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The distribution of myofibroblasts and CD34-positive stromal cells in normal renal pelvis and ureter and their cancers

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Summary. In this article, we examined the distribution of myofibroblasts and CD34-positive stromal cells in normal renal pelvis and ureter and their cancers using immunohistochemistry. Eighteen tumors and normal tissues apart from the main tumor were examined. In the wall of normal renal pelvis and ureter, no myofibroblasts were observed through all layers, but CD34-positive stromal cells were observed in the deep area of lamina propria, muscular layer and adventitia. In the stroma of renal pelvic and ureteral cancers, myofibroblasts were distributed in fifteen tumors and were absent in three tumors. All three tumors containing no myofibroblasts in the stroma were non-invasive type and all invasive cancers contained myofibroblasts in the stroma. CD34-positive stromal cells were consistently absent in the stroma of cancers, irrespective of the invasiveness. Finally, myofibroblasts are major stromal components in renal pelvic and ureteral cancers, particularly in invasive cancers, and CD34-positive stromal cells are consistently absent or lost in the stroma of their cancers. These findings suggest that the invasion of renal pelvic and ureteral cancers may cause the phenotypic change of stromal cells.

Key words: Renal pelvis, Ureter, CD34-positive stromal cell, Myofibroblast

Introduction

Myofibroblasts are distributed in various normal organs and pathological conditions (Powell et al., 1999). CD34-positive stromal cells, known also as vascular adventitial fibroblastic cells or dendritic interstitial cells, have been reported not only in various normal organs, but also in some pathological conditions (Yamazaki and Eyden, 1995, 1996a,b, 1997; Nakayama et al., 1999, 2000, 2003, 2004; Papadas et al., 2001; Soma et al, 2001; Barth et al., 2002a-c; Kuroda et al., 2004a-c). However, to the best of our knowledge, there are no reports on the distribution of myofibroblasts and CD34-positive stromal cells in normal renal pelvis and ureter and their cancers. In an attempt to understand the stromal reaction in malignant lesions of renal pelvis and ureter, we investigated the distribution of myofibroblasts and CD34-positive stromal cells using an immunohistochemical method and discuss their role.

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Materials and methods

Tissue specimens

Eighteen specimens from fifteen patients with renal pelvic and ureteral cancers, surgically resected between 1998 and 2004, were chosen from the pathology files of the Department of Pathology, Kochi Medical School, Kochi University and an affiliated hospital. Normal specimens, apart from the neoplasm of each case were used for comparison. The sex ratio (male:female) of the patients with these tumors was 7:8. The mean age and age range of the patients were 72.6 years and 58 to 84 years, respectively. Surgically resected specimens were fixed in 10% neutral formalin and embedded in paraffin. Tissue sections were cut to a thickness of 3 μm and stained with hematoxylin-eosin. With each neoplasm, items including the tumor size, projection pattern, the presence of invasion, histological type, nuclear grade and the depth of invasion were evaluated. The histological type of urothelial neoplasms was performed on the basis of recent classification of bladder cancer (Epstein et al., 1998; Murphy et al., 2004).

Immunohistochemistry and its interpretation

Immunohistochemistry was performed using a

streptavidin-biotin immunoperoxidase technique. Antibodies employed in the present study are summarized in Table 1. Vascular smooth muscle cells and endothelial cells were used as internal positive controls for alpha-smooth muscle actin (ASMA) and high molecular weight caldesmon (h-CD), and CD34 and CD31 immunostains, respectively.

High molecular weight caldesmon (h-CD) is a well-developed smooth muscle actin-specific antibody and is not generally expressed in myofibroblasts (Ueki et al, 1987; Ceballos et al., 2000; Watanabe et al., 2000). Therefore, we classified stromal cells positive for both ASMA and h-CD as smooth muscle cells, and ASMA-positive and h-CD-negative cells as myofibroblasts. Furthermore, we interpreted stromal cells positive for both CD34 and CD31 as vascular endothelial cells and CD34-poisitve and CD31-negative stromal cells as CD34-positive stromal cells (Kuroda et al., 2004a-c). The distribution density of myofibroblasts and CD34-positive stromal cells was semi-quantatively evaluated as follows: -, negative, +, a small number, positive (less than 10 positive cells /high power field (HPF) on average of 5 HPFs); ++, a moderate to large number, positive (more than 11 positive cells /HPF on average of 5 HPFs). To exclude the influence of the secondary inflammation in the stroma, the site lacking the significant inflammation was selected for the evaluation of immunohistochemistry.

Double immunostaining

Double immunostaining of all specimens was performed for CD34 and ASMA in order to elucidate the relationship of CD34-positive stromal cells and myofibroblasts. Sections were treated with 0.3% hydrogen peroxide/methanol for 10 min at room temperature (RT) and incubated overnight with anti-CD34 antibody. The sections were then incubated with peroxides-conjugated mouse gig and rabbit gig (Simple stain PO-MAX (multi), Nichirei, Tokyo, Japan) for 1 h at RT and immersed in 0.2% DAB and 0.1% hydrogen peroxide in 0.05M Tris buffer. After washing with PBS, the sections were incubated for 2 h at RT with biotinylated rabbit and anti-mouse IgG F(ab')2 fragment (Dako Cytomation, Glostrup, Denmark), and alkaline phosphatase-conjugated streptavidin (Nichirei, Tokyo, Japan) for 30 min at RT. To visualize this reaction, the sections were stained with Fast blue.

Results

Distribution of myofibroblasts and CD34-positive stromal cells in normal renal pelvis and ureter

No myofibroblasts were observed in any layers of normal renal pelvis and ureter (Fig. 1a). On the other hand, CD34-positive stromal cells were observed in the deep area of lamina propria, the muscular layer and the adventitia (Fig. 1b). Particularly, the adventitia contained many CD34-positive stromal cells.

The relationship between various pathological parameters and the distribution density of myofibroblasts and CD34-positive stromal cells in renal pelvic and ureteral cancers

These results are summarized in Table 1. CD34-positive stromal cells were consistently absent in the stroma of cancers (Fig. 2a), irrespective of the tumor size, projection, the presence of invasion, histological type, nuclear grade, depth of invasion and presence of myofibroblasts. In contrast, myofibroblasts were

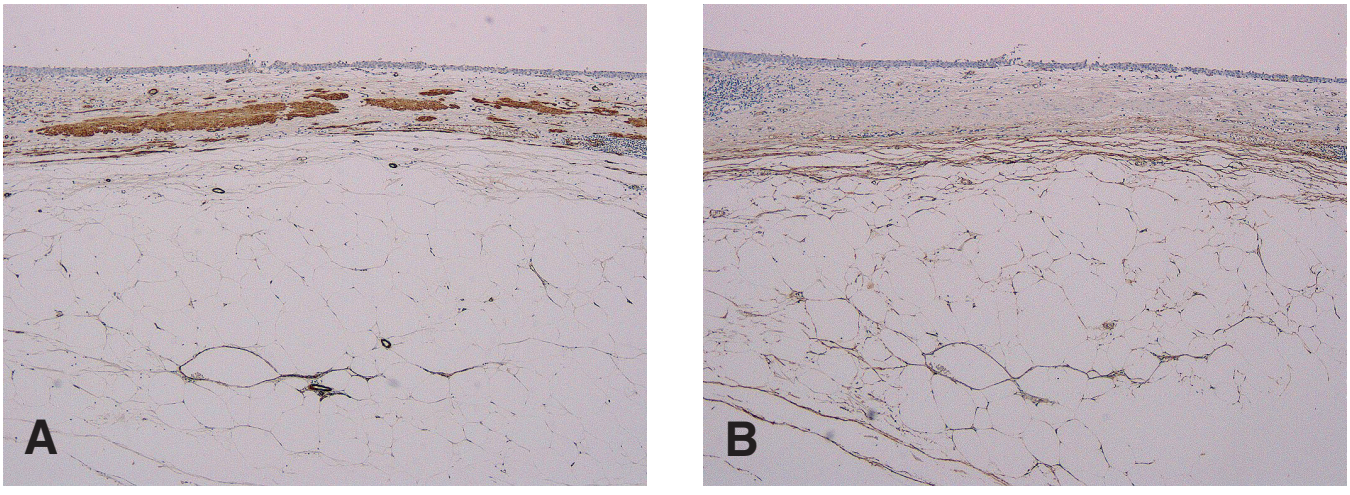

Fig. 1. The distribution of myofibroblasts and CD34-positive stromal cells in normal renal pelvis and ureter. **A.** Immunostaining of alpha-smooth muscle actin (ASMA). Myofibroblasts are completely absent in all layers. **B.** Immunostaining of CD34. CD34-positive stromal cells are present in the deep area of lamina propria, the muscular layer and adventitia. x 10

Table 1. Antiboies employed in the present study.

ANTIGEN	CLONE	DILUTION	SOURCE	ANTIGEN RETRIEVAL
ASMA	1A4	01:50	Dako Cytomation, Glostrup, Denmark	-
h-caldesmon	h-CD	01:50	Dako Cytomation, Glostrup, Denmark	microwave
CD34	MY-10	01:20	Beckton Dickinson, CA, USA	pronase
CD31	JC/70A	01:20	Dako Cytomation, Glostrup, Denmark	pronase
E-cadherin	6F9	01:10	PROGEN. Heidelberg, Germany	pronase
TGF-ß1	9016.2	01:50	R&D systems. Inc., MN, USA	pronase
TGF-ß receptor	polyclonal	01:50	Upstate biotechnology, NY, USA	microwave

Table 2. Distribution of stromal cells in renal pelvic and ureteral cancers.

PATIENT NO.	TUMOR NO.	SIZE	HISTOLOGY	TUMOR DEPTH	MYOFIBROBLAST	CD34(+)- CELL
1	1	0.2cm	CIS	m	-	-
	2	1.0cm	CIS	m	-	-
2	3	0.4cm	CIS	m	+	-
3	4	4.0cm	papillary ca, low grade	m	+	-
4	5	0.5cm	papillary ca, low grade	m	+	-
	6	0.4cm	papillary ca, low grade	m	-	-
	7	1.0cm	papillary ca, low grade	lp	++	-
5	8	1.7cm	invasive SCC>UC	s	++	-
6	9	2.5cm	invasive ca	ad	++	-
7	10	2.5cm	invasive ca	lp	+	-
8	11	4.2cm	papillary ca, high grade	ad	++	-
9	12	2.7cm	papillary ca, low grade	lp	+	-
10	13	7.0cm	papillary ca, low grade	lp	++	-
11	14	2.8cm	papillary ca, low grade	lp	+	-
12	15	1.6cm	papillary ca, low grade	lp	++	-
13	16	3.0cm	papillary ca, low grade	lp	++	-
14	17	4.0cm	papillary ca, low grade	lp	++	-
15	18	3.0cm	papillary ca, high grade	ml	++	-

CIS, carcinoma in situ; m, mucosa; lp, lamina propria; ml, muscle layer; ad, adventitia, s, surrounding tissue; -, negative, +, positive, a small number, ++, positive, a moderate to large number.

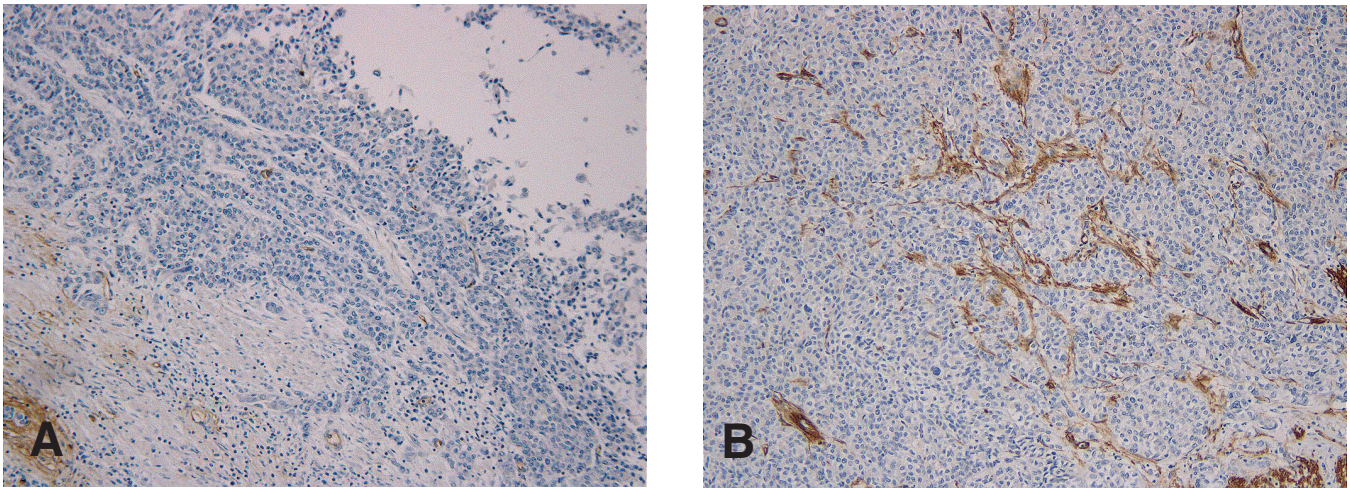

Fig. 2. The distribution of myofibroblasts and CD34-positive stromal cells in renal pelvic and ureteral cancers. **A.** Immunostaining of CD34. CD34-positive stromal cells were consistently absent in the stroma of cancers. **B.** Immunostaining of ASMA. Myofibroblasts are observed in the stroma of invasive cancer. x 25

distributed in fifteen tumors (Fig. 2b) and were absent in three tumors. All three tumors containing no myofibroblasts in the stroma did not show the invasion into the lamina propria. There seemed to be no significant relationship between the distribution density of myofibroblasts and other pathological parameters.

Other immunohistochemical findings

Normal renal pelvic mucosa showed a positive reaction for E-cadherin, but was negative for TGF-ß1 and TGF-ß receptor. In renal pelvic cancer, cancer cells showed the reduced expression for E-cadherin, compared with normal mucosa. In renal pelvic cancer, neoplastic cells were focally positive for TGF-ß1, and inflammatory cells and myofibroblasts were positive for TGF-ß receptor.

Double immunostaining

No stromal cells expressing both CD34 and ASMA were seen in the stroma of invasive carcinomas.

Discussion

Regarding the stromal cells in renal pelvis and ureter, there are no descriptions on myofibroblasts and CD34-positive stromal cells. Therefore, this is the first report on myofibroblasts and CD34-positive stromal cells in renal pelvis and ureter.

In normal renal pelvis and ureter, no myofibroblasts were observed anywhere. In contrast, myofibroblasts consistently appeared in the stroma of invasive carcinomas. Additionally, myofibroblasts existed in the stroma of half of the cases of examined non-invasive carcinoma. Therefore, myofibroblasts seem to appear with the progression of carcinoma and be a major stromal component in invasive renal pelvic and ureteral cancers. The increase of myofibroblasts is associated with up-regulation of TGF-ß1 and down-regulation of E-cadherin. These findings in renal pelvic and ureteral cancers resemble to some extent those of laryngeal or hypopharyngeal cancer (Kojc et al., 2005). Further examinations will be required to elucidate the significance of the appearance of myofibroblasts in non-invasive renal pelvic and ureteral cancers.

In normal renal pelvis and ureter, CD34-positive stromal cells were chiefly distributed in the deep area of lamina propria, muscular layer and adventitia. It is well known that CD34-positive stromal cells are actually fibroblasts (fibrocytes) (Barth et al., 2002a-c). In contrast, CD34-positive stromal cells were consistently absent in the stroma of carcinomas. Chauhan et al. reported that the loss of CD34 in the stroma of ductal carcinoma in situ of the breast was significantly more frequent in high grade tumors than in low or intermediate grade ones (Chauhan et al., 2003). Barth et al. reported that normal cervical stroma and the stroma adjacent to cervical intraepithelial neoplasia (CIN) III showed a dense network of cytoplasmic process of CD34-positive cells and early stroma invasion by squamous carcinoma showed focal loss of network of cytoplasmic process of CD34-positive cells (Barth et al., 2002c). On the basis of our results, CD34-positive stromal cells seem to be absent or disappear, irrespective of the invasiveness of carcinoma, nuclear grade or other pathological parameters. However, further examination of cases with non-invasive carcinomas will be needed for the evident conclusion. Totally, our results seem to be similar to those by Barth et al. Namely, there seems to be a reverse correlation on the distribution of stromal cells in normal renal pelvis and ureter and their cancers. Therefore, fibroblasts or CD34-positive stromal cells may be activated with the cancer progression and transformed into myofibroblasts, as previously suggested (Tuxhorn et al., 2001; Barth et al., 2002c). However, we found no stromal cells expressing both CD34 and ASMA in the present study. This finding may imply that the phenotypic switching occurs rapidly. Through this phenotypic switching, myofibroblasts may play an important role in the stromal response of the host for invasive renal pelvic and ureteral carcinoma. Tumor cells may also recruit circulating CD34-positive cells derived from myeloid precursors and convert them into myofibroblasts (Ruiter et al., 2002; Kojc et al., 2005). Therefore, it is possible that CD34-positive stromal cells in renal pelvis and ureter may be also derived from myeloid precursors. Further examination will be required to elucidate the role of myofibroblasts and CD34-positive stromal cells in renal pelvis and ureter.

In conclusion, myofibroblasts appear in the stroma with the progression of renal pelvic and ureteral cancers and CD34-positive stromal cells are consistently absent in the stroma of invasive carcinoma.

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