteinase matrilysin is a target of beta-catenin transactivation in intestinal tumors*, Oncogene, 1999, 18(18):2883–2891.

[60] Zhang T, Otevrel T, Gao Z, Gao Z, Ehrlich SM, Fields JZ, Boman BM, *Evidence that APC regulates survivin expression: a possible mechanism contributing to the stem cell origin of colon cancer*, Cancer Res, 2001, 61(24):8664–8667.

[61] Takeda K, Yasumoto K, Takada R, Takada S, Watanabe K, Udono T, Saito H, Takahashi K, Shibahara S, *Induction of melanocyte-specific microphthalmia-associated transcription factor by Wnt-3a*, J Biol Chem, 2000, 275(19):14013–14016.
[62] Roose J, Huls G, van Beest M, Moerer P, van der Horn K, Goldschmeding R, Logtenberg T, Clevers H, *Synergy between tumor suppressor APC and the beta-catenin-Tcf4 target Tcf1*, Science, 1999, 285(5435):1923–1926.
[63] Yasumoto K, Takeda K, Saito H, Watanabe K, Takahashi K, Shibahara S, *Microphthalmia-associated transcription factor interacts with LEF-1, a mediator of Wnt signaling*, EMBO J, 2002, 21(11):2703–2714.
[64] Yan D, Wiesmann M, Rohan M, Chan V, Jefferson AB, Guo L, Sakamoto D, Caothien RH, Fuller JH, Reinhard C, Garcia PD, Randazzo FM, Escobedo J, Fantl WJ, Williams LT, *Elevated expression of axin2 and hnkd mRNA provides evidence that Wnt/beta-catenin signaling is activated in human colon tumors*, Proc Natl Acad Sci U S A, 2001, 98(26):14973–14978.
[65] Doglioni C, Piccinin S, Demontis S, Cangi MG, Pecciarini L, Chiarelli C, Armellin M, Vukosavljevic T, Boiocchi M, Maestro R, *Alterations of beta-catenin pathway in non-melanoma skin tumors: loss of alpha-ABC nuclear reactivity correlates with the presence of beta-catenin gene mutation*, Am J Pathol, 2003, 163(6):2277–2287.
[66] Fukumaru K, Yoshii N, Kanzaki T, Kanekura T, *Immunohisto-chemical comparison of beta-catenin expression by human normal epidermis and epidermal tumors*, J Dermatol, 2007, 34(11):746–753.
[67] El-Bahrawy M, El-Masry N, Alison M, Poulsom R, Fallowfield M, *Expression of beta-catenin in basal cell carcinoma*, Br J Dermatol, 2003, 148(5):964–970.
[68] Saldanha G, Ghura V, Potter L, Fletcher A, *Nuclear beta-catenin in basal cell carcinoma correlates with increased proliferation*, Br J Dermatol, 2004, 151(1):157–164.
[69] Boonchai W, Walsh M, Cummings M, Chenevix-Trench G, *Expression of beta-catenin, a key mediator of the WNT signaling pathway, in basal cell carcinoma*, Arch Dermatol, 2000, 136(7):937–938.
[70] Brinkhuizen T, van den Hurk K, Winnepenninckx VJ, de Hoon JP, van Marion AM, Veeck J, van Engeland M, van Steensel MA, *Epigenetic changes in basal cell carcinoma affect SHH and WNT signaling components*, PLoS One, 2012, 7(12):e51710.
[71] Oh ST, Kim HS, Yoo NJ, Lee WS, Cho BK, Reichrath J, *Increased immunoreactivity of membrane type-1 matrix metalloproteinase (MT1-MMP) and β-catenin in high-risk basal cell carcinoma*, Br J Dermatol, 2011, 165(6):1197–1204.
[72] Madan V, Lear JT, Szeimies RM, *Non-melanoma skin cancer*, Lancet, 2010, 375(9715):673–685.
[73] Choi HN, Kim KR, Lee JH, Park HS, Jang KY, Chung MJ, Hwang SE, Yu HC, Moon WS, *Serum response factor enhances liver metastasis of colorectal carcinoma via alteration of the E-cadherin/beta-catenin complex*, Oncol Rep, 2009, 21(1):57–63.
[74] Wang L, Li CL, Wang L, Yu WB, Yin HP, Zhang GY, Zhang LF, Li S, Hu SY, *Influence of CXCR4/SDF-1 axis on E-cadherin/β-catenin complex expression in HT29 colon cancer cells*, World J Gastroenterol, 2011, 17(5):625–632.

Corresponding author
Ovidiu Simion Cotoi, Lecturer, MD, PhD, Department of Cell and Molecular Biology, University of Medicine and Pharmacy of Tîrgu Mureş, 38 Gheorghe Marinescu Street, 540141 Tîrgu Mureş, Romania; Phone +40740–026 393, e-mail: ovidiu.cotoi@umftgm.ro

Received: June 8, 2013

Accepted: November 25, 2013