Expression of p53, D2-40 and α-smooth muscle actin in different histological subtypes of facial basal cell carcinoma

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Abstract
Although, generally BCC grows slowly and is minimally invasive, tumors developed in the head and neck region behave more aggressively with deep tissue invasion, recurrence and even local or distant metastases, causing significant morbidity or mortality. Recently, numerous studies have been conducted in order to identify new prognostic markers of BCC aggressiveness, but the results are not consistent. Thus, we were interested here in the immunohistochemical investigation of p53, D2-40 and α-SMA expression in the aggressive forms (eight infiltrative-morpheaform, six micronodular and six metatypical cases) versus superficial facial BCCs (five cases). As results, we first noticed that p53, D2-40 and α-SMA expression varied between different types of investigated BCCs. The highest reactivity was observed in metatypical subtype for the D2-40. p53 was mainly expressed in the micronodular BCC subtype and on overall, the tumor reactivity to this marker correlated directly with the reactivity for the other two used biomarkers. The infiltrative-morpheaform facial BCCs were peculiar more reactive to α-SMA. For all three investigated markers, regardless the histological subtype, the tumor reactivity was higher at the advancing edge, and in addition, at this level we noticed a D2-40 and α-SMA stromal reactivity for some cases of the more aggressive BCC subtype (peculiar in metatypical subtype). Thus, we concluded that in order to identify the most aggressive forms of facial BCCs it is useful to investigate these three markers, and this is even more important as they can all constitute therapeutic targets.

Keywords: basal cell carcinoma, p53, D2-40, α-smooth muscle actin, immunohistochemistry, histological subtypes.

Introduction
Basal cell carcinoma (BCC) is the most common worldwide cutaneous cancer [1] with up to 70% of primary BCCs occurring in head or neck region [2]. In this location, BCC has proved to behave more aggressively with deep invasion, recurrence, and regional and distant metastasis [3, 4]. Recently numerous studies have been conducted in order to identify new prognostic markers of BCC aggressiveness, but the results are not consistent [5–10].

In about 50% of all human cancers, the p53 protein is either lost or mutated in a way that compromises its function leading to gain-of-function towards cell migration, invasion, and metastasis [11–17]. On one hand, it seems that loss of p53 is implicated in the loosening of cell–cell junctions and the loss of epithelial integrity, while the mutant p53 are very potent inducers of a metastatic phenotype [16].

It is well known that the chronic exposure to sunlight is responsible for p53 mutation in cutaneous carcinogenesis, playing an important role in the development of BCCs [18–20]. Thus, p53 was regarded as a possible candidate to predict the aggressive behavior in BCC, but the results reported by several investigators are contradictory [21–25].

On the other hand, podoplanin is a mucin-type transmembrane glycoprotein first described in lymphatic endothelium [26] and its formalin-resistant epitope is recognized by D2–40 monoclonal antibody [27]. Although many of its functions are not fully known, human podoplanin was proved to be involve in cancer cell migration, invasion, metastasis, malignant progression [28–30], platelet aggregation [31], tissue development and repair [32, 33].

Moreover, tumor invasiveness involves both tumor cell ability to migrate into surrounding tissue and the proinvasive changes in the surrounding stroma [34]. Responsible for the cell motility are microfilaments, mainly actin, which is sparsely found in normal epithelial cells. It was shown that during malignant transformation of some carcinomas occurs an alteration in the staining pattern of intracellular microfilaments [35]. Thus, Gabbiani et al. found a minimally actin expression in normal epithelial cells while in BCCs and squamous cell carcinomas its expression was more obvious at the edge of the growing tumors and in isolated tumor cells surrounding blood vessels or lymphatic vessels [36]. Also, Tsukamoto et al. reported actin expression within the stroma and tumor nests of some sclerosing, adenoid, solid and superficial BCCs [37].
Given the above data, we were interested here in the immunohistochemical investigation of p53, D2-40 and α-SMA expression in the aggressive types (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 [38]. The BCC growth pattern, assigned as described [39] 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, there 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 forehead (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 distilled 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 Dako Cytomation 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 1:50 for p53 (DO-7, monoclonal mouse anti-human, Dako, Redox, Romania, code M7001), 1:200 for podoplanin (D2-40, monoclonal mouse anti-human, Dako, Redox, Romania, code M3619) and 1:50 for Smooth Muscle Actin (1A4, monoclonal mouse anti-human, Dako, Redox, Romania, code M0851). The primary antibodies were amplified with biotinylated species-specific secondary 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 assessment of the immunostaining, we used the immunoreactive score (IRS) of the Remmele and Stegner [40]. 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 points 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. The reactivity for all three markers was assessed in tumor cells taking into account all cellular localizations of this marker (membranous, cytoplasmic and nuclear). The stromal reactivity was assed semi-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, Apiadro, Bucharest, Romania) equipped with a 5-megapixel cooled CCD camera and the Image ProPlus AM57 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 for all subtypes were compared utilizing ANOVA and Student’s t-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 p53

In all investigated BCC specimens, we found p53 immunoreactivity in the normal epidermis keratinocytes adjacent to the tumors (Figure 2A). Also, we noticed p53 expression in the tumor associated preneoplastic lesions, especially in the dysplastic lesions (Figure 2B).

In this study, p53 expression was recorded in 21 (84%) cases. P53 expression was not diffusely distributed in all areas of the tumors. The highest reactivity was noticed in the peripheral palisading zone and in the deeper advancing edges of the tumors (Figure 2C).

Also, some differences in p53 reactivity were recorded according to histological BCC subtype. Thus, the micronodular subtype appear to be the most reactive BCC subtype, all six investigated cases being positive and also showing the highest IRS scores (score 9). The reaction pattern was diffuse in the tumor micronodules (Figure 2C). The lowest p53 immunoreactivity was recorded in the morpheaform BCC subtype, two of the eight investigated cases being negative. The reduced p53 staining in these cases was obvious especially at the invasive front in the infiltrative cases (Figure 1D). In the metatypical subtype, p53 reactivity was present both in the areas with squamous and basal cells differentiations (Figure 2E). Somewhat, lower p53 reactivity was noticed in the most differentiated tumor squamous areas. In the superficial BCC cases, p53 staining was recorded in four of the five investigated cases, the highest intensity being noticed at the advancing edge (Figure 2F).

Immunohistochemical expression of D2-40

In the normal skin fragments from the resection margins, we observed a D2-40 reactivity confined to a subgroup of the basal cells in epidermis, in the basal cell layer of the outer root sheath of hair follicles, in a subgroup of basal cells at the follicular infundibulum, at the peripheral germinative cells of the sebaceous glands, and in the endothelium of dermal lymphatic vessels (Figure 2A). A constant observation in all BCCs cases was the focal D2-40 reactivity of the basal cells from the above epidermis or immediately adjacent to the tumor (Figure 2B).
In tumor BCCs fragments, the reactivity was noticed in 12 (48%) cases. The highest reactivity was noticed in metatypical subtype with four positive cases. The reaction was noticed around the areas with squamous cells differentiation (Figure 2C). The maximum-recorded IRS score was 6. In two cases, which were deeply invasive, we also noticed a stromal D2-40 reactivity (most probably at the level of the tumor-associated myofibroblasts) in the close vicinity of the invasive tumor islands (Figure 2D).

On the second place as reactivity was the superficial BCC type with three positive cases. The reaction was focally with some positive cells predominantly in the periphery (Figure 2E). From the eight infiltrative-morpheaform BCC subtype, only in three cases we noticed a weak positive reaction in the cytoplasm of some tumor cells from the periphery of proliferative structures (Figure 2F).

Figure 1 – Immunohistochemical reactivity to p53: (A) Positive reaction in the normal epidermis keratinocytes adjacent to the facial BCC tumors. DAB (brown), ×100; (B) Positive reaction in the epidermis dysplastic lesions associated to facial BCC tumors. DAB (brown), ×100; (C) The highest tumor reactivity was noticed in the peripheral palisading zone and in the deeper advancing parts of the investigated BCCs. Micronodular subtype. DAB (brown), ×100; (D) The p53 immunoreactivity in the morpheaform BCC subtype was obvious especially at the invasive front in the infiltrative cases. DAB (brown), ×200; (E) Positive reaction both in the areas with squamous and basal cells differentiations of metatypical BCC subtype. DAB (brown), ×100; (F) Positive reaction in the superficial BCC, mainly at the invasive front. DAB (brown), ×100.
Figure 2 – Immunohistochemical reactivity to D2-40: (A) Positive reaction in the basal cell layer of the outer root sheath of hair follicles, in a subgroup of the basal cells at the follicular infundibulum, and at the peripheral germinative cells of the sebaceous glands. DAB (brown), ×40; (B) Positive reaction in a subgroup of the basal cells from the above epidermis or immediately adjacent to facial BCC tumors. DAB (brown), ×100; (C) The highest tumor reactivity was noticed in the metatypical subtype, especially around the areas with squamous cells differentiation. DAB (brown), ×100; (D) The stromal D2-40 reactivity in a metatypical noticed in the close vicinity of the invasive tumor islands (most probably at the level of the tumor associated myofibroblasts). DAB (brown), ×100; (E) Positive reaction in the superficial BCC type that was confined to few peripheral tumor cells. DAB (brown), ×200; (F) Positive reaction in the infiltrative-morpheaform BCC, restricted to few tumor cells from the periphery of proliferative structures. DAB (brown), ×200.

Last in terms of reactivity was the micronodular subtype, where from the six investigated cases the D2-40 staining was present in two cases with minimum IRS score (1). Generally, the tumor cell reactivity was lower than that of basal cells in adjacent epidermis or tumor lymphatic vessels endothelium. In all investigated tumor specimens, we noticed D2-40 reactivity in the endothelium of tumor lymphatic vessels.

Immunohistochemical expression of α-SMA

In the normal skin fragments from the resection margins, we observed an α-SMA reactivity confined to
erector pili muscle, fibroblasts of the tissue sheath surrounding hair follicles in anagen phase, myoepithelial cells encompassing eccrine glands (Figure 3A), pericytes around blood vessels, and vascular smooth muscle. In addition, weak diffuse α-SMA immunoreactivity was noticed in epidermis keratinocytes adjacent to tumor proliferations (Figure 3B).

In tumor BCCs fragments, the reactivity was noticed in 12 (48%) cases. The highest reactivity was noticed in the infiltrative-morpheaform BCC subtype, all eight investigated cases being positive to α-SMA. The highest IRS score was 9 and the lowest was 3. The staining pattern was cytoplasmic diffuse both inside and at the periphery of proliferative structure with more intense reaction in the periphery (Figure 3C). In three cases, we noticed a positive α-SMA reaction in the stroma around tumor proliferative units. The second most reactive BCC

Figure 3 – Immunohistochemical reactivity to α-SMA: (A) Positive reaction in the myoepithelial cells encompassing eccrine glands. DAB (brown), ×100; (B) Weak diffuse positive reaction in epidermis keratinocytes adjacent to a superficial BCC. DAB (brown), ×200; (C) Positive reaction in infiltrative-morpheaform BCC subtype with more intense reaction in the periphery. DAB (brown), ×100; (D) Positive reaction in micronodular BCC subtype that was more obvious inside the tumor micronodules. DAB (brown), ×200; (E) Weak positive reaction in the metatypical BCC type with predominantly focal staining pattern, especially in the periphery of tumor nodules and at the invasive front. DAB (brown), ×200; (F) A strong stromal reaction at the invasive front of a metatypical BCC case. DAB (brown), ×40.
subtype was the micronodular with four positive cases. The highest IRS score was 6 and the lowest was 2. The staining pattern was focal or diffuse and was more obvious inside the tumor micronodules (Figure 3D). In two cases, especially at the advancing edge, we noticed a weak α-SMA reaction in the adjacent stroma. The lowest α-SMA tumor reactivity was recorded by us in the metatypical subtype. The reaction was present in four cases with score 2 as the highest IRS score. The staining pattern was predominantly focal, especially in the periphery of tumor nodules and at the invasive front (Figure 3E). In all investigated cases, we noticed a strong stromal reaction at the invasive front (Figure 3F). In the superficial BCC subtype, four cases were positive and one negative. The IRS scores were small (score 1 and 2). The staining pattern was diffuse with some intensification inside tumor proliferative units. In none of the investigated cases, we did not notice any α-SMA reaction in the adjacent stroma. The stromal α-SMA positivity, regardless histological BCC subtype, was noticed in the cytoplasm of certain fusiform cells, with centrally-located oval nuclei, that morphologically resemble myofibroblasts. Also, regardless histological BCC subtype, in all investigated cases we noticed α-SMA reaction in the tumor-associated blood vessels (pericytes around small blood vessels and vascular smooth muscle).

Statistically, although the ANOVA testing did not show any significant global differences for the p53 marker, the Student’s t-test showed a higher expression in the micronodular variant compared to metatypical (p<0.017), infiltrative-morpheaform (p=0.003), and superficial forms (p=0.019) (Figure 4). Globally, the ANOVA test has showed for the D2-40 reactivity a significant difference between the semiquantitative scores (IRS score) of the four BCCs histological subtype, F(3,46)=21.231, p=0.001, with the metatypical showing for the Student’s t-test a higher reactivity only when compared to micronodular form (p=0.0038) (Figure 4). Regarding the α-SMA reactivity, the ANOVA test did not show any significant global differences but the Student’s t-test showed a higher expression in the infiltrative-morpheaform BCC subtype compared to metatypical (p=0.0002), micronodular (p=0.0022), and superficial forms (p=0.0009) (Figure 4).

The Pearson test showed a low direct correlation between the D2-40 and p53 IRS scores [r(21)=0.272], p<0.05, and a moderate direct correlation between p53 and α-SMA IRS scores [r(21)=0.339], p<0.05 (Figure 5).

Figure 4 – Comparative statistics of the averages of all expression levels. ANOVA testing showed a significant difference between the semiquantitative scores (IRS score) of the four BCCs histological subtype only for D2-40 tumor reactivity. P<0.001 for all tests.

Discussion

Although, generally BCC growths slowly and is minimally invasive, tumors developed in the head and neck region behave more aggressively with deep tissue invasion, recurrence and even local or distant metastases, causing significant morbidity or mortality [3, 4]. There is no single specific biomarker available for distinguishing aggressive BCCs from non-aggressive tumors, but some studies pointed out that growth pattern and the histological subtype of the tumor can be related to its aggressive behavior [3, 41, 42]. In this setting, we aimed to determine whether there is any relationship between p53, D2-40 and α-SMA expression in aggressive type (infiltrative-morpheaform, micronodular and metatypical) versus superficial facial BCCs.

p53 expression

Several studies have shown p53 expression in pre-cancerous skin lesions and on chronically sun-exposed normal skin suggesting that p53 mutation is an early

Figure 5 – Correlation analysis showed a low direct correlation between the D2-40 and p53 IRS scores, and a moderate direct correlation between p53 and α-SMA IRS scores on Pearson testing (r≥0.272, r≥0.339, p≤0.05).
at the opposite pole were the morpheaform tumors that had the lowest p53 reactivity.

Most authors have suggested a significant relation between p53 expression and aggressiveness of BCCs [22, 24, 52, 53, 59]. On the contrary, Healy et al. found no significant correlation between p53 reactivity and recurrence of BCC [6]. A somehow controversial topic is the relationship between biological behavior of BCCs and their histopathological features. In the study design by De Rosa et al., p53 expression correlated with dedifferentiation of BCC suggesting that increased p53 immunoreactivity might be an indicator of increased aggressiveness for this tumor [24]. Auepemkiate et al. found a significant correlation between p53 immunoreactivity and the solid variant of BCC and an infiltrative growth pattern [21]. Koseoglu et al. reported that 81.2% of nodular BCC growth pattern were p53 negative, while 40% of cases with the infiltrative growth pattern were immunoreactive for p53 [49]. In fact, some studies have shown that the infiltrative BCC growth pattern are more frequent associated with deeper invasion and high recurrence rates compared with other subtypes [60, 61].

On the other hand, Healy et al. failed to confirm any association between p53 immunoreactivity and tumor aggressiveness in BCCs [6]. Also, Demirkan et al. do not shown any significant correlation between p53 expression and the rate of recurrence, pattern and diameter of BCC [23].

Podoplanin

Podoplanin seems to mediate tumor invasion by modulating actin remodeling of the cytoskeleton of tumor cells [29]. This effect may be due by increasing ERM-protein phosphorylation and/or by increasing the Rho GTPases activities [28, 29, 62]. Podoplanin was found to be expressed by tumor cells in various human cancers, such as squamous cell carcinoma (of the oral cavity, esophagus, larynx, lung, cervix, and skin) [28, 29, 63, 64], mesothelioma [65], germ cell tumors [66–68], tumors of the central nervous system [69] and some subtypes of vascular tumors [70–72]. Vicki et al. reported that 90% of human squamous cell carcinomas expressed podoplanin mostly as one-cell layer at the invasion front [29].

Several studies have shown that podoplanin was expressed by a subgroup of the basal cells in normal epidermis, in the basal cell layer of the outer root sheath of hair follicles and by the peripheral germinative cells of the sebaceous glands, and that the majority of squamous cell carcinomas and primary skin adnexal carcinomas also positive for this antibody [68, 73, 74].

Data on the podoplanin immunoreactivity in skin BCCs are contradictory. Thus, Ishida et al. have showed that D2-40 was focally expressed in up to 65% of investigated BCCs and the authors suggested that this reactivity could reflect the differentiation toward the outer root sheath of hair follicles [75]. Also, the authors noticed that there was no significant correlation between the histological subtypes of BCCs and the incidences of immunoreactivity to D2-40. On the contrary, Kanner et al. found that D2-40 reactivity was present only in one of the seven investigated cases [76]. Plaza et al. reported D2-40 expression in 22.2% of investigated BCCs and primarily located in the basal layer of the tumor nests [77]. These data were in contradiction with those regarding D2-40 reactivity in trichoepitheliomas, with 95.5% of these tumors expressing the antibody and not only in the basal layer, but also in the suprabasal layer of the tumor cells. The authors concluded that D2-40 could represent a potential marker in the distinction of trichoepitheliomas from BCCs, and the expression of these markers in a subset of BCCs can reflect the potential of these tumors for multilinear differentiation [76]. Tebcherani et al. reported that only 6% of 307 investigated BCCs were immunoreactive to D2-40 with 4% cases presenting a peripheral layer of tumor cell nests reactivity and 2% with diffuse tumor cell D2-40 expression [78].

Our investigation found focal D2-40 staining in 48% of basal cell carcinomas with the metatypical subtype as the most reactive and the micronodular as the subtype with the lowest reactivity. Generally, the staining pattern was focally with some positive cells predominantly in the periphery of tumor proliferative units. The tumor staining intensity was lower than that confined to a subgroup of the basal cells from adjacent epidermis or from that recorded in the tumor associated lymphatic vessels endothelium.

α-SMA

Studies that have investigated the role of α-SMA as a marker of aggressiveness of BCC has been focused both on its expression in the tumor and in the surrounding stroma.

Regarding tumor expression, some authors found that α-SMA staining tends to be more prominent in the aggressive histological variants of BCC [37, 79, 80]. Thus, Tsukamoto et al. reported an overall actin reactivity of 47% with the solid, adenoid and sclerosing BCC types expressing more frequently, and in greater amount, than cystic, keratotic and superficial types [37]. Somewhat, similar results were also obtained by us, in our study, being recorded a tumor reactivity of 48% from the investigated BCCs cases. We also noticed that tumors with the highest reactivity belonged to infiltrative-morpheaform BCC subtype, followed by the micronodular tumors, the staining pattern being diffuse but with more intense reaction at the tumor advancing edge. Low immunoreactive scores were obtained in the metatypical and superficial BCC tumors. Instead, Christian et al. recorded that only 40% of investigated BCC were α-SMA positive with the micronodular and morpheaform as the most reactive BCC types [79]. The authors found that actin staining was present in 66% of micronodular, 62% of morpheaform, and 0% of nodular BCC, concluding that actin expression in micronodular BCC may be a marker for aggressive invasion.

Law et al. comparing the actin reactivity in seven purely nodular BCCs versus 13 nodular-infiltrative BCCs found that actin expression was more prominent in the nodular component of mixed nodular-infiltrative BCC (85%), when compared with purely nodular BCC (28%) [80]. The authors suggested that nodular components of these two BCCs subtypes are different, and actin expression in the nodular components may be associated with potential invasiveness. Uzquiano et al. reported actin staining in three of 12 (25%) of the nodular BCC, all 10 of the infiltrative BCC, and three of 10 of the
metastatic BCC [81]. The authors suggested that increased actin expression may contribute to local invasiveness, especially in the infiltrative BCC subtype, but its reactivity is lost in the metastatic phenotype. The role that actin in tumor cells plays in tumor biology is not known, the authors hypothesized that actin within tumor cells increases cell mobility, allowing an increased invasion. The tumor actin altered expression may be triggered by a cytokine (such as basic fibroblast growth factor), which has an autocrine effect on the individual BCC cells leading to increased actin synthesis, motility, and invasion [79]. On the contrary, in a more recently study, Adegboyega et al. found that α-SMA tumor expression irrespective of its extent does not show any correlation with tumor aggressiveness [82].

The α-SMA stromal staining was observed by Christian et al. and the authors concluded that this reactivity is most probably due to the presence of stromal myofibroblasts [79]. These are involved in tumor invasion by stromelysin-3 secretion, a metalloproteinase that degrades the stromal matrix [83]. In fact, Suster et al. have hypothesized that actin-containing stromal myofibroblasts facilitate motility and invasiveness of BCC tumor cells [84]. Law et al. found actin expression in the stroma of 62% mixed nodular-infiltrative BCC and no expression in the stroma of any of the purely nodular BCC cases [81]. It seems that BCC cells are capable of induction of myofibroblastic stromal changes in surrounding tissues by secretion of some cytokines. Moreover, Adegboyega et al. showed that only the stromal expression of α-SMA is important in determining the biology and aggressiveness of the BCC [82]. Similar results were reported by Motegi et al., the authors suggesting that the induction of TGF-β from BCC cells or infiltrating cells may induce epithelial to mesenchymal transition of tumor cells, and the differentiation from fibroblasts to myofibroblasts localized around tumors [85]. Authors also suggested that the presence of myofibroblasts surrounding the BCC nest could be one of hallmarks of the aggressiveness of BCC.

In our study, α-SMA stromal reactivity was noticed in only 44% of investigated BCCs, with all metatypical cases being positive and with the most obvious reaction at the tumor invasion front. In addition, we must keep in mind that are several reports of BCC cases being positive and with the most obvious reaction to α-SMA [84, 86, 87].

Statistically, in our study, the only marker that showed significant immunoreactive differences between different histological variants of facial BCCs was D2-40 with metatypical subtype as the most reactive. For the other two used markers, we noticed that micronodular subtype has the highest p53 reactivity while for α-SMA staining the infiltrative-morpheform subtype was the most reactive. When we look at the correlation between tumor immunoreactivity of these biomarkers, we observed a moderate direct correlation between p53 and α-SMA IRS scores, and a low direct correlation between the D2-40 and p53 tumor reactivity.

Conclusions

The expression of p53, D2-40 and α-SMA varied between different types of investigated BCCs. The highest reactivity was observed in metatypical subtype for the D2-40, p53 was mainly expressed in the micronodular BCC subtype and generally, the tumor reactivity to this marker showed a direct correlation with the reactivity for the other two used biomarkers. The infiltrative-morpheform facial BCCs were peculiar more reactive to α-SMA. For all three investigate markers, regardless histological subtype, the tumor reactivity was higher at the advancing edges, and in addition, at this level we noticed a D2-40 and α-SMA stromal reactivity for some cases of the more aggressive BCC subtype (peculiar in metatypical subtype). Thus, we concluded that in order to identify the most aggressive forms of facial BCCs, it is useful to investigate these three markers, especially that they can constitute viable therapeutic targets.

Contribution Note

All authors contributed equally to the manuscript.

References

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