

The distribution pattern of myofibroblasts in the stroma of human bladder carcinoma depends on their invasiveness

N. Shimasaki¹, N. Kuroda², E. Miyazaki², Y. Hayashi², M. Toi², M. Hiroi², H. Enzan² and T. Shuin¹

¹Department of Urology, Program of Tumor Biology and Regulation and

²Department of Pathology, Program of Bioregulation and Genetics, Kochi Medical School, Kochi University, Kochi, Japan

Summary. The presence of myofibroblasts has been elucidated in the stroma of neoplasm of various organs. In the present article, we studied the distribution of myofibroblasts in the stroma of bladder carcinoma. Twenty-five surgical resected bladder tumors (urothelial carcinoma, n=21; combined urothelial carcinoma and adenocarcinoma, n=2; sarcomatoid squamous cell carcinoma, n=1; combined urothelial carcinoma and squamous cell carcinoma, n=1) were selected and we evaluated the distribution of myofibroblasts using immunohistochemical, electron and immunoelectron microscopic techniques. Immunohistochemically, the distribution pattern of myofibroblasts in invasive and non-invasive carcinomas were predominantly fascicular and reticular forms, respectively. Moreover, myofibroblasts around bladder carcinoma cells were confirmed by electron microscope. Understanding the distribution pattern of myofibroblasts in the stroma of bladder carcinoma may provide available information about the presence of carcinoma invasion.

Key words: Human bladder carcinoma, Myofibroblast

Introduction

The presence of myofibroblasts has been detected in the stroma of the neoplasm of several organs including oral cavity, pharynx, larynx, breast, pancreas, liver and colon (Ooi et al., 1997; Chauhan et al., 2003; Barth et al., 2004; Illemann et al., 2004; Kuroda et al., 2004). Generally, a large number of myofibroblasts has been observed in the stroma of invasive carcinomas of some organs (Lagace et al., 1985; Kuroda et al., 2004), but myofibroblasts also appear in the stroma of non-invasive

cancer (Barth et al., 2002; Chauhan et al., 2003). In the urological field, we recently identified the participation of myofibroblasts in the capsular formation of human renal parenchymal neoplasm (Shimasaki et al., 2005). Therefore, in the present study, we examined the distribution of myofibroblasts in bladder carcinoma and discussed its significance.

Materials and methods

Tissue specimens

Hematoxylin-eosin-stained preparations from all bladder tumors surgically resected (total cystectomy or trans-urethral bladder tumor resection) at Kochi Medical school and affiliated hospitals between 2000 and 2004 were histologically divided according to recent WHO classification (Eble et al., 2004). Among them, twenty-five bladder tumors were selected for this study. Histologically, these tumors were subdivided into urothelial carcinoma (n=21), combined urothelial carcinoma and adenocarcinoma (n=2), sarcomatoid squamous cell carcinoma (SCC)(n=1) and combined urothelial carcinoma and SCC (n=1). The sex ratio (male:female) of the patients with these tumors was 21:4. The mean age and age range of the patients were 72 and 48-88 years, respectively.

Routine histological procedures

Resected specimens were fixed with 10% formaldehyde and embedded with paraffin. Sections were cut with 3 µm and stained with hematoxylin and eosin.

Immunohistochemistry and its interpretation

Immunohistochemistry was performed using a streptavidin-biotin immunoperoxidase technique. Antibodies employed in the present study are

Offprint requests to: Dr N. Kuroda, Department of Pathology, Program of Bioregulation and Genetics, Kochi Medical School, Kochi University, Kohasu, Oko-cho, Nankoku City, Kochi 783-8505, Japan. e-mail: nkuroda@med.kochi-u.ac.jp

summarized in Table 1. We classified stromal cells positive for both alpha-smooth muscle actin (ASMA) and h-caldesmon (h-CD) as smooth muscle cells of lamina propria and muscular layer, and ASMA-positive and h-CD-negative cells as myofibroblasts. Thus, the distribution of myofibroblasts in bladder carcinoma was evaluated.

Electron microscopy

Three samples extracted from surgically resected bladder carcinomas were immediately fixed with 2.5% glutaraldehyde and post-fixed with 1% osmium tetroxide. After processing and embedding in epoxyresin, ultrathin sections stained with uranyl acerate were examined under an electron microscope (JEM 100S; JEOL Ltd, Tokyo, Japan).

Immunoelectron microscopy

Tissue samples obtained from three bladder carcinomas, as described above, were fixed by immersion in a periodate-lysine-paraformaldehyde solution for 24 hr. Frozen sections (20 µm) were cut

from the material after incubation in a mixed solution of phosphate-buffered saline and sucrose. The tissue sections from the samples were subsequently incubated with anti-ASMA and analyzed immunohistochemically using the procedures above with the addition of prefixation in 0.5% glutaraldehyde. The sections were processed and embedded in epoxy resin. Ultrathin sections stained with lead citrate were examined under an electron microscope.

Results

Histology

Bladder carcinomas were divided into invasive type (n=6) and non-invasive type (n=19) according to the invasion into lamina propria. Urothelial carcinoma (n=21) was subdivided into four tumors of invasive type and seventeen tumors of non-invasive type. Combined urothelial carcinoma and adenocarcinoma (n=2) included one tumor of invasive type and one tumor of non-invasive type. Sarcomatoid SCC (n=1) and combined urothelial carcinoma and SCC (n=1) showed invasive type.

Table 1. Antibodies employed in the present study.

ANTIGEN	CLONE	DILUTION	SOURCE	TREATMENT
ASMA	1A4	1:50	Dako Cytomation, Glostrup, Denmark	-
h-caldesmon (h-CD)	h-CD	1:50	Dako Cytomation, Glostrup, Denmark	microwave

ASMA, alpha-smooth muscle actin.

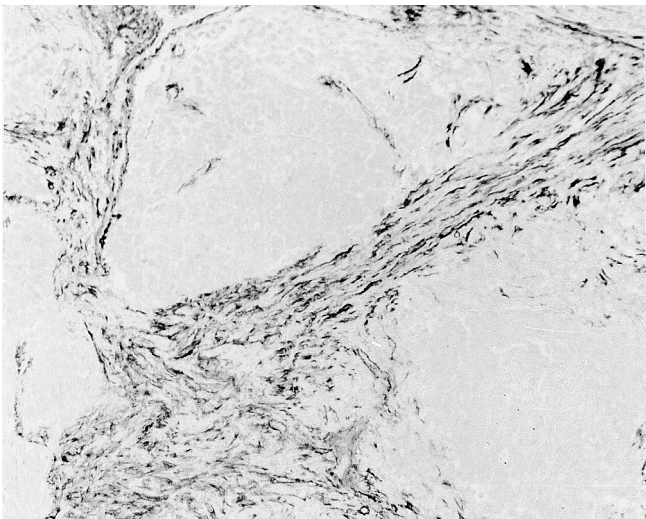


Fig. 1. Immunohistochemical finding of ASMA in invasive bladder carcinoma. The fascicular distribution pattern of ASMA-positive myofibroblasts is seen. x 50

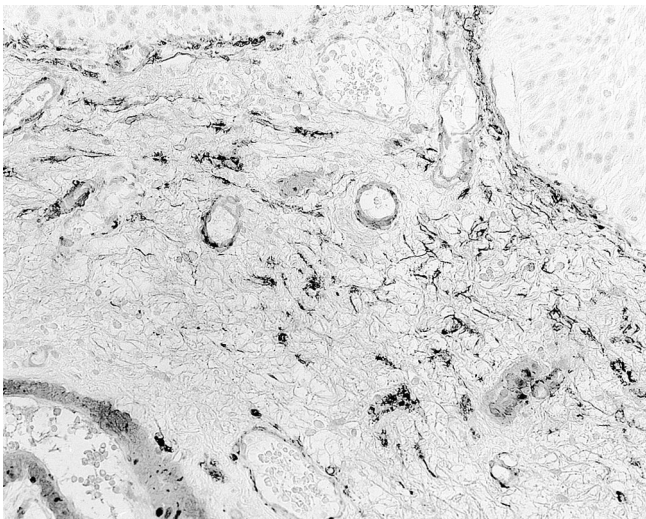


Fig. 2. Immunohistochemical finding of ASMA in non-invasive bladder carcinoma. The reticular distribution pattern of ASMA-positive myofibroblasts is observed. x 50

Immunohistochemical findings

Immunohistochemical results are summarized in Table 2. Irrespective of the invasiveness, the stroma of all bladder carcinomas contained myofibroblasts. The distribution of myofibroblasts of all six tumors of invasion type showed fascicular pattern (Fig. 1). In the distribution of myofibroblasts among nineteen tumors of non-invasion type bladder carcinoma, one tumor showed the fascicular pattern and eighteen tumors showed the reticular pattern (Fig. 2). Statistically, there was a significant relationship between the reticular distribution pattern of myofibroblasts and non-invasive bladder carcinoma, and between the fascicular distribution pattern of myofibroblasts and invasive bladder carcinoma ($p < 0.01$).

Ultrastructural findings

Stromal cells around the cluster of bladder carcinoma cells possessed a well-developed Golgi apparatus and rough endoplasmic reticulum in the cytoplasm, and many myofilaments and dense bodies were also observed (Fig. 3). These findings showed the myofibroblastic nature in stromal cells (Schurch et al., 1998).

Immunoelectron microscopic findings

Filamentous structures of stromal cells around bladder carcinoma cells showed a positive reaction for ASMA (Fig. 4).

Discussion

In the invasion into lamina propria of bladder carcinoma, the stromal responses, including retraction artifact, inflammatory cells, hypocellular or loose stroma and pseudosarcomatous stroma may be observed. Although the stroma response may be sometimes absent, desmoplastic or sclerotic stroma may be also frequently observed. Regarding the component of stromal cells in bladder carcinoma, spindle cells have been regarded as fibroblasts for a long time. To the best of our knowledge, there have been no descriptions on the presence of myofibroblasts in the stroma of human bladder carcinoma. The differentiation from fibroblasts into myofibroblasts is basically modulated by cancer-derived cytokines, such as TGF- β . In the present study, we elucidated for the first time that myofibroblasts appeared in the stromal response of human bladder carcinoma. Therefore, we suggest that urothelial carcinoma cells

Table 2. Summary of the relationship between the distribution pattern of myofibroblasts and invasiveness into lamina propria of bladder carcinoma.

INFILTRATING TYPE (NUMBER)	DISTRIBUTION PATTERN	
	fascicular	reticular
invasion type (n=6)	6*	0*
non-invasion type (n=19)	1*	18*
		*P < 0.01

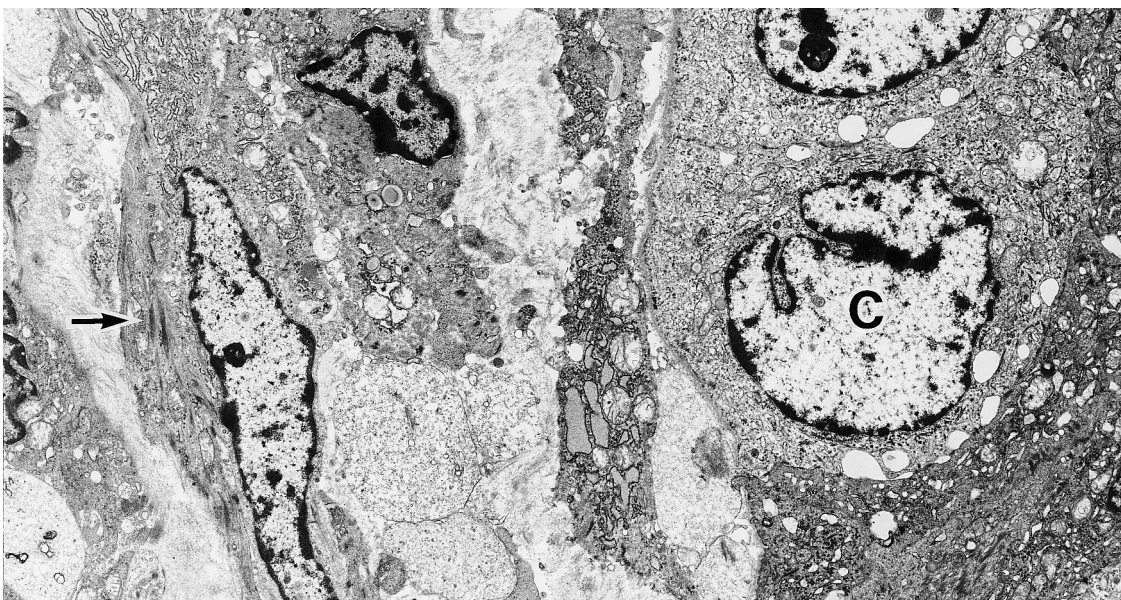


Fig. 3. Ultrastructural finding of stromal cells in invasive bladder carcinoma. Stromal cells have many myofilaments and dense bodies (arrows) in the cytoplasm. C: cancer cells. x 2,000



Fig. 4. Immunoelectron microscopic finding of ASMA of stromal cells in invasive bladder carcinoma. Cytoplasmic filamentous structures are reactive for ASMA. x 2,000

may produce cytokines such as TGF- β , which makes fibroblasts differentiate into myofibroblasts in the lamina propria (De Wever and Mareel, 2003). There are some reports that numerous myofibroblasts appear in the stroma of invasive cancer in various organs (Lagace et al., 1985; Kuroda et al., 2004, 2005). In contrast, myofibroblasts also appear in the stroma of non-invasive carcinoma (Barth et al., 2002; Chaunhan et al., 2003). In non-invasive breast carcinoma, the number of myofibroblasts in the stroma depends on the nuclear grade of cancer cells (Chauhan et al., 2003). We found that myofibroblasts appeared in human bladder carcinoma, irrespective of the invasion beyond the mucosa. However, it is difficult to evaluate the relationship between the number of myofibroblasts and nuclear grade because of the low number of bladder carcinoma cases in the present study. The reticular and fascicular distribution patterns of myofibroblasts were predominantly observed in the stroma of non-invasive and invasive bladder carcinomas, respectively. These findings suggest that the invasion beyond the mucosa may evoke the change of distribution of myofibroblasts. We suggest that the change of the distribution of myofibroblasts associated with the carcinoma invasion may exhibit the change of the number of myofibroblasts or the compression of stromal cells by carcinoma cells. Thus, the evaluation of the distribution pattern of

myofibroblasts may be useful for the estimation of the invasion of bladder carcinoma, even in small biopsy specimens.

On the other hand, there are two ASMA-positive cells in the lamina propria of urinary bladder cancer. One is muscularis mucosa and the other is myofibroblast. For the discrimination of these cells, the immunohistochemistry of h-CD is very important. h-CD is a marker of well-differentiated smooth muscle cells and no myofibroblasts generally express h-CD. Pathologists should keep in mind that ASMA-positive cells in the lamina propria may be myofibroblasts but not muscularis mucosa.

In conclusion, myofibroblasts appear in the stroma of human bladder carcinoma, irrespective of the invasion, but the distribution pattern of myofibroblasts is different between non-invasive and invasive bladder carcinomas. Namely, the reticular pattern is predominantly observed in the non-invasive carcinoma and the fascicular pattern is chiefly seen in the invasive carcinoma. Understanding the distribution pattern of myofibroblasts may be helpful in evaluating the invasion of bladder carcinoma.

Acknowledgements. We are grateful to Mr. Tadatashi Tokaji, Ms. Hisayo Yamasaki and Ms. Kanako Yamaoka, Department of Pathology, Kochi Medical School, for their excellent technical assistance.

References

- Barth P.J., Ramaswamy A. and Moll R. (2002). CD34+ fibrocytes in normal cervical stroma, cervical intraepithelial neoplasia III, and invasive squamous cell carcinoma of the cervix uteri. *Virchows Arch.* 441, 564-568.
- Barth P.J., Züschröder T.S., Ramaswamy A. and Moll R. (2004). CD34+ fibrocytes, α -smooth muscle antigen-positive myofibroblasts, and CD117 expression in the stroma of invasive squamous cell carcinomas of the oral cavity, pharynx, and larynx. *Virchows Arch.* 444, 231-234.
- Chauhan H., Abraham A., Phillips R.A., Pringle J.H., Walker R.A. and Jones J.L. (2003). There is more than one kind of myofibroblasts: analysis of CD34 expression in benign, in situ, and invasive breast lesions. *J. Clin. Pathol.* 56, 271-276.
- De Wever O. and Mareel M. (2003). Role of tissue stroma in cancer cell invasion. *J. Pathol.* 200, 429-447.
- Eble J.N., Sauter G., Epstein J. and Sesterhenn I. (2004). *World Health Organization Classification of Tumors. Pathology and Genetic Tumors of the Urinary System and Male Genital Organs.* IARC Press, Lyon.
- Illemann M., Hansen U., Nielsen H.J., Anderson P.A., Hoyer-Hansen G., Lund L.R., Dano K. and Nielsen B.S. (2004). Leading-edge myofibroblasts in human colon cancer express plasminogen activator inhibitor-1. *Am. J. Clin. Pathol.* 122, 256-265.
- Kuroda N., Toi M., Nakayama H., Miyazaki E., Yamamoto M., Hayashi Y., Hi M. and Enzan H. (2004). The distribution and role of myofibroblasts and stromal cells in normal pancreas and various pancreatic lesions. *Histol. Histopathol.* 19, 59-67.
- Kuroda N., Nakayama H., Miyazaki E., Toi M., Hiroi M. and Enzan H. (2005). The distribution of CD34-positive stromal cells and myofibroblasts in colorectal carcinoid tumor. *Histol. Histopathol.* 20, 27-33.
- Lagace R., Grimaud J.A., Schurch W. and Seemayer T.A. (1985). Myofibroblastic stromal reaction in carcinoma of the breast: variations of collagenous matrix and structural glycoprotein. *Virchows Arch. Pathol. Anat.* 408, 49-59.
- Ooi L.P.J., Crawford D.H.G., Gotley D.C., Clouston A.D., Strong R.W., Gobe G.C., Halliday J.W., Bridle K.R. and Ramm G.A. (1997). Evidence that "myofibroblast-like" cells are the cellular source of capsular collagen in hepatocellular carcinoma. *J. Hepatol.* 26, 798-807.
- Schurch W., Seemayer T.A. and Gabbiani G. (1998). The myofibroblast. A quarter century after its discovery. *Am. J. Surg. Pathol.* 22, 141-147.
- Shimasaki N., Kuroda N., Guo L., Jin Y., Miyazaki E., Hayashi Y., Toi M., Hiroi M., Enzan H. and Shuin T. (2005). The participation of myofibroblasts in the capsular formation of human conventional and chromophobe renal cell carcinomas. *Histol. Histopathol.* 20, 67-73.

Accepted September 7, 2005