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Research Article

The role of GLI1, YAP, CTGF and E-cadherin in the pathogenesis of basal cell carcinoma – our preliminary results

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Abstract. Introduction: UV radiation is the most important risk factor for the development of basal cell carcinomas (BCC). Hedgehog (Hh), Hippo and Wnt- β-catenin pathways are implicated in the pathogenesis of BCC. GLI1 is the effector of the Hh pathway. YAP is involved in the Hippo pathway and is controlled by E-cadherin through β-catenin. All are interconnected and also connected to the Wnt pathway. CTGF is a target molecule of YAP. Objective: The aim of this study was to analyze the expression of GLI1, YAP, CTGF and E-cadherin in surgically excised BCCs and the relationships between these molecules. Also, we attempted to identify the eventual differences between tumors developing on skin with choric versus intermittent sun exposure. Material and method: We analyzed 46 BCCs, among which 15 were on chronic sun exposed areas and 15 on intermittently sun exposed skin. On these tumors we performed immunohistochemical staining for GLI1, YAP, CTGF and E-cadherin and characterized it as staining intensity and percentage of positive cells.Results: We found that YAP and CTGF but not GLI1 were present in all BCCs. E-cadherin's expression was heterogeneous in tumor cells. The expression of GLI1 was positively correlated with the expression of YAP as staining intensity (correlation coefficient=0.31, p=0.03). There was a negative correlation between E-cadherin and YAP as percentage of positive cells (correlation coefficient=-0.32, p=0.03). The pattern of immunostaining of these molecules did not depend on the histological subtype or the type of sun exposure.Conclusion: Our preliminary results confirm the presence of GLI1, YAP, CTGF and E-cadherin in the studied tumors. Further studies on larger tumor sets are necessary to establish the role of these molecules as prognostic factors in BCC.

Key Words: basal cell carcinoma, pathogenesis, immunohistochemistry

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Introduction

Basal cell carcinoma (BCC) is the most common form of skin cancer in people with fair-skin phototype. The most important risk factor is exposure to ultraviolet (UV) radiation. However, BCC sometimes occurs in non-sun-exposed areas such as the genital area. The type of UV exposure seems to play a role in the development of BCC so that people with intermittent exposure to UV radiation mostly develop superficial BCC (Verkouteren et al 2019). The mechanism of BCC carcinogenesis by activating the Hedgehog (Hh) molecular signaling pathway has been extensively studied. Mutations at this level are mostly UV-induced and/or mutations in the PTCH1 gene (Pellegrini et al 2017). 90% of BCCs show up-regulation of Hh (Pellegrini et al 2017). The canonical pathway is regulated by PTCH-SMO-SUFU, leading to the translocation of GLI (glioma-associated oncogene) factors into the nucleus (Pellegrini et al 2017). In BCC, mutations that affect the canonical Hh pathway but also mutations that lead to non-canonical pathway activation, increase GLI1 expression (Pellegrini et al 2017).

There is FDA-approved systemic medication for the treatment of locally aggressive or metastatic carcinomas that cannot be cured by other therapeutic methods; these drugs target SMO. However, 30% of BCCs treated with SMO inhibitors do not respond or develop resistance (Bonilla et al 2016). Solutions could consist of combined therapies targeting the signaling cascade more downstream of SMO (Pellegrini et al 2017).

Recently, in addition to mutations in PTCH1, SMO, SUFU, TP53 (all of which lead to stimulation/upregulation of the Hh pathway) in BCC, mutations in PTPN14 and LATS1 have also been identified (Bonilla et al 2016).

YAP (yes-associated protein) is a protein involved in the Hippo signaling pathway. The Hippo/YAP pathway is interrupted at multiple points in the BCC formation process, including PTPN14 and LATS1 (Pellegrini et al 2017). To date, no connection has been found between the histological-pathological

subtype and the genetic profile (Peris et al 2019). The Hippo pathway has been shown to be upregulated in BCC (Bonilla et al 2016, Pellegrini et al 2017). Hippo inactivation leads to YAP activation, its nuclear localization and cell proliferation through Wnt (Debaugnies et al 2018) and CCN1 (Quan et al 2014). It has recently been shown that, independent of Hippo, YAP also responds to mechanical stimuli triggered by tissue stiffness (Akladios et al 2017, Debaugnies et al 2018).

YAP targets include GLI2, CCN1 and connective tissue growth factor (CTGF / CCN2) (Akladios et al 2017). Activated YAP mediates stroma remodeling by CTGF and increases stiffness via paracrine signaling (Quan et al 2014). However, CTGF does not directly regulate YAP for keratinocyte proliferation (Quan et al 2014).

In mice, BCC induction by SMO mutations reveals increased YAP (Debaugnies et al 2018) and remodeling of the extracellular matrix in terms of ROCK-modulated fibrosis (Akladios et al 2017). It has also been shown that, by activating β-catenin, YAP stimulates GLI2 and cell proliferation (Akladios et al 2017). YAP and Hh are positively and mutually regulated in epidermal homeostasis (Akladios et al 2017).

E-cadherin is an important transmembrane protein in cell-cell interactions (such as movement blocking and growth inhibition) through β-catenin-dependent mechanisms and others. Thus, it has a role in tumor invasion, and its expression is low in aggressive and infiltrative tumors (Bartoš et al 2015, N-G Kim et al 2011). Results are contradictory in BCC. Some authors report a decrease in E-cadherin expression in infiltrative BCC, while others report no histologically different subtypes (Bartoš et al 2015).

Homophilic extracellular binding of E-cadherin plays a role in density-dependent YAP regulation, in a β-catenin and LATS dependent manner, preventing the nuclear translocation of YAP. This inhibits cell proliferation (N-G Kim et al 2011).

Wnt signaling determines the nuclear localization of β-catenin and thus promotes stem cell maintenance and cell growth. In the development of BCC via the Hh pathway, Wnt is essential as it regulates GLI1 expression (HS Kim et al 2019, FK Noubissi et al 2014, F Noubissi et al 2018). Wnt signaling is induced by Hh activation (Calonje et al 2005).

Histopathologically, it has been observed that BCC is characterized by a special type of stroma compared to other epithelial malignancies, which would be a possible explanation for the "non-metastatic" capacity (Bartoš et al 2015, Micke et al 2007). However, BCC cells show a degree of changes characteristic of the epithelial-mesenchymal transition (EMT) (Lee et al 2004). The mechanisms involved in the EMT are not fully elucidated, but most pathogenetic pathways result in decreased levels of E-cadherin (Tucci et al 2013).

Numerous factors can contribute to the development of different histological forms of BCC, with a possible impact on the formation of subsequent BCCs (Lovatt et al 2005).

Cancer cells, along with some tumor stem cells coexist with an active stromal microenvironment, fibroblasts and immune cells, regulating each other through various mechanisms. A very recent article demonstrates that BCC tumor cells are highly dependent on these interactions (Mendez et al 2020).

GLI1, the Hh effector, is the signature molecule of one of the most phylogenetically conserved signaling pathways. YAP is essential for stem cell population and BCC proliferation. YAP interactions with the micro-environment are done by CTGF (Quan et al 2014). YAP is regulated by surrounding factors through E-cadherin (N-G Kim et al 2011).

In addition, sun exposure alone would not explain the frequency of BCCs in certain areas or their histology. Studies have shown that there may be other contributing factors such as the quality of cell-matrix interactions (Heckmann et al 2002).

This pilot study aimed to analyze GLI1, YAP, CTGF and E-cadherin expression and the relationship between these molecules in basal cell carcinomas to bring more knowledge to the process of carcinogenesis. There are few data on the clinical expression of these molecules, and the relationships between the pathogenetic pathways involved are being investigated. In addition, given the major contribution of UV in the development of BCC, we aimed to identify possible clinical and pathological differences between tumors developed on skin characterized by chronic sun exposure as opposed to those that occur in areas with intermittent sun exposure.

Material and methods

This is a retrospective, observational study, performed in the Dermatology Clinic of Cluj County Emergency Clinical Hospital. It was performed on inpatients undergoing surgery for multiple BCCs between January 2012 and October 2015, on skin with chronic exposure to sunlight and on non-sun-exposed body area. Patients were selected from 961 hospitalizations for excisions of non-melanoma skin tumors (Figure 1).

Subjects who were included in the study signed an informed consent form.

The study protocol was approved by the Ethics Commission of "Iuliu Hatieganu" University of Medicine and Pharmacy Cluj-Napoca.

Inclusion criteria for groups A and B were as follows: (1) patient undergoing conventional surgical excision for the first recurrence of a previously excised BCC; (2) patient whose initially excised tumor had free histological margins. Exclusion criteria were as follows: (1) patient whose excised tumor had a histopathological result of squamous cell carcinoma or benign tumor; (2) patient with initial or recurrent BCC with at least one positive histological margin; (3) patient with initial tumor or recurrent tumor with basal squamous histological subtype or perineural invasion; (4) patient treated with Mohs surgery; (5) patient with subsequent tumor recurrence; (6) patient who did not present for follow-up between November 2017 and November 2018. We found 14 patients who met the inclusion criteria, of which 8 also met the exclusion criteria. From these 8 patients we analyzed initial BCCs (group A - recurrent tumors) and recurrent tumors (group B - non-recurrent tumors).

Inclusion criteria for groups C (tumors on sun-exposed skin) and D (tumors on non-sun-exposed skin) were as follows: (1) patient with surgical excision for one tumor in a sun-exposed area (nose, paranasal (nasolabial groove), ear, preauricular, rest of face, neck (including nape), scalp, anterior thorax, posterior thorax, upper limbs) and one tumor in a non-sun-exposed area (retroauricular, lower limbs, abdomen, lumbar area, genitals) simultaneously or in consecutive steps; (2) patient with both excisions between January 2012 and October 2015. Exclusion criteria were as follows: (1) patient with tumor recurrence detected

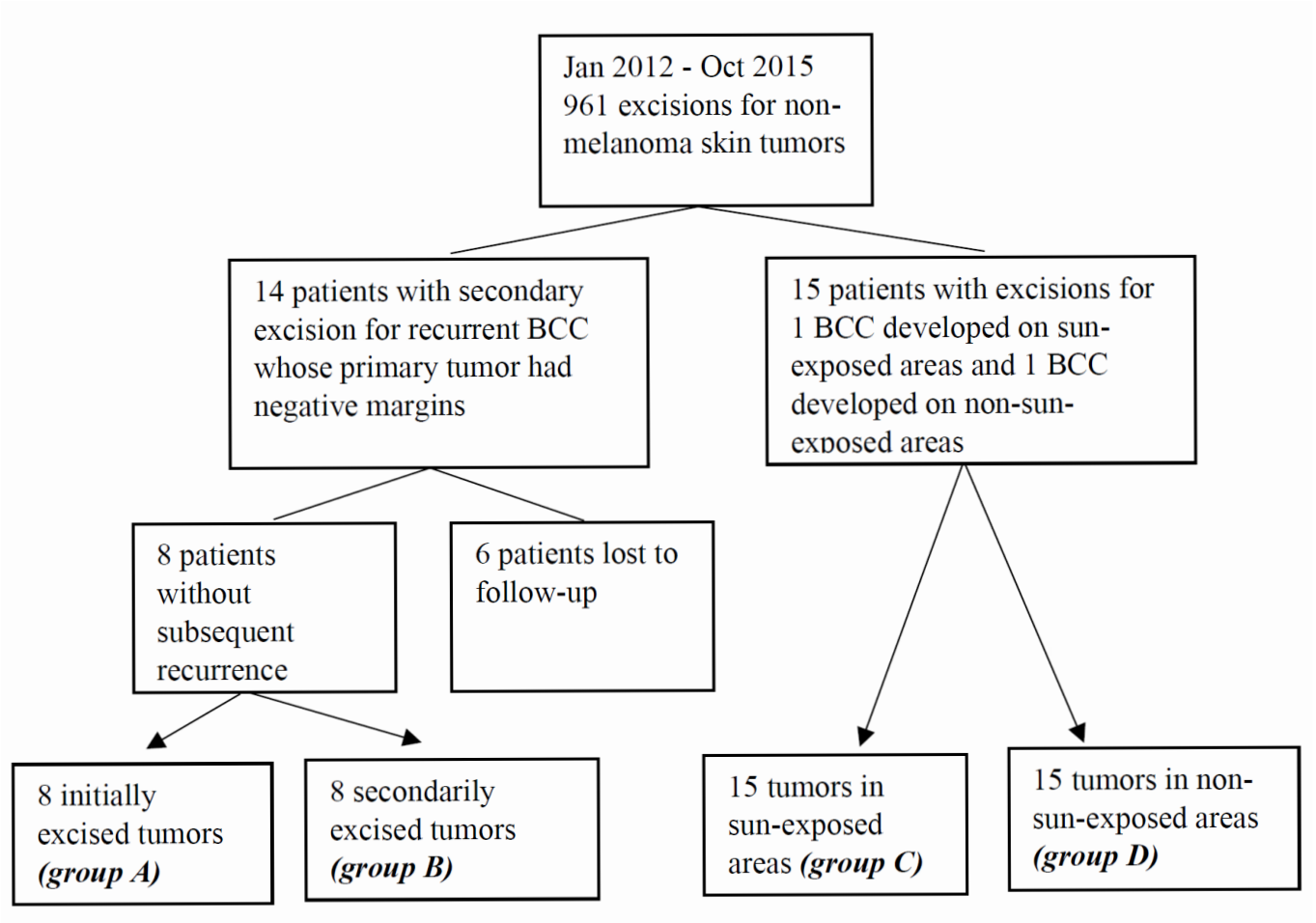

Fig. 1. Selection of patients and tumors included in the study

at subsequent periodic examinations; (2) patient who underwent surgical treatment using the Mohs technique; (3) patient with tumors with basal squamous histological subtype or perineural invasion; (4) patient with more than 2 excised tumors throughout the study period; (5) patient who did not present for follow-up visit between November 2017 and November 2018. Thus, out of 961 hospitalizations, 15 patients with 30 tumors were selected. The following parameters were analyzed for the selected tumors: time from excision (years), time until recurrence (years), location, size (cm), main morphology (chosen in order of aggression: infiltrative - sclerodermiform - nodular - superficial - cystic, noting that the micronodular subtype was associated with the infiltrative subtype), Clark's level, Breslow's depth, the lateral excision margin and the deep excision margin (mm). Breslow's depth index is defined by the distance in mm from the granular layer of the epidermis to the deepest point of invasion. For immunohistochemical analysis, 5 µm sections were extracted from formalin-fixed paraffin-embedded specimens representing highly cancerous areas. These were stained with E-cadherin antibodies (Clone EP700Y, Roche, Ventana; BenchMark Ultra, standard CC1, 16 min primary antibody incubation, OptiView amplification); anti-YAP antibodies (rabbit monoclonal EP1675Y, abcam, 1/200 dilution), GLI1 antibodies (rabbit polyclonal ab, ThermoFischer Scientific, 5µg/ml), CTGF/CCN2 antibodies (rabbit polyclonal ab, Novus Biologicals; 1/50 dilution), and slides were analyzed using Olympus BX43 microscope, 10x and 40x objectives. Staining was expressed as percentage (average of 5 HPF) and intensity (0 – no staining, 1 – weak staining, 2 – moderate staining, 3 – strong staining).

Statistical analysis was performed using MedCalc® Statistical Software version 19.6 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2020). Quantitative data were expressed by median and 25th and 75th percentiles (non-normal distribution), and qualitative data by frequency and percentage. Comparisons between groups were made using the Man-Whitney or the chi-square test, depending on the situation. The correlations between groups were checked using Spearman's rank correlation coefficient. A p-value less than 0.05 was considered statistically significant.

Forty-six tumors (groups A, B, C and D) were analyzed by immunohistochemistry (Figure 1) to analyze the correlations between GLI1, YAP, CTGF and E-cadherin expression. To evaluate the histopathological and immunolabeling differences between chronically sun-exposed and intermittently exposed tumors, 30 paired tumors from the same patient were analyzed, of which 15 on sun-exposed areas (group C) and 15 on non-sun-exposed areas (group D).

Results

There were no cases of metastatic tumors or tumors of the genital area.

Regarding the immunoexpression of molecules (see Table 1), GLI1 is present in 35/46 tumors (76.1%), being poorly expressed in 10.0% (5.0-23.75) of cells (see Table 1). YAP is present in

Table 1. Immunoexpression and distribution of GLI1, YAP, CTGF and E-cadherin in tumors and adjacent tissues

Molecule		GLI1	YAP	CTGF	E-cadherin
Expression	**Tumors N (%)**	35 (76.1)	46 (100)	46 (100)	45 (97.2)
	Tumor cells* (%)	10.0 (5.0-23.75)	97.5 (72.5-100)	70.0 (20.0-90.0)	20.0 (10.0-38.75)
	Intensity*	1.0 (1.0-1.0)	3.0 (2.0-3.0)	2.0 (1.0-2.0)	2.0 (1.0-2.0)
Distribution	**Tumor cells**	Cytoplasm and nucleus	Cytoplasm and nucleus	Nucleus	Cell membrane (heterogenous)
	Stroma	Absent	Cytoplasm: in capillary's endothelial cells	Nucleus	Absent
	Adjacent tissue	Nucleus: weak, focal	Cytoplasm: basal layer	Nucleus	Cell membrane

* median (percentiles 25-75)

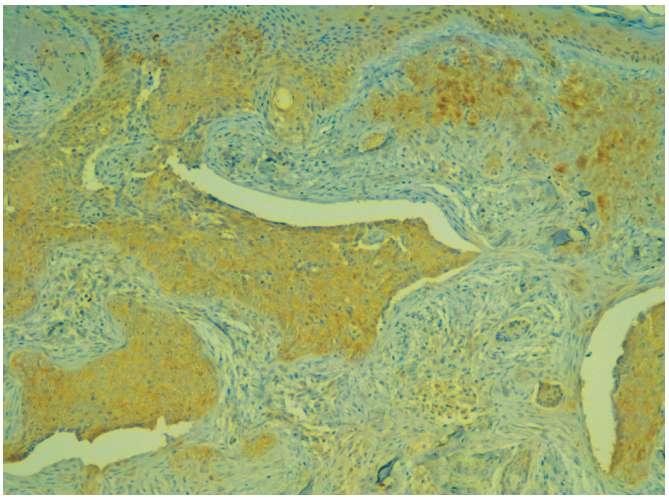

Fig. 2. Cytoplasmic and nuclear GLI1 expression in tumor cells, weak intensity, 40x

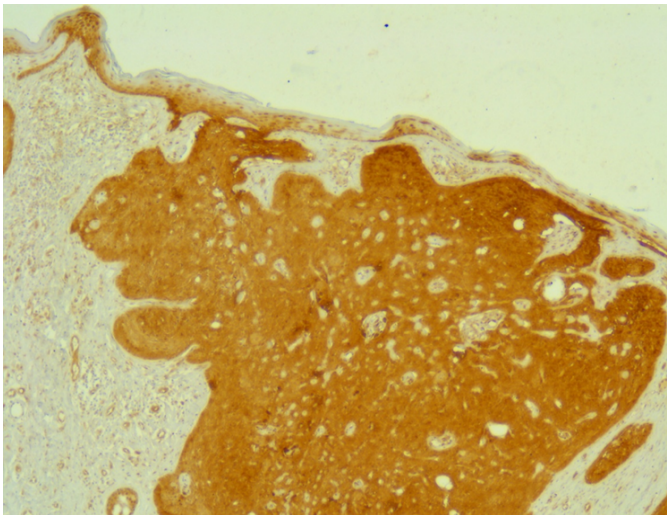

Fig. 3. Cytoplasmic and nuclear YAP expression in tumor cells, strong intensity, basal or absent in the adjacent cytoplasmic epidermis, 40x

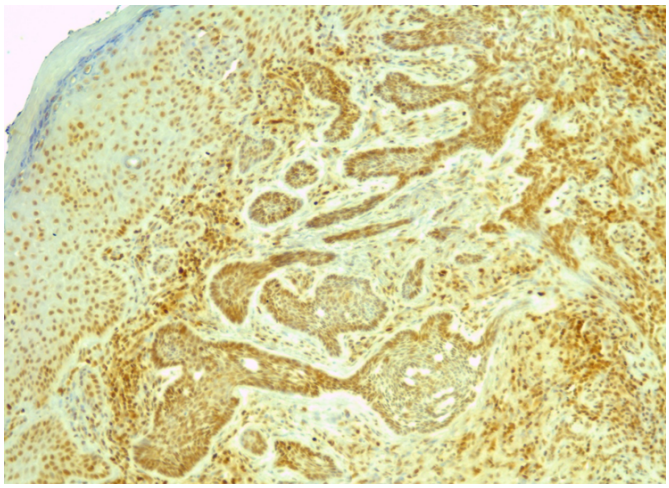

Fig. 4. Nuclear CTGF expression in the tumor islands and in the adjacent stroma, more intense in the stroma, 40x

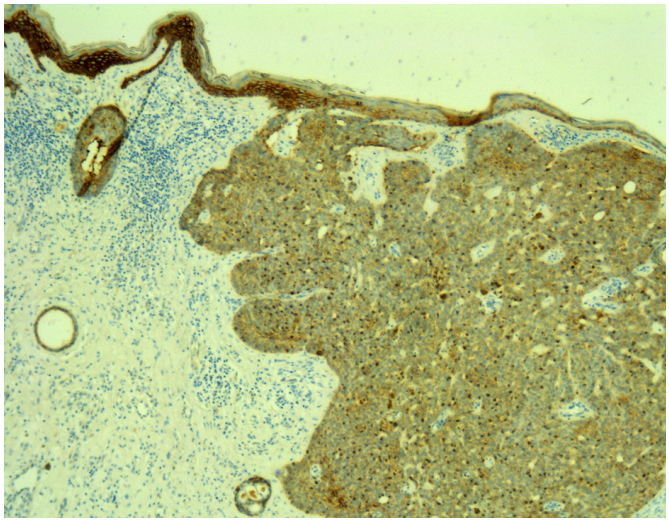

Fig. 5. Membrane-bound E-cadherin. Loss of expression was observed in the tumor in contrast to the adjacent epidermis in all cases, 40x

all tumors, with strong intensity in 97.5% (72.5-100) of cells. CTGF is present in all BCCs, with moderate intensity in 70.0% (20.0-90.0) of cells. E-cadherin was expressed in 45/46 (97.2%) tumors, showing moderate intensity in 20.0% (10.0-38.75) of cells. E-cadherin was absent in a single tumor (2.2%) located retroauricularly (Breslow 4, Clark 5), where YAP was expressed on a scale of 3 in terms of intensity in all cells, GLI1 was weakly expressed in 5% of cells, and CTGF was moderately expressed in 20% of cells. For the qualitative distribution of molecules in tissue, see Table 1 and Figures 1,2,3,4.

Correlation between the expression of the molecules can be seen in table 2. A moderate indirect correlation was observed between YAP and E-cadherin regarding the percentage of cells expressing these molecules. That is, as there were more cells

Table 2. Correlation between GLI1, YAP, CTGF and E-cadherin immunoexpression in tumors

		GLI1	YAP	CTGF	E-cadherin
GLI1	**Correlation coefficient**	1	# 0.25 § 0.31	# 0.14 § 0.23	# -0.21 § -0.27
	p-value	-	# 0.08 § 0.03 *	# 0.33 § 0.12	# 0.15 § 0.06
YAP	**Correlation coefficient**	# 0.25 § 0.31	1	# -0.03 § -0.13	# -0.32 § -0.15
	p-value	#0.08 § 0.03 *	-	# 0.83 § 0.39	# 0.03 § 0.30
CTGF	**Correlation coefficient**	# 0.14 § 0.23	# -0.03 § -0.13	1	# 0.19 § -0.08
	p-value	#0.33 § 0.12	# 0.83 § 0.39	-	# 0.20 § 0.58
E-cadherin	**Correlation coefficient**	# -0.21 §-0.27	# -0.32 § -0.15	# 0.19 § -0.08	1
	p-value	# 0.15 § 0.06	# 0.03 * § 0.30	# 0.20 § 0.58	-

- correlation regarding the percentage of tumor cells that express the molecules; § - correlation regarding the intensity of immunoexpression in tumor cells

Table 3. Histological characteristics of tumors in non-sun-exposed and sun-exposed areas

	N (%)	Non-sun exposed	Sun-exposed	p-value
Histological subtypes	Superficial 2 (6.7%)	1 (6.7%)	1 (6.7%)	0.38
	Nodular 25 (83.3%)	13 (86.7%)	12 (80%)	
	Infiltrative 2 (6.7%)	0 (0%)	2 (13.3%)	
	Cystic 1 (3.3%)	1 (6.7%)	0 (0%)	
	Pigmented 5 (16.7%)	1 (6.7%)	4 (26.7%)	0.33
	Ulceration 21 (70%)	10 (66.7%)	11 (73.3%)	1
Lateral margin (mm)		1.5 (1.0-2.0)	1.0 (0.7-1.8)	0.04
Deep margin (mm)		1.4 (1.0-2.9)	1.6 (1.0-2.0)	0.95
Breslow's depth		1.2 (0.3-2.6)	1.2 (1.0-2.0)	0.92
Clark's level		3.0 (2.0-4.0)	3.0 (3.0-4.0)	0.14

expressing YAP, the number of cells expressing E-cadherin decreased. (p=0.03) In terms of expression intensity, the trend was also negative, but not statistically significant. (p=0.30)

There was a direct, positive correlation between YAP and GLI1; their expression levels increased proportionally. Regarding the percentage of positive cells, the difference was not statistically significant. (p=0.08) But in terms of expression intensity, YAP and GLI1 increased proportionally. (p=0.03)

In addition, there was a negative correlation between GLI1 and E-cadherin in terms of cell percentage (p=0.15) and staining intensity. (p=0.06)

Neither the percentage of stained cells nor the intensity of CTGF expression was correlated with the other molecules studied. As a percentage, there was a trend towards a positive correlation with E-cadherin. (p=0.20) In terms of intensity, there was a trend towards a weak direct correlation with GLI1. (p=0.12)

There were no differences between the expressions of the molecules in different histological subtypes.

In terms of tumor localization, tumors in sun-exposed areas were found mainly on the cheeks, paranasal sinuses, scalp, anterior thorax, 10 (66.6%), and those in non-sun-exposed areas were located largely on the abdomen and lumbar area, 9 (60.0%).

The sizes of excised tumors from sun-exposed areas compared to non-sun-exposed areas were similar, 0.7 cm (0.6-1.0) and 1 cm (0.5-2.0). (p=0.44)

The distribution of histological subtypes (see Table 3) was somewhat similar, and most tumors were nodular, ≥80%. (p=0.38)

Microscopically, pigmentation was observed in only 1 (6.7%) of non-sun-exposed tumors and in 4 (26.7%) of tumors in sun-exposed areas. (p=0.33). The presence of ulceration at the microscopic level was found in about 70% of cases, similarly distributed between groups. (p=1.00).

The distance between the tumor and the nearest lateral surgical margin was 1.0 mm (0.7-1.8) in the case of tumors in sun-exposed areas, significantly closer than in the case of tumors in non-sun-exposed areas, respectively 1.5 mm (1.0-2.0). (p=0.04)

Table 4. Expression of molecules in tumors in non-sun-exposed and sun-exposed areas

Molecule		Non-sun-exposed	Sun-exposed	p-value
GLI1	**intensity**	1.0 (1.0-2.0)	1.0 (0.0-1.0)	0.6
	percentage	10 (1.0-20.0)	7.5 (0.0-16.25)	0.97
YAP	**intensity**	2.0 (2.0-3.0)	2.0 (1.75-3.0)	0.64
	percentage	100.0 (80.0-100.0)	90.0 (67.5-100.0)	0.35
CTGF	**intensity**	2.0 (2.0-3.0)	2.0 (1.75-3.0)	0.79
	percentage	75.0 (60.0-90.0)	80.0 (17.5-91.25)	0.63
E-cadherin	**intensity**	1.0 (1.0-2.0)	2.0 (1.75-2.0)	0.2
	percentage	20.0 (5.0-40.0)	30 (10.0-52.5)	0.75

The deep margin was similar in the two groups, 1.6 and 1.4, respectively, p=0.95.

Breslow's depth was similar in the 2 groups, 1.2 mm, p=0.92; whereas Clark's level was slightly higher in the case of sun-exposed tumors, 3 (3.0-4.0) compared to 3 (2.0-4.0) in the case of non-sun-exposed tumors, p=0.14.

The differences in the expression (both percentage of stained cells and staining intensity) of the studied molecules between tumors in sun-exposed areas and tumors in non-sun-exposed areas were not statistically significant (see Table 4). There is only a weak trend towards a reduced intensity of E-cadherin in sun-exposed areas.

Discussion

Of the 3 subfamilies of glioma-associated oncogenes (GLI), GLI1 is the only transcriptional activator whose nuclear localization is considered the signature of the Hh activation pathway (Tanese et al 2018).

According to a recent study, although GLI1 is specifically higher in BCC compared to other skin cancers, there is no difference in GLI1 expression in different histological subtypes of BCC (HS Kim et al 2019). In addition, according to the study conducted by Tanese et al. on skin tumors in Japan, 98.2% of BCCs have high nuclear GLI1 expression regardless of histological subtype. The positivity was predominant in tumor islands, the overlying tissues being negative for GLI1, suggesting the specificity of the antibody (Tanese et al 2018). These results are inconsistent with our study in which 22.9% of the BCCs studied did not express GLI1, and when present, GLI1 expression was low (both in terms of percentage of stained cells and intensity). Regarding intracellular localization, our results are consistent with data from the literature, GLI1 being present in the cytoplasm or nucleus of tumor cells, with weak and focal expression in the nucleus of the adjacent tissue. Nuclear GLI1 immunoexpression could be explained by the fact that activation of the Hh pathway translocates GLI1 into the nucleus (Pellegrini et al 2017). It was absent in the tumor stroma.

As stated in the current literature, YAP activation occurs either following the inactivation of the Hippo pathway or due to tissue stiffness. YAP has a critical role in maintaining stem cell population and BCC proliferation (Quan et al 2014). In tumors induced by SMO mutations, there is an increase in nuclear YAP levels (Debaugnies et al 2018) and the remodeling of the extracellular matrix is present (Akladios et al 2017). Moreover, YAP and Hh pathway modulate each other in a positive manner in epidermal homeostasis (Akladios et al 2017). This was also observed in our study, where there was a direct correlation between YAP expression and GLI1 expression mainly in terms of intensity, but also as percentage of stained cells, even if it was not statistically significant.

YAP was present in all tumors in our study, with high expression in terms of intensity and percentage of stained cells. This is consistent with data from the literature showing a high expression of YAP in BCC, both in the cytoplasm and in the nucleus. YAP phosphorylation retains the molecule in the cytoplasm, and it has been found as an oncogene in solid tumors (pulmonary and colonic adenocarcinoma, medulloblastoma); and it promotes epidermal cell growth in the skin (Quan et al 2014). Another study reported the nuclear localization of YAP in BCC and signatures of the YAP pathway in BCC of any histological type (Debaugnies et al 2018) similarly to our study where CTGF, a direct target gene of YAP, was present in all BCCs, although sometimes poorly expressed. In our study, in terms of intracellular localization, YAP was localized to both the cytoplasm and the nucleus of tumor cells. In the stroma it was present on capillaries in the cytoplasm, probably due to tumor neoangiogenesis. Cytoplasmic basal location was found in the adjacent epidermis; being present in immature cells, probably playing a role in maintaining stem cell population (Quan et al 2014).

CTGF, also known as CCN2, is a direct target gene of YAP, which induces stroma modeling by increasing its stiffness, without influencing keratinocyte proliferation (Quan et al 2014).

In our study, CTGF would have been expected to be negatively correlated with E-cadherin due to the binding of both molecules to the stroma. On the contrary, in the present study there is a weak trend towards a positive correlation between CTGF and E-cadherin. And CTGF was not correlated with the other molecules. Instead, it was present in the cell nucleus both in the tumor islands and in the stroma and adjacent skin. A more intense expression was found in the stroma, corresponding to data from the literature (Quan et al 2014).

Tumor cells correlate strongly with the active stroma. In the case of BCC, the stroma seems to have particular characteristics, according to a recent article, this being one of the factors that contribute to the non-metastatic status due to the presence of special fibroblasts (Mendez et al 2020).

There is a certain degree of epithelial-mesenchymal transition in BCC (Bartoš et al 2015) and, although the underlying mechanisms of this transition have not yet been elucidated, most of them lead to reduced E-cadherin expression (Tucci et al 2013). E-cadherin is an important molecule in intercellular adhesion, being responsible for the preservation of tissue micro-architecture. Its presence is generally low in locally infiltrative tumors,

the homogeneous or heterogeneous expression pattern also being important (Bartoš et al 2015). E-cadherin expression was low-moderate in the tumors in the present study, in terms of both staining intensity and percentage of stained cells. It was always found in the membrane, in the tumor islands in a heterogeneous manner independent of tumor aggressiveness, but absent in the stroma, and present on the cell membranes of adjacent tissues. There was a decrease in E-cadherin expression compared to the surrounding tissues in all cases, similar to data in the literature, the loss of expression being higher in infiltrative tumors.

A study on BCC of the eyelid reported the absence of E-cadherin in nodular and adenoid tumors and moderate expression in the morpheaform subtype. (Bălăşoiu et al 2015) In our group, E-cadherin did not show differences depending on histological subtypes and it was absent in a single tumor. This retroauricular tumor on thin skin was a nodular BCC type, thick and invading the subcutaneous tissue, with high expression of YAP, low CTGF expression and very low GLI1 expression. Compared to the study conducted by Balasoiu et al., the tumors in our study were predominantly nodular, 83.3%, and the rest were superficial, infiltrative and cystic.

In our study, there was a weak trend towards a decrease in the intensity of E-cadherin in tumors on chronically sun-exposed skin. There was a moderate negative correlation between YAP and E-cadherin. There were no statistically significant differences in terms of expression intensity. However, the percentage of E-cadherin negative cells increased with higher YAP expression. Studies in the literature show that YAP is modulated by environmental factors and by E-cadherin (N-G Kim et al 2011). In the studied tumors, there was also a trend towards a negative correlation between the intensity of GLI1 and E-cadherin, similarly to YAP, which is to be expected since YAP and GLI1 are positively correlated. The same trend is observed for the percentage of positive cells, even if it is not statistically significant. Data from the literature link GLI1 expression to the Wnt/β-catenin pathway in the development of BCC by maintaining stem cell population, (HS Kim et al 2019, FK Noubissi et al 2014, F Noubissi et al 2018) and Wnt signaling is induced by Hh, (Calonje et al 2005) whose final product is GLI1.

In BCC, membrane-bound β-catenin levels are lower than in normal epidermis, with membrane localization prevailing in aggressive tumors (Rajabi et al 2019). In other studies, E-cadherin expression is correlated with β-catenin in squamous cell carcinomas and actinic keratoses (Saenz-Sardà et al 2018). In our study, an indirect demonstration was observed through the greater loss of E-cadherin expression in aggressive tumors (eg infiltrative) compared to the neighboring epidermis.

This study also aimed at the differences between tumors in chronically sun-exposed areas and intermittently sun-exposed areas. For this purpose, sun-exposed areas were considered as chronic exposure, and non-sun-exposed areas as intermittent exposure. Among the tumors studied, there were no tumors in the genital area. In our study we found that the mean value for Breslow's depth was 1.2, but Clark's level was slightly higher on sun-exposed areas than on non-sun-exposed areas, probably due to thinner dermis on sun-exposed areas, and thus a deeper tumor spread.

Moreover, tumors on non-sun-exposed areas have the closest lateral margin from the tumor islands at a greater distance than those on sun-exposed areas, probably due to a greater ease of excision than in sun-exposed areas and/or easier tissue mobilization. Regarding the expression of the molecules of interest: GLI1, YAP, CTGF and E-cadherin, there were no significant differences between tumors regarding the type of sun exposure. There was only a slight trend towards a decrease in E-cadherin expression in the case of sun-exposed areas. On the other hand, there were no differences in expression between histological subtypes. However, there were over 80% nodular carcinomas, so a different activation difference of the signaling pathways between subtypes is not completely ruled out.

This study's limitation is the low number of analyzed tumors, as this is a pilot study and the tumors were selected on particular criteria.

Conclusion

In conclusion, the molecular profile of the molecules involved in the signaling pathways that have a role in the development and differentiation of BCCs, GLI1, YAP, CTGF and E-cadherin, did not depend on the histological subtype or the type of sun exposure. Tumors on sun-exposed areas had a closer lateral margin probably due to a smaller macroscopic margin to preserve healthy tissue. The Hh pathway was positively correlated with YAP in BCC. YAP was present in the cytoplasm of new tumor vessels. Further studies on more tumors are needed to clarify the role of these molecules in the pathogenesis of BCC.

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