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Peritumoral Clefting and Expression of MMP-2 and MMP-9 in Basal Cell Carcinoma of the Skin

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Abstract. *Background/Aim: Peritumoral clefting is one of the main histologic features of basal cell carcinoma of the skin (BCC). The aim of the study was to analyze the expression of MMP-2 and MMP-9 both in cells of basal cell carcinoma and in the adjacent stroma and to correlate the findings of immunohistochemical analysis with the presence of peritumoral clefting. Patients and Methods: The study was made on archival material comprising 48 cases of BCC. These were scanned for the presence of peritumoral clefts. The results of immunohistochemical staining for MMP-2 and MMP-9 were determined semiquantitatively using immunohistochemical staining index (ISI). Results: Peritumoral retractions were found in 40 BCC cases. Positive immunohistochemical reaction for MMP-2 in tumor cells was found in 47 cases and in all cases in the adjacent stroma. Positive immunostaining for MMP-9 in BCC tumor cells was observed in 37 cases and in all cases in the adjacent stroma. There was no statistically significant association between peritumoral retractions and expression of MMPs. A statistically significant correlation was found in the expression of both MMP-2 and MMP-9 between the tumor and the stroma. Conclusion: Tumor cells elaborate MMP-2 and -9, but they also produce some other factors that may induce production of MMPs in adjacent stromal cells. The role of MMPs in the development of peritumoral clefts could not be confirmed.*

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Basal cell carcinoma (BCC) is the most frequent human malignant tumor (1, 2). A significantly increased incidence of BCC has been observed in Europe, United States and Australia, especially among Caucasians (3, 4). Basal cell carcinoma is characterized by a slow, locally invasive growth leading to destruction of the surrounding structures, but usually not showing a very aggressive behavior, thus it seldom causes metastases or mortality (1-4).

Peritumoral clefts (retraction clefting) are empty spaces arround tumor nests that may be seen in different tumor types (5-8). The exact mechanism of their evolution is still controversial, although the loss of basal epithelial cells, the lower expression of adhesion molecules and the higher expression of proteins involved in extracellular matrix remodeling most probably have a major role. Extensive studies of peritumoral clefting have been made on breast and prostate cancer, where their occurrence has been shown to have both diagnostic and prognostic significance (8, 9).

Retraction clefting may be useful in the differential diagnosis between basal cell carcinoma and adnexal skin tumors (7).

The extracellular matrix (ECM) constantly undergoes a tightly regulated remodeling process in order to preserve tissue homeostasis (10). It is known that ECM can have different roles: it can localize tumor growth in normal conditions, or induce tumor cell proliferation in others. Matrix metalloproteinases (MMPs) lead to proteolytic degradation and thus remodeling of ECM, but more importantly, they are thought to participate in all steps of carcinogenesis – loss of tumor cohesiveness, basal membrane

degradation, cell migration and blood vessel wall penetration (11-12). Some MMPs are specific for certain tissues. Metalloproteinases 2 and 9 (MMP-2 and MMP-9) have a major role in the digestion of basement membrane type IV collagen and play an important role in the progression of BCC (13-14).

According to some studies, expression of MMP-2 and MMP-9 is significantly higher in basal cell carcinomas than in adjacent normal tissue (15, 16).

This study analyzed the expression of MMP-2 and MMP-9 both in basal cell carcinoma cells of the skin and in the adjacent stroma and correlated the findings of immunohistochemical analysis with the presence of peritumoral clefting.

Patients and Methods

Patient's selection and clinical data. The study was made on archival material taken from files of the Department of Pathology, School of Medicine, University of Zagreb, Croatia, comprising 48 consecutive cases of basal cell carcinoma diagnosed by the same dermatologist in the period from 2010 to 2018.

Histopathology. Hematoxylin and eosin stained tissue sections from tumors were available for review in all cases. The slides were scanned for the presence of peritumoral retractions under low magnification (x40) and further analyzed under medium (x100) and high (x400) magnification by light microscopy. We considered peritumoral retractions the empty spaces that partially or completely encircle tumor nests and separate them from the adjacent stroma. Artefactual clefts resulting from thermal damage of material were not considered to be peritumoral retractions.

Immunohistochemical staining was performed using standard procedures on a DAKO TechMate Horizon automated immunostainer (DAKO, Copenhagen, Denmark). The pretreatment of sections was performed using Dako PT link (deparaffinization, rehydration and epitope retrieval). After blocking the endogenous peroxidase activity by 5 min incubation with 3% hydrogen peroxide, the sections were incubated at room temperature with a primary monoclonal mice antibody against MMP-2 (code ab86607, Abcam, Cambridge, UK; dilution 1:100) for 30 min and a rabbit monoclonal antibody against MMP-9 (code ab76003, Abcam, Cambridge, UK, dilution 1:100. This was followed by incubation with the labeled polymer (EnVision HRP; Dako, Glostrup, Dennmark). Color was developed by incubation with 3,3'-diaminobenzidine tetrahydrochloride and slides were counterstained by hematoxylin. Normal placental tissue (MMP-2) and normal lung tissue (MMP-9) were used as a positive control. For negative control, primary antibody was omitted.

The results of immunohistochemical analysis were determined semiquantitatively using immunohistochemical staining index (ISI), obtained by multiplying the percentage of positive cells (PPC) and staining intensity (SI), as previously described (17). The percentage of positive cells (PPC) was scored as 0 for no positive cells, 1 for up to 10% positive cells, 2 for >10-50% positive cells and 3 for more than 50% positive cells, while SI was scored as 0 for no staining, 1 for weak staining, 2 for moderate staining and 3 for strong staining. The immunohistochemical staining index was labeled as follows: 0=zero; 1-3=low; 4-6=moderate and 9=high.

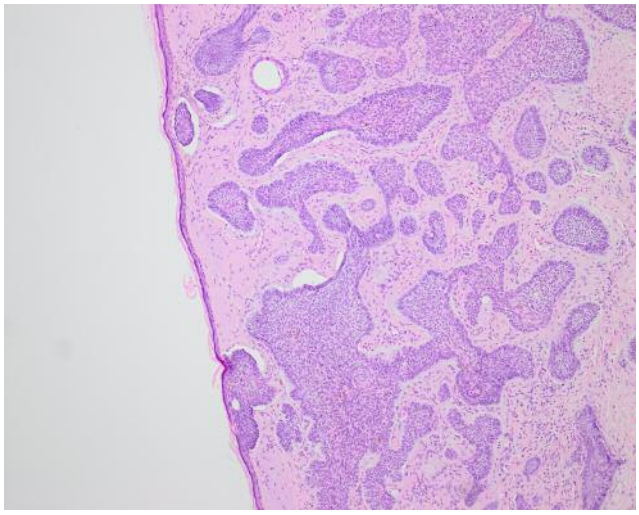

Figure 1. *Peritumoral retraction clefting in basal cell carcinoma of the skin (HE×100).*

Statistical methods. Statistical analysis was done using Mann-Whitney *U*-test. $p<0.05$ was considered to be statistically significant. The analysis was made using IBM SPSS Statistics software version 21.0 and GraphPad Prism version 8.0.0.

Results

Peritumoral retractions were found in 40 BCC cases, while there were no clefts observed in 8 cases (Figure 1).

Positive immunohistochemical reaction for MMP-2 in tumor cells was found in 47 cases of BCC (38 showing weak ISI and 9 moderate ISI) and in all cases in the adjacent stroma (21 showing weak ISI and 27 moderate ISI) (Figure 2A). Positive immunostaining for MMP-9 in BCC tumor cells was observed in 37 (77.1%) cases (all showing weak ISI), while in the adjacent stroma 25 (52.1%) weak ISI and 23 (47.9%) moderate ISI with significant difference compared to tumor expression, $p<0.001$ (Figure 2B). The results are shown in Table I.

In evaluating MMP-2 and MMP-9 expression in the peritumoral stroma, we observed that the inflammatory cells, which are often present within it, were also stained.

There was no statistically significant correlation between the expression of MMP-2 and MMP-9 (in the tumor or stroma) and the presence of peritumoral retractions (p ranged from 0.16 to 0.82).

Statistically significant correlation was found in the expression of both MMP-2 and MMP-9 between the tumor and the stroma. MMP-2 expression was stronger in the stroma (mean ISI 1.56) than in the tumor (mean ISI 1.17, $p<0.01$). MMP-9 expression was also stronger in the stroma than in the tumor cells (mean ISI 1.48 *versus* 0.77 in the tumor, $p<0.01$).

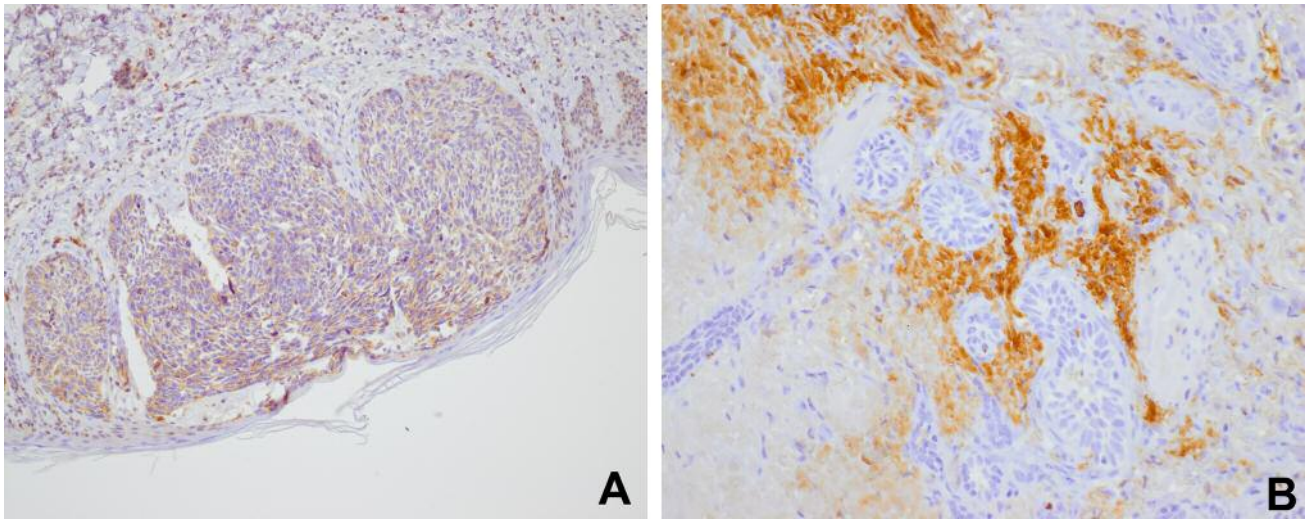

Figure 2. *Immunohistochemical expression of matrix metalloproteinase 2 (MMP-2) and MMP-9 in basal cell carcinoma of the skin. (A) The staining intensity of MMP-2 was moderate and the percentage of positive cells was >50%, making a moderate immunohistochemical staining index (A; MMP-2, ×200). (B) The staining intensity of MMP-9 was strong, but the percentage of positive cells was 10-50%, making the moderate immunohistochemical staining index (B; MMP-9 ×400).*

Discussion

BCC is microscopically characterized by nests of basaloid cells showing peripheral palisading, surrounded by peritumoral empty spaces that are usually regarded as artifacts due to fixation of specimens. However, there are other suggestions regarding the cause of peritumoral clefting (5, 6, 18). Acs *et al*. suggested that retraction clefting represents prelymphatic spaces in cases of breast carcinoma (8). There are recent studies showing the presence of peritumoral clefts *in vivo* by reflectance confocal microscopy (19, 20). Ghita *et al*. examined superficial BCC using this method and observed dark spaces surrounding the tumor nests (20). However, the cause of this phenomenon is still unknown.

Matrix metalloproteinases may be predominantly expressed in the epithelial tumor component or in the stroma, the latter probably being induced by tumor infiltration, direct cell-cell contact or secretion of growth factors, whether tumor-derived or released from the degraded ECM (10). Beside the aforementioned, the difference between results of studies analyzing MMP expression in cancer is caused by the usage of different tissues (blood, urin, cancer tissue of various localization, grade and stage), different methodology (mRNA, proenzyme or active protein measurement), different quantification of results and different antibodies or protocols.

Considering the role of MMPs in ECM degradation, it is plausible to assume that their expression should be higher in invasive tumors *versus* noninvasive ones or normal tissues.

Table I. *The presence of peritumoral retractions and immunohistochemical staining index (ISI) of MMP-2 and MMP-9 in basal cell carcinoma of the skin.*

	Tumor	Stroma	*p*-Value
Peritumoral retractions			
None	8 (16.7%)		
Present	40 (83.3%)		
MMP-2 ISI			
Negative	1 (2.1%)	0 (0.0%)	<0.001
Low	38 (79.2%)	21 (43.8%)	
Moderate	9 (18.7%)	27 (56.2%)	
High	0 (0.0%)	0 (0.0%)	
MMP-9 ISI			
Negative	11 (22.9%)	0 (0.0%)	<0.001
Low	37 (77.1%)	25 (52.1%)	
Moderate	0 (0.0%)	23 (47.9%)	
High	0 (0.0%)	0 (0.0%)	

Gozdzialska *et al*. observed a significantly higher expression of MMP-2 and MMP-9 mRNA and a significantly lower expression of collagen IV mRNA in BCCs than in normal tissue adjacent to tumor (15). O'Grady *et al*., in their study on the expression of MMP-2 and MMP-9 in non-melanoma skin cancers, observed no MMP-2 expression in cells of basal cell carcinoma, but a stromal expression in all of their 38 BCC cases (21). They found MMP-9 expression in the stroma of all samples and in the epithelium of all but two BCC cases (21). MMP-9 expression was reported to be increased in BCC cells as well as in peritumoral stroma in a study by Monhian

et al. (13). They suggested that factors produced by tumor cells induce MMP elaboration in adjacent tissue (13).

In our study, we found positive immunohistochemical expression for MMP-2 in tumor cells in 98% of cases, for MMP-9 in 77% cases, and in the adjacent stroma for MMP-2 and -9 in all cases, but we did not find a statistically significant correlation between the expression of MMPs and peritumoral clefts. A statistically significant stronger expression of the analyzed markers was found in the immediate peritumoral stroma, which in most of the cases contained inflammatory cells. Our results suggest that tumor cells elaborate MMP-2 and -9, however, it seems that they also produce some other factors that may induce production of MMPs in adjacent stromal cells.

Conclusion

Peritumoral retractions are present in basal cell carcinoma of the skin. Using immunohistochemical staining for MMP-2 and MMP-9, we confirmed that the majority of tumoral nests with retractions showed positive staining for MMP-2 and -9 in tumor cells as well as in adjacent stromal tissue. However, there was no statistically significant association between peritumoral retractions and the expression of analyzed MMPs. Therefore, we were not able to confirm the role of MMPs in the development of peritumoral clefts of BCC. Further studies on larger groups of patients may shed some light on the cause of this phenomenon in BCCs as well as in other tumor types characterized by the same feature.

Conflicts of Interest

The Authors have no conflicts of interest to declare regarding this study.

Authors' Contributions

I.M. and D.L.D have examined and selected the patients and drafted the manuscript. A.M. and T.R.Dz. performed histological and immunohistochemical analysis. I.P. did statistical analysis. T.R.Dz. and B.K. drafted the manuscript and did the final editing.

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