Rom J Morphol Embryol 2022, 63(2):383–393 ISSN (print) 1220–0522, ISSN (online) 2066–8279 doi: 10.47162/RJME.63.2.09

ORIGINAL PAPER

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

Immunohistochemical expression of p53, Ki67, α-SMA, CD44 and CD31 in different histological subtypes of basal cell carcinoma

ANCA COJOCARU[1,2], CARROL BÎRJOVANU[3], ANA-MARIA CIUREA[4], DRAGOŞ NICULESCU[5], OLGUŢA-ANCA ORZAN[2,6], ANA ION[2], DRAGOŞ OVIDIU ALEXANDRU[7], IONICA PIRICI[8], ELENA JANINA VÎLCEA[9], ELENA-ALEXANDRA MARINESCU[10], MARIUS EUGEN CIUREA[10]

[1] *PhD Student, Doctoral School, University of Medicine and Pharmacy of Craiova, Romania*

[2] *Department of Dermatology, Elias Emergency University Hospital, Bucharest, Romania*

[3] *Department of Ophthalmology, Dr. Carol Davila Central Military Emergency University Hospital, Bucharest, Romania*

[4] *Department of Oncology, University of Medicine and Pharmacy of Craiova, Romania*

[5] *Department of Orthopedics, University of Medicine and Pharmacy of Craiova, Romania*

[6] *Department of Dermatology, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania*

[7] *Department of Medical Informatics and Biostatistics, University of Medicine and Pharmacy of Craiova, Romania*

[8] *Department of Human Anatomy, University of Medicine and Pharmacy of Craiova, Romania*

[9] *Department of Machine Construction Technology, Faculty of Industrial Engineering and Robotics, Politehnica University of Bucharest, Romania*

[10] *Department of Plastic Surgery, University of Medicine and Pharmacy of Craiova, Romania*

Abstract

Basal cell carcinoma (BCC) is a common, locally invasive tumor that arises within sun-damaged skin and rarely develops on the palms and soles or mucous membranes. Men generally have higher rates of BCC than women. Incidence also increases with age and the median age of diagnosis is 68 years old. Mortality from BCC is rare and cases of aggressive, local destructive, metastatic BCCs are more likely from tumors with aggressive histopathological (HP) patterns. The aim of this study was to investigate and correlate the immunohistochemical expression of p53, Ki67, alpha-smooth muscle actin (α-SMA), cluster of differentiation (CD)44 and CD31 with both aggressive and nonaggressive types of BCCs. In our study, we observed a varied staining pattern for p53, with the highest reactivity noticed in the peripheral palisading zone. The staining pattern for Ki67 was similar to p53, with a more pronounced reaction in the periphery of the tumor. We found different Ki67 and p53 expression among the various subtypes of BCC. The CD31 reactivity, mostly seen in the stroma, was positive in all BCCs and varied significantly between its different HP subtypes. Regarding stromal expression of α-SMA, the adenoid and basosquamous types had the most intense reaction in our study. The CD44 tumor expression was correlated in our study to the aggressive pattern of BCCs.

Keywords: basal cell carcinoma, p53, Ki67, α-SMA, CD44, CD31.

Introduction

Keratinocyte carcinomas, basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), are the most frequent skin cancers in humans and the incidence of both continues to rise. In individuals with fair skin, approximately 75–80% of malignancies are BCCs.

BCC is a common, locally invasive tumor that arises within sun-damaged skin and rarely develops on the palms and soles or mucous membranes. Men generally have higher rates of BCC than women. Incidence also increases with age and the median age of diagnosis is 68 years old.

Mortality from BCC is rare and cases of aggressive, local destructive, metastatic BCC are more likely from tumors with aggressive histopathological (HP) patterns: morpheaform, basosquamous. Perineural invasion may also be an indicator of aggressive disease [1–3].

BCC is a frequently diagnosed cancer with variable HP subtypes: nodular, the most common subtype; superficial, the most common subtype in younger age groups; morpheaform, the biological behavior is more aggressive; basosquamous, histological features of both BCC and SCC; micronodular, destructive behavior and high rates of recurrence and adenoid [4, 5].

Exposure to ultraviolet radiation (UVR) can cause mutations in the *p53* gene, which is the most frequent genetic abnormality in skin cancers. These mutations can be identified in approximately 20% of melanomas, 50% of BCCs, and more than 90% of SCCs [6].

Ki67 is frequently used as a marker of cell proliferation and can be detected only in dividing cells. It is highly expressed in malignant tissues [7].

Alpha-smooth muscle actin (α-SMA), an isoform of actin, is a marker of epithelial-to-mesenchymal transition and can predict aggressive behavior in BCC [8].

CD44, an adhesion glycoprotein, is used as a marker for cancer stem cell in epithelial tumors [9].

Platelet endothelial cell adhesion molecule-1 (PECAM-1), also known as cluster of differentiation (CD)31, is a transmembrane glycoprotein and a specific and sensitive marker of vascular differentiation [10].

Aim

The aim of this study was to investigate and correlate the immunohistochemical (IHC) expression of p53, Ki67, α-SMA, CD44 and CD31 with both aggressive and non-aggressive types of BCC.

Materials and Methods

We investigated a number of 68 cases of BCC from the Clinic of Plastic Surgery, Emergency County Hospital, Craiova, Romania, of which 17 cases of adenoid subtype, eight cases of basosquamous subtype, 10 cases of micronodular subtype, five cases of morpheaform subtype, 14 cases of nodular subtype and 14 cases of superficial subtype.

Selected formalin-fixed, paraffin-embedded (FFPE) tissue blocks were processed and fresh 4 μm-thick sections were cut and stained with Hematoxylin–Eosin (HE) or prepared for immunohistochemistry. For the later, the sections were deparaffinized in xylene, rehydrated in increasing concentration of alcohol series, processed for antigen retrieval by microwaving in 0.1 M citrate buffer pH 6 for 20 minutes, incubated in 1% hydrogen peroxide in distilled water for 30 minutes to block the endogenous peroxidase activity, and further kept for another 30 minutes in 3% skimmed milk in phosphate-buffered saline (PBS) to block unspecific antigenic binding sites. The primary antibodies were incubated on the slides at 4°C for 18 hours (p53, mouse, diluted as 1:50; Ki67, mouse, diluted as 1:100; α-SMA, mouse, diluted as 1:100; CD44, mouse, diluted as 1:50; CD31, mouse, diluted as 1:100 – Dako, Glostrup, Denmark), and the next day the signal was amplified for 60 minutes utilizing a goat anti-mouse peroxidase polymer-based system (Nichirei Bioscience, Tokyo, Japan). The signal was then detected with 3,3'-Diaminobenzidine (DAB) (Nikirei- Bioscience) and the slides were coverslipped in DPX (Sigma-Aldrich, St. Louis, MO, USA) after a Hematoxylin counterstaining. Images have been acquired utilizing a Nikon Eclipse 55i microscope (Nikon Europe B.V., Amsterdam, The Netherlands) equipped with a 5-megapixel cooled charge-coupled device (CCD) camera and the Image-Pro Plus AMS7 software (Media Cybernetics Inc., Buckinghamshire, UK), and for semi-quantitative purposes, the same illumination and exposure conditions have been employed for all captures. Imaging data were saved as uncompressed *.*tiff* files and a common red, green, blue (RGB) signature profile was utilized to automatically detect DAB signal and quantify it as area and integrated optical density (IOD) for α-SMA and CD44. For p53 and Ki67, positive nuclei were manually counted and reported as percentages from the total number of nuclei in those respective tumor areas (20× area). For CD31, the number of vessels was manually counted and reported.

All recorded data were analyzed in MS Excel. For each tumor subtype, data were expressed as average ± standard deviation (SD), and the results were compared utilizing analysis of variance (ANOVA) testing.

This paper followed the general ethical guidelines of scientific research and has been approved by the Local Ethics Committee.

Results

IHC expression of p53

In the normal epidermis and underlying dermis adjacent to the tumors, we noticed flattened dermo–epidermal junction and papillary dermis with lymphocytes, plasma cells, fibroblasts, and positive reaction to p53 may be seen in the nuclei of the *stratum basale* of the epidermis, as well as in the nuclei of the cellular component found in the dermis (fibroblasts, lymphocytes, plasma cells and a few eosinophils) (Figure 1A).

At the tumoral level, the highest expression was observed in the superficial subtype of BCC, with a homogenous strong positive reaction to p53 seen throughout the tumoral basaloid lobules projecting from the *stratum basale* of the epidermis, with irregular, well-defined margins without signs of invasion in the underlying dermis. The peripheral layer of cells pertaining to the basaloid nests display a typical palisading architecture. Cytologically, the cells within the tumor have large, hyperchromatic nuclei with scarce cytoplasm. The overlying epidermis shows atrophy. An inflammatory infiltrate, mainly composed of lymphocytes, may be seen in the dermis adjacent to the basaloid lobules. A positive reaction to p53 may also be seen in the epidermis, as well as in the papillary dermis, nevertheless, the reaction is much fainter than the one observed in the nuclei of the cells comprising the basaloid lobules (Figure 1B).

Regarding nodular BCC, we noticed a solid, large, relatively circumscribed basaloid lobule surrounded by a thin layer of collagen bundles pertaining to the adjacent stroma. Typical palisading basaloid cells may be seen at the periphery of the lobule, however, in the center, the cells display a chaotic arrangement. The nuclei are large, hyperchromatic, and relatively uniform throughout the tumor. The peripheral layer of basaloid cells showed marked positive reaction to p53, compared to the cells located in the center of the lobule (Figure 1C).

In the adenoid BCC, we observed a positive reaction to p53 within the basaloid cells forming gland-like structures, with some of the nuclei showing a more pronounced staining than the others (Figure 1D).

The morpheaform subtype, observed as basaloid strands with extensive spread into a sclerotic stroma made of tightly packed collagen fibers, had a strong positive reaction to the p53 nuclear marker in the basaloid strands (Figure 1E).

The micronodular subtype is seen as multiple small nests made of basaloid cells with occasional palisading at the periphery. The basaloid nests extend into the surrounding stroma. A positive reaction to p53 is present especially in the nuclei of the basaloid cells at the periphery of the micronodules (Figure 1F).

The lowest p53 expression was observed in the basosquamous carcinoma, with a few scattered basaloid cells within the nests show a faint positive reaction to p53. Few foci of squamous differentiation may be seen within the basaloid nests, in which palisading at the periphery is also present. A small cleft between one of the nests and the adjoining stroma may be observed (Figure 1G).

Comparing the percentages of p53-marked nuclei in the total number of nuclei, between the different types of BCCs, we found that there are no significant differences between the values obtained for control areas (ANOVA p=0.516>0.05), but there are highly significant differences (ANOVA p=0.000<0.001) between different areas of the tumor, which proves that the mutational status of p53 differs depending on the type of BCC. In addition, there were significant differences between each HP subtype and the corresponding peritumor control areas. From this point of view, superficial and nodular subtypes of BCC had more p53-positive cells compared to the other types (Figure 1H).

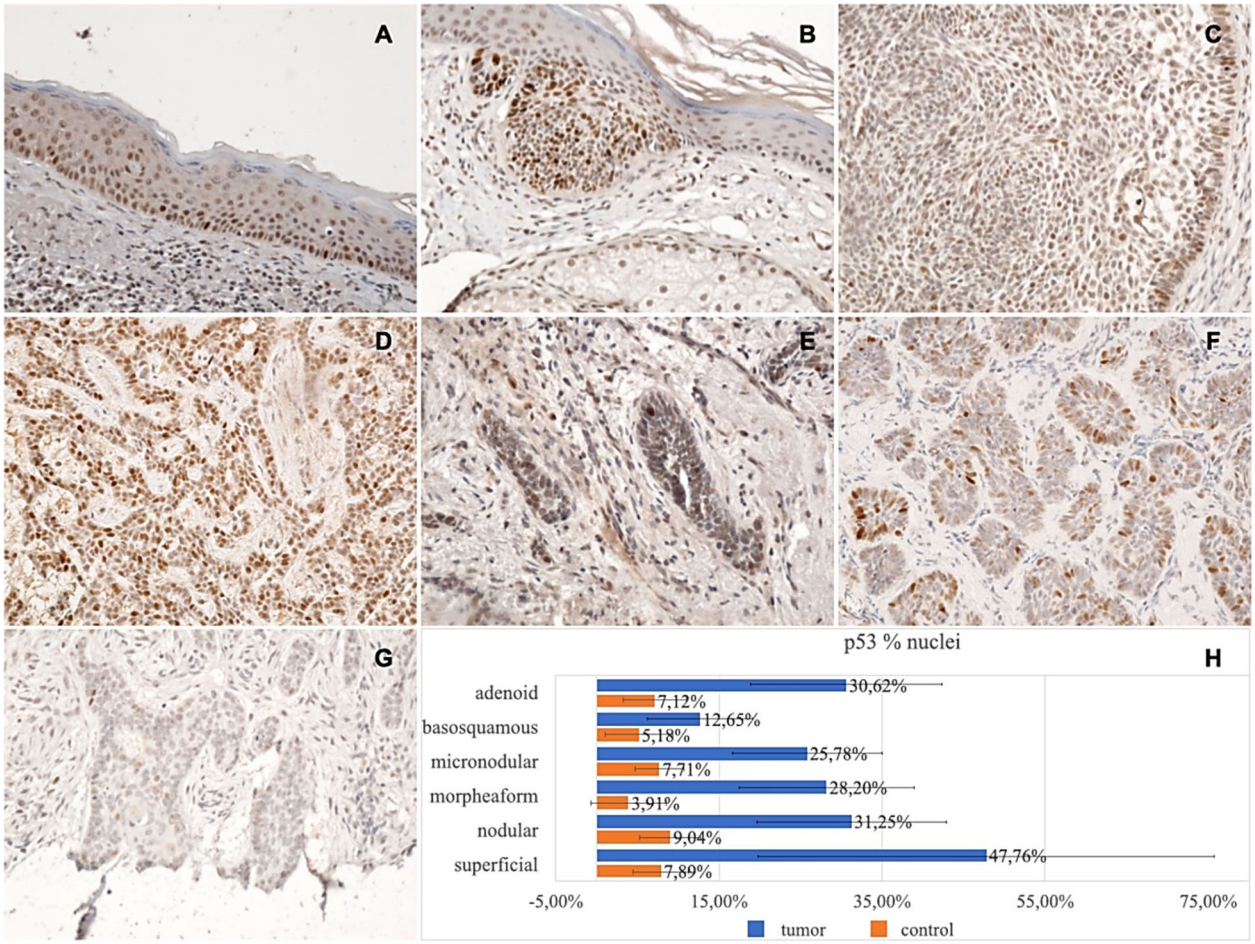

Figure 1 – ***p53 immunoexpression in: (A) Control; (B) Superficial BCC; (C) Nodular BCC; (D) Adenoid BCC; (E) Morpheaform BCC; (F) Micronodular BCC; (G) Basosquamous carcinoma. (H) Percentage values of p53 immunoreactive nuclei in tumor versus control in various BCC subtypes; error bars represent standard deviation of the means. Anti-p53 antibody immunomarking: (A–G) ×200. BCC: Basal cell carcinoma.***

IHC expression of Ki67

In the normal epidermis and the neighboring dermis surrounding the tumor, we noticed positive reaction to the Ki67 nuclear marker in the germinative layer of the epidermis. The cellular component of the dermis is represented by a few lymphocytes, plasma cells and fibroblasts (Figure 2A).

Considering BCC subtypes, the highest expression was observed in the superficial BCC, with positive reaction within the basaloid nest with a homogenous distribution in the center, as well as in the periphery of the lobule. The basaloid nest is relatively well-defined, isolated, large, and closely attached to the overlying epidermis. Palisading of the basaloid cells may be seen at the periphery, as well as a more chaotic arrangement in the center (Figure 2B).

The nodular BCC has a positive, pronounced reaction to Ki67 especially in the nuclei located at the periphery of the basaloid lobule, as well as in the nuclei of the cells pertaining to the basaloid strand. A less marked positive reaction to Ki67 may also be observed in the center of the basaloid lobule, which has an irregular shape and in which the typical pattern of palisading at the periphery is less defined (Figure 2C).

In the adenoid BCC, basaloid cells form multiple pseudoglandular structures of different shapes and sizes. The stroma around the gland-like structures is scarce and mucinous. The positive reaction to Ki67 is heterogenous within the basaloid pseudoglands, with some of the nuclei showing pronounced positivity, while others displaying a weaker positive reaction (Figure 2D).

The morpheaform subtype had a positive reaction to Ki67 within some of the nuclei of the delicate strands comprised of basaloid cells encased in a sclerotic, dense stroma (Figure 2E).

The micronodular subtype had the lowest reactivity with Ki67-positive reaction in only a few of the nuclei pertaining to the basaloid micronodules, which are extending into the adjoining stroma. Palisading may still be observed (Figure 2F).

Basosquamous carcinoma shows basaloid lobules representative for a BCC separated by a thin layer of stroma

with apparent retraction spaces between the collagen fibers and the lobules. The larger nest shows a biphasic aspect, with foci of squamous differentiation, some of them in particular displaying hyperkeratosis with orthokeratosis and possibly keratin formation. There is a positive heterogenous reaction to Ki67 in only some of the nuclei: some cells show a strong staining, while others display a rather pale appearance (Figure 2G).

Analyzing the number of Ki67 marked nuclei from the total number of nuclei in the analyzed area, between the different types of studied carcinomas, we found that there were no significant differences between the values obtained for the control areas (ANOVA p=0.302>0.05), but there are significant differences (ANOVA p=0.009<0.05) between areas of the tumor, which proves that the indices of cell division are different for each tumor type. Furthermore, there were significant differences between each HP subtype and the corresponding control areas surrounding the tumor. Superficial and nodular subtypes had also the highest levels of marker expression in this case as well (Figure 2H).

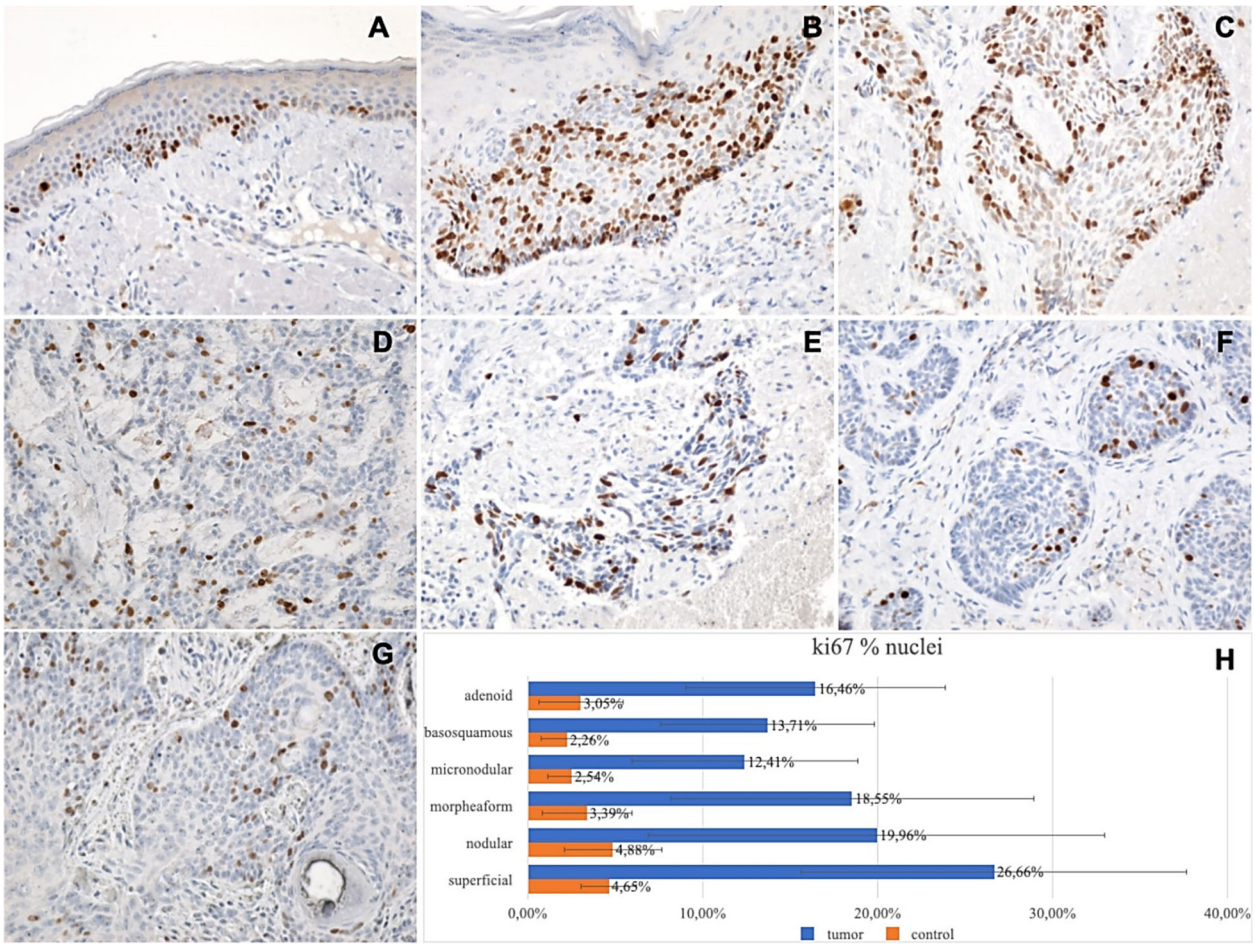

Figure 2 – ***Ki67 immunoexpression in: (A) Control; (B) Superficial BCC; (C) Nodular BCC; (D) Adenoid BCC; (E) Morpheaform BCC; (F) Micronodular BCC; (G) Basosquamous carcinoma. (H) Percentage values of Ki67 immunoreactive nuclei in tumor versus control in various BCC subtypes; error bars represent standard deviation of the means. Anti-Ki67 antibody immunomarking: (A–G) ×200. BCC: Basal cell carcinoma.***

IHC expression of CD31

A positive reaction to CD31 may be seen in the papillary dermis adjacent to the tumors. A uniform thinning of the epidermis in which a few necrotic keratinocytes reside may be observed. The dermal papillae and the rete ridges intertwine to establish the dermo–epidermal junction (Figure 3A).

Regarding superficial BCC, described as a basaloid lobule projecting from an atrophic epidermis into the underlying papillary dermis, a positive reaction to CD31 is mostly seen in the stroma, in the endothelial cells padding the dermal capillaries (Figure 3B).

The nodular BCC had the strongest positive reaction to CD31 mostly seen in the stroma, corresponding to the endothelial cells padding the stromal vessels. The stroma surrounding the basaloid lobule is highly cellular, with a great number of inflammatory cells (lymphocytes, plasma cells, monocytes) dispersed between the fibroblasts. The representative peripheral palisading of the basaloid cells can also be noticed (Figure 3C).

Within the stroma separating the pseudoglands of adenoid BCC, a marked positive reaction for CD31 is seen. Basaloid cells form reticular, gland-like structures, some of them in close contact, others separated by a rather mucinous stroma (Figure 3D).

A heterogenous expression of CD31 is seen only in some parts of the stroma in the case of morpheaform BCC (Figure 3E). The lowest CD31 expression was observed in the micronodular subtype, with a positive reaction mostly in the endothelium of the stromal capillaries (Figure 3F).

The basosquamous carcinoma is defined by basaloid, well-defined lobules with a squamous component extending into the surrounding stroma. The main basaloid lobule

presents with a circular area in which the cells become flatter, a feature which may suggest squamous differentiation. A retraction space between one of the basaloid lobules and the stroma is present. A positive reaction to CD31 is mostly seen in the stroma, with no positive reaction within the basaloid lobules (Figure 3G).

Comparing the number of CD31 marked vessels from the total number of vessels in the studied area, among the different subtypes of BCCs, we found that there are no significant differences between the values obtained for the control areas (ANOVA p=0.098>0.05), but there are significant differences (ANOVA p=0.049<0.05) between areas of the tumor, which proves the different sensitivity of CD31 depending on the type of carcinoma. Nodular and basosquamous subtypes had more CD31-positive vessels compared to other forms (Figure 3H).

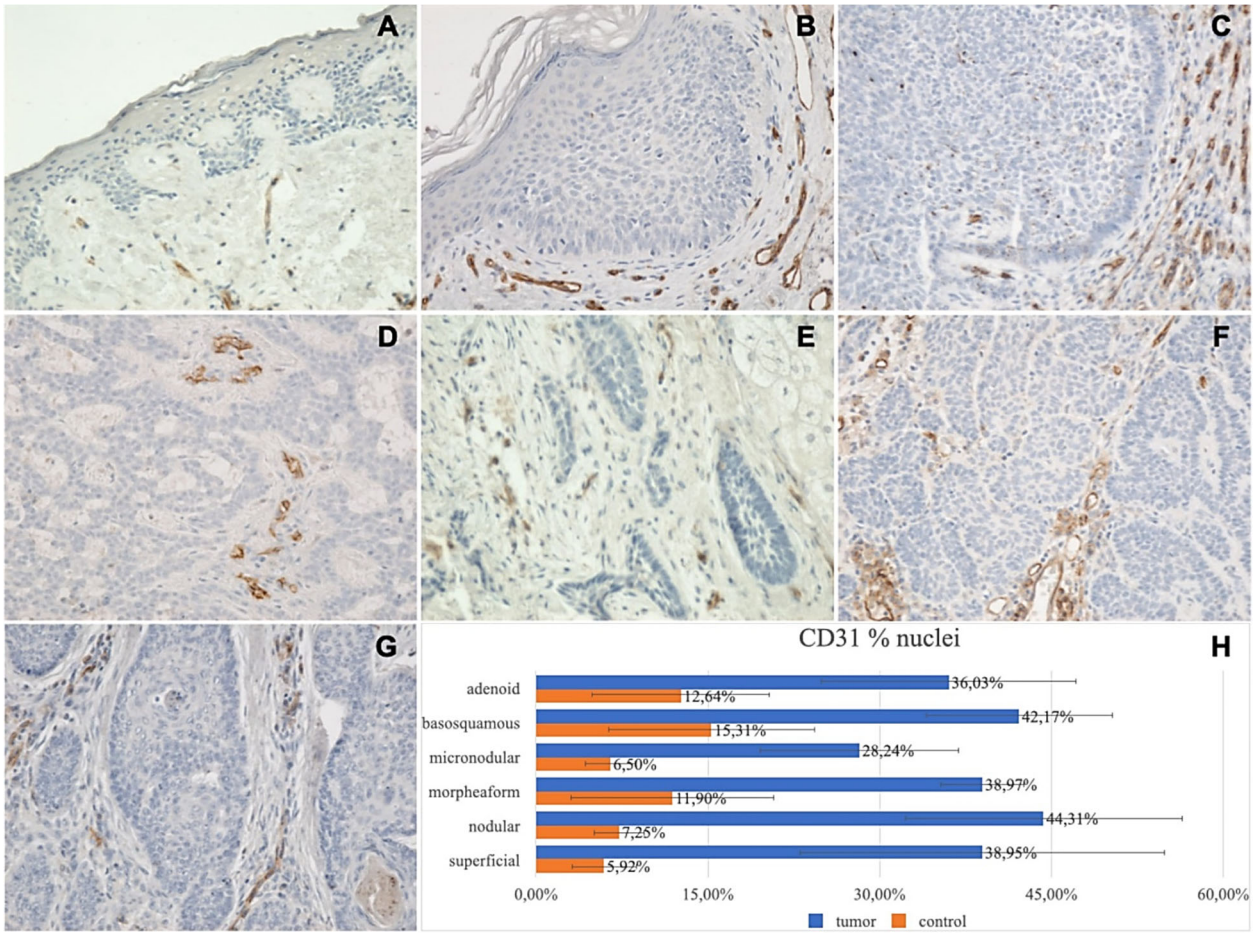

Figure 3 – ***CD31 immunoexpression in: (A) Control; (B) Superficial BCC; (C) Nodular BCC; (D) Adenoid BCC; (E) Morpheaform BCC; (F) Micronodular BCC; (G) Basosquamous carcinoma. (H) Percentage values of CD31 immunoreactive vessels in tumor versus control in various BCC subtypes; error bars represent standard deviation of the means. Anti-CD31 antibody immunomarking: (A–G) ×200. BCC: Basal cell carcinoma; CD31: Cluster of differentiation 31.***

IHC expression of α-SMA

The study of α-SMA expression revealed positive reaction in the papillary dermis surrounding BCCs, mostly around the dermal capillaries. Within the epidermis, there are a few apoptotic keratinocytes. The underlying papillary dermis shows a pronounced cellular component, with lymphocytes, plasma cells, monocytes (Figure 4A).

A positive reaction to SMA is encountered in the fibroblasts and myofibroblasts of the peritumoral stroma, as well as in the vascular smooth muscle cells. A fainter positive reaction to SMA may be observed in the basaloid lobule of the superficial subtype originating from the lower part of the epidermis. The papillary dermis is rich in small blood vessels located mostly around the basaloid lobule (Figure 4B).

Regarding the nodular subtype, a positive reaction to SMA is observed within the stroma surrounding the basaloid lobules, particularly in the right lower part of the image, most likely staining vascular smooth muscle cells pertaining to the stromal microvessels. At the periphery of the lobule, there are portions in which the basaloid cells form a palisade (Figure 4C).

A pronounced, positive reaction for SMA is observed within the stroma of adenoid subtype, probably due to the presence of myofibroblasts, which are activated fibrillogenic cells. The stroma is scarce between basaloid lobules, which have pseudoglandular pattern (Figure 4D).

In the morpheaform subtype, the dense, sclerotic stroma surrounding the basaloid strands shows a strong positive reaction to SMA. The basaloid cell within the strands, however, do not stain positive to SMA (Figure 4E).

Compared to the above-mentioned subtypes of BCC, some of the cells in the basaloid nests of micronodular subtype show positive staining for SMA. A positive reaction to SMA is also observed within the stroma. Nests comprised of basaloid cells form a palisade at the periphery. However, there is a chaotic arrangement of the cells in the center of the micronodules (Figure 4F).

Basosquamous carcinoma shows basaloid nests, with foci of squamous differentiation. These are surrounded by thin strands of stroma. A notable, positive reaction to SMA with homogenous staining is seen within the stroma adjacent to the basaloid nests (Figure 4G).

Comparing the expression levels of *α*-SMA, expressed mainly in myofibroblasts, from the total analyzed area, between the different types of studied carcinomas, we found that there are no significant differences between the values obtained for the control areas (ANOVA p=0.248>0.05), but there is highly significant difference between the values obtained for the areas in the tumor (ANOVA p=0.000 <0.001), and highly significant differences (ANOVA p=0.000<0.001) between the areas in the stroma. The highest values were recorded for tumor epithelium in the micronodular and superficial forms, the lowest values being recorded for morpheaform subtype. On the other hand, regarding stromal expression, the highest *α*-SMA reactivity was recorded by the adenoid and basosquamous types (Figure 4H).

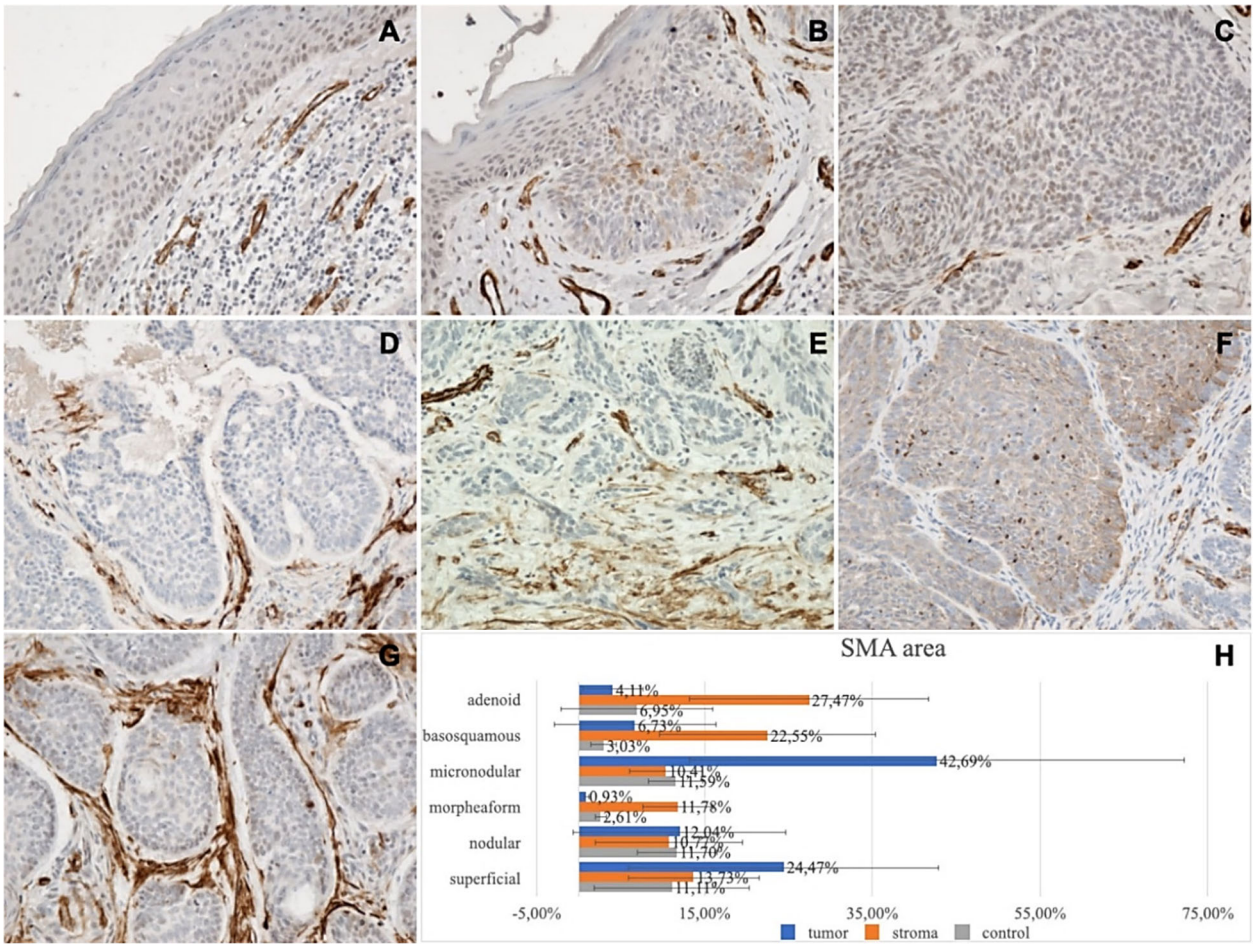

Figure 4 – ***α-SMA immunoexpression in: (A) Control; (B) Superficial BCC; (C) Nodular BCC; (D) Adenoid BCC; (E) Morpheaform BCC; (F) Micronodular BCC; (G) Basosquamous carcinoma. (H) Percentage values of α-SMA immunoreactive tumor area versus immunoreactive stromal area versus control; error bars represent standard deviation of the means. Anti-α-SMA antibody immunomarking: (A–G) ×200. α-SMA: Alpha-smooth muscle actin; BCC: Basal cell carcinoma.***

IHC expression of CD44

Considering CD44 reactivity in the area adjacent to the tumor, keratinocytes show a positive reaction, the stain strongly highlighting their contour and shape. A positive reaction to CD44 is also seen among the fibroblasts within the dermis. The epidermis shows a reduction in the number of keratinocyte layers, particularly at the level of the *stratum spinosum*, which results in an overall thinning of the epidermis. The dermo–epidermal junction is rather flattened, with no rete ridges and an isolated dermal papilla. Within the papillary dermis, inflammatory cells (lymphocytes, plasma cells, a few monocytes, and neutrophils) and numerous fibroblasts may be found (Figure 5A).

The superficial subtype appears as a large, isolated lobule, with irregular, well-circumscribed margins emerging from the *stratum basale* of the epidermis. The underlying dermis contains fibroblasts and scattered macrophages and lymphocytes. Regarding CD44 expression, the *stratum spinosum* of the peritumoral epidermis stands out showing a positive reaction to CD44, as well as the cellular component of the surrounding stroma, compared to the basaloid lobule, in which a notable positive reaction to CD44 is not found (Figure 5B).

A positive reaction to the CD44 surface marker is seen among the fibroblasts of the peritumoral stroma, rather than in the basaloid nest of nodular subtype. Within it, the basaloid cells at the center display a chaotic arrangement, however, at the periphery, they form the typical palisade. The peritumoral stroma is rich in fibroblasts (Figure 5C).

Regarding adenoid subtype, a faint, positive reaction to

CD44 on the surface of the basaloid cells may be observed, compared to the surrounding stroma, in which a less pronounced staining is seen. Basaloid cells are arranged in a pseudoglandular, reticular pattern, with delicate gland-like structures that are either in close contact or separated by a thin layer of mucinous stroma containing a few fibroblasts (Figure 5D).

A positive reaction to CD44, with notable surface staining, is observed within the basaloid cells of the morpheaform subtype nests and strands. Nevertheless, the positive reaction to CD44 within the stroma is less pronounced. The surrounding stroma is dense and numerous fibroblasts are present (Figure 5E).

The stromal fibroblasts show a strong positive reaction to CD44 compared to the basaloid cells of the micronodular nests. The solid basaloid nests have different shapes and sizes and irregular margins which coalesce to form larger structures (Figure 5F).

The basosquamous carcinoma is described as large lobules, with a biphasic appearance, with both a basaloid morphology, as well as foci of squamous differentiation. At the periphery of some lobules, the basaloid cells form a palisade. Similar to the morpheaform subtype presented above, there is a positive reaction to CD44 with marked staining of the basaloid cells compared to the surrounding stroma, which displays a weaker reaction to CD44 (Figure 5G).

The CD44 adhesion molecule was expressed in both tumor cells and stromal elements. Comparing the percentage value of CD44 signal area from the total analyzed area, between the different BCC subtypes, we found that there are significant differences between the values obtained for the control areas (ANOVA p=0.005<0.05) and for those in the tumor (ANOVA p=0.003<0.05), and that there are highly significant differences (ANOVA p=0.000<0.001) between the areas in the stroma. Regarding tumor cells, the highest CD44 expression was recorded for the morpheaform and basoquamous subtypes, while in the case of stromal expression, the highest expression of CD44 was recorded for the adenoid and morpheaform subtypes (Figure 5H).

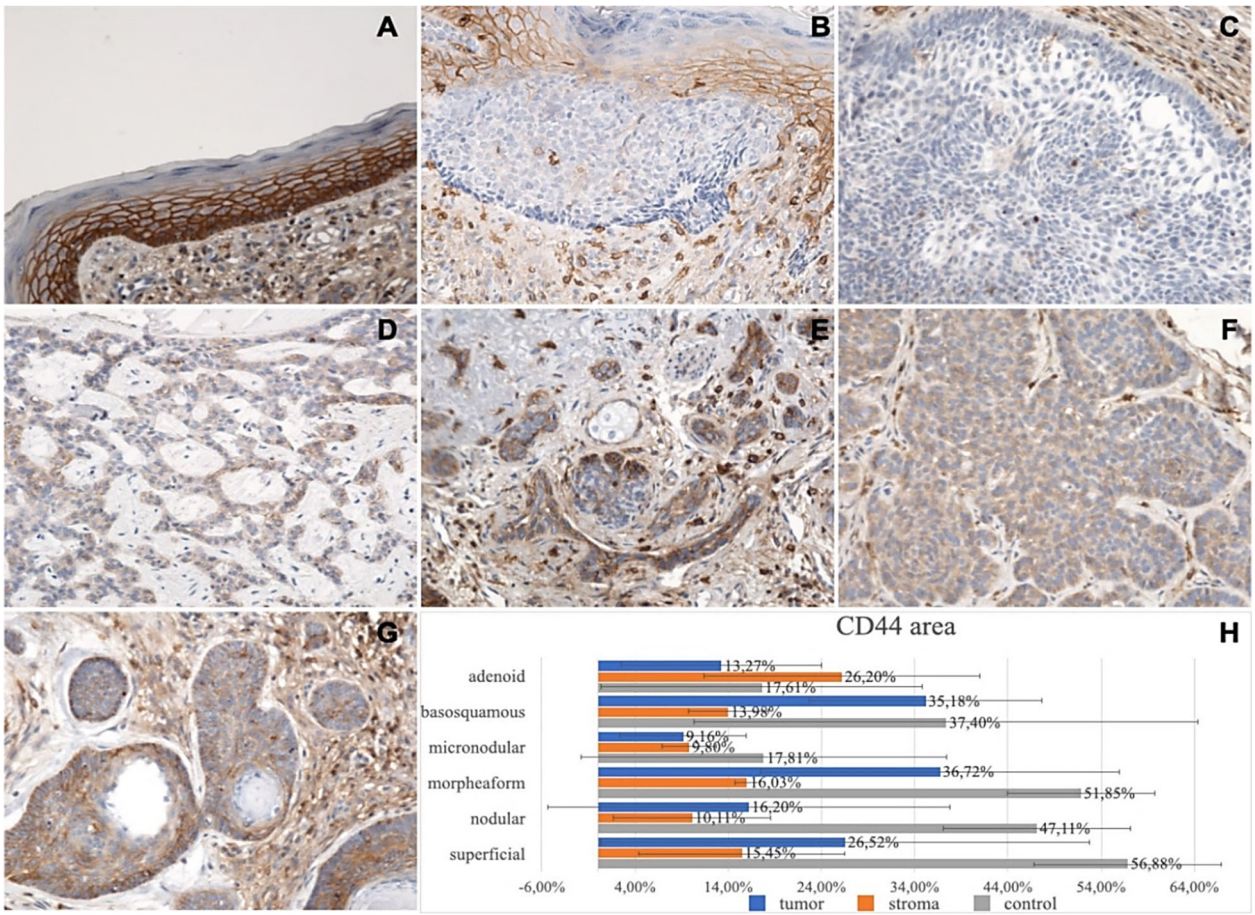

Figure 5 – ***CD44 immunoexpression in: (A) Control; (B) Superficial BCC; (C) Nodular BCC; (D) Adenoid BCC; (E) Morpheaform BCC; (F) Micronodular BCC; (G) Basosquamous carcinoma. (H) Percentage values of CD44 immunoreactive tumor area versus immunoreactive stromal area versus control; error bars represent standard deviation of the means. Anti-CD44 antibody immunomarking: (A–G) ×200. BCC: Basal cell carcinoma; CD44: Cluster of differentiation 44.***

Discussions

BCC is the most common skin cancer. Considering that metastasis is rare, it has an excellent prognosis but can cause local destruction [11, 12].

To evaluate the biological behavior of BCC, the architectural pattern is the only indicator of histological prognosis. Therefore, depending on the growth pattern, BCC can be divided into two broad categories: non-aggressive (indolent) and aggressive. Indolent subtypes, including superficial and nodular BCC, are described as well demarcated nodules, either small or large, with peripheral palisading. Aggressive subtypes, including morpheaform, micronodular and metatypical, are less demarcated and have mitotic activity, increased cell necrosis and deeper growth [13–18].

Because of its contribution in maintaining genetic integrity, p53 is known as the "guardian of the genome". In BCC carcinogenesis, the mutations in the Hedgehog (Hh) pathway are primarily involved. Besides these mutations, the tumor protein p53 (*TP53*) gene is also one of the most frequently mutated in BCC. The *p53* mutation is closely correlated with UVR exposure, its over expression can be found not only in cancers, but also in premalignant skin lesions and on longtime sun-exposed normal skin. Therefore, *p53* mutation can be considered an early modification in skin carcinogenesis [19–25].

Similar to other studies, we found positive reaction to p53 in the epidermal keratinocytes adjacent to the tumor and also in the cellular component of the dermis [26, 27]. This may be due to the BCC affinity for sun-exposed areas [28].

In our study, we observed a varied staining pattern for p53, with the highest reactivity noticed in the peripheral palisading zone. Histologically, we remarked that the superficial and nodular subtypes were the most reactive, followed by the adenoid subtype. By contrast, the basosquamous carcinomas had the lowest p53 reactivity. Consequently, we found no relation between p53 reaction and BCC aggressiveness as the indolent types had a more intense reactivity.

Other authors have also found no correlation between p53 expression and aggressiveness of BCCs [29, 30]. Likewise, Karagece Yalçin & Seçkın failed to report any significant link between p53 reactivity and aggressive patterns of BCC [31].

On the contrary, several studies have shown significantly higher expression of p53 in the aggressive category [32–34]. In other studies, the micronodular subtype had the most intense p53 immunoreactivity, while the morpheaform subtype was the least reactive [35].

The biological behavior of tumors is substantially influenced by proliferation markers. The Ki67 nuclear antigen is primarily associated with cell proliferation and is only expressed in cycling cells [36].

Regarding normal epidermis adjacent to the tumor, we observed that the positive reaction to Ki67 was present in only a few of the keratinocytes. In other studies, Ki67 immunoreactivity was also limited to a small number of keratinocytes in the normal skin [37].

In our study, the staining pattern for Ki67 was similar to p53, with a more pronounced reaction in the periphery of the tumor. Similar to other studies, we found different Ki67 expression among the various subtypes of BCC [38]. We noticed that the superficial subtype was the most reactive, followed by the nodular and morpheaform subtypes. At the opposite pole, the micronodular subtype had the lowest reactivity. Considering that non-aggressive subtypes showed a higher reactivity to Ki67 than aggressive subtypes, no connection between Ki67 reactivity and BCC aggressive growth patterns could be established.

Kramer *et al.* also found no correlation between the proliferative index and the aggressive patterns of BCC [39].

Other studies reported that Ki67 expression was positive in all of the BCC subtypes but varied in intensity between recurrent BCCs and non-recurrent ones, with a higher expression of Ki67 in the recurrent cases [40, 41].

Horlock *et al.* observed that the morpheaform, infiltrating and superficial subtypes of BCC had the most intense Ki67 reactivity and highlighted the association between high levels of proliferation and BCC subtypes [42].

Tumor angiogenesis has an important role in tumor growth and also in determining its invasive behavior. There is considerable data suggesting that angiogenesis is essential in switching from hyperplasia to invasive growth. CD31, also known as PECAM-1, is a sensitive and specific marker of endothelial differentiation [43].

In the epidermis overlying BCCs, we observed positive reaction to CD31 in the papillary dermis, suggesting an upregulation in the vascular cell adhesion system. Kikuchi *et al.* also described CD31 immunoreactivity of the endothelial cells located in the dermis around the BCCs [44].

In the current study, the CD31 reactivity, mostly seen in the stroma, was positive in all of the BCCs subtypes and varied significantly between the different HP subtypes, with the nodular, basosquamous and morpheaform subtypes as the most reactive. The micronodular subtype had the lowest reactivity. The aim of the study conducted by Chin *et al.* was to investigate if the different behavior of BCCs and SCCs, could be explained by their different pattern of vascularization. To achieve that, they used IHC staining for CD31 and found a significant difference in their angiogenic patterns. Similar to our findings, in the case of BCCs, they reported positive reaction to CD31 only in the stroma, with no intratumoral blood vessels. Regarding SCC reactivity to CD31, they noticed positive reaction both in the stroma and intratumoral. They correlated these differences with these tumors' different behavior regarding both metastatic and invasiveness potential [43].

The basosquamous and morpheaform variants, also known as aggressive subtypes, are more likely to recur than the non-aggressive ones [45, 46]. Regarding tumoral aggressive behavior, some authors found that the average value of microvessel count, determined by CD31, was significantly higher in the recurrent group of BCCs [40].

To establish if angiogenic rate is related with biological behavior of BCCs, Staibano *et al.* examined it using anti-Factor VII antibody, a less sensitive marker for angiogenesis than CD31, and their study reported a link between tumor vascularization and the BCC aggressiveness [47].

The existence of myofibroblast differentiation, identified by α-SMA reactivity, has been described as a potential marker for BCC aggressiveness in previous studies. Actin is occasionally found in normal epithelial cells and plays an important role in cell motility [48]. Regarding the surrounding tissues of BCCs, we found α-SMA reactivity in the papillary dermis.

To determine if there is a link between α-SMA immunoreactivity and the invasive behavior of BCC, previous studies analyzed its expression both in the surrounding stroma and in the tumor [35].

Adegboyega *et al.* highlighted that stromal expression of α-SMA is an accurate marker of aggressiveness in BCC, while nonaggressive types of BCC express α-SMA in the tumor cells [49].

Regarding stromal expression, the adenoid and basosquamous types had the most intense reaction in our study. Similar results were also reported by other authors,

with all basosquamous cases being positive [35]. Motegi *et al.* also suggested that the stromal reactivity to α-SMA could be an indicator of aggressive behavior and reported its expression in 67% of micronodular subtypes and 62% of morpheaform subtypes compared with 0% of nodular subtypes [50]. Law *et al.* compared actin expression in seven nodular subtypes *versus* 13 nodular-infiltrative subtypes and reported no reactivity in the stroma of the nodular BCCs and positive reaction in 62% of nodular-infiltrative BCCs stroma [51].

Christian *et al.* indicated that the presence of α-SMA in the surrounding stroma of BCC or in the tumor cells, may be an indicator of aggressive behavior. The authors related the presence of α-SMA in more than half of the cases of micronodular and morpheaform subtypes and in none of the cases of nodular subtype [52].

The α-SMA tumor expression was correlated by some authors with the aggressive histological subtypes of BCC [51, 53]. In our study, we noticed that tumors with the highest expression belonged to micronodular subtypes, followed by superficial and nodular tumors. The lowest immunoreactivity was obtained in the morpheaform subtype.

Tsukamoto *et al.* noticed the highest actin reactivity in the adenoid, solid and sclerosing subtypes [53]. Uzquiano *et al.* related actin reactivity in three of 12 of the nodular subtypes, three of 10 of the metastatic BCCs and in all cases of infiltrative BCCs [54].

CD44, a transmembrane glycoprotein, is known as a marker for cancer stem cells. Hyaluronic acid (HA) is the main ligand for CD44. The activation of various signaling pathways, a result of the activation of CD44 by its ligand, leads to cell adhesion, proliferation, invasion, and migration. The standard CD44 (CD44s) and its isoforms (CD44v) seem to play a role in promoting tumorigenesis and, therefore, may be a target for cancer therapy [55].

Previous studies suggested that CD44v promotes cell migration and proliferation in the case of head and neck SCC and has also been associated with resistance to Cisplatin therapy in these cases [56, 57].

Karvinen *et al.* reported a completely different CD44 expression in BCCs *versus* SCCs, describing a low level or total absence of CD44 in BCCs, and suggested that this may be due to their different capacity of metastasis. However, the study did not consider the HP subtypes of BCC [58].

As previously reported, we found CD44 expression in the area adjacent to the tumors, in epidermis keratinocytes and dermis fibroblasts [59]. Except for adenoid types of BCC, the normal epidermis adjacent to the tumors showed higher CD44 expression compared to the tumor epithelium. These differences suggested a decreasing trend of CD44 expression in the tumor epithelium *versus* surrounding epidermis. Other authors also highlighted reduced staining for CD44 in BCC samples compared to the normal epidermis [58, 60]. On the contrary, in the study leaded by Milosevic *et al.*, the expression of CD44 was higher in tumor cells compared to normal epidermis and margins [61].

We analyzed CD44 expression for the different subtypes of BCC, both in the tumor and in the surrounding stroma. The CD44 tumor expression was correlated in our study to the aggressive pattern of BCCs. Thus, the highest level of CD44 was observed in the morpheaform and basosquamous subtypes of BCC. In contrast, the micronodular subtype had the lowest expression of CD44. On the other hand, regarding stromal expression, the most intense reaction was noticed in the adenoid and morpheaform subtypes. The micronodular subtype had the lowest reaction in this case as well.

Dingemans *et al.*, using an antigen retrieval procedure, found that CD44 expression in tumor areas is lower compared to the normal epidermis. In the same study, CD44 expression was linked to BCCs growth patterns. Similar to our study, they found higher CD44 expression in cases with infiltrative and adenoid growth pattern compared to cases with nodular and superficial growth pattern [62].

Conclusions

The expression of p53, Ki67, CD31, α-SMA and CD44 varied between the different subtypes of BCC. The highest reactivity was noticed in superficial and nodular subtypes for p53 and Ki67 markers. The nodular subtype also had the highest CD31 expression, followed by basosquamous subtype. Regarding α-SMA and CD44 tumor expression, the highest levels were observed in the aggressive subtypes of BCC and therefore demonstrated a tendency to indicate the severity of BCC. Thus, our study suggests that IHC investigation of these markers may be useful for the development of a new targeted therapy.

Conflict of interests

The authors declare that they have no conflict of interests.

References

[1] McDaniel B, Badri T, Steele RB. Basal cell carcinoma. 2022 Sep 19. In: StatPearls [Internet]. StatPearls Publishing, Treasure Island, FL, USA, 2022 Jan–. PMID: 29494046 Bookshelf ID: NBK482439

[2] Fagan J, Brooks J, Ramsey ML. Basal cell cancer. 2022 Aug 20. In: StatPearls [Internet]. StatPearls Publishing, Treasure Island, FL, USA, 2022 Jan–. PMID: 29261882 Bookshelf ID: NBK470301

[3] Sherwani Y, Aldana PC, Khachemoune A. Squamous and basal cell carcinoma with perineural invasion: pathophysiology and presentations. Int J Dermatol, 2022, 61(6):653–659. https://doi.org/10.1111/ijd.15817 PMID: 34370865

[4] Bungărdean RM, Şerbănescu MS, Streba CT, Crişan M. Deep learning with transfer learning in pathology. Case study: classification of basal cell carcinoma. Rom J Morphol Embryol, 2021, 62(4):1017–1028. https://doi.org/10.47162/RJME.62.4.14 PMID: 35673821 PMCID: PMC9289702

[5] Pampena R, Parisi G, Benati M, Borsari S, Lai M, Paolino G, Cesinaro AM, Ciardo S, Farnetani F, Bassoli S, Argenziano G, Pellacani G, Longo C. Clinical and dermoscopic factors for the identification of aggressive histologic subtypes of basal cell carcinoma. Front Oncol, 2021, 10:630458. https://doi.org/10.3389/fonc.2020.630458 PMID: 33680953 PMCID: PMC7933517

[6] van der Pols JC, Xu C, Boyle GM, Parsons PG, Whiteman DC, Green AC. Expression of p53 tumor suppressor protein in sun-exposed skin and associations with sunscreen use and time spent outdoors: a community-based study. Am J Epidemiol, 2006, 163(11):982–988. https://doi.org/10.1093/aje/kwj137 PMID: 16624969

[7] Li LT, Jiang G, Chen Q, Zheng JN. Ki67 is a promising molecular target in the diagnosis of cancer (review). Mol Med Rep, 2015, 11(3):1566–1572. https://doi.org/10.3892/mmr.2014.2914 PMID: 25384676

[8] Sánchez-Ramírez D, Medrano-Guzmán R, Candanedo-González F, De Anda-González J, García-Rios LE, Pérez-Koldenkova V, Gutiérrez-de la Barrera M, Rodríguez-Enríquez S, Velasco-Velázquez M, Pacheco-Velázquez SC, Piña-Sánchez P, Mayani H, Gómez-Delgado A, Monroy-García A, Martínez-Lara AK, Montesinos JJ. High expression of both desmoplastic

stroma and epithelial to mesenchymal transition markers associate with shorter survival in pancreatic ductal adenocarcinoma. Eur J Histochem, 2022, 66(1):3360. https://doi.org/10.4081/ejh.2022.3360 PMID: 35174683 PMCID: PMC8883614

[9] Biddle A, Gammon L, Fazil B, Mackenzie IC. CD44 staining of cancer stem-like cells is influenced by down-regulation of CD44 variant isoforms and up-regulation of the standard CD44 isoform in the population of cells that have undergone epithelial-to-mesenchymal transition. PLoS One, 2013, 8(2): e57314. https://doi.org/10.1371/journal.pone.0057314 PMID: 23437366 PMCID: PMC3577706

[10] Pusztaszeri MP, Seelentag W, Bosman FT. Immunohistochemical expression of endothelial markers CD31, CD34, von Willebrand factor, and Fli-1 in normal human tissues. J Histochem Cytochem, 2006, 54(4):385–395. https://doi.org/10.1369/jhc.4A6514.2005 PMID: 16234507

[11] Kim DP, Kus KJB, Ruiz E. Basal cell carcinoma review. Hematol Oncol Clin North Am, 2019, 33(1):13–24. https://doi.org/10.1016/j.hoc.2018.09.004 PMID: 30497670

[12] Basset-Seguin N, Herms F. Update in the management of basal cell carcinoma. Acta Derm Venereol, 2020, 100(11): adv00140. https://doi.org/10.2340/00015555-3495 PMID: 32346750

[13] Stanoszek LM, Wang GY, Harms PW. Histologic mimics of basal cell carcinoma. Arch Pathol Lab Med, 2017, 141(11): 1490–1502. https://doi.org/10.5858/arpa.2017-0222-RA PMID: 29072946

[14] Moon D, Randall G, Higgins S, Sutton AV, Wysong A. Misclassification of aggressive basal cell carcinoma subtypes and implications for management. Dermatol Surg, 2021, 47(5): 593–598. https://doi.org/10.1097/DSS.0000000000002954 PMID: 33905389

[15] Requena C, Serra-Guillén C, Sanmartín O. Histologically aggressive basal cell carcinoma with particular emphasis on galeal infiltration of the scalp. Actas Dermosifiliogr, 2022, 113(6):575–582. https://doi.org/10.1016/j.ad.2022.01.026 PMID: 35339267

[16] Wetzel M, Strickley J, Haeberle MT, Brown TS. Depth of invasion of aggressive and nonaggressive basal cell carcinoma. J Clin Aesthet Dermatol, 2019, 12(3):12–14. PMID: 30988868 PMCID: PMC6440705

[17] Cerci FB, Kubo EM, Werner B. Comparison of basal cell carcinoma subtypes observed in preoperative biopsy and Mohs micrographic surgery. An Bras Dermatol, 2020, 95(5):594–601. https://doi.org/10.1016/j.abd.2020.04.005 PMID: 32693938 PMCID: PMC7563020

[18] Matsushita S, Nakamura Y, Tanaka R, Araki R, Yamamura K, Yoshioka M, Inoue A, Komori T, Saito S, Teramoto Y, Nakamura Y, Fujisawa Y, Aoki M. Prediction of the invasive level of basal cell carcinomas in the facial area: analysis of 718 Japanese cases. J Dermatol Sci, 2020, 99(3):152–157. https://doi.org/10.1016/j.jdermsci.2020.07.001 PMID: 32811698

[19] Loureiro JB, Abrantes M, Oliveira PA, Saraiva L. P53 in skin cancer: from a master player to a privileged target for prevention and therapy. Biochim Biophys Acta Rev Cancer, 2020, 1874(2): 188438. https://doi.org/10.1016/j.bbcan.2020.188438 PMID: 32980466

[20] Marei HE, Althani A, Afifi N, Hasan A, Caceci T, Pozzoli G, Morrione A, Giordano A, Cenciarelli C. p53 signaling in cancer progression and therapy. Cancer Cell Int, 2021, 21(1):703. https://doi.org/10.1186/s12935-021-02396-8 PMID: 34952583 PMCID: PMC8709944

[21] Holick MF. Sunlight, UV radiation, vitamin D, and skin cancer: how much sunlight do we need? Adv Exp Med Biol, 2020, 1268:19–36. https://doi.org/10.1007/978-3-030-46227-7_2 PMID: 32918212

[22] Ke Y, Wang XJ. TGFβ signaling in photoaging and UV-induced skin cancer. J Invest Dermatol, 2021, 141(4S):1104–1110. https://doi.org/10.1016/j.jid.2020.11.007 PMID: 33358021 PMCID: PMC7987776

[23] Lee JW, Ratnakumar K, Hung KF, Rokunohe D, Kawasumi M. Deciphering UV-induced DNA damage responses to prevent and treat skin cancer. Photochem Photobiol, 2020, 96(3): 478–499. https://doi.org/10.1111/php.13245 PMID: 32119110 PMCID: PMC7651136

[24] Bauer A, Haufe E, Heinrich L, Seidler A, Schmitt J. Neues zum berufsbedingten Hautkrebs – Basalzellkarzinom und solare UV-Exposition [Update on occupational skin cancer-basal cell carcinoma and solar UV exposure]. Hautarzt, 2021, 72(6): 484–492. https://doi.org/10.1007/s00105-021-04818-0 PMID: 33884436

[25] Yang SH, Liu CT, Hong CQ, Huang ZY, Wang HZ, Wei LF, Lin YW, Guo HP, Peng YH, Xu YW. Autoantibodies against p53, MMP-7, and Hsp70 as potential biomarkers for detection of nonmelanoma skin cancers. Dis Markers, 2021, 2021:5592693. https://doi.org/10.1155/2021/5592693 PMID: 34336006 PMCID: PMC8289574

[26] Kim SA, Ryu YW, Kwon JI, Choe MS, Jung JW, Cho JW. Differential expression of cyclin D1, Ki-67, pRb, and p53 in psoriatic skin lesions and normal skin. Mol Med Rep, 2018, 17(1):735–742. https://doi.org/10.3892/mmr.2017.8015 PMID: 29115643 PMCID: PMC5780150

[27] Loureiro JB, Ribeiro R, Nazareth N, Ferreira T, Lopes EA, Gama A, Machuqueiro M, Alves MG, Marabini L, Oliveira PA, Santos MMM, Saraiva L. Mutant *p53* reactivator SLMP53-2 hinders ultraviolet B radiation-induced skin carcinogenesis. Pharmacol Res, 2022, 175:106026. https://doi.org/10.1016/j.phrs.2021.106026 PMID: 34890775

[28] Dika E, Scarfì F, Ferracin M, Broseghini E, Marcelli E, Bortolani B, Campione E, Riefolo M, Ricci C, Lambertini M. Basal cell carcinoma: a comprehensive review. Int J Mol Sci, 2020, 21(15):5572. https://doi.org/10.3390/ijms21155572 PMID: 32759706 PMCID: PMC7432343

[29] Healy E, Angus B, Lawrence CM, Rees JL. Prognostic value of Ki67 antigen expression in basal cell carcinomas. Br J Dermatol, 1995, 133(5):737–741. https://doi.org/10.1111/j.1365-2133.1995.tb02748.x PMID: 8555026

[30] Demirkan NC, Colakoglu N, Düzcan E. Value of p53 protein in biological behavior of basal cell carcinoma and in normal epithelia adjacent to carcinomas. Pathol Oncol Res, 2000, 6(4):272–274. https://doi.org/10.1007/BF03187330 PMID: 11173659

[31] Karagece Yalçin U, Seçkın S. The expression of p53 and COX-2 in basal cell carcinoma, squamous cell carcinoma and actinic keratosis cases. Turk Patoloji Derg, 2012, 28(2):119–127. https://doi.org/10.5146/tjpath.2012.01110 PMID: 22627629

[32] Bolshakov S, Walker CM, Strom SS, Selvan MS, Clayman GL, El-Naggar A, Lippman SM, Kripke ML, Ananthaswamy HN. *p53* mutations in human aggressive and nonaggressive basal and squamous cell carcinomas. Clin Cancer Res, 2003, 9(1): 228–234. PMID: 12538474

[33] Ansarin H, Daliri M, Soltani-Arabshahi R. Expression of p53 in aggressive and non-aggressive histologic variants of basal cell carcinoma. Eur J Dermatol, 2006, 16(5):543–547. PMID: 17101476

[34] Koseoglu RD, Sezer E, Eyibilen A, Aladag I, Etikan I. Expressions of p53, cyclin D1 and histopathological features in basal cell carcinomas. J Cutan Pathol, 2009, 36(9):958–965. https://doi.org/10.1111/j.1600-0560.2008.01204.x PMID: 19187116

[35] Mercuţ R, Ciurea ME, Mărgăritescu C, Popescu SM, Crăiţoiu MM, Cotoi OS, Voinescu DC. Expression of p53, D2-40 and α-smooth muscle actin in different histological subtypes of facial basal cell carcinoma. Rom J Morphol Embryol, 2014, 55(2): 263–272. PMID: 24969973

[36] Alaeddini M, Etemad-Moghadam S. Cell kinetic markers in cutaneous squamous and basal cell carcinoma of the head and neck. Braz J Otorhinolaryngol, 2022, 88(4):529–532. https://doi.org/10.1016/j.bjorl.2020.07.010 PMID: 32972865 PMCID: PMC9422672

[37] Caldwell CJ, Hobbs C, McKee PH. The relationship of Ki67 and involucrin expression in proliferative, pre-neoplastic and neoplastic skin. Clin Exp Dermatol, 1997, 22(1):11–16. https://doi.org/10.1046/j.1365-2230.1997.d01-232.x PMID: 9330045

[38] Deng M, Marsch AF, Petronic-Rosic V. Molecular variations in histologic subtypes of basal cell carcinoma. Skinmed, 2017, 15(4):265–268. PMID: 28859735

[39] Kramer E, Herman O, Frand J, Leibou L, Schreiber L, Vaknine H. Ki67 as a biologic marker of basal cell carcinoma: a retrospective study. Isr Med Assoc J, 2014, 16(4):229–232. PMID: 24834759

[40] Yerebakan O, Ciftçioglu MA, Akkaya BK, Yilmaz E. Prognostic value of Ki-67, CD31 and epidermal growth factor receptor expression in basal cell carcinoma. J Dermatol, 2003, 30(1): 33–41. PMID: 12598707

[41] Flordelis JO, Shen YC, Wu YH. Basaloid tumors arising from seborrheic keratosis: malignant basal cell carcinoma or benign basaloid follicular hamartomatous proliferation? J Cutan Pathol, 2020, 47(3):207–218. https://doi.org/10.1111/cup.13607 PMID: 31682281
[42] Horlock NM, Wilson GD, Daley FM, Richman PI, Sanders R. Cellular proliferation characteristics of basal cell carcinoma: relationship to clinical subtype and histopathology. Eur J Surg Oncol, 1997, 23(3):247–252. https://doi.org/10.1016/s0748-7983(97)92508-7 PMID: 9236901
[43] Chin CWS, Foss AJE, Stevens A, Lowe J. Differences in the vascular patterns of basal and squamous cell skin carcinomas explain their differences in clinical behaviour. J Pathol, 2003, 200(3):308–313. https://doi.org/10.1002/path.1363 PMID: 12845626
[44] Kikuchi A, Sakuraoka K, Shimizu H, Nishikawa T. Immunohistochemical evaluation of epidermis overlying basal cell carcinomas. Br J Dermatol, 1993, 128(6):644–649. https://doi.org/10.1111/j.1365-2133.1993.tb00260.x PMID: 7687854
[45] Bartoš V, Pokorný D, Zacharová O, Haluska P, Doboszová J, Kullová M, Adamicová K, Péč M, Péč J. Recurrent basal cell carcinoma: a clinicopathological study and evaluation of histomorphological findings in primary and recurrent lesions. Acta Dermatovenerol Alp Pannonica Adriat, 2011, 20(2):67–75. PMID: 21993704
[46] Szewczyk MP, Pazdrowski J, Dańczak-Pazdrowska A, Golusiński P, Majchrzak E, Luczewski L, Marszałek S, Silny W, Golusiński W. Analysis of selected recurrence risk factors after treatment of head and neck basal cell carcinoma. Postepy Dermatol Alergol, 2014, 31(3):146–151. https://doi.org/10.5114/pdia.2014.40959 PMID: 25097485 PMCID: PMC4112263
[47] 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. https://doi.org/10.1016/s0046-8177(96)90400-1 PMID: 8698314
[48] Krum Dos Santos AC, Lima D, Marchiori Bakos R, da Silva Cartell A, Rangel Bonamigo R. Stromal α-smooth muscle actin positivity is associated with high-risk basal cell carcinomas. Am J Dermatopathol, 2021, 43(12):e137–e140. https://doi.org/10.1097/DAD.0000000000002007 PMID: 34231496
[49] Adegboyega PA, Rodriguez S, McLarty J. Stromal expression of actin is a marker of aggressiveness in basal cell carcinoma. Hum Pathol, 2010, 41(8):1128–1137. https://doi.org/10.1016/j.humpath.2009.12.014 PMID: 20381122
[50] Motegi S, Yamada K, Ishikawa O. Twist1 in tumor cells and α-smooth muscle actin in stromal cells are possible biomarkers for metastatic giant basal cell carcinoma. J Dermatol, 2013, 40(8):661–663. https://doi.org/10.1111/1346-8138.12188 PMID: 23683049
[51] Law AM, Oliveri CV, Pacheco-Quinto X, Horenstein MG. Actin expression in purely nodular *versus* nodular-infiltrative basal cell carcinoma. J Cutan Pathol, 2003, 30(4):232–236. https://doi.org/10.1046/j.0303-6987.2003.054.x PMID: 12680952
[52] Christian MM, Moy RL, Wagner RF, Yen-Moore A. A correlation of alpha-smooth muscle actin and invasion in micronodular basal cell carcinoma. Dermatol Surg, 2001, 27(5):441–445. https://doi.org/10.1046/j.1524-4725.2001.00200.x PMID: 11359490
[53] Tsukamoto H, Hayashibe K, Mishima Y, Ichihashi M. The altered expression of alpha-smooth muscle actin in basal cell epithelioma and its surrounding stroma: with special reference to proliferating cell nuclear antigen expression and adenoid differentiation. Br J Dermatol, 1994, 130(2):189–194. https://doi.org/10.1111/j.1365-2133.1994.tb02898.x PMID: 7907224
[54] Uzquiano MC, Prieto VG, Nash JW, Ivan DS, Gong Y, Lazar AJF, Diwan AH. Metastatic basal cell carcinoma exhibits reduced actin expression. Mod Pathol, 2008, 21(5):540–543. https://doi.org/10.1038/modpathol.3801051 PMID: 18223552
[55] Chen C, Zhao S, Karnad A, Freeman JW. The biology and role of CD44 in cancer progression: therapeutic implications. J Hematol Oncol, 2018, 11(1):64. https://doi.org/10.1186/s13045-018-0605-5 PMID: 29747682 PMCID: PMC5946470
[56] Reategui EP, de Mayolo AA, Das PM, Astor FC, Singal R, Hamilton KL, Goodwin WJ, Carraway KL, Franzmann EJ. Characterization of CD44v3-containing isoforms in head and neck cancer. Cancer Biol Ther, 2006, 5(9):1163–1168. https://doi.org/10.4161/cbt.5.9.3065 PMID: 16855392
[57] Wang SJ, Wreesmann VB, Bourguignon LYW. Association of CD44 V3-containing isoforms with tumor cell growth, migration, matrix metalloproteinase expression, and lymph node metastasis in head and neck cancer. Head Neck, 2007, 29(6):550–558. https://doi.org/10.1002/hed.20544 PMID: 17252589
[58] Karvinen S, Kosma VM, Tammi MI, Tammi R. Hyaluronan, CD44 and versican in epidermal keratinocyte tumors. Br J Dermatol, 2003, 148(1):86–94. https://doi.org/10.1046/j.1365-2133.2003.05028.x PMID: 12534600
[59] Hartmann-Petersen S, Tammi RH, Tammi MI, Kosma VM. Depletion of cell surface CD44 in nonmelanoma skin tumours is associated with increased expression of matrix metalloproteinase 7. Br J Dermatol, 2009, 160(6):1251–1257. https://doi.org/10.1111/j.1365-2133.2009.09031.x PMID: 19222463
[60] Baum HP, Schmid T, Schock G, Reichrath J. Expression of CD44 isoforms in basal cell carcinomas. Br J Dermatol, 1996, 134(3):465–468. PMID: 8731670
[61] Milosevic M, Lazarevic M, Toljic B, Simonovic J, Trisic D, Nikolic N, Petrovic M, Milasin J. Characterization of stem-like cancer cells in basal cell carcinoma and its surgical margins. Exp Dermatol, 2018, 27(10):1160–1165. https://doi.org/10.1111/exd.13755 PMID: 30033544
[62] Dingemans KP, Ramkema MD, Koopman G, Van Der Wal AC, Das PK, Pals ST. The expression of CD44 glycoprotein adhesion molecules in basal cell carcinomas is related to growth pattern and invasiveness. Br J Dermatol, 1999, 140(1):17–25. https://doi.org/10.1046/j.1365-2133.1999.02602.x PMID: 10215763

Corresponding authors

Ana-Maria Ciurea, MD, PhD, Department of Oncology, University of Medicine and Pharmacy of Craiova, 2 Petru Rareş Street, 200349 Craiova, Dolj County, Romania; Phone +40749–885 996, e-mail: ana.ciurea@umfcv.ro

Dragoş Niculescu, Lecturer, MD, PhD, Department of Orthopedics, University of Medicine and Pharmacy of Craiova, 2 Petru Rareş Street, 200349 Craiova, Dolj County, Romania; Phone +40769–638 286, e-mail: dragos.niculescu@umfcv.ro

Received: May 10, 2022

Accepted: November 9, 2022