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Prognostic significance of epithelial-mesenchymal transition phenotypes in upper urinary tract urothelial carcinoma

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ABSTRACT

Epithelial-mesenchymal transition (EMT) is a process which epithelial cells gain mesenchymal phenotype such as motility and invasiveness. We investigated the role of EMT in upper urinary tract urothelial carcinoma (UTUC). The patient cohort included 93 cases of UTUC treated with radical nephroureterectomy. Tissue microarrays were constructed from formalin-fixed paraffin-embedded tissue blocks. Immunohistochemical staining was performed for E-cadherin, vimentin, and smooth muscle actin to evaluate the EMT status. Interpretation criteria were defined for the staining results and EMT phenotypes were assigned as wild type, incomplete type (loss of E-cadherin and negative for vimentin), and complete type (loss of E-cadherin and positive for vimentin). The loss of E-cadherin and vimentin-expression was observed in 76 (81.7%) and 10 (10.8%) cases, respectively, yielding EMT phenotypes comprised of 17 cases (18.3%) of wild type, 66 cases (71.0%) of incomplete type, and 10 cases (10.8%) of complete types. In survival analyses, wild type showed statistically significant association with longer extra-bladder recurrence free survival ($p < .001$) and overall survival ($p < .001$). In multivariate analyses, complete type was an independent prognostic factor for extra-bladder recurrence free survival and overall survival. EMT phenotype based on the combination of EMT-related markers may provide a useful prognostic marker for UTUC patients.

1. Introduction

Urothelial carcinoma (UC) is the ninth most common cancer worldwide [1]. A vast majority of UC (90–95%) occurs in urinary bladder while only 5–10% of all UC develops in upper urinary tract including pyelocalyceal system and ureter [2]. Because of the predominance of urinary bladder urothelial carcinoma (UBUC) and histologic similarity of UBUC to upper tract urothelial carcinoma (UTUC), the understanding of the UTUC has been extrapolated from UBUC in many aspects. However, in recent studies, UBUC and UTUC revealed practical, anatomical, biological and molecular differences leading to the awareness of these carcinomas as two distinct entities [3,4]. Unlike UBUC, UTUC was associated with anomalies in the activity of specific regulatory proteins [5,6], and patients with Lynch syndrome have a higher incidence of UTUC [7]. Radical nephroureterectomy with bladder cuff excision is the standard treatment for UTUC. Although the prognosis of early stage UTUC is excellent, the 5-year survival rate of pT3 cases is lowered to around 40% [8]. A variety of other clinicopathologic factors including lymphovascular invasion (LVI) have been investigated for prognostic and predictive implication in UTUC but none has been proved of robust clinical utility [9–12].

Epithelial-mesenchymal transition (EMT) is a process during which epithelial cells lose their cell-to-cell adhesion and gain the ability to migrate and invade. EMT is one of the important processes not only in the tumor biology but also in the various physiologic process, including embryogenesis, would healing, inflammation, and fibrosis [13]. During the EMT, tumor cells are expected to repress their epithelial phenotype and sequentially upregulate mesenchymal marker expression, such as vimentin, fibronectin, and smooth muscle actin, and surrounding tumor microenvironment may also be remodeled to facilitate the invasion of tumor cells [14]. EMT is primarily regulated through signaling pathways affecting E-cadherin mediated intercellular adhesion and the cytoskeleton. E-cadherin is a transmembrane protein that plays a role as a physical bond between epithelial cells, so its function is essential for the

Abbreviations: UC, urothelial carcinoma; UBUC, urinary bladder urothelial carcinoma; UTUC, upper urinary tract urothelial carcinoma; LVI, lymphovascular invasion; EMT, epithelial mesenchymal transition; IHC, immunohistochemical staining; BcFS, urinary bladder cancer free survival; RFS, extra-bladder recurrence free survival; OS, overall survival; TMA, tissue microarrays; IR, immunoreactivity

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integrity of epithelia [15]. Loss of E-cadherin expression has been established as a feature of EMT in various carcinomas [16–18]. In UTUC, the effects of alteration of E-cadherin or other EMT markers on prognosis have been studied and have shown diverse results [19–22].

Because EMT is a complex and multi-step process, tumor cells in the EMT process can show a wide spectrum of phenotypes and it is logical to assume that there may be tumor cells in transitive state which have lost the epithelial phenotype but have not yet acquired the mesenchymal property. In addition, since EMT is a reversible process, epithelial markers can be re-expressed in advanced cancer cells which has already obtained mesenchymal phenotypes [23,24].

In this study, we investigated the effect of EMT on progression and prognosis in UTUC using immunohistochemical staining (IHC) of E-cadherin, vimentin, and smooth muscle actin on tissue microarray sections. To denote tumor cells with intermediate subtypes between typical epithelial and mesenchymal phenotypes, we used the EMT classification method proposed by Sung et al. [25] in a study for esophageal carcinoma.

2. Materials and methods

2.1. Patients selection

A retrospective study was conducted in 93 patients who underwent radical nephroureterectomy for UTUC during the period from January 2000 to December 2005 at the Department of Pathology of Samsung Medical Center, Seoul, Republic of Korea. Cases with previous history of UBUC were excluded from the study. Pathology reports and histologic slides were reviewed by two pathologists (JC and GYK) to evaluate morphologic features such as histologic grade (WHO Classification of Tumours of the Urinary System and Male Genital Organs, 2016 [26]), growth pattern (papillary or infiltrating), LVI, depth of invasion (pT status), and nodal metastasis (pN status). (American Joint Committee on Cancer cancer staging manual, 8th edition [27]) Clinical information including age, sex, and follow up data was retrieved from medical charts. The clinicopathological characteristics of the patients are summarized in Table 1. Of the 93 patients, non-invasive carcinoma (pTa) was 12 cases (12.9%). Most of the cases were of conventional type when examined histologic type, but some cases showed squamous differentiation (11 cases, 11.8%) and micropapillary feature (2 cases, 2.2%), but no inverted growth pattern was observed. Lymph node metastasis was found in 5 cases among 28 lymph node-containing specimens. Follow up data included urinary bladder cancer free survival (BcFS), extra-bladder recurrence free survival (RFS), and overall survival (OS). Survival data was available in all patient. The mean follow-up period was 63.8 months (range, 3.0–133.5) and 34 patients died of UTUC during the follow-up period.

2.2. Construction of tissue microarray

Tissue microarrays (TMA) were constructed using a manual tissue arrayer (Beecher Instruments, Sun Prairie, WI, USA). Two cores with a diameter of 2 mm-tissue were obtained from the most representative tumor areas (preferentially, central area and invasive front) of formalin-fixed, paraffin-embedded tissue blocks and were arranged in TMA blocks. Hematoxylin-eosin staining (H&E) of the TMA sections was performed for tissue confirmation.

2.3. Immunohistochemical staining and evaluation

Sections of TMA were cut to a thickness of 4 μm and immunohistochemically labeled with E-cadherin (mouse monoclonal antibody, 1:50, Invitrogen, Carlsbad, CA, USA), vimentin (mouse monoclonal antibody, 1:200, DAKO, Glostrup, Denmark), and smooth muscle actin (mouse monoclonal antibody, 1:100, DAKO) with autoimmunostainer, BenchMark XT automated stainer (Ventana Medical Systems, Inc, Tucson, AZ), according to manufacturer's protocol.

For evaluation of IHC of E-cadherin, we devised a new scoring method. The scoring system is composed of following 4 categories: score 0, totally negative; score 1, partial membranous staining

Table 1
Correlations of clinicopathologic parameters with epithelial-mesenchymal transition phenotypes.

	Epithelial-mesenchymal transition phenotype						Total	p value
	Wild (%)		Incomplete (%)		Complete (%)			
Age (years)								.273*
< 65	7	(14.0)	39	(78.0)	4	(8.0)	50	
≥65	10	(23.3)	27	(62.8)	6	(14.0)	43	
Sex								.010*
Male	17	(23.9)	49	(69.0)	5	(7.0)	71	
Female	0	(0.0)	17	(77.3)	5	(22.7)	22	
Histologic grade								.000†
Low	14	(30.4)	32	(69.6)	0	(0.0)	46	
High	3	(6.4)	34	(72.3)	10	(21.3)	47	
Growth pattern								.002*
Papillary	15	(21.7)	51	(73.9)	3	(4.3)	69	
Infiltrating	2	(8.3)	15	(62.5)	7	(29.2)	24	
LVI								.694*
Absent	15	(17.4)	62	(72.1)	9	(10.5)	86	
Present	2	(28.6)	4	(57.1)	1	(14.3)	7	
pT status								.018†
pTa	3	(25.0)	9	(75.0)	0	(0.0)	12	
pT1	6	(24.0)	19	(76.0)	0	(0.0)	25	
pT2	2	(15.4)	10	(76.9)	1	(7.7)	13	
pT3	6	(14.0)	28	(65.1)	9	(20.9)	43	
pN status								.070†,‡
pNx	13	(20.0)	46	(70.8)	6	(9.2)	65	
pN0	4	(17.4)	17	(73.9)	2	(8.7)	23	
pN1	0	(0.0)	3	(60.0)	2	(40.0)	5	

LVI, lymphovascular invasion.
* Pearson's chi-square test.
† Chi-square test using linear by linear association.
‡ p-value was analyzed only in pN0 and pN1 groups.