

Immunohistochemical Expression of Calponin in Cutaneous Basal Cell Carcinoma

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ABSTRACT Calponin is an actin filament-associated protein significantly involved in the regulation of the cellular motility. Some data have indicated that overproduction of calponin in basal cell carcinoma (BCC) of the skin may be responsible for local tumor invasiveness and more aggressive biological behavior. We studied the immunohistochemical expression of calponin in a set of cutaneous BCCs, in order to clarify whether the presence of calponin in cancer cells may be a predictor of invasive tumor growth. The study group consisted of 37 primary BCCs categorized into a non-infiltrative subgroup (5 superficial, 16 nodular subtypes) and infiltrative subgroup (9 nodular-infiltrative, 7 infiltrative subtypes). A specific monoclonal antibody against calponin was used for staining. Expression of calponin in tumor tissue was found in 72.9% (27/37) of the cases, though staining intensity was relatively weak. In superficial, nodular, nodular-infiltrative, and infiltrative BCC subtypes, calponin positivity was found in 80% (4/5), 75% (12/16), 66.7% (6/9), and 71.5% (5/7), respectively. We did not confirm a significant correlation between expression of calponin and given, non-infiltrative, and infiltrative BCC subgroups. Furthermore, we found seven BCCs (18.9%) with striking immunoreactivity for calponin in adjacent peritumorous stroma. There was a significant association between stromal immunoreactivity for calponin and tumor growth histomorphology being positive only in BCCs with infiltrative growth features. Our study has shown that neoplastic cells in cutaneous BCC commonly produce calponin regardless of histological subtype. Expression of calponin in tumor tissue was not associated with the aggressive tumor phenotype. However, since some BCCs with infiltrative growth patterns strongly expressed calponin in peritumorous stroma, this finding could more reliably reflect the biological behavior of cancer and should be better explained in the future.

KEY WORDS: basal cell carcinoma, calponin, immunohistochemistry

INTRODUCTION

Basal cell carcinoma (BCC) of the skin is currently the most common malignancy in humans (1-3). In contrast to most other cancers, it generally has a favourable clinical course, growing slowly and expanding only locally (1-3). However, some cases show aggressive behavior, rapidly infiltrating deeper tissue structure, and sometimes (although very rarely) giving rise to metastatic spread (1,2). Although many various molecular markers have been studied in cutaneous BCC (4), it is still not clearly understood which

of them are directly responsible for aggressive tumor behavior, and, conversely, which potentially prevent cancer cells to metastasize. Because BCC has a purely epithelial origin, it naturally expresses a broad spectrum of epithelial biomarkers, such as cytokeratins or E-cadherin. Interestingly, this neoplasia may also exhibit some mesenchymal/myoepithelial markers, e.g. alpha-smooth muscle actin (5-9) and calponin (5,10), which are unusual for "ordinary" carcinomas. Both these cytoplasmic proteins possess contractile

properties and are thus significantly involved in the regulation of cell kinetics and are largely responsible for cellular motility. Since normal epithelial cells have not a marked tendency to move, alpha-smooth muscle actin (α -SMA) and calponin are only sparsely found in their cytoplasm. On the other hand, their intracytoplasmic overproduction generally suggests an increased cellular mobility and hence a higher invasive potential of malignant tumor cells. Cytoskeletal reorganizations, especially alterations of contractile tension generated by the actin-myosin complex, are of central importance in the development of the phenotype of morphologically transformed neoplastic cells with invasive behavior. Of the proteins described above, α -SMA has been more intensively studied in cutaneous BCC (5,6,7,9), including our recent study (8). However, to the best of our knowledge, there are only two studies (5,10) dealing with the expression of calponin in BCC of the skin. Since these papers gave contradictory results, this topic is still far from clear. Therefore, we focused on immunohistochemical analysis of calponin expression in a set of cutaneous BCCs in order to clarify whether the presence of calponin in cancer cells may be a predictor of invasive tumor growth.

PATIENTS AND METHODS

Patients and tumor specimens

Biopsy samples from 37 chosen cases of primary cutaneous BCCs from various topographic locations were included in this study. They were obtained from 23 subjects (17 men, 6 women) aged 35-91 years (mean 73.5 years). All patients were treated at the clinical departments of the Faculty Hospital in Zilina (Slovakia) and all biopsy samples were histopathologically investigated at the Department of Pathology in the Faculty Hospital in Zilina during the year 2014. For the purpose of this study, we selected a set of representative samples of cutaneous BCCs including four histomorphological subtypes: superficial (5 cases), nodular (16 cases), mixed nodular-infiltrative (9 cases), and pure infiltrative (7 cases). They were divided into two separate subgroups for statistical analysis. The first subgroup was comprised of 21 indolent (non-infiltrative) BCC subtypes (superficial and nodular). The second subgroup was comprised of 16 BCCs with an (at least focal) infiltrative growth pattern (mixed nodular-infiltrative and infiltrative subtypes). Only samples with enough tumor tissue in the paraffin-embedded blocks to harvest appropriate slides for immunohistochemistry were chosen.

Immunohistochemistry

Biopsy samples were routinely processed and immunohistochemically stained for calponin according to the manufacturer's instructions. Representative 4 μ m tissue sections on silanized slides were baked for 2 hours in an oven at 56 °C. The sections were then deparaffinized in xylene, rehydrated in a series of descending ethanol concentrations, and treated with microwaves in a Dako Target Retrieval Solution (0.01 M citrate buffer, pH 6.0; Glostrup, Denmark) for 20 minutes. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 5 minutes. Subsequently, specific monoclonal mouse anti-human antibodies against calponin (clone CALP, code M3556, DAKO, dilution 1:50) was used for staining. After incubation at ambient temperature for 20 minutes, post primary antibodies were applied and an immunoreaction was visualized by means of the DAB (3,3'-diaminobenzidine) detection chromogen solution (Dako REAL™ EnVision™ Detection System, code K5007). Slides were counterstained with Mayer's hematoxylin, dehydrated, mounted, and finally evaluated using a light microscope. Positive reactions on the myoepithelial layer of eccrine glands served as internal control.

Data interpretation and statistical analysis

After including a total percentage of immunolabelled tumor cells, according to Lee *et al.* (10), we adopted the following four-titre semiquantitative scoring system: negative score 0 (<5% positive cells), score 1+ (5-24% positive cells), score 2+ (25-49% positive cells), and score 3+ (\geq 50% positive cells). We considered a tumor to be calponin-positive if it had a score

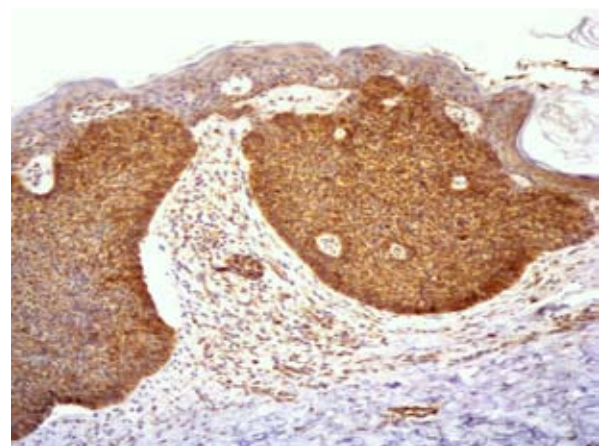


Figure 1. Diffuse expression of calponin (score 3+) within tumor tissue in superficial basal cell carcinoma (BCC) (clone calponin, DAKO, original magnification \times 120).

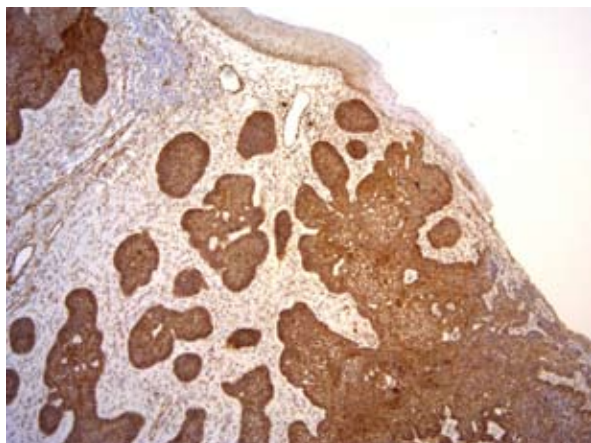


Figure 2. Diffuse expression of calponin (score 3+) within tumor tissue in nodular basal cell carcinoma (BCC) (clone calponin, DAKO, original magnification $\times 120$).

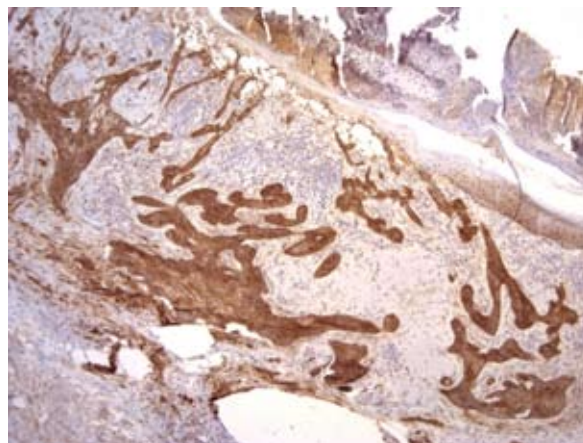


Figure 3. Diffuse expression of calponin (score 3+) within tumor tissue in infiltrative basal cell carcinoma (BCC) (clone calponin, DAKO, original magnification $\times 120$).

of 1+, 2+, or 3+. If the proportion of stained cells did not reach at least 5% (score 0), the result was classified as negative. In addition, we also evaluated adjacent peritumorous stroma for calponin expression, simply categorized as positive or negative. Data were collected in a databank, using a SPSS Statistics software. For the statistical analysis, the chi-square test was employed and a P value of <0.05 was considered statistically significant.

RESULTS

In our series, calponin was expressed in 27 BCCs (72.9%) showing cytoplasmic staining within cancer cells. No nuclear or membranous immunoreactivity was detected. In superficial, nodular, nodular-infiltrative, and infiltrative BCC subtypes, calponin positivity (defined as present in $\geq 5\%$ of immunolabelled tumor cells) was found in 80% (4/5), 75% (12/16), 66.7% (6/9), and 71.5% (5/7), respectively (Figure 1, Figure 2, Figure 3). Within the positive group, a wide quantitative range of calponin expression was found. Overall, there were sixteen cases (59.3%) with a score of 1+, four cases (14.8%) with a score of 2+, and seven (25.9%) cases with a score of 3+. We did not find a statistically significant correlation between immunohistochemical expression of calponin (present versus absent) and given, non-infiltrative, and (at least focally) infiltrative BCC subgroups ($P=0.6$). In mixed nodular-infiltrative BCC cases, the percentage of calponin-positive tumor tissue in both structural components seemed to be about the same. As for staining intensity, the vast majority of BCCs showed weak cytoplasmic immunoreactivity and, although some

cases contained areas with a more pronounced staining, it virtually never reached the degree observed in normal myoepithelial cells of the eccrine glands.

In addition to calponin expression in tumor tissue, we registered seven BCCs (18.9%) with strong immunoreactivity for calponin in adjacent peritumorous stroma. All these cases manifested histomorphologically (at least focal) infiltrative growth features (three nodular-infiltrative subtypes and four infiltrative subtypes). Among them, five (71.4%) were calponin-positive in tumor tissue and the remaining two (28.6%) were composed of only calponin-negative tumor cell populations (Figure 4). In contrast to epithelial cancer cells, however, stromal cells positive for calponin showed much stronger staining identical to that of

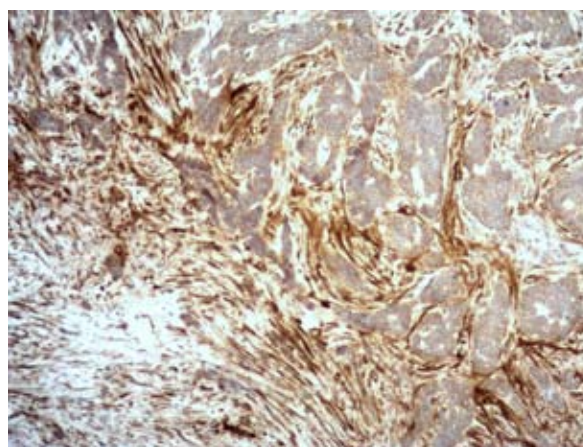


Figure 4. Strong expression of calponin in the peritumorous stroma in infiltrative basal cell carcinoma (BCC). Tumor nests are virtually negative (score 0) (clone calponin, DAKO, original magnification $\times 120$).

Table 1. A summary of the immunohistochemical findings in the set of 37 basal cell carcinoma (BCC) we investigated

BCC subtype	n	Calponin expression in tumor tissue	Calponin expression in stroma
superficial	5	score: 0	1 (20 %)
		score: 1+	0
		score: 2+	2
		score: 3+	2
nodular	16	score: 0	4 (25 %)
		score: 1+	9
		score: 2+	1
		score: 3+	2
nodular-infiltrative	9	score: 0	3 (33.3 %)
		score: 1+	3
		score: 2+	1
		score: 3+	2
infiltrative	7	score: 0	2 (28.5 %)
		score: 1+	4
		score: 2+	0
		score: 3+	1

the myoepithelial layer of the eccrine glands. Moreover, we observed a significant association between stromal immunoreactivity for calponin and tumor growth histomorphology being positive only in BCCs with infiltrative growth features ($P < 0.001$). There was no correlation between calponin expression (present versus absent) in tumor tissue and peritumorous stroma ($P = 0.3$). A summary of the immunohistochemical findings in our set of BCCs investigated is presented in Table 1.

DISCUSSION

Calponin was first isolated from chicken gizzard smooth muscle cells as a 34 kDa actin- and calmodulin-binding protein with a proposed function in the regulation of smooth muscle contraction (11). At present, it is defined as a family of actin filament-associated proteins expressed in both smooth muscle and non-smooth muscle cells (12). Three isoforms have been identified in the vertebrates: a basic calponin (h1-calponin), a neutral calponin (h2-calponin), and an acidic calponin (h3-calponin) (12). These isoforms have distinct patterns of cell type-specific expression, indicating their functional diversities corresponding to the specific cellular microenvironment and/or the activity of different cell types (12). In routine biopsy practice, immunohistochemical detection of calponin principally serves to confirm smooth muscle and myoepithelial cell differentiation. Monoclonal anti-human calponin antibodies (clone CALP), which are most commonly used in standard histopathological examination, stain positively with differentiated visceral and vascular smooth muscle cells, myoepithelial cells in the lobules, ducts, and galactophorous tissue

of normal human breasts, as well as periacinar and periductal myoepithelial cells of the salivary glands. Thus, in addition to some mesenchymal neoplasms, calponin is expressed in benign or malignant human tumors of myoepithelial origin, for example in the salivary glands (13,14), in the breast (15), or in the skin (16).

The results of our present study showed that immunohistochemical expression of calponin in tumor tissue occurred in 72.9% of all BCC cases. Therefore we may conclude this is a common feature, although staining intensity as such is relatively weak. This finding is remarkable, because BCC of the skin does not exhibit smooth muscle or myoepithelial differentiation. Lee *et al.* (10), who first described this phenomenon, presumed that BCC cells which produce calponin might acquire the myoepithelium-like contractile feature and hence enhance their invasiveness. They studied 32 cutaneous BCCs consisting of 18 cases of nodular and 14 cases of infiltrative or mixed nodular-infiltrative subtypes. They found positive immunoreactivity of tumor cells for calponin in 18.7% of all cases investigated. However, while calponin expression was found in only one (5.5%) case of nodular BCC, in the infiltrative component, it was found in 5 (35.7%) of 14 infiltrative or mixed nodular-infiltrative subtypes. This difference was statistically significant. On the basis of these observations it may be hypothesized that aberrant (over) production of calponin in BCC cells is responsible for local invasiveness of the tumor and more aggressive biological behavior. In a later study, Uzquiano *et al.* (5) analyzed an identical number of cases consisting of 10 metastatic BCCs, 12 nodular BCCs, and 10 infiltrative BCCs. They used

the same cut-off level of staining positivity ($\geq 5\%$) as Lee *et al.* (10). In spite of that, they did not find a statistically significant difference in the expression of calponin among the distinct BCC subtypes. In their series, calponin immunoreactivity was found in 50% of nodular BCCs, 60% of infiltrative BCCs, and 30% of metastatic BCCs.

In our analysis of 37 primary cutaneous BCCs, we found that immunohistochemical expression of calponin in tumor tissue did not correlate with more aggressive tumor phenotypes. These findings are similar to the results reported by Uzquiano *et al.* (5) and discrepant to those of Lee *et al.* (10). On the other hand, we observed an association between infiltrative growth features of cancer and strong expression of calponin in peritumorous stromal cells. Since normal fibroblasts do not tend to produce calponin, such findings indicate their transformation into myofibroblasts, probably induced by molecular interactions between neoplastic cells and the surrounding stroma. There is now increasing evidence that biological behavior of cutaneous BCC is modulated by specific epithelial-mesenchymal interplay (17,18). Bearing in mind the stroma-dependency as a characteristic feature of this malignancy, it can be hypothesized that the growth pattern of BCC may be influenced by calponin production in the stromal myofibroblasts around the tumor nests, rather than in the neoplastic cells alone. Active myofibroblasts may facilitate tumor cells invasion into the connective tissue through increased motility, as well as through the release of metalloproteinases, which degrade the surrounding mesenchymal matrix. Until now, there have been no reports about calponin expression in the peritumorous stroma in cutaneous BCC, since both papers above (5,10) did not mention it. We thus had no opportunity to compare our results with other observations. Therefore, we believe that this area represents a promising subject for further studies.

CONCLUSION

Our study demonstrated that neoplastic cells in cutaneous BCC commonly produce calponin regardless of histological subtypes. We also found that expression of calponin in tumor tissue was not associated with aggressive tumor phenotypes. Therefore, immunohistochemical application of anti-calponin antibodies alone does not seem to be a useful prognostic marker for this malignancy. However, since some BCCs with infiltrative growth patterns strongly expressed calponin in peritumorous stroma, this finding may more reliably reflect the biological behavior of cancer and should be better explained in the

future. Further research is needed to elucidate the mechanism and role of calponin in BCC biology.

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