의학 박사학위 논문

Impact of Caveolin-1 Expression and Microvessel Density on the Prognosis of Upper Urinary Tract Transitional Cell Carcinoma

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조 대 성

Impact of Caveolin-1 Expression and Microvessel Density on the Prognosis of Upper Urinary Tract Transitional Cell Carcinoma

by

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- ABSTRACT -

Impact of Caveolin-1 Expression and Microvessel Density on the Prognosis of Upper Urinary Tract Transitional Cell Carcinoma

Purpose: Caveolin-1 is a principal component of caveolae membranes and plays a regulatory role in several signal pathways. It may also be crucial in the organization of caveolae of endothelial cells and in regulating endothelial cell differentiation and angiogenesis. Caveolin-1 has been reported to be dysregulated in various human cancers, but the pattern of dysregulation and presumably the role of caveolin-1 appear to vary with tumor type. Caveolin-1 has not previously been studied in upper urinary tract transitional cell carcinoma (UUT-TCC). In this study, I investigated the relationship of caveolin-1 expression and microvessel density (MVD), a reflection of angiogenesis, with prognosis in patients with UUT-TCC.

Materials and Methods: Formalin-fixed, paraffin-embedded tissue sections of UUT-TCC from 98 patients who had undergone radical nephroureterectomy were stained immunohistochemically with specific antibodies against caveolin-1 and CD34. Caveolin-1 immunostaining was semi-quantitatively evaluated based on proportion (percentage of positive cells) and intensity, and then immunoreactive scores for each case were generated by multiplying the values for the two parameters. A score of 0 was considered as negative, while all other scores were considered as positive. The expression patterns of caveolin-1 and MVD were compared with the various clinicopathological

variables, including T stage, N stage, nuclear grade, tumor location, previous history of bladder cancer, coexisting bladder cancer at diagnosis, bladder cancer recurrence during follow-up, and cancer-specific survival.

Results: Caveolin-1 expression correlated significantly with T stage ($p<0.001$) and grade ($p=0.036$). MVD correlated significantly with T stage ($p<0.001$), N stage ($p=0.002$), and grade ($p<0.001$). Caveolin-1 expression was significantly associated with MVD ($p=0.015$). The survival rate of patients with positive caveolin-1 expression or high MVD was significantly lower than that of patients with negative caveolin-1 expression or low MVD, respectively ($p<0.0001$, $p=0.0283$). Univariate analyses indicated that T stage, grade, caveolin-1 expression and MVD were significant prognostic factors for cancer-specific survival. Multivariate analyses indicated that T stage and caveolin-1 expression were independent prognostic factors for cancer-specific survival.

Conclusions: Increased expressions of caveolin-1 and MVD are associated with poor prognosis in UUT-TCC. Furthermore, caveolin-1 expression was associated with MVD. These results suggest that caveolin-1 may play an important role in the progression of UUT-TCC by regulating tumor angiogenesis.

Key Words: Carcinoma, Transitonal cell, Urinary tract, Caveolin-1, Angiogenesis, Prognosis

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I. INTRODUCTION

Upper urinary tract transitional cell caricinoma (UUT-TCC) is an uncommon disease, accounting for 4.5-9% of all renal tumors and 5-6% of all urothelial tumors (Iborra et al., 2003; Ataus et al., 2006). Although the majority of bladder TCCs are superficial, UUT-TCCs are invasive in over 50% of cases (Kang et al., 2003). van der Poel et al. reported that thinner muscular and submucosal layers and absence of serosa in the upper urinary tract could explain the higher invasiveness of UUT-TCC compared to the bladder cancer (van der Poel et al., 2005). Non-invasive pTa and pT1 UUT-TCCs after surgery show survival rates comparable to those for similar staged bladder cancers. However, invasive UUT-TCCs are associated with worse prognosis compared to invasive bladder cancers with 5-year survival rate ranging from 30 to 60% (Kang et al., 2003; Tawfiek and Bagley, 1997; Cheng et al., 2000; Stein et al., 2001). Although patient characteristics for this disease and the influence of tumor stage and grade on the prognosis were well defined in previous studies, few series has systematically analyzed patterns of relapse and potential prognostic factors. Thus, other factors accurately predicting the biological potential of this malignancy are required to select candidates for adjuvant therapy who are at high risk of tumor progression.

Caveolae are flask-shaped invaginations of the plasma membrane that are described as structures resembling 'little caves' due to their appearance as 50- to 100-nm vesicular invagination of the plasma membrane by transmission electron microscopy (Okamoto et al., 1998; Praton, 1996). The functions of caveolae include signal transduction, vesicular trafficking, lipid homeostasis, and angiogenesis (Williams and Lisanti, 2004; Sonveaux et al.,

2004). Caveolins are chief structural proteins that are both necessary and sufficient for the formation of caveolae membrane domain. Molecular cloning has identified three distinct caveolin genes, caveolin-1, caveolin-2, and caveolin-3 (Glenney and Soppet, 1992; Scherer et al., 1995; Scherer et al., 1996; Tang Z et al., 1996). Caveolin-1 and caveolin-2 are co-expressed in most cell types, while the expression of caveolin-3 is muscle-specific (Okamoto et al., 1998; Song et al., 1996). Thus, endothelial cells and fibroblasts have caveolin-1 and caveolin-2, while cardiac myocytes and skeletal muscle fibers express caveolin-3. In contrast, smooth muscle cells express all three caveolins.

Recent studies using caveolin-deficient mouse models dramatically showed that caveolae and caveolins play an important role in various human pathological conditions including cancer, diabetes, bladder dysfunction, muscular dystrophy and especially those related to the cardiovascular system such as atherosclerosis, cardiac hypertrophy, cardiomyopathy, pulmonary hypertension, and smooth muscle cell proliferation (Williams and Lisanti, 2004). In regard to cancer, controversies exist on the role of caveolin-1. Caveolin-1 is suppressed in some cancer and functions as tumor suppressor gene, whereas it is upregulated in another cancers and associated with cancer progression and metastasis (Edelson et al., 1997; Hino et al., 2003; Campbell et al., 2003; Yang et al., 1999; Kato et al., 2002). However, there is no previous study about caveolin-1 expression in UUT-TCC. Thus, investigating caveolin-1 expression in UUT-TCC and its potential role in tumor progression should be of important value.

Tumor angiogenesis is indispensable for tumor growth, progression, and metastasis by providing nutrients and oxygen for metabolism and removal of resultant waste products. In addition, tumor-induced blood vessels are fragile

and highly permeable, with a discontinuous basement membrane. Therefore, tumor metastases are easier than normal blood vessels by tumor invasion. Microvessel density (MVD), a histologic measure of tumor angiogenesis, is associated with metastasis and clinical outcome in several types of cancer (Sharma et al., 2005; Blood and Zetter, 1990). Moreover, caveolin-1 was reported to directly influence the angiogenic process for tumor progression and metastasis (Sonveaux et al., 2004). However, studies about microvessel density in UUT-TCC are limited (Nakanishi et al., 1997; Inoue et al., 2002; Zhang et al., 2001).

The purpose of this study is to investigate the relationship of caveolin-1 expression and MVD with prognosis in patients with UUT-TCC.

II. MATERIALS AND METHODS

A. Patients and specimens

Formalin-fixed, paraffin-embedded, archival surgical specimens from 98 patients (76 men and 22 women, mean age 61.7 years, range 33-85 years) diagnosed with UUT-TCC were assessed. All patients had undergone radical nephroureterectomy at Ajou University Hospital and Yonsei Medical Center between November 1994 and February 2004. Tumors were staged using the TNM classification of American Joint Committee on Cancer (AJCC) (Greene et al., 2002), and graded according to the World Health Organization (WHO)/International Society of Urological Pathology (ISUP) grading criteria (Epstein et al., 1998).

B. Immunohistochemistry

Paraffin-embedded blocks were sectioned at a 4㎛, deparaffinized and rehydrated. After microwave pretreatment in citrate buffer (pH 6.0) for antigen retrieval, slides were immersed in 3% hydrogen peroxide for 15 min to block the endogenous peroxidase activity. The sections were blocked with blocking reagent in Cap-Plus™ Detection Kit (ZYMED, San Francisco, CA, USA) for 10 min. Sections were then incubated with mouse monoclonal antibody to caveolin-1 (clone 2297, diluted 1:200; BD Biosciences, San Diego, CA, USA) or CD34 (diluted 1:350; Neomarkers, San Francisco, CA, USA) for 1 hour at 37℃ in a humidified chamber. After a second incubation with a biotinylated secondary antibody, slides were incubated with peroxidase-conjugated streptavidin (Cap-Plus™ Detection Kit). Reaction products were visualized by immersing slides in diaminobenzidine tetrachloride and finally counterstained with Mayer's hematoxylin. Positive staining of

smooth muscle cells or endothelium, known to be abundant in caveolin-1, provided an internal positive control for caveolin-1 immunostaining. Negative controls were obtained by omitting the primary antibody.

C. Evaluation of immunohistochemistry

1. Caveolin-1

Caveolin-1 expression was based on the presence of cytoplasmic and/or membranous staining. Caveolin-1 immunostaining was semi-quantitatively evaluated based on the proportion (percentage of positive cells) and intensity, according to the methods described by Sinicrope et al. with minor modifications (Sinicrope et al., 1995). The proportion of caveolin-1 positive cells was divided into five score categories: (0) ≤ 10%; (1) 11-25%; (2) 26-50%; (3) 51-75%; and (4) > 75% positive cells. Caveolin-1 intensity was also classified into four categories as (0) negative, (1) weak, (2) moderate (same intensity of smooth muscle cells) and (3) strong (Fig. 1). A caveolin-1 score for each case was generated by multiplying the values for the two variables. A score of 0 was considered as negative, while all other scores were considered as positive.

2. MVD

MVD was determined with CD34-stained slides using the procedure of Weidner et al. (Weidner et al., 1993). Individual microvessels were counted in the area of highest vascularity at ×200 in three selected microscopic fields. Any brown-staining endothelial cell or cluster that was separated from other nearby microvessels was counted. Large anastomosing sinusoidal vessels were counted as a single vessel. Large vessels with thick muscular walls were excluded from the count. The microvessel count was expressed as the mean number of vessels in the selected area (Fig. 2).

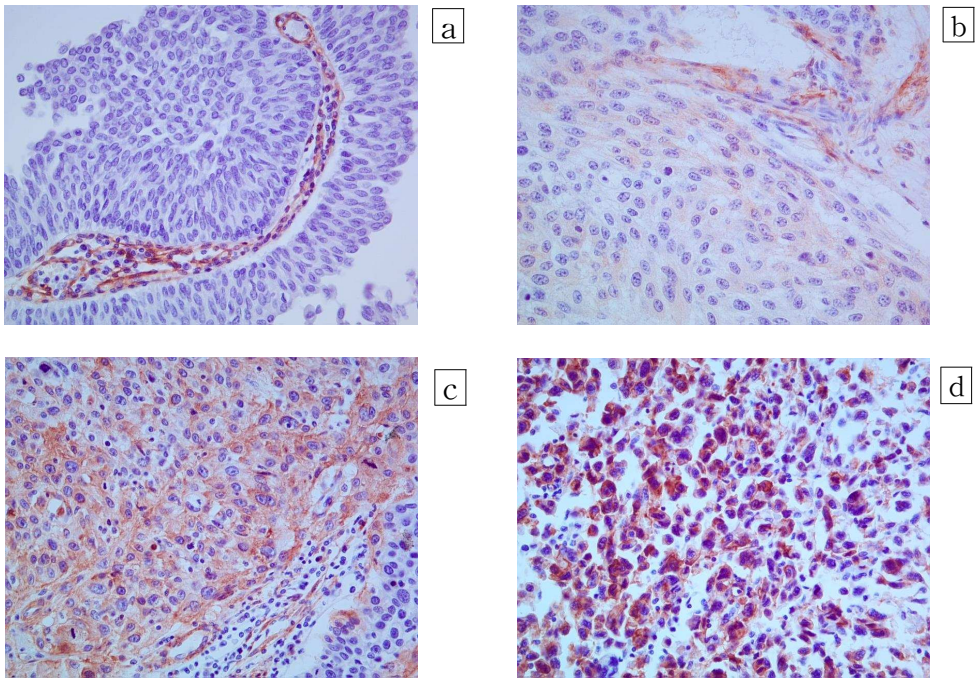

Fig. 1. Immunohistochemical staining for caveolin-1 in upper urinary tract transitional cell carcinoma tissues (×400).

a) Cancer cells are not stained (caveolin-1 intensity 0).

b) Smooth muscle cells are stained as an internal control. Cancer cells are stained weakly compared to an internal control (intensity 1).

c) Cancer cells are stained as the same intensity as internal control (intensity 2).

d) Cytoplasms of cancer cells are strongly stained for caveolin-1 (intensity 3).

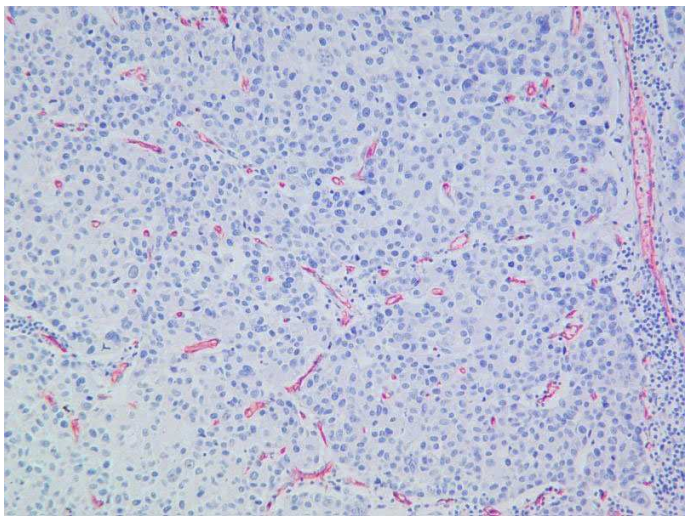

Fig. 2. Immunohistochemical staining for CD34 in upper urinary tract transitional cell carcinoma tissues (×200).

D. Statistical analysis

Either the chi-square test or the independent sample t-test was used to analyze the correlation between caveolin-1 expression or MVD and clinicopathological variables, including T stage, N stage, nuclear grade, tumor position, previous history of bladder cancer, coexisting bladder cancer at diagnosis, and bladder cancer recurrence during follow-up. The relationship between caveolin-1 and MVD was evaluated by independent sample t-test. For univariate and multivariate analyses, the median value of MVD was chosen as the cut-off point for high or low expression. The cancer-specific survival calculations were illustrated with Kaplan-Meier curves, and univariate and multivariate analyses were performed using the log-rank test or the Cox proportional hazards regression model. The values of $p<0.05$ were considered to be statistically significant in all of the analyses.

III. RESULTS

The clinicopathological characteristics of the 98 patients are summarized in Table 1.

Table 1. Clinicopathological data of 98 patients with upper urinary tract transitional cell carcinoma

Characteristics	No. of patients (%)
T stage	
T1	64 (65.3)
T2	9 (9.2)
T3	21 (21.4)
T4	4 (4.1)
N stage	
N0	90 (91.8)
N1	5 (5.1)
N2	3 (3.1)
Grade	
Low (G1+G2)	40 (40.8)
High (G3)	58 (59.2)
Tumor location	
Renal pelvis	56 (57.1)
Ureter	42 (42.9)
Previous history of bladder cancer	
No	91 (92.9)
Yes	7 (7.1)
Coexisting bladder cancer	
No	89 (90.8)
Yes	9 (9.2)
Bladder cancer recurrence	
No	63 (64.3)
Yes	35 (35.7)

There were no synchronous distant metastases in 98 patients. Seventy patients were disease-free at a median follow-up of 52.5 months (range,

12-162 months). The other 28 patients had metachronous metastases at a median of 28 months (range, 4-86 months) after nephroureterectomy. Twenty-seven patients died during follow-up period.

The expressions of caveolin-1 in the 98 patients are summarized in Table 2.

Table 2. Caveolin-1 expression of 98 patients with upper urinary tract transitional cell carcinoma

Intensity	Proportion					Total
	0	1	2	3	4	
0	86	0	0	0	0	86
1	2	4	1	0	0	7
2	0	1	0	1	0	2
3	0	1	2	0	0	3
Total	88	6	3	1	0	98

Of the 98 sections, positive immunostaining for caveolin-1 was observed in 10 (10.2%) and the staining was focal in most cases. The mean MVD as assessed by CD34 was 57.1 (median, 53; range, 19-197). Caveolin-1 expression correlated significantly with T stage ($p<0.001$) and grade ($p=0.036$), but not with N stage ($p=0.149$), tumor location ($p=0.847$), previous history of bladder cancer ($p=0.711$), coexisting bladder cancer at diagnosis ($p=0.211$), or bladder cancer recurrence during follow-up ($p=0.691$) (Table 3). MVD correlated profoundly with T stage ($p<0.001$), N stage ($p=0.002$), and grade ($p<0.001$), but not with tumor location ($p=0.115$), previous history of bladder cancer ($p=0.706$), coexisting bladder cancer at diagnosis ($p=0.544$), or bladder cancer recurrence during follow-up ($p=0.882$) (Table 3).

Table 3. Relationship between caveolin-1 expression or microvessel density and clinicopathological variables in 98 patients with upper urinary tract transitional cell carcinoma

Variables	No. of patients (%)	Caveolin-1 expression (%)	p-value	MVD[a]	p-value
T stage					
Superficial (T1)	64 (65.3)	1 (1.6)	<0.001*	44.31±18.65	<0.001*
Invasive (T2-T4)	34 (34.7)	9 (26.5)		81.15±28.45	
N stage					
N0	90 (91.8)	8 (8.9)	0.149	54.50±25.05	0.002*
N1+N2	8 (8.2)	2 (25.0)		86.25±47.09	
Grade					
Low (G1+G2)	40 (40.8)	1 (2.5)	0.036*	45.08±21.62	<0.001*
High (G3)	58 (59.2)	9 (15.5)		65.38±29.84	
Tumor location					
Renal pelvis	56 (57.1)	6 (10.7)	0.847	60.79±33.03	0.115
Ureter	42 (42.9)	4 (9.5)		52.17±20.35	
Previous history of bladder cancer					
No	91 (92.9)	9 (9.9)	0.711	57.40±29.08	0.706
Yes	7 (7.1)	1 (14.3)		53.14±20.55	
Coexisting bladder cancer					
No	89 (90.8)	8 (9.0)	0.211	57.65±29.44	0.544
Yes	9 (9.2)	2 (22.2)		51.56±16.60	
Bladder cancer recurrence					
No	63 (64.3)	7 (11.1)	0.691	57.41±30.51	0.882
Yes	35 (35.7)	3 (8.6)		56.51±24.86	

*Statistically significant

[a]MVD: microvessel density

The caveolin-1 expression was significantly associated with MVD (p=0.015). A Kaplan-Meier survival curve showed that the survival of patients with positive caveolin-1 expression or high MVD was significantly worse than that of patients with negative caveolin-1 expression or low MVD respectively (p<0.0001, p=0.0283) (Fig. 3, 4).

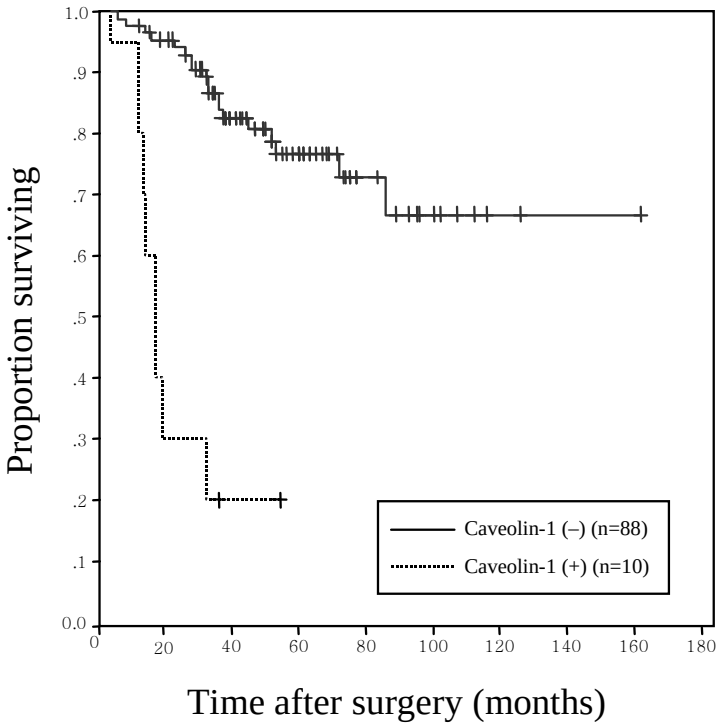

Fig. 3. Kaplan-Meier cancer-specific survival curves according to caveolin-1 expression. The survival rate of patients with caveolin-1 positive tumors was significantly lower than that of patients with caveolin-1 negative tumors (p<0.0001).

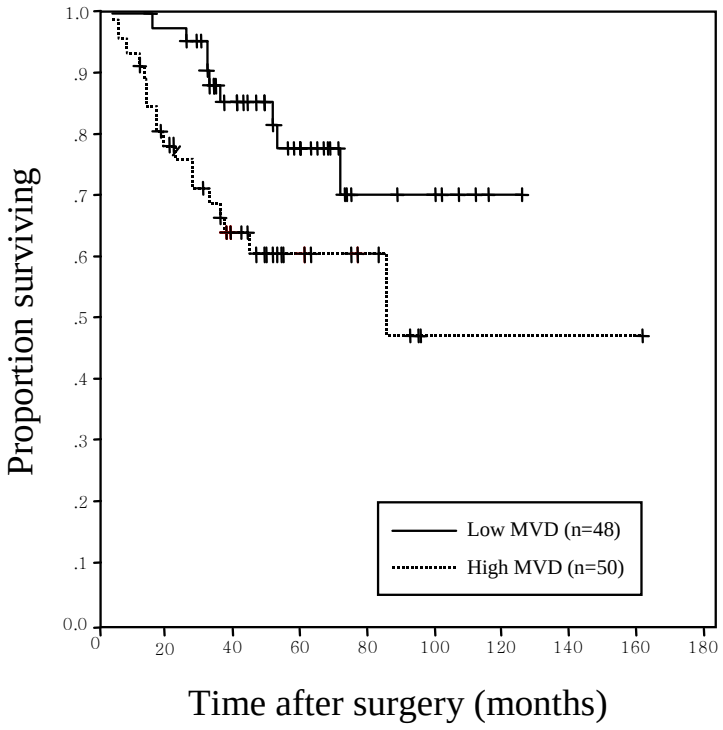

Fig. 4. Kaplan-Meier cancer-specific survival curves according to microvessel density (MVD). The survival rate of patients with high MVD tumors was significantly lower than that of patients with low MVD tumors (p=0.0283).

The univariate analyses identified T stage, grade, caveolin-1 expression, and MVD as significant prognostic factors for cancer-specific survival, whereas the multivariate analyses indicated that T stage (p=0.029) and caveolin-1 expression (p=0.002) were independent prognostic factors (Table 4).

Table 4. Univariate and multivariate survival analysis of 98 patients with upper urinary tract transitional cell carcinoma

Variables	Univariate	Multivariate	
	p value	Hazards ratio	p value
T stage	<0.0001*	3.443	0.029*
N stage	0.0682	1.435	0.571
Grade	0.0172*	1.496	0.499
Tumor location	0.8689	1.402	0.459
Previous history of bladder cancer	0.3393	0.816	0.793
Coexisting bladder cancer	0.7997	0.826	0.787
Bladder cancer recurrence	0.3694	1.551	0.297
Caveolin-1	<0.0001*	5.239	0.002*
MVD[a]	0.0283*	0.716	0.556

*Statistically significant

[a]MVD: microvessel density

IV. DISCUSSION

The exact role of caveolin-1 in cancers is still controversial. Some reports suggested that caveolin-1 could function as a tumor suppressor gene. Engelman et al. reported that genes for caveolin-1 and caveolin-2 were localized to human chromosome 7q31.1 in close proximity to the D7S522 locus, a region commonly deleted in a variety of human cancers, including squamous cell carcinoma, prostate cancer, renal cell carcinoma, ovarian adenocarcinoma, colon carcinoma, and breast cancer (Engelman et al., 1998). Edelson et al. observed that allelic loss at 7q31.3 occurred in all grades and stages of invasive ovarian carcinomas but not in borderline ovarian tumors. Hence, they concluded that the inactivation of tumor suppressor gene in this region was an early event in ovarian tumorigenesis and borderline and invasive tumors were separate entities (Edelson et al., 1997). Hino et al. found that caveolin-1 transfected human breast cancer cell line MCF-7 cells showed less proliferation than the vector control and concluded that caveolin-1 gene might influence the tumor suppressor efficacy in MCF-7 cells (Hino et al., 2003). Williams et al. reported that caveolin-1 knockout mice showed acceleration of multifocal dysplastic mammary lesion development and thought caveolin-1 might play an important in vivo role in suppressing early tumor development (Williams et al., 2003). These reports suggested the strong evidence supporting a tumor suppressive role of caveolin-1.

However, the idea that caveolin-1 act exclusively as tumor suppressor gene was upset by a contradictory report demonstrating potential role of caveolin-1 as oncogene. Ayala et al. reported that growth factor-induced phosphorylation of serine 80 on caveolin-1 led to secretion of the protein which could act in an autocrine or paracrine loop to protect the cancer cells

from apoptosis and it particularly facilitated perineural invasion of prostate cancer cells (Ayala et al., 2006). Drab et al. observed that caveolin-1 knockout mice could not form spontaneous tumors but had pulmonary defect and vascular dysfunction and this result confounded the classification of caveolin-1 as tumor suppressor gene (Drab et al., 2001). In addition, several studies have reported overexpression of caveolin-1 in various types of cancers. Campbell et al. showed that elevated immunoexpression of caveolin-1 was a predictor of poor disease-free survival in renal cell carcinoma, suggesting that cell signalling pathway involving caveolin-1 might have importance in tumor progression (Campbell et al., 2003). A recent report from the author's institution also demonstrated that increased expression of caveolin-1 was associated with metastasis and a worse prognosis in clear cell renal cell carcinoma (Joo et al., 2004). Yang et al. showed that caveolin-1 expression was associated with high Gleason score, positive surgical margin, and lymph node involvement in prostate cancer (Yang et al., 1999). Furthermore, they found that caveolin-1 immunoreactivity independently predicted a shorter time to disease progression after surgery in lymph node-negative cancers. Fine et al. showed that the expression of caveolin-1 was elevated in most of adenocarcinoma but not in normal colonic epithelium and adenomas (Fine et al., 2001). However, there was no statistical correlation between elevated caveolin-1 expression and T stage or metastasis. Kato et al. found that overexpression of caveolin-1 was associated with lymph node metastasis and a worse prognosis after surgery in esophageal squamous cell carcinoma and suggested that overexpression of caveolin-1 might be a marker for lymph node metastasis and poor prognosis after surgical resection (Kato et al., 2002). Based on these results, it is assumed that caveolin-1 could not initiate tumorigenesis, but act as an important factor for tumor

progression and metastasis in several cancers.

In recent studies, several reports described biphasic differential expression of caveolin-1 according to different histologic subtypes of cancers or tumor progression. Sunaga et al. reported that the majority of small cell lung carcinomas lost caveolin-1 expression through promoter methylation, major mode of inactivation of many tumor suppressor genes in human cancers, while the majority of non-small cell lung cancers retained caveolin-1 expression through FAK phosphorylation and Ra1A expression of caveolin-1 (Sunaga et al., 2004). Kato et al. showed that the loss of caveolin-1 regulation resulted in tumor extension and dedifferentiation of lung adenocarcinoma, whereas caveolin-1 overexpression was correlated with tumor extension in squamous cell carcinoma of the lung (Kato et al., 2004). They suggested that these reciprocal functions of caveolin-1 were due to different activation states of the different domains of caveolin-1 and altered interactions with different binding partners. Hung et al. found that caveolin-1 expression was increased in primary oral squamous cell carcinomas but decreased in metastatic oral squamous cell carcinomas of lymph node (Hung et al., 2003). According to these results, they concluded that caveolin-1 might play an oncogenic or anti-oncogenic role that was regulated in a complicated way at different malignant stages and tumor progression of oral squamous cell carcinoma in lymph node did not require caveolin-1 involvement. As described above, caveolin-1 expression was variable according to tumor type and the exact mechanism regulating caveolin-1 remained unclear. Therefore, further exploration of its mechanisms in global carcinogenesis is warranted.

For TCC of the urinary tract, there were some reports about caveolin-1 expression in bladder TCC but no study was accomplished in UUT-TCC. Rajjayabun et al. found that caveolin-1 expression was correlated with tumor

stage and grade but not with tumor multiplicity, recurrence, progression, or survival in bladder cancer patients (Rajjayabun et al., 2001). Fong et al. showed that caveolin-1 and caveolin-2 were detected in some urothelial carcinoma, but not in nonneoplastic urothelium (Fong et al., 2003). They also found that elevated expression of caveolin-1 and caveolin-2 in urothelial carcinoma correlated with tumor grade and squamous differentiation, suggesting the possible role of caveolin-1 and caveolin-2 in tumor progression and squamous differentiation.

In this study, caveolin-1 expression correlated significantly with T stage and grade. The survival of patients with positive caveolin-1 expression was significantly worse than that of patients with negative caveolin-1 expression. Also, caveolin-1 expression was an independent prognostic factor for cancer-specific survival. These results suggest that caveolin-1 expression is associated with tumor progression and poor prognosis in UUT-TCC.

Caveolin-1 is also involved in angiogenesis. It is an important component of caveolae in endothelial cells (Okamoto et al., 1998; Woodman et al., 2003). In addition, caveolin-1 and caveolin-1 scaffolding domain are important in regulating endothelial cell differentiation/tubule formation, a prerequisite in the process of angiogenesis (Liu et al., 2002). Sonveaux et al. reported that in caveolin-1 knockout mice, vascular endothelial growth factor (VEGF) stimulation, nitric oxide (NO) production and endothelial tube formation were dramatically abrogated when compared with caveolin-1 wild type mice (Sonveaux et al., 2004). They also found that exogenous caveolin transfection in caveolin-1 knockout mice restored VEGF-induced NO production and concluded that caveolae would play a critical role in ensuring the coupling between VEGF receptor-2 stimulation and downstream mediators of angiogenesis. Studies of several malignancies showed that MVD, a reflection

of tumor angiogenesis, was associated with metastasis and patient's outcome. Furthermore, neoangiogenesis was often a significant independent prognostic indicator of both overall and disease-free survival (Sharma et al., 2005).

To my knowledge, there were only three reports about MVD in UUT-TCC. Nakanishi et al. evaluated both the expression of PDGF B-chain mRNA and angiogenesis in UUT-TCC from 91 patients and showed that there was no significant relationship between the expression of PDGF B-chain mRNA or MVD and the clinicopathological variables, metastasis, or prognosis (Nakanishi et al., 1997). Therefore, they concluded that expression of PDGF B-chain mRNA and MVD seemed to be of no real value in metastasis or prognosis of UUT-TCC. Inoue et al. evaluated the significance of angiogenic factors such as MVD, VEGF, basic fibroblast growth factor (bFGF), matrix metalloproteinase type 2 (MMP-2) and type 9 (MMP-9), interleukin-8 (IL-8), and E-cadherin as a predictor for prognosis from 55 patient with UUT-TCC. They found that increased MVD, elevated expression level of MMP-9 and MMP-2, and a higher MMP-9:E-cadherin ratio (M:E ratio) were associated with poor prognosis and M:E ratio was an independent prognostic factor for cancer-specific survival (Inoue et al., 2002). However, MVD was not an independent prognostic factor. Zhang et al. analyzed cell proliferation, as detected by Ki-67 staining, MVD, and apoptotic index from 42 patients with UUT-TCC. They found close associations of both proliferation index and MVD with tumor stage and of apoptotic index with tumor grade (Zhang et al., 2001). They also demonstrated a strong relationship between proliferation index and MVD, but did not show associations of apoptotic index with proliferation index or MVD in UUT-TCC. From these results, it was suggested that the higher activity of cell proliferation with rich neovascularization might be related to a higher

malignant potential of the cancer.

In the present study, MVD was significantly correlated with T stage, N stage, and grade. The survival of patients with higher MVD was significantly worse than that of patients with lower MVD. In addition, MVD was a significant prognostic factor for cancer-specific survival in univariate analysis. Therefore, the significance of MVD in UUT-TCC is comparable to that in breast and prostate cancers. Moreover, there was a significant positive correlation between MVD and caveolin-1 expression.

In conclusion, these results suggest that caveolin-1 may play an important role in the progression of UUT-TCC and angiogenesis may be mediated by the action of caveolin-1 in the progression of UUT-TCC. Therefore, it is likely that caveolin-1 may be an integral part of prognostic factors for cancer progression and metastasis in UUT-TCC.

V. CONCLUSIONS

Increased expressions of caveolin-1 and MVD are associated with poor prognosis in UUT-TCC. Furthermore, caveolin-1 expression is significantly associated with MVD. These results suggest that caveolin-1 may play an important role in the progression of UUT-TCC by regulating tumor angiogenesis.

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- 국문 요약 -

상부요로 이행세포암종에서 Caveolin-1 발현 및 미세혈관밀도의 예후에 대한 영향

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목적: Caveolin-1은 세포막에 존재하는 소포의 주요 구조단백질로서 여러 신호전달경로에서 조절역할을 하며, 혈관 내피세포의 분화 및 혈관형성의 조절에 관여하는 것으로 알려져 있다. 최근에 caveolin-1의 이상 발현이 각종 악성 종양과 연관된다고 보고되고 있지만 종양마다 양상이 달라서 그 역할에 대해서는 논란의 여지가 있으며, 상부요로 이행세포암종에서는 아직 연구된 바 없다. 이에 저자는 상부요로 이행세포암종에서 caveolin-1의 발현과 혈관형성의 척도가 되는 미세혈관밀도 (microvessel density; MVD)를 분석하여 예후와의 연관성을 알아보고자 하였다.

재료 및 방법: 상부요로 이행세포암종으로 근치적 신요관전적출술을 시행받은 환자 중 추적관찰이 가능하였던 98명의 보관된 파라핀 포매 조직을 이용하여 caveolin-1과 CD34에 대한 면역조직화학염색을 시행하였다. Caveolin-1의 발현 정도는 염색부분 (proportion)과 염색강도 (intensity)에 따라 반정량적으로 점수화하였고, 염색점수는 각각의 예에서 염색부분과 염색강도의 값을 곱하여 계산하였다. 염색점수가 0인 경우를 음성으로 정하였고, 그 이상의 염색점수를 가질 때 caveolin-1 발현 양성이라고 정하였다. Caveolin-1의 발현 및 미세혈관밀도와 T 병기, N 병기, 분화도, 종양의 위치, 방광암의 과거력, 진단당시 방광암 동반유무, 추적관찰 중 방광암 발생유무 및 암특이생존율 등의 임상병리학적 변수들과의

연관성을 분석하였다.

결과: Caveolin-1의 발현은 T 병기 ($p<0.001$) 및 분화도 ($p=0.036$)와 통계학적으로 유의한 연관성을 보였고, MVD는 T 병기 ($p<0.001$), N 병기 ($p=0.002$) 및 분화도 ($p<0.001$)와 통계학적으로 유의한 연관성을 보였다. Caveolin-1의 발현은 미세혈관밀도와 통계학적으로 유의한 연관성을 보였다 ($p=0.015$). Kaplan-Meier 생존곡선에서 caveolin-1 발현 양성인 환자는 caveolin-1 발현 음성인 환자에 비해 유의하게 생존율의 감소를 보였고 ($p<0.0001$), 미세혈관밀도가 높은 환자는 미세혈관밀도가 낮은 환자에 비해 유의하게 생존율의 감소를 보였다 ($p=0.0283$). 암특이생존율에 대한 단변량 분석에서 T 병기, 분화도, caveolin-1의 발현 및 미세혈관밀도가 통계학적으로 유의한 예후인자였고, 다변량 분석에서는 T 병기 및 caveolin-1의 발현이 독립적인 예후인자였다.

결론: Caveolin-1의 발현 및 미세혈관밀도의 증가는 상부요로 이행세포암종에서 불량한 예후와 연관이 있었고, caveolin-1의 발현과 미세혈관밀도 사이에는 유의한 연관성이 있었다. 따라서 caveolin-1은 상부요로 이행세포암종의 진행에 있어서 중요한 역할을 하며, 그 기전 중에는 혈관형성이 관여하리라 생각한다.

핵심되는 말: 이행세포암종, 상부요로, Caveolin-1, 미세혈관밀도, 예후