Myofibroblasts reaction in urothelial carcinomas

AURORA ALEXA, FLAVIA BADERCA, RODICA LIGHEZAN,
D. IZVERNARIU

Department of Histology,
"Victor Babes" University of Medicine and Pharmacy, Timisoara

Abstract
The myofibroblast is a connective tissue cell with intermediate features between the fibroblast and the smooth muscle cell and unknown origin, which normally is present in only a few organs, but with increased incidence in malignancies. The patterns of myofibroblastic reaction may be synchronous, metachronous and mixed. The presence of the myofibroblasts has been demonstrated into the stroma of breast carcinomas, particularly in firm, retracted tumors with no inflammatory infiltrate. The present literature lacks data regarding the presence and the behavior of the myofibroblasts in urothelial carcinomas. Fifty-nine urothelial carcinoma specimens from patients admitted into the Urology Clinic of the Emergency County Hospital of Timisoara between 1999 and 2004 were stained with usual HE stain for the morphological diagnosis and immunohistochemically stained with smooth muscle actin, vimentin, and desmin for the detection of myofibroblasts. In biopsies sampled from normal urinary bladder and in urothelial carcinomas of the superior urinary tract Ta, we have not noticed any cells with myofibroblast morphology or immunophenotype. In Ta tumors, no matter the differentiation grade, we have not noticed myofibroblasts neither between the tumor cells nor at distance. The myofibroblasts were identified in seven of the 26 (26.92%) tumors in T1 stage. In T2 and T3 stage tumors the number of myofibroblasts differs from case to case, being significantly higher in tumors with high differentiation grade, G3.

Keywords: myofibroblasts, urothelial carcinomas, immunohistochemistry, vimentin, actin.

Introduction
The myofibroblast is a connective tissue cell with a particular immunophenotype, characterized by simultaneous expression of vimentin, smooth muscle actin, desmin and myosin heavy chain. Their classification is made upon the expression patterns of the intermediate filaments and myosin: VA (express actin and vimentin), VAD (vimentin, actin, and desmin), VADM (express all the four markers). From this point of view, the myofibroblast has intermediate features between the fibroblast and the smooth muscle cell. In classical morphology, it is a cell with acidophyllic cytoplasm and intensely stained, indented nucleus. Sometimes, longitudinal filaments may be noticed in the peripheral areas of the cytoplasm. Its identification, and moreover, the quantification of the myofibroblast reaction are extremely difficult, if not impossible, in plain morphology.

Normally, the myofibroblast is present only in a few organs, but, in malignancies, its incidence grows significantly. However, the presence of the myofibroblasts has been extensively studied only in breast cancer, with three reaction patterns: synchronous, metachronous and mixed myofibroblastic reaction with the tumor proliferation. The intimate mechanisms of myofibroblastic hyperplasia are not well known. The origin of the myofibroblast is also subject for debate, with three hypotheses so far: the fibroblast, the smooth muscle cell and the pericyte. The significance of the myofibroblast reaction in malignant tumors is even less known. Both myofibroblasts and fibroblasts may originate from the bone marrow, especially during injuries [1, 2].

During tumor growth, the interaction between the tumor and the host is most often characterized by desmoplastic reaction. The role of this desmoplastic reaction is not clearly understood, although the modifications induced by the tumor in the host organ’s stroma may contribute to tumor invasion [3].

Normally, the interaction between normal epithelium and the stromal connective tissue helps maintaining the tissue integrity. In cancer, the interaction between cancer cells and the surrounding stroma is modified by several growth factors, such as TGFβ (transforming growth factor) and PDGF (platelet derived growth factor) and results in abnormal stroma formation, disruption of the tissue integrity followed by invasion and finally by metastases [4].

The circulant fibroblasts have the most important role in the tumor stroma development [5], and there are studies that show that, in adequate conditions, the fibroblasts may transform into myofibroblasts [6]. The role of the myofibroblasts in cancer is not fully known. There are a few studies that hypothesize that the myofibroblasts are a barrier of the tumor cells against the immune response, thus increasing their metastatic ability [7]. On the other hand, the patients with encapsulated hepatocellular carcinomas have a better survival than those with non-encapsulated tumors. It has been shown that some myofibroblast-like cells (positive for smooth muscle actin) are the source of the
collagen from the capsule of hepatocellular carcinomas [8]. Moreover, TGFβ1, which is produced by non-tumor cells at the tumor–stroma interface, may contribute to maintaining the myofibroblastic phenotype, thus forming the tumor capsule [8].

In rectal adenocarcinomas, the type of the stroma is associated with the prognosis: the tumors with myxoid or immature stroma have more myofibroblasts and worse prognosis [9].

The tumor myofibroblasts origin is still controversial. One source of myofibroblasts may be the epithelial cells, through epithelial–mesenchymal cells transition [10].

The presence of the myofibroblasts has been demonstrated into the stroma of breast carcinomas, particularly in firm, retracted tumors with no inflammatory infiltrate. The present literature lacks data regarding the presence and the behavior of the myofibroblasts in urothelial carcinomas.

Material and Methods

Fifty-nine urothelial carcinoma specimens from patients admitted into the Urology Clinic of the Emergency County Hospital of Timisoara between 1999 and 2004 were primary processed for the morphological diagnosis and immunohistochemistry.

Primary processing included fixation in 4% buffered formalin, paraffin embedding and sectioning at 3–5 microns thickness. The sections were dewaxed and rehydrated in successive baths of benzene and alcohol, and then stained with Hematoxylin and Eosin for the morphological diagnosis or pretreated for immunohistochemistry.

Immunohistochemical staining began by antigen retrieval in citrate buffer at pH 6, 20 minutes in the microwave oven, followed by endogenous peroxidase blocking with hydrogen peroxide, and then the staining: primary antibody (vimentin clone V9, smooth muscle actin clone 1A4 and desmin clone DO7) for 30 minutes, secondary biotinylated antibody for 10 minutes, then the streptavidin–peroxidase complex for 10 minutes, followed by activated chromogen (diaminobenzidine – DAB) and by nuclear counterstaining with modified Lillie’s Hematoxylin. After staining, the sections had been dehydrated and mounted with Canada balm.

Results

By examining the sections stained with the methods above, we have not noticed myofibroblasts into the lamina propria of the normal urethers resected from the patients with cancer. The same was true for the intraepithelial lesions (dysplasia and in situ carcinoma) (Figure 1).

On these sections, we have noticed that is mandatory to apply all of the three methods, in order to avoid the confusion between myofibroblasts and pericytes and smooth muscle cells from small blood vessel walls.

In biopsies sampled from normal urinary bladder and in urothelial carcinomas of the superior urinary tract Ta, we have not noticed any cells with myofibroblast morphology or immunophenotype.

In vimentin stained slides, only the fibroblasts from lamina propria were positive, and on those stained with anti smooth muscle actin the smooth muscle cells from the muscularis mucosae, blood vessels wall and muscularis propria were positive.

In Ta tumors, no matter the differentiation grade, we have not noticed myofibroblasts neither between the tumor cells nor at distance. The myofibroblasts were identified in seven of the 26 (26.92%) tumors in T1 stage. All of the myofibroblasts that we have noticed in these cases were of VA type, positive to vimentin and smooth muscle actin. The myofibroblasts were present at the tumor–stroma interface and between the tumor cells, characterizing a synchronous reaction (Figure 2).

In T2 and T3 stage tumors the number of myofibroblasts differs from case to case, being significantly higher in tumors with high differentiation grade, G3.

In these cases, two types of myofibroblastic reaction were met – synchronous, in which the myofibroblasts were noticed between the tumor cells, and metachronous, in which the myofibroblasts were at distance from the tumor cells.

Figure 1 – In situ carcinoma. Smooth muscle actin immunostaining. Smooth muscle cells from the blood vessels walls, muscularis mucosae (discontinue, arrow) and muscularis are positive (100×).

Figure 2 – Myofibroblasts moderate in number, arranged between the islands of tumor cells. Smooth muscle actin immunostaining (400×).
As for the number of myofibroblasts, out of the 21 cases of stage T2 urothelial carcinoma, 14 (66.66%) had presented a high density of myofibroblasts into the stroma, concentrated near the tumor proliferation front and between the tumor cells (Figure 3). In these cases, on usually stained slides, we have noticed the stromal hypercellularity and the presence of a high number of collagen fibers.

In these cases (stage T2 and T3 tumors), the VA type myofibroblasts (positive for smooth muscle actin and vimentin) had both synchronous (Figure 4), and metachronous reaction. VAD type myofibroblasts, with complete phenotype (positive to desmin, smooth muscle actin and vimentin), were present only at distance from the tumor cells, characterizing a metachronous reaction (Figure 5).

Out of the 10 patients with T3 urothelial carcinoma, the myofibroblasts were identified in six cases (60%). The myofibroblastic reaction was synchronous and metachronous, composed by VA and VAD, in approximately equal proportions. We have not noticed significant differences between T2 and T3 urothelial carcinomas. The myofibroblastic reaction was not correlated with the differentiation grade either. The percentages of the cases with myofibroblastic reaction, as well as the type of the myofibroblasts detected are presented in Table 1.

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Cases with Mfb</th>
<th>VA</th>
<th>VAD</th>
</tr>
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<tbody>
<tr>
<td>Ta (n=11)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T1 (n=26)</td>
<td>7 (26.92%)</td>
<td>S</td>
<td>0</td>
</tr>
<tr>
<td>T2 (n=21)</td>
<td>14 (66.66%)</td>
<td>S + M</td>
<td>M</td>
</tr>
<tr>
<td>T3 (n=10)</td>
<td>6 (60%)</td>
<td>S + M</td>
<td>S + M</td>
</tr>
</tbody>
</table>

Mfb – myofibroblasts; 0 – absent myofibroblasts; S – synchronous myofibroblastic reaction, M – metachronous myofibroblastic reaction.

The morphology of the myofibroblasts was similar for all three antibodies used: vimentin (Figure 6), smooth muscle actin (Figure 7) and desmin (Figure 8).
They were elongated or star shaped cells, with interconnected cytoplasmic processes, which create the sensation of a network. These distribution and arrangement were present in both synchronous and metachronous reactions. The aspect is less obvious in the synchronous myofibroblastic reaction, in which the myofibroblasts have the tendency of forming fascicles between tumor cells (Figure 9).

The metachronous reaction was characterized by the absence of intratumoral myofibroblasts and by massive proliferation at distance from the tumor proliferation front. A band of connective tissue devoid of myofibroblasts may be noticed between the tumor and the area with massive proliferation of those cells. This aspect was noticed mostly in the cases with locally invasive, poorly differentiated tumors without lymph node metastases.

Discussion

When it was discovered, in 1971, by Gabbiani G et al. [11], the myofibroblast was considered only as a modulation form of the fibroblast. Nowadays, this cell is individualized as having both features of fibroblast and smooth muscle cell. It is rarely noticed in normal human tissues, with the exception of the intestinal villi, and the capsule of the kidney and the spleen. The myofibroblasts are very high in number in healing wounds.

Its origin is controversial, with the fibroblast, the pericyte and smooth muscle cell hypothesized as precursors. No matter its origin, it has an indented nucleus, a high number of cytoplasmic processes which establish nexus type connections with the neighboring cells, thus forming a contractile network. Its cytoplasm is packed with actin filaments, and electron microscopy identifies actin filaments, dense bodies and rough endoplasmic reticulum. The specific and non specific organelles point towards its major functions: contraction and collagen synthesis. This organization pattern explains the involvement of the myofibroblast in wound healing, as well as its presence in desmoplastic stroma of the tumors.

The myofibroblastic reaction is well studied in some malignant tumors (breast cancer, for example), but there is no study published so far regarding their presence in the urinary tract tumors. The observations on other malignant tumor have shown the existence of two types of tumor-associated myofibroblasts reaction: synchronous, in which the myofibroblasts are located between the invasive malignant cells, and metachronous, in which the diffuse myofibroblastic proliferation is located at a certain distance of the tumor proliferation front. Some of the recent data mention the favorable prognosis of the cases with metachronous reaction, correlated with survival.

Our results point out the fact that only immunohistochemistry is suitable for the certain identification of myofibroblasts. We have not noticed myofibroblasts in plane urothelial lesions and Ta tumors, but they are present in invasive tumors, no matter of their differentiation grade. We have not found any reference in the literature regarding this issue, but our results suggest that the presence of the myofibroblasts may be
considered as an individual prognostic factor in the urothelial carcinomas of the upper urinary tract, because they point to the desmoplastic reaction in invasive tumors. Now, we could not discriminate between the prognostic value of the synchronous versus metachronous reaction, the latter having apparently a higher prognostic value, but without statistical significance ($p=0.23$).

Conclusions

The myofibroblastic reaction characterizes the invasive tumors in T2 and T3 stages, being noticed in 16 out of the 18 cases. The myofibroblasts are absent in Ta tumors and rare in T1 tumors. The myofibroblastic density is not correlated with the tumor differentiation grade. The myofibroblastic density is an individual prognostic factor, their presence is predictive for lymph node metastases, and is always associated with the desmoplastic reaction.

References


Corresponding author

Dragoș Izvernariu, Assistant Professor, MD, Department of Histology, “Victor Babes” University of Medicine and Pharmacy, 2 Eftimie Murgu Square, 300041 Timișoara, Romania; Phone +40723–382 389, e-mail: dragos_izve@yahoo.com

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