ORIGINAL PAPER

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

The prognostic value of CXCR4, α-SMA and WASL in upper lip basal cell carcinomas

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Abstract

Lip cancers account for 10–12% of the total head and neck cancers and, although squamous cell carcinoma is by far the most common lower lip cancer, the basal cell carcinoma (BCC) seems to be more common for the upper lip. Most BCCs have a clinically indolent behavior, but there are also local aggressive and/or metastatic cases, with the incidence of such cases being estimated at about 1–10% of all cases of BCC. Many of the molecular mechanisms underlying this aggression are still unknown, which is why our study aimed to investigate the potential prognosis of a few markers, such as C-X-C chemokine receptor type 4 (CXCR4), alpha-smooth muscle actin (α-SMA) and Wiskott–Aldrich syndrome like (WASL) in upper lip BCCs. For this purpose, 24 basocellular cancers with this localization have been investigated immunohistochemically, histopathologically belonging to the next varieties: superficial, nodular, micronodular, adenoid cystic, keratotic, sclerodermiform and mixed. Regardless of the histopathological subtype, for all invasive cases we have recorded an increased reactivity of the three markers especially in the invasion front, reactivity also present at the stroma level, especially at the stroma–parenchyma interface. The most intense immunoreactivity was obtained for the micronodular and sclerodermiform subtypes, confirming their biological behavior to be more aggressive than the rest of the investigated strains. All these results confirm the prognostic value of the CXCR4/α-SMA/WASL panel in assessing the biological behavior of the upper lip BCC.

Keywords: upper lip, basal cell carcinoma, prognostic, CXCR4, α-SMA, WASL.

Introduction

Lip cancers make up 10–12% of the total head and neck cancers [1], the lips being considered as the most common localization of oral cancer, with an incidence ranging between 25–65% of total oral cancers [2]. However, although squamous cell carcinoma is by far the most common cancer of the lower lip, in the upper lip the basal cell carcinoma (BCC) appears to be more common [3, 4]. BCC is generally the neoplasm developed in older people [5], but it can also develop in young people living in sunny regions [6, 7], as well as those carrying gene mutations leading to albinism [8], basal cellular syndrome, Bazex–Dupré–Christol syndrome, Rombo syndrome and *xeroderma pigmentosum* [9].

Although the mortality rate of this cancer is low, its high incidence induces significant costs in terms of its morbidity, with significant direct and indirect financial costs and implicitly affecting the quality of life of these patients [10]. Most BCCs have a clinically indolent behavior, but there are also local aggressive and/or metastatic cases, with the incidence of such cases being estimated at about 1–10% of all cases of BCC [11].

If the clinical and histopathological (HP) factors that dictate the adoption of an aggressive behavior from this type of tumor are known enough [12], the same cannot be said about the molecular factors with a potential prognostic role in BCC evolution [13]. Thus, BCC was said to be one of the most mutated human cancers, most mutations being determined by exposure to ultraviolet radiation [14]. In addition, many of the cases of BCC present mutations in the Hedgehog pathway, especially the patched 1 (*PTCH1*) gene [15], and in a more limited number of cases, a pathogenic role seems to be played by the p53 tumor suppressor gene [16]. That is why recent investigations are centered on the study of those biological processes with certain implications in adopting aggressive behavior by BCC. In this respect, our study also aimed at investigating the prognostic role of markers like C-X-C chemokine receptor type 4 (CXCR4), alpha-smooth muscle actin (α-SMA) and Wiskott–Aldrich syndrome like (WASL) in upper lip BCC.

Materials and Methods

The group of this study comprised a number of 24 BCC cases, aged between 42 and 83 years, with upper lip localization, diagnosed and operated during the 2012–2016 period, in the Clinics of Oral and Maxillofacial Surgery, Ear, Nose and Throat (ENT) Surgery, Plastic Surgery and Dermatology, Emergency County Hospital, Craiova, Romania. For the immunohistochemical processing, the corresponding HP blocks from the archives of the Laboratory of Pathological Anatomy of the same Hospital

were used. From the archived paraffin blocks, serial sections with a thickness of 4 μm were made, which were then applied to electrostatically charged glass slides. After drying at laboratory temperature, the sections were dewaxed and rehydrated. The immunohistochemical study was an enzyme detection type one, using LSAB2 technique (Labeled Streptavidin–Biotin 2 System) and Dako kit (Redox, Romania – K0675). The antibodies used in this study and their main features are shown in Table 1.

Table 1 – ***The antibodies, clone, dilution, antigenic recovery and controls used***

Antibody	Clone / Producer	Dilution	Antigen breakout	External positive control
CXCR4	Rabbit, polyclonal / Thermo Scientific	1:500	Citrate, pH 6	Squamous carcinoma
α-SMA	Mouse, monoclonal 1A4 / Dako	1:50	Citrate, pH 6	Colon
WASL	Rabbit, polyclonal / Sigma-Aldrich	1:75	Citrate, pH 6	Colon

CXCR4: C-X-C chemokine receptor type 4; α-SMA: Alpha-smooth muscle actin; WASL: Wiskott–Aldrich syndrome like.

The visualization of the reactions was done with the Dako (Redox, Romania – K3468) chromogenic kit of 3,3'-Diaminobenzidine (DAB). The counterstaining was done with Bio-Optica Mayer's Hematoxylin (Tunic, Romania – M06002), after which the sections were dehydrated, clarified and mounted. For validation of the reactions, we used positive external controls and negative external controls by omitting the primary antibody.

As a method for immunohistochemical reactions' quantification, we used the immunoreactive score (IRS) method created by Remmele & Stegner, consisting in examining at ×40 objective at least five tumor areas with maximum tumor reactivity (set at ×10 objective), by determining the percentage of immunoreactive tumor cells multiplied by the intensity of immunoreactions [17]. The percentage scores of tumor cells' immunoreactivity were: 1 (less than 25% marked cells), 2 (26–49% marked cells), 3 (50–74% marked cells), and 4 (over 75% marked cells), and the immunoreactivity intensity was rated as: 1 (weak), 2 (moderate), and 3 (strong). Therefore, IRS varied between 1 and 12. The presence of stromal immunoreactivity in this study was evaluated only qualitatively, by notifying the presence or absence of markers' reactivity and identifying the cellular sources of this marker.

The images were captured using the Nikon Eclipse 55i microscope, equipped with a 5-megapixel cooling camera and Image-Pro Plus software. For the statistical analysis, Student's *t*, analysis of variance (ANOVA), χ^2 (*chi*)-square and Pearson's *r* tests were used applying the Statistical Package for the Social Sciences (SPSS) 10 software. To statistically test the probability of association between the different classes of descriptive categorical variables in this study, we compiled contingency tables with these data, and subsequently used *chi*-square test. The results were considered statistically significant when $p<0.05$. For comparisons on several variables of interest, we used the ANOVA test.

Results

Of the 24 subjects in this study, a total of 12 were less than 60 years old and 12 over 60 years old. Of these, 13 were females and 14 males. In 22 of the 24 cases, the tumor was identified on the upper lip skin, and in only two cases, it was localized on the red of the lip. Histopathologically, the following subtypes were identified: superficial – two (8.33%) cases, nodular – seven (29.17%) cases, adenoid cystic – five (20.84%) cases, micronodular – three (12.5%) cases, sclerodermiform – two (8.33%) cases, keratotic – two (8.33%) cases, mixed – three (12.5%) cases (Table 2).

Table 3 shows the IRS obtained for each case, for each of the three markers used.

Table 2 – ***Distribution of immunohistochemically investigated cases according to histopathological subtype and main epidemiological characteristics***

Histopathological subtypes	Age [years]		Gender		Topography		Total
	<60	*>60*	*Female*	*Male*	*Skin*	*Vermilion*	
Superficial	1	1	1	1	2	0	2
Nodular	5	2	2	5	6	1	7
Adenoid cystic	1	4	3	2	5	0	5
Micronodular	1	2	2	1	2	1	3
Sclerodermiform	1	1	1	1	2	0	2
Keratotic	2	0	2	0	2	0	2
Mixed	1	2	2	1	3	0	3
No. of cases (%)	12 (50)	12 (50)	13 (54.2)	11 (45.8)	22 (91.6)	2 (8.4)	24 (100)

Table 3 – ***IRS according to the histopathological BCC subtype on each case and for each antibody used (CXCR4, α-SMA, WASL)***

Histopathological subtype of BCC	Marker	Cases – IRS							Average IRS
		Case No. 1	*Case No. 2*	*Case No. 3*	*Case No. 4*	*Case No. 5*	*Case No. 6*	*Case No. 7*	
Superficial (2 cases)	CXCR4	1	2						1.5
	α-SMA	3	3						3
	WASL	2	3						2.5
Nodular (7 cases)	CXCR4	2	2	3	4	2	6	6	3.57
	α-SMA	3	6	3	4	4	4	3	3.86
	WASL	3	6	6	9	6	6	6	6

Histopathological subtype of BCC	Marker	Cases – IRS							Average IRS
		Case No. 1	*Case No. 2*	*Case No. 3*	*Case No. 4*	*Case No. 5*	*Case No. 6*	*Case No. 7*	
Adenoid cystic (5 cases)	CXCR5	1	2	3	4	3			2.6
	α-SMA	3	4	4	6	4			4.2
	WASL	3	3	6	6	3			4.2
Micronodular (3 cases)	CXCR5	3	6	9					6
	α-SMA	6	8	8					7.33
	WASL	6	9	12					9
Sclerodermiform (2 cases)	CXCR5	6	9						7.5
	α-SMA	8	6						7
	WASL	9	12						10.5
Keratotic (2 cases)	CXCR6	3	6						4.5
	α-SMA	4	6						5
	WASL	6	9						7.5
Mixed (3 cases)	CXCR6	2	3	6					3.67
	α-SMA	6	8	6					6.67
	WASL	6	9	9					8

IRS: Immunoreactive score; BCC: Basal cell carcinoma; CXCR4: C-X-C chemokine receptor type 4; α-SMA: Alpha-smooth muscle actin; WASL: Wiskott–Aldrich syndrome like.

CXCR4

In the tumor lesions adjacent epidermis, the reactivity for CXCR4 was present in the spinous layer, with cytoplasmic and membranous pattern, the reactivity progressively increasing from the parabasal layer to the granulous one. Reactivity was more obvious especially in areas with associated hyperplasia lesions (Figure 1A). CXCR4 was also positive at the level of the adnexal skin structures, respectively with the maximum reactivity in the hair follicle bulb, with a slightly lower intensity in the external sheath keratinocytes and, respectively, in the sebaceous glands and in the exocrine sweat glands. Reactivity to this marker also occurred in the endothelial cells of blood vessels and some of the inflammatory cells present in the dermis or tumor stroma.

The maximal reactivity (*i.e.*, 7.5) was recorded in the cases of sclerodermiform type BCC (Figure 1B). The maximum intensity was observed especially at the invasion front and especially in the case of an associated inflammatory infiltrate.

The second place concerning reactivity was the micronodular form, with a minimum of reactivity of 3 and a maximum of 9 and an average of IRS for CXCR4 of 6. The tumor reactivity was higher in the invasion front (Figure 1C) compared to the surface area. The pattern of reactivity was cytoplasmic and nuclear, more obvious at the invasion front.

Then there were cases of keratotic BCC, with an average IRS of 4.5 for CXCR4. Reactivity was more evident in the keratinized cells at the center of the proliferation and less in the keratinic pearls (Figure 1D).

A lower reactivity was noted in cases of nodular form of BCC, the average of IRS being 3.57. Reactivity was more pronounced, especially at the level of neoplastic cells inside the proliferation compared to those at the periphery (Figure 1E).

A slightly higher reactivity compared to the nodular variant was observed in mixed BCC cases, with an IRS average of 4.5. This reactivity was observed especially in the sclerodermiform and, respectively, micronodular component of the mixed forms (Figure 1F).

Among the cases with low reactivity were those belonging to the adenoid cystic variant, the registered IRS average being of 2.6. Higher reactivity was evident in solid areas compared to glandular or cystic areas.

The last place as reactivity was the two cases of superficial BCC, which showed IRS of 1 and 2, respectively. A weak cytoplasmic label for CXCR4 was observed, especially in the interior of the tumor proliferation.

α-SMA

The only reported reactivity for the α-SMA marker in the structures adjacent to tumor proliferation was in the erector hair muscles, in the vascular pericytes or in the arteriolar smooth muscles, as well as in the myoepithelial cells of the sweat glands. In the case of tumor proliferations, maximal recorded reactivity was for the micronodular variant (IRS 7.33) and for the sclerodermiform type (IRS 7), respectively (Table 3). A similar reactivity was noted for mixed cases (IRS 6.67) and a lower one was recorded in the keratotic variant (IRS 5). The lowest reactivity was recorded in cases of superficial BCC (IRS 3) and nodular form (IRS 3.85), respectively. Regardless of the HP subtype, the reactivity was higher in invasive cases, especially at the invasion front. In addition, we have also reported a reactivity from the stromal myofibroblasts associated with tumor proliferation, more evident in the nodular and sclerodermiform variants of BCC.

The maximum tumor reactivity was recorded in cases of micronodular BCC, especially in the invasion front (Figure 2A). The pattern of the reaction was a cytoplasmic perimembranous one and more evident especially within the neoplastic islets and not at their periphery. A similar reactivity was also observed for mixed cases, where the reactivity was higher in the micronodular and sclerodermal component compared to the pure nodular component.

In cases of sclerodermiform BCC, the reactivity was obvious in the invasion front (Figure 2B). In the keratotic variant, we have noticed the absence of the α-SMA reaction in the areas of squamous differentiation. In the adenoid cystic variant, the reactivity was somewhat higher in the tumor cells that delimitated the pseudoglandular and cystic structures (Figure 2C). In the nodular variant,

the reactivity was higher at the proliferation level in the invasion front and lower to the tumor surface (Figure 2D). Within the invasive tumors, an intense positive myofibroblastic reaction was also noted in the α-SMA marker (Figure 2E). Reactivity in cases of superficial BCC was particularly present in peripheral cells (Figure 2F).

WASL

Adjacent to tumor proliferation, we have noticed the presence of nuclear and cytoplasmic reactivity for WASL in the epidermis and adnexal glands. At the level of the epidermis, the reactivity was limited to the basal, parabasal layer and to the lower 2/3 of the spinous layer, the reactivity being much more evident in the areas of hyperplasia. Regarding the reactivity of the annexes, a very intense mark was observed in the hair follicles, followed by the sebaceous glands and the sweat glands, respectively. A high reactivity was also noted in fibroblasts, vascular endothelium and inflammatory cells in the dermis and hypodermis.

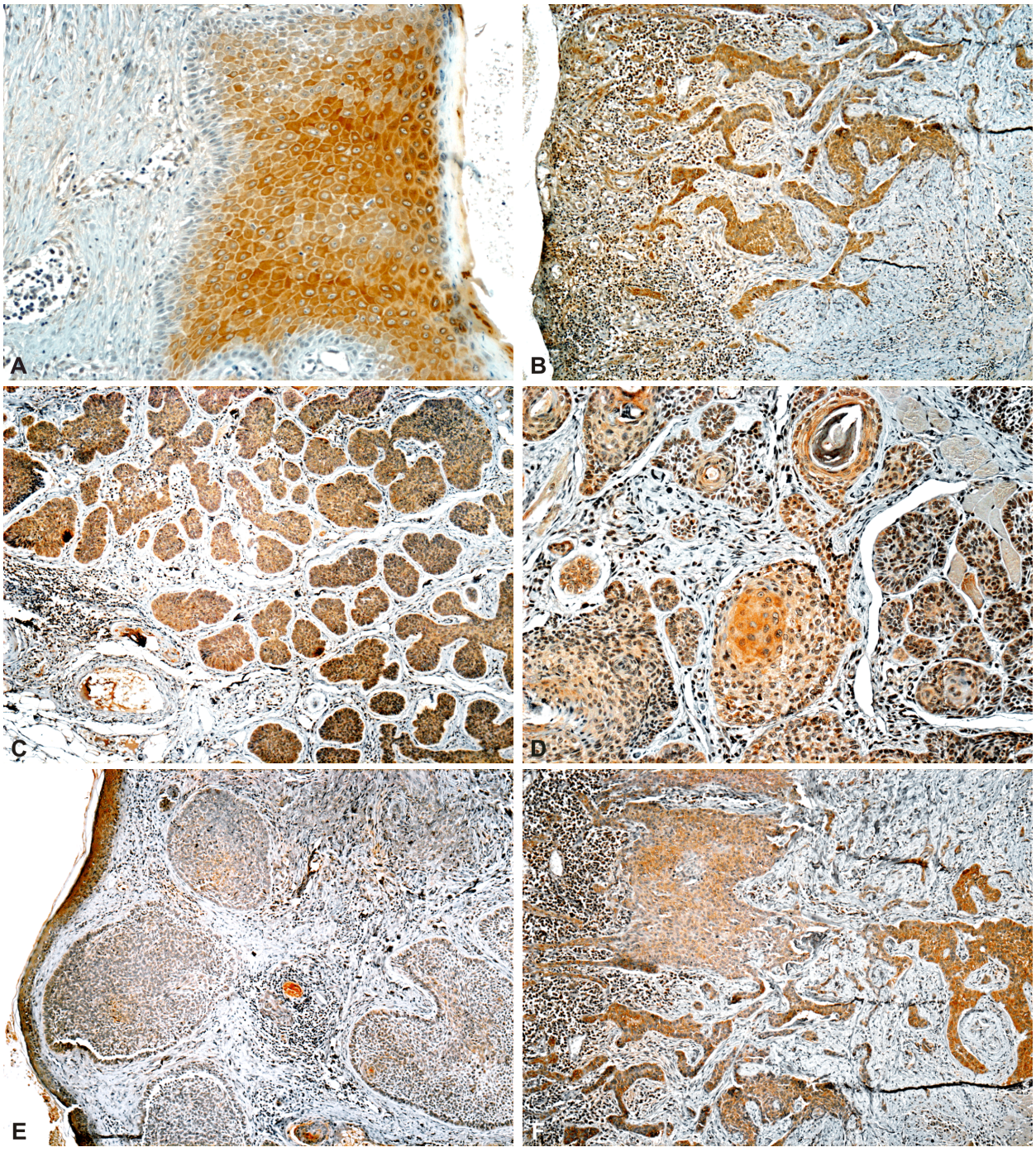

Figure 1 – ***(A) Cytoplasmic reactivity of spinous layer cells to CXCR4 in the acanthosis zone of an associated hyperplasia lesion; (B) Sclerodermiform BCC – reactivity to CXCR4, especially in the invasion front; (C) Micronodular BCC – reactivity to CXCR4 in the invasion front; (D) Keratotic BCC – CXCR4 reactivity especially in keratinized neoplastic cells; (E) Nodular BCC – CXCR4 reactivity especially within tumor proliferation; (F) Nodular and sclerodermiform mixed BCC – CXCR4 reactivity, especially in the sclerodermiform component. IHC-DAB staining: (B, C and E) ×100; (A, D and F) ×200. CXCR4: C-X-C chemokine receptor type 4; BCC: Basal cell carcinoma; IHC: Immunohistochemistry; DAB: 3,3'-Diaminobenzidine.***

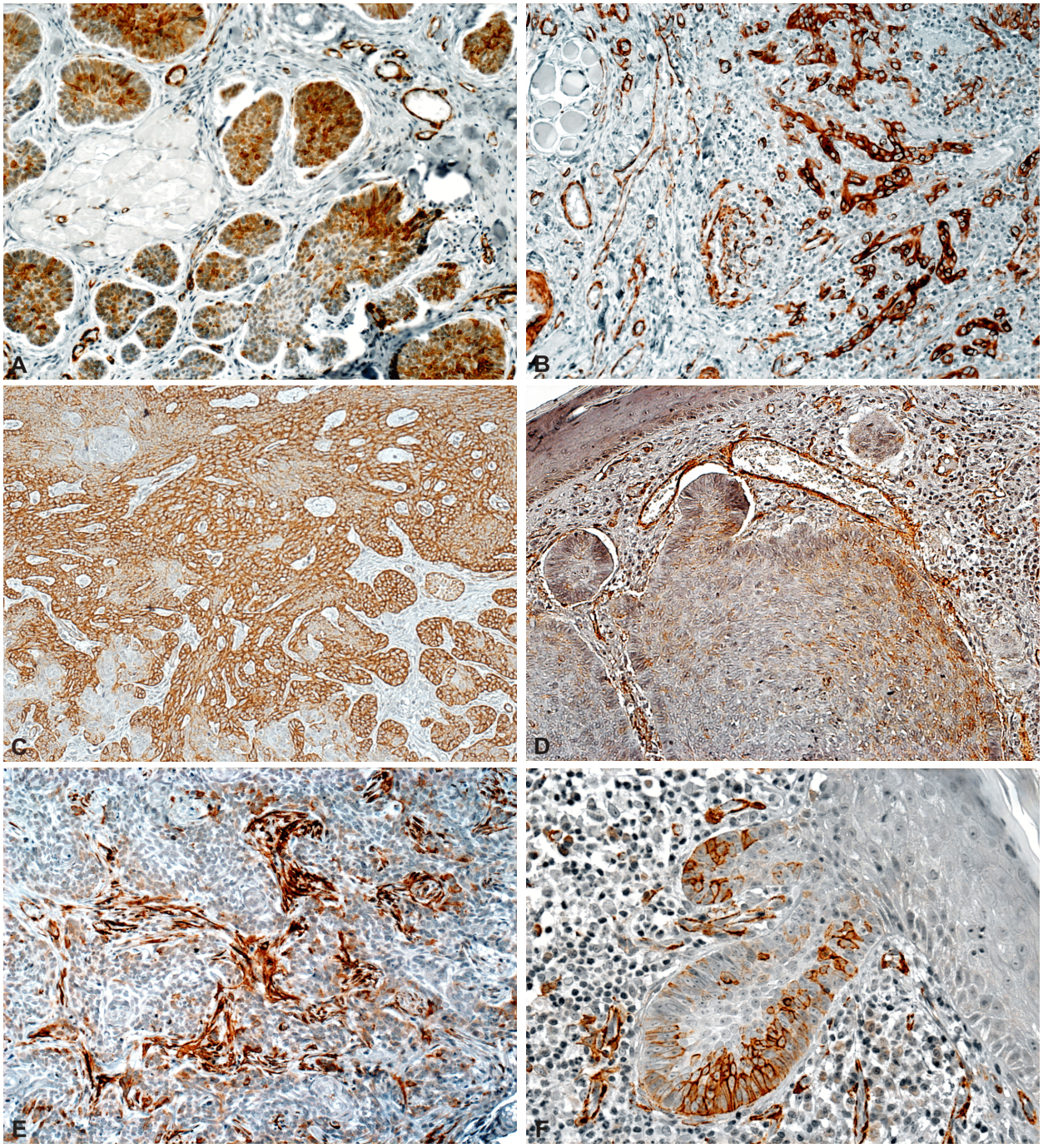

Figure 2 – ***(A) Micronodular BCC – reactivity to α-SMA in the tumor proliferation in the invasion front; (B) Sclerodermiform BCC – reactivity to α-SMA, especially at the proliferation level of the invasion front; (C) Adenoid cystic BCC – reactivity to α-SMA, which is more accentuated in tumor cells that delimitate pseudoglandular and cystic structures; (D) Nodular BCC – reactivity to α-SMA, which is weaker in tumor cells from the periphery of proliferations than in the surface area of the tumors; (E) Nodular BCC – intensive reactivity to α-SMA from the myofibroblastic reaction at the periphery of tumoral proliferations; (F) Superficial BCC – reactivity to α-SMA predominant in peripheral tumor cells. IHC-DAB staining: (A–E) ×200; (F) ×400. BCC: Basal cell carcinoma; α-SMA: Alpha-smooth muscle actin; IHC: Immunohistochemistry; DAB: 3,3'-Diaminobenzidine.***

In terms of tumor lesions, maximal reactivity was observed in sclerodermiform BCCs, where an IRS of 10.5 was recorded, followed by the micronodular variant with an IRS of 9 and somewhat more distant, in cases with mixed BCCs with IRS 8 and keratotic type cases with IRS 7.5 (Table 3). Minimum reactivity was recorded in superficial BCCs (IRS 2.5) and adenoid cystic form (IRS 4.2).

The maximum tumor reactivity was recorded in cases of sclerodermiform BCC (IRS 10.5) in some cases the score being IRS 9 and in the others, IRS 12. Reactivity was more intense in front compared to the superficial area of the tumor (Figure 3A). The pattern of reactivity was cytoplasmic and nuclear.

In the second place as tumor reactivity was a micronodular variant, the recorded scores in the three cases being IRS 6, IRS 9 and IRS 12. Also, here we have noticed the same reactivity difference between the front and the superficial regions of the tumor with a higher

reactivity on the invasion front (Figure 3B). The pattern of the reaction appears to be predominantly cytoplasmic, more intense to the extent of tumor proliferation. Reactivity was also observed from stromal fibroblasts, blood vessels and inflammatory cells in the tumor stroma. A similar reactivity was noted in the mixed variant, where the mean score was IRS 8, the intensity of the reaction being higher in the front and especially in the sclerodermal and micronodular components (Figure 3C).

An intermediate reactivity was presented in the keratotic version, where we recorded IRS 6 and IRS 9, respectively. On the level of the areas with squamous differentiation and especially where keratosis pearls were formed, reactivity for WASL was absent (Figure 3D). Here again, a higher reactivity was noted in invasive cases, especially at the front and with a slightly higher intensity towards the periphery of tumor proliferations. For nodular BCC, the minimum score was IRS 3, and the maximum was IRS 9, with an average IRS 6. Also, here the reactivity was higher in the profound regions of tumors and especially in invasive cases (Figure 3E). The overall adenoid cystic variant had a lower reactivity, the average score being IRS 4.2. Reactivity for WASL was greater in the deep regions of invasive tumors, having the tendency to intensify towards the tumor stroma interface. The weakest reactivity was observed in cases of superficial BCC, the average score being IRS 2.5. The reactions pattern was cytoplasmic and nuclear with a tendency to increase to the center of tumor proliferation, but far below the intensity of the adjacent stromal immunoreactivity (Figure 3F).

The results of the statistical study on the reactivity for the three markers used in the seven investigated BCC variants

In the Figure 4, the IRS for the three investigated markers (CXCR4, α-SMA and WASL) are given for each of the seven BCC HP variants taken in the study. Analyzing the data presented for the CXCR4 marker, we did not notice the existence of statistically significant differences ($p<0.05$) between the various HP investigated BCC subtypes, instead, for the α-SMA marker there were correlations between the superficial type and the micronodular variety ($p=0.011$), sclerodermiform ($p=0.046$) and mixed ($p=0.045$), between the nodular type and micronodular variants ($p=0.006$), sclerodermiform ($p=0.054$) and mixed ($p=0.04$) and between the adenoid cystic type and the micronodular variety ($p=0.026$), and for the WASL marker there were significant differences between superficial type and micronodular variants ($p=0.036$), sclerodermiform ($p=0.013$) and mixed ($p=0.123$), as well as between the adenoid cystic type and the sclerodermiform variety ($p=0.022$). Thus, at least for the α-SMA and WASL markers there were significant differences in immunoreactivity between the so-called less aggressive forms (superficial, nodular and cystic adenoid) and those aggressive ones (micronodular, sclerodermiform and mixed), where the higher IRS were recorded.

By comparing the results of the IRS scores for each of the three markers but for each HP BCC subtype investigated, we have established the existence of significant correlations only for the nodular and adenoid cystic types. In the nodular variant, we have found a weak reversed correlation between CXCR4 and α-SMA ($r=-0.29$), a poor direct correlation between α-SMA and WASL ($r=0.27$) and a moderate direct correlation between CXCR4 and WASL ($r=0.318$). For the adenoid cystic variety, we have obtained strong direct correlations between the three markers (CXCR4 and α-SMA, $r=0.88$; α-SMA and WASL, $r=0.66$; CXCR4 and WASL, $r=0.72$). Therefore, at least in the case of the adenoid cystic variant, the immunoreactivity for the three markers was directly correlated. A slightly weaker correlation was obtained for the nodular variant and we did not obtain statistically significant correlations for the aggressive variants (micronodular, sclerodermiform and mixed) of studied BCCs.

Discussions

Basal cell carcinoma has a low metastatic potential, in literature values quoted for metastatic incidence ranging from 0.0028% to 0.55% [18, 19]. Consecutive tumors may develop quite frequently, with relapses being more common in the first post-operative year. The risk of developing new tumors in the first three post-operative years is 27% to 44%, increasing to 50% in five years, and to 90% at 10 years after the first intervention [18, 20]. Although there are generally slow growing tumors, some of them may have a faster rate of development and may invade adjacent structures [21].

Over time, a series of studies have been conducted that have attempted to explain the locally aggressive character of some of the BCCs and the metastatic potential of some of these cases. Thus, several abnormalities have been incriminated in several biological processes, including: apoptosis, intercellular adhesions and epithelial–mesenchymal transition [21, 22]. In this regard, our immunohistochemical study also aims to investigate several markers proven to be involved in the local invasiveness of several human cancers.

CXCR4

CXCR4 is the stromal cell-derived factor-1 chemokine receptor (SDF-1, also known as CXCL12), the SDF-1/CXCR4 signaling pathway governing the directional cell migration and is involved in a few biological processes, including: cardiovascular apparatus morphogenesis, hematopoiesis, immune response and cancers metastasis. In the skin, CXCR4 has been shown to be overexpressed by keratinocytes in burn proliferation, and inhibition of this receptor appears to increase the rate of after burn skin wounds re-epithelialization [23]. In addition, the same authors showed that the receptor was overexpressed in post-combustion skin wounds and by other cells, like the immune cells of the associated inflammatory infiltrate and the endothelial cells of the adjacent vessels. More recently, it was shown that this SDF-1/CXCR4 signaling pathway would play an important pathogenic role in chronic inflammatory skin diseases, such as psoriasis and therefore its inhibition could be a new therapeutic strategy for patients with such diseases [24].

In our study, we have recorded a good tumor reactivity, the immunoreactivity scores ranging from 1 to 9, with reactivity differences depending on the HP variant. Thus, the highest scores were obtained for the sclerodermal type (IRS 7.5) and for the micronodular type (IRS 6), the most

aggressive forms of BCC. On the opposite side were the cases of superficial BCC (IRS 1) and adenoid cystic BCC (IRS 2.6), respectively. An intermediate reactivity was shown in the keratotic (IRS 4.5) and nodular (IRS 3.57) forms. Regardless of the HP subtype, the reactivity was higher in the invasive forms in the hypoderm and the underlying striated muscle, while there are differences between the superficial and the profound areas of tumors with a maximum reactivity for the latter. The immunoreactivity pattern was cytoplasmic and nuclear, particularly visible at the invasion front of the sclerodermal and micronodular forms, and more as an inflammatory reaction was associated with the front. In nodular and superficial forms, tumor reactivity was predominantly cytoplasmic and more evident in tumor cells within neoplastic proliferations.

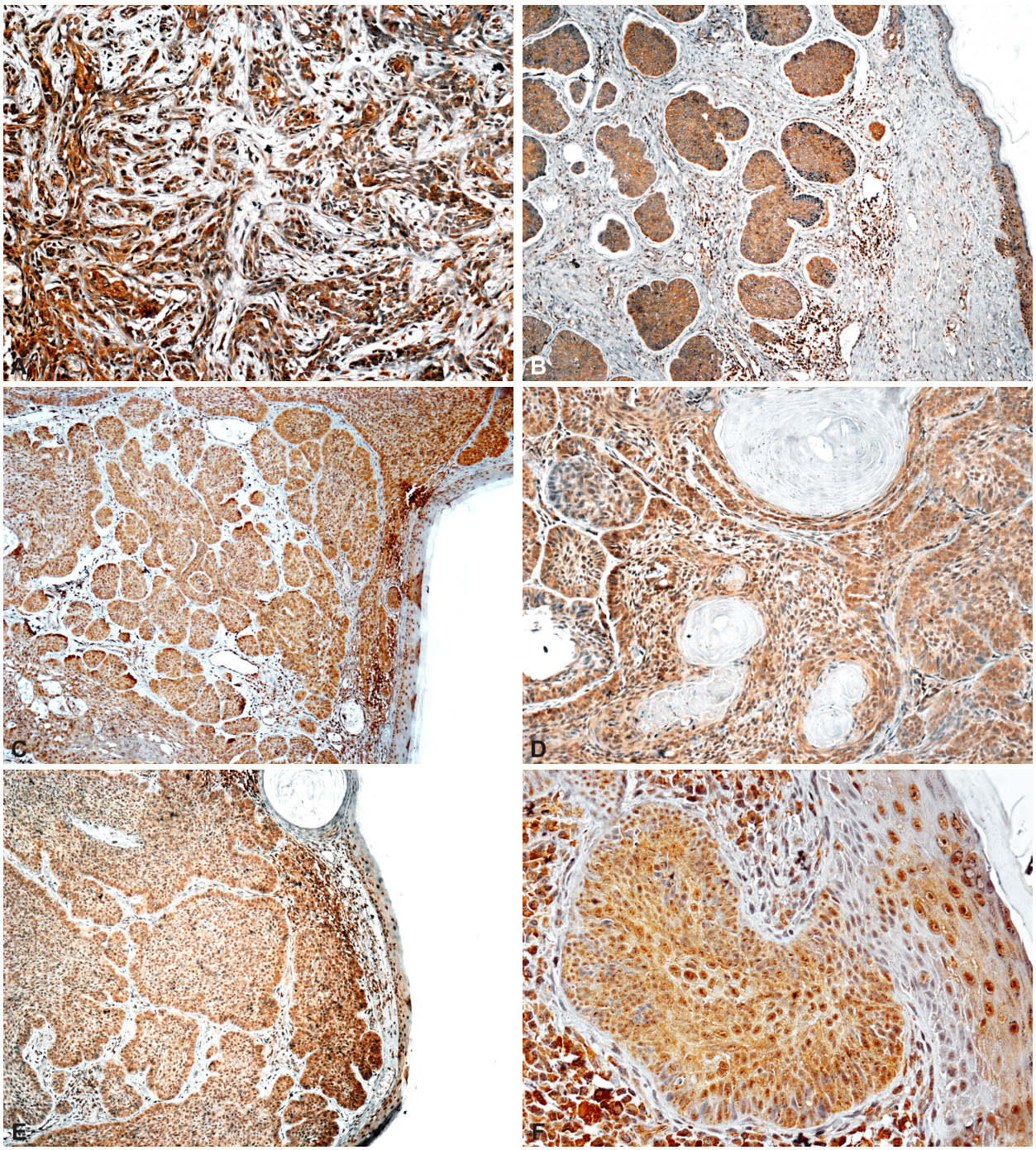

Figure 3 – ***(A) Sclerodermiform BCC – reactivity for WASL at the level of the tumor surface proliferations; (B) Micronodular BCC – reactivity for WASL at the surface level of the tumor proliferations; (C) Nodular-micronodular mixed BCC – reactivity for WASL higher in the micronodular tumor component; (D) Keratotic BCC – reactivity for WASL in tumor proliferation with the exception of squamous differences and especially of keratosis pearls; (E) Nodular BCC – reactivity for WASL at tumor surface proliferations in the superficial tumor region; (F) Superficial BCC – reactivity for WASL at the level of tumor proliferations with a stronger mark on the center of proliferation. Tumor reactivity is lower compared to peritumoral inflammatory infiltrate. IHC-DAB staining: (B, C and E) ×100; (A and D) ×200; (F) ×400. BCC: Basal cell carcinoma; WASL: Wiskott–Aldrich syndrome like; IHC: Immunohistochemistry; DAB: 3,3'-Diaminobenzidine.***

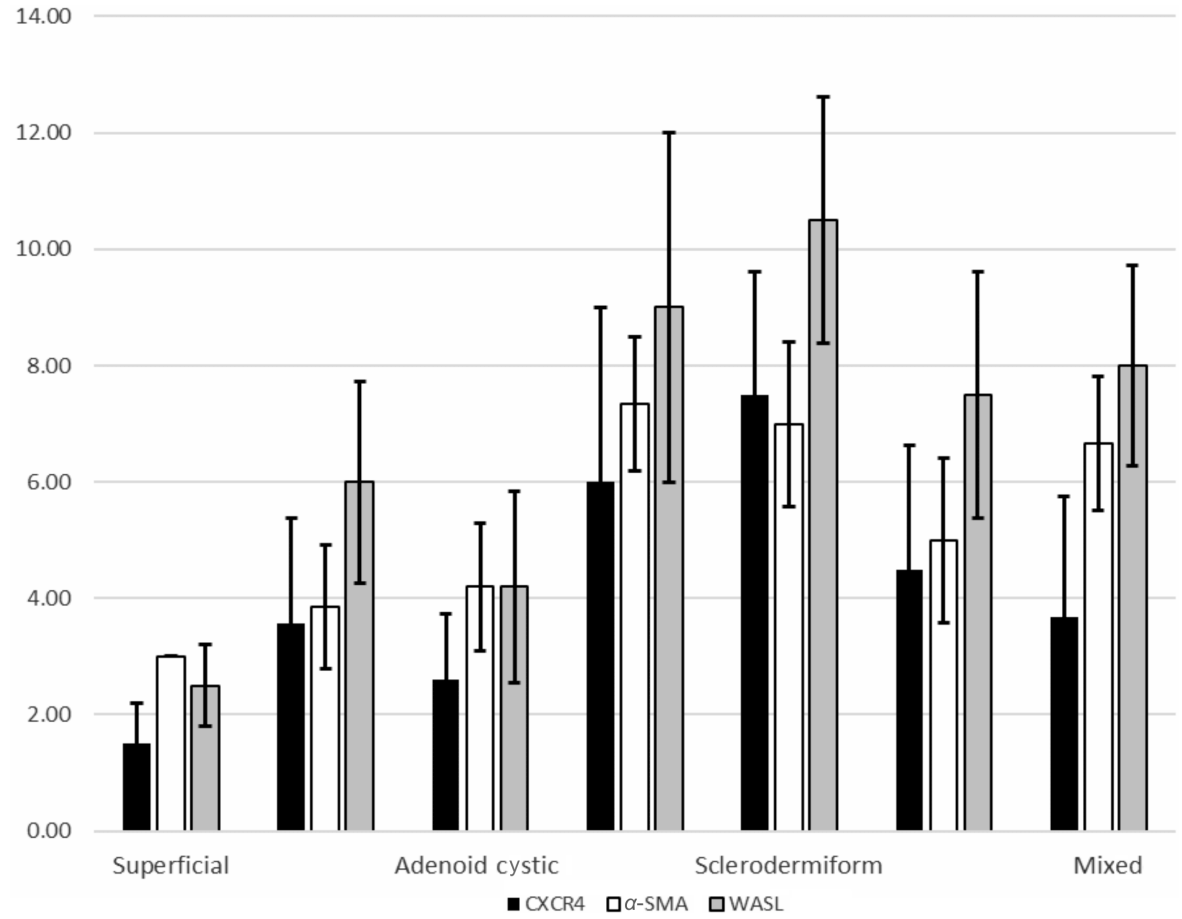

Figure 4 – ***Graphic representation of mean IRS for CXCR4, α-SMA, and WASL for each of the BCC studied specimens. Error bars are the standard deviation. IRS: Immunoreactive score; CXCR4: C-X-C chemokine receptor type 4; α-SMA: Alpha-smooth muscle actin; WASL: Wiskott–Aldrich syndrome like; BCC: Basal cell carcinoma.***

In a study of actinic keratosis, BCC, squamous cell carcinoma and normal skin, Basile *et al.* have noted that there was an overexpression of the CXCR4 receptor in squamous carcinomas compared to actinic keratosis and BCC [25]. The authors concluded that overexpression of this receptor is associated with cancers with a high metastatic potential, like squamous carcinoma. However, in a study published in 2006, Chen *et al.* showed that this receptor is much better expressed in the nodular ulcerative and sclerosant forms of BCC, respectively, compared to the rest of the HP forms [26]. These forms also have a much higher local aggressivity potential, which can be explained by the overexpression of the CXCR4 receptor that would mediate the overexpression of the matrix metalloproteinase (MMP)-13 [27]. Moreover, in another study, CXCR4 overexpression would be responsible for tumorigenesis and tumor angiogenesis in cutaneous BCCs [28]. In a more recent study of 80 cases of BCC, overexpression of the CXCR4 receptor was observed in 70% of the studied cases, this expression correlating significantly with the size of the tumors (tumors larger than 2 cm) and with the HP subtype (invasive type being more immunoreactive than non-invasive one) [29].

α-SMA

α-SMA is a protein involved in cellular processes such as motility, structural preservation and maintenance of their integrity. It is also a marker of myofibroblastic differentiation, this protein being involved in the development of contractile force during skin healing and in fibro-contractile diseases [30]. In addition, a number of studies have demonstrated the activation of carcinoma-associated stromal fibroblasts and their transdifferentiation to a myofibroblastic type by overexpression of *α*-SMA at their level in a wide variety of carcinomas, including squamous carcinoma of the head and neck [31]. It would appear that stromal fibroblasts associated with carcinomas would create at the extracellular matrix level "traces" that carcinoma cells would follow in the local invasive evolution of these types of malignant neoplasms [32]. On the other hand, the presence of myofibroblasts in the oral cancers stroma, including the tongue, has been shown to be associated with poor prognosis in various studies [33–35]. At the same time, it has been shown that evaluating the *α*-SMA expression at the invasion front level in squamous carcinoma of the head and neck would have prognostic value for the survival of these patients [36].

In our study, the *α*-SMA tumor immunoreactivity was an average one in comparison to the other markers used in the study, with scores ranging from 3 to 8, with the highest mean in micronodular BCC cases (IRS 7.33), followed by the scleroderma form (IRS 7) and the mixed one (IRS 6.67), respectively. The lowest reactivity was recorded in cases of superficial BCC (IRS 3) and nodular ones (IRS 3.87). Regardless of the HP subtype, cytoplasmic

reactivity was greater in invasive cases, especially at the invasion front and inside the neoplastic proliferations. In addition, we have also reported a reactivity from stromal myofibroblasts associated with tumor proliferation, more evident in invasive nodular and sclerodermiform BCCs.

Investigating different varieties of BCC, Christian *et al.* found out that α-SMA expression was met in 66% of cases of micronodular BCC, 62% of morpheaform cases and none of cases of nodular BCC [37]. In addition, in half of the cases of α-SMA-positive micronodular carcinoma, invasion of fascia or even muscle and perineural invasion were observed. The authors concluded that α-SMA expression is a marker of invasiveness of micronodular forms of BCC. In another study, increased stromal expression of α-SMA concomitantly with a decrease of bcl-2 oncoprotein expression would be highly suggestive for the aggressive behavior of BCC cases [38]. Additionally, Law *et al.* showed that there are differences in α-SMA reactivity in the nodular component of the nodular infiltrative BCC and the pure nodular variant, and that such a profile would justify the invasive potential of the mixed form [39]. Another study found that immunoreactivity for α-SMA would increase from nodular to infiltrative/morpheaform types, but reactivity would decrease in metastatic cases, which would mean direct involvement in the local invasiveness of this protein, but its expression would be lost when the tumor would acquire a metastatic phenotype [40]. On the other hand, Pilloni *et al.* showed that BCC cases smaller than 3 cm express this protein, thus allowing the identification of a subgroup of small BCCs with aggressive potential [41]. In another study, comparing the α-SMA immunoreactivity between the aggressive and non-aggressive forms of BCC, it was noted that while the reactivity of the tumor cells is similar, stromal reactivity was limited only to aggressive cases, the latter constituting a useful predictive marker of the aggressiveness of such cases of BCC [42].

WASL

WASL is a protein involved in the transduction of signals from membrane receptors to the actinic cytoskeleton. Recent studies have shown that this protein acts directly or by association with the cell division control protein 42 (Cdc42), known as the actinic filament regulator or by association with the actin-related proteins 2/3 (Arp2/3) cytoskeleton organizer complex [43]. At the same time, by binding to actin monomers and the Arp2/3 complex, it is involved in the formation of invadopodium precursors, an actin rich membrane organotin associated with extracellular matrix degradation in the cancer invasion and metastasis process [44].

In our study, the tumor immunoreactivity for WASL was high, approaching the values for Twist1 and cyclooxigenase-2 (COX-2), with scores ranging from IRS 2 to 12. The most intense immunoassay was recorded in sclerodermiform type (IRS 10.5), micronodulary type (IRS 9) and mixed type (IRS 8). The smallest values were obtained in superficial (IRS 2.5) and adenoid cystic (IRS 4.2) types. Regardless of the HP subtype, reactivity for WASL was greater in the deep regions of local invasive tumors, particularly towards the invasion front and the periphery of tumor proliferation. The pattern of reactions was cytoplasmic and nuclear, and reactivity was also present in stromal fibroblasts/myofibroblasts, vascular endothelial cells and inflammatory cells in intra- and peritumoral infiltrates.

In ameloblastoma, a tumor characterized by a high local aggression, the overexpression of the neural (N)-WASP protein was demonstrated especially in the polycystic form and especially the invasive front [45]. In lung cancer, the metastasis process has been shown to be inactivated by growth arrest-specific protein 7 (GAS7) that co-operates with N-WASP in fibronectin/integrin/focal adhesion kinase (FAK) control that regulates tumor cell motility [46]. On the other hand, breast cancer has shown that overexpression of microRNA (miR)-142-3p molecule is responsible for the suppression of expression of a number of factors involved in cytoskeletal function, including WASP, and as a consequence the invasiveness of breast cancer cells is inhibited [47]. The glioma pathogenesis-related protein 1 (GLIPR1) regulates the migration and invasion of neoplastic cells from the partial glioblastoma *via* the N-WASP protein [48]. In oral squamous carcinomas, it was observed that the small protein profilin-2 can be used as both as a potential suppressor and a prognostic factor considering its involvement in the regulation of WASP/Arp2/3 signaling, the major motility cell regulation pathway [49]. A reduction of the invasiveness of head and neck squamous cell carcinomas was reported after administering the miR-375 molecule, by preventing the formation of invasive membranes [50]. In the minireview published by Alblazi & Siar, overexpression of actinic cellular motric regulatory proteins, including WASP and N-WASP proteins, was highlighted in the oro-facial region tumors, which would be responsible for: (*1*) promoting invasion and metastasis, (*2*) association with a poor prognosis in laryngeal cancers, (*3*) increased persistence and metastasis in salivary glands cystic adenoid carcinomas, (*4*) its prognostic value of increased cancer risk from premalignant lesions of the oral mucosa, and (*5*) increasing the local invasive potential of the jaw bone ameloblastoma [51].

Conclusions

Tumor cells in the upper lip BCC had an intrinsic potential for local invasion, as evidenced by the high IRS obtained for CXCR4, especially in the sclerodermal and micronodular forms. In addition, a WASL+/α-SMA+ profile was also observed in the same BCC HP varieties suggesting the existence of an intrinsic motricity in the tumor cells, especially in those in the invasion front. At the same time, for the same HP varieties, an intensive immunoreactivity was also observed for all markers investigated in the stromal tumor micromedium, which would suggest the existence of certain parenchymal–stromal interactions having a role in facilitating the local invasiveness of the upper lip BCC. The use of a CXCR4/α-SMA/WASL has prognostic value allowing the identification of BCC cases with the greatest local invasive potential.

Conflict of interests

The authors declare that they have no conflict of interests.

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Received: October 30, 2017

Accepted: September 28, 2018