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Reappraisal of clinicopathological relevance of RON expression in upper tract urothelial carcinoma

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Abstract

Purpose: Aberrant activation and cross-talk of recepteur d'origine nantais (RON) and epidermal growth factor receptor (EGFR) family receptors have been reported in several human malignancies, including urinary bladder. However, the clinical significance of RON and EGFR family receptors in patients with upper tract urothelial carcinoma (UTUC) remains unexplored.

Materials and methods: Immunohistochemical staining for RON, EGFR family member was performed on serial sections of archival tissue specimens from 124 patients with UTUC who underwent nephroureterectomy plus bladder cuff resection. Clinicopathological characteristics were retrospectively reviewed, and cancer-specific survival was determined by medical records. The associations between clinicopathological variables, expression status of receptors, and cancer-specific survival were analyzed. T24 cell sublines and one published Cornell's series were utilized for gene set enrichment analysis.

Results: With a median follow-up duration of 50 months (range: 1–177 months), 41 UTUC-related deaths were recorded. RON, EGFR, human epidermal growth factor receptor 2 (HER2/neu), and human epidermal growth factor receptor 3 (HER3) expressions were positive in 45 of 124 (36.3%), 20 of 124 (16.1%), 13 of 96 (13.5%), and 26 of 96 (27.1%) tumors, respectively. Both univariate and multivariate Cox regression analyses showed that stage (hazard ratio 1.85; 95% confidence interval, 1.13–3.00; $P = 0.014$) and RON expression (hazard ratio, 1.95; 95% confidence interval, 1.04–3.66; $P = 0.038$) were independent poor prognostic factors for disease-specific overall survival. Gene set enrichment analysis results showed that RON expression predicts gene enrichment with disease metastasis (normalized enrichment score, 1.89, $P = 0.008$).

Conclusion: RON expression was an independent predictor of poor cancer-specific survival. UTUC with RON/HER2 coexpression exhibits the potential of metastatic behavior and the worst outcome.

Keywords: EGFR, prognosis, RON, urothelial carcinoma

1. Introduction

Upper tract urothelial carcinoma (UTUC) consists of urothelial carcinoma (UC) derived from renal pelvis or ureter. Unlike those derived from urinary bladder, UTUC is not common in urological malignances[1,2]. UTUCs may be detected incidentally, with symptoms such as microscopic or gross hematuria and flank pain. Systemic symptoms (anorexia, weight loss, and bone pain) prompt the probability of metastatic diseases[2]. There were 3 different molecular characteristics of UTUCs according to the risk factors or etiologies, including conventional UTUC probably caused by cigarette smoking, hereditary Lynch syndrome-associated caused by DNA repair gene alteration, and aristolochic acid nephropathy–associated UTUC caused by Chinese herb consumption[3]. In Western countries, UTUCs are not common and account approximately for 5% to 10% of entire UCs and

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Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

The study has obtained the approval and institutional oversight of the Institutional Review Board for the Protection of Human Subjects at National Cheng Kung University Hospital (IRB No. A-ER-103-012).

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bladder cancer about 90% to 95%[2]. Compared with those in Western countries or Japan[4], there is a relatively higher incidence and unusual distribution of UTUC in Taiwan[5,6]. It might result from both high incidence of chronic arsenism and aristolochic acid nephropathy in Taiwan[7]. Accordingly, there were many unusual clinicopathological presentations in Taiwan cohort including younger, female predominant, more prior history of chronic kidney disease, bladder cancer history, less smoking, less high-grade or less high-stage diseases, and less lymphovascular invasion but more multifocal and squamous differentiation compared with Japanese cohort[8]. Interestingly, there is no significant difference in oncological outcomes (disease recurrence or mortalities)[8]. One decade ago, the majority of interest in treatment was focused on urinary bladder UC and mainly cisplatin-based therapy[1]. Recently, several promising therapies have raised the interest in treating UC regardless of bladder or upper tract with single or combination, including immune checkpoint inhibitors[9], antibody-drug conjugate (ADC)[10,11], and fibroblast growth factor receptor–targeted therapy[12]. Although these clinical trials enrolled both UTUC and bladder UC, these subgroup analyses regarding UC site showed inconsistent results[13]. The reasons include smaller patient populations and anatomical, etiological, or genetic differences[14]. Therefore, it is mandatory to reinvestigate the clinical relevance of influential molecules in UTUC.

The recepteur d'origine nantais (RON) is an oncogenic receptor tyrosine kinase, also known as macrophage stimulating 1 receptor, and belongs to the mesenchymal-epithelial transition (MET) proto-oncogene family[15]. RON presents with homo- or heterodimer structures and is expressed on the ciliated epithelia of the mucociliary transport apparatus of the lung, or renal tubular cells where it binds with macrophage stimulating protein through β-subunit and induces downstream biological processes, including cellular proliferation, cell survival and epithelial-mesenchymal transition (EMT)[16,17]. RON has been reported to be expressed in several human malignancies, including breast cancers[18], colorectal cancers[19], ovarian cancers[20], and bladder cancers[21]. Our previous studies have shown that RON can bind with heterologous receptors in human bladder cancer, such as MET[21] or epidermal growth factor receptor (EGFR)[22]. Coexpression of both RON and MET predicts a worse survival in human bladder cancer[21]. In collaboration with EGFR, RON can translocate into the nucleus and serve as a transcriptional factor to execute several cellular functions[22]. Despite these advances, only a few studies focus on the role of RON expression in human UTUC[23]. Therefore, it is mandatory to reappraise the clinicopathologic relevance of RON expression in UTUC patients.

2. Materials and methods

2.1. Study population and clinicopathological characteristics

This study was performed in accordance with and conforming to the Declaration of Helsinki. With the approval and institutional oversight of the Institutional Review Board for the Protection of Human Subjects at National Cheng Kung University Hospital (A-ER-103-012), archival UTUC specimens that were eligible and available for immunohistochemical staining were obtained between 1988 and 2010 from 124 patients who underwent radical nephroureterectomy with bladder cuff resection. Clinical characteristics of the enrolled patients were retrospectively obtained from chart records, and pathological characteristics of the tumors including gross morphology, histological grade, and pathological stage were utilized after being reevaluated by a senior experienced pathologist. Patients were followed with regular cystoscopic surveillance and computerized tomography for the detection of recurrence and metastasis. The observation time was the interval between diagnosis and the last contact, either due to UTUC-related death or the last follow-up. Data were censored at the last follow-up for patients who were considered to be alive or patients who died of other causes.

2.2. Immunohistochemistry of RON, EGFR, HER2/neu, and HER3

The procedure of immunohistochemistry (IHC) was adapted from our previous study[18,24,25]. Briefly, serial tissue sections were managed with deparaffinization, rehydration, and autoclaving, then treated with 3% H_2O_2/methanol and skimmed milk in phosphate buffer saline subsequently. For reducing nonspecific background staining, slides were preincubated with 0.3% bovine serum albumin and then with the primary anti-RON (EP1132Y, ab52927, Abcam, Cambridge, United Kingdom) (1:100 dilution), anti-EGFR (clone 31G7, Triton Diagnostics, Alameda, California) (1:10 dilution), anti-HER2/neu (BioGenex Laboratories, San Ramon, California) (1:20 dilution), and anti-HER3 (RTJ-2, Santa Cruz Biotechnology) (1:100 dilution). Parallel staining without primary antibody served as a negative control and human liver or spleen section as a positive control. With a 1-hour incubation of secondary antibodies, the immunostaining was developed using an horseradish peroxidase IHC System (BioGenex Super sensitive Polymer kit) and then counterstained with hematoxylin.

The degree of immunostaining intensity was analyzed blindly by one senior pathologist (N.-H.C.). In brief, those either with membranous or nuclear staining were categorized as positive, either + (10–50% of tumor cells) or ++ (>50%) expression, and those without any immunostaining or <10% of tumor cells were thought as negative expression[18,25] (Fig. 1).

2.3. T24 cell lines and reverse transcription polymerase chain reaction

Human bladder cancer cell line T24 and its metastatic sublines (T24-P and T24-B), kindly provided by Professor Jer-Tsong Hsieh[26] were used for the current study. All the cells were maintained in the Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and 50 μg/mL gentamicin at 37 °C in a humidified atmosphere of 5% CO_2.

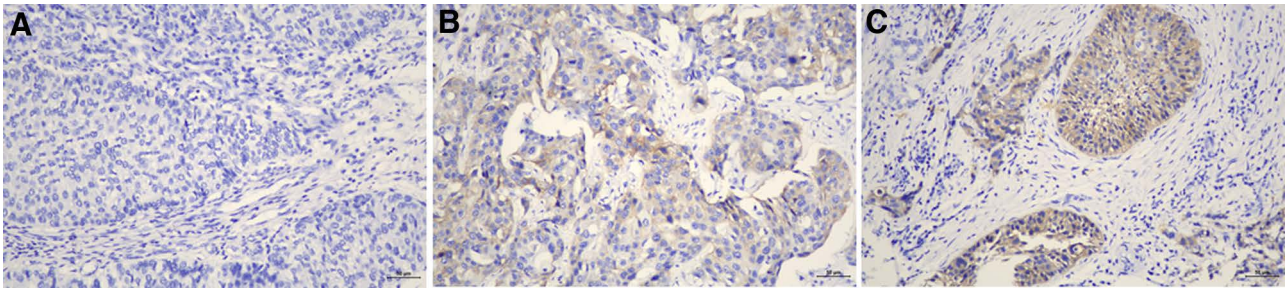

Figure 1. RON immunohistochemistry. (A) negative, (B) +, and (C) ++. ×200. RON, recepteur d'origine nantais.

Table 1

Clinicopathological characteristics of the study population.

Parameters	Total (N = 124)	RON (+) (n = 45)	RON (–) (n = 79)	*P*
Age, y	64.7±10.2	64.8 ± 10.3	64.7±10.3	0.845
Male gender (%)	51 (41.1%)	20 (44.4%)	31(39.2%)	0.571
History of bladder tumor (%)	32 (25.8%)	14 (31.1%)	18 (22.8%)	0.308
Prenephrectomy renal function (%)				0.080
eGFR ≥ 60 mL/min/1.73 m^2	61 (49.2%)	28 (62.2%)	32 (40.5%)	
eGFR < 60 mL/min/1.73 m^2	32 (25.8%)	8 (17.8%)	24 (30.3%)	
Dialysis	14 (11.3%)	4 (8.9%)	11 (13.9%)	
Not known	17 (13.7%)	5 (11.1%)	12 (15.2%)	
Primary tumor location (%)				0.930
Ureter	71 (57.3%)	26 (57.8%)	45 (57.0%)	
Renal pelvis	53 (42.7%)	19 (42.2%)	34 (43.0%)	
Multifocality (%)	26 (21.0%)	8 (17.8%)	18 (22.8%)	0.510
Gross morphology				0.392
Papillary	112 (90.3%)	42 (93.3%)	70 (88.6%)	
Nonpapillary	12 (9.7%)	3 (6.7%)	9 (11.4%)	
Tumor grade, high (%)	67 (54.0%)	27 (60.0%)	40 (50.6%)	0.314
Stage (%)				0.071
pTa-pT1	53 (42.7%)	15 (33.3%)	38 (48.1%)	
pT2	19 (15.3%)	11 (24.4%)	8 (10.1%)	
pT3	25 (20.2%)	8 (17.8%)	17 (21.5%)	
pT4 or N+	27 (21.8%)	11 (24.4%)	16 (20.3%)	
Follow-up months, median (range)	51.5 (1–177)	40 (2–170)	55.5 (1–177)	0.181
Recurrence (%)	31 (25.0%)	10 (22.2%)	21 (26.6%)	0.590
Distant metastasis (%)	27 (21.8%)	14 (31.1%)	13 (16.5%)	0.057
Chemotherapy (%)	27 (21.8%)	14 (31.1%)	13 (16.5%)	0.057

Abbreviation: eGFR, estimated glomerular filtration rate; RON, recepteur d'origine nantais.

Values are expressed as the number (%) or mean ± standard deviation. The case numbers of variables with missing data were specifically annotated.

Cells were seeded in 6- or 10-cm dishes overnight and refreshed in the medium the next day. Total RNA was extracted using the TRIzol (Invitrogen, California) method according to the manufacturer's protocol and then reverse transcribed with High Capacity cDNA Reverse Transcription Kits (Applied Biosystems, California). The resulting cDNA was used for polymerase chain reaction (PCR) in triplicate and data collection was performed in a Smart Cycler 2 PCR system (Cepheid, California). All samples were amplified simultaneously in duplicates in a one-assay run. The $-\Delta\Delta C_t$ method[27] was utilized to measure relative changes in mRNA levels examined by the quantitative reverse transcriptase PCR (qRT-PCR) experiments, after normalizing the transcript levels of each gene by the levels of β-actin as an internal control. The used primers for RON mRNA expression are 5'- GTCAATGGGACTGAGTGTCTGC-3' (Forward) and 5'- TCTCTGTACTGGAAGGTCCAGG -3' (Reverse).

2.4. Gene set enrichment analysis

The mRNA expression levels from UTUC patients were downloaded from the website cBioPortal for cancer genomics (cBioPortal for Cancer Genomics). Two series of UC were utilized, one UTUC from Cornell's study[28] and the other bladder cancer for *The Cancer Genome Atlas*[29]. Gene set enrichment analyses were adapted as previously described[30]. Briefly, the high and low expression levels of RON were stratified by the medium expression level and were performed using the Molecular Signatures Database (MSigDB) v5.2. The normalized enrichment score (NES) was calculated and used to assess how significantly a certain gene set is enriched as an indicated dataset.

2.5. Statistical analysis

Categorical variables were presented as number (%), while numerical variables were presented as mean ± standard deviation. Fisher exact test and Mann-Whitney-Wilcoxon test were used to compare differences between groups with varying receptor expression status. The associations between cancer-specific survival and clinicopathologic variables were assessed using the Kaplan-Meier method and the log-rank test. The statistical analyses were performed using IBM SPSS Statistics version 25 and GraphPad Prism 8.3.0, with a 2-sided approach, and statistical significance was determined at a threshold of 0.05.

3. Results

3.1. Clinicopathologic characteristics of the cohort

Our cohort comprised 124 patients with a median age of 64.7 years (range: 22–90), and male patients accounted for 41.1% (51/124). About one-fourth of the patients exhibit deteriorated renal function (n = 32) and 11.3% (n = 14) are on dialysis. The majority of primary tumors were located in the ureter, accounting for 57.3% (71/124) of cases. High-grade tumors were observed in 54.0% of cases, while 57.3% presented with muscle-invasiveness. Twenty-six (21%) tumors were multifocal and 12 (9.7%) were nonpapillary in morphology. With a median follow-up duration of 51.5 months (range, 1–177 months), any urinary tract recurrence occurred in 31 (25%) of cases, while distant metastasis was observed in 27 (21.8%) of cases. Subsequent systemic chemotherapy either adjuvant or salvage was performed in 27 (21.8%) of cases. In the point of data analysis, 41 (33.1%) UTUC-related deaths were recorded (Table 1).

3.2. RON, EGFR, HER2, HER3 expression and clinicopathologic correlation

All the enrolled patients exhibited both RON and EGRF immunostaining, while only 96 patients exhibited HER2 and HER3 immunostaining. IHC analysis of specimens revealed positive expression of RON, EGFR, HER2, and HER3 in 36.3% (45/124), 16.1% (20/124), 13.5% (13/96), and 27.1% (26/96) of

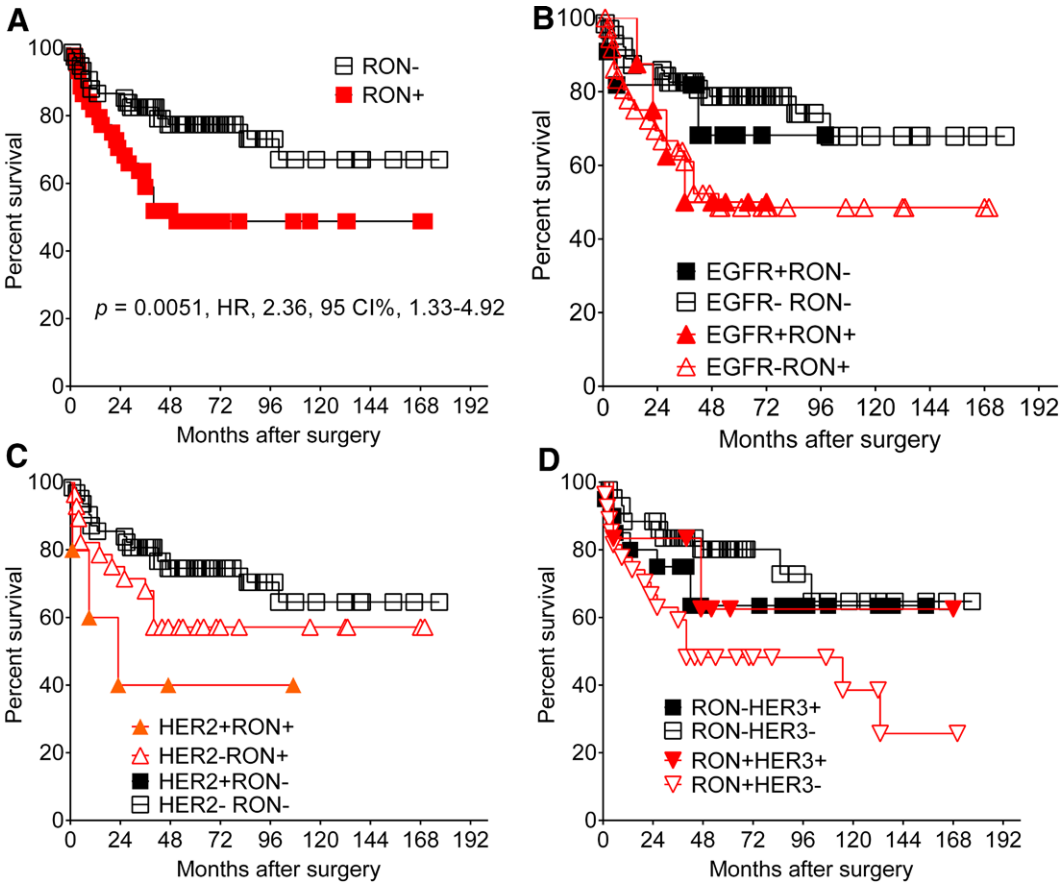

Figure 2. Disease-specific survival according to RON, EGFR, HER2/neu, and HER3 expression. (A) RON expression or not, (B) EGFR and RON expression, (C) HER2/neu and RON expression, and (D) HER3 and RON expression. EGFR, epidermal growth factor receptor; HER2/neu, human epidermal growth factor receptor 2; HER3, human epidermal growth factor receptor 3; RON, recepteur d'origine nantais.

tumors, respectively. RON expression was borderline associated with more risk of advanced or development of distant metastases (P = 0.071 and 0.057, respectively) (Table 1). EGFR expression was significantly associated with a history of urinary bladder tumor (P = 0.011) (Supplementary Table 1, http://links.lww.com/URSC/A65). HER2 expression was significantly associated with an increased risk of muscle-invasive disease (Fisher exact test, P = 0.028) and distant metastasis (chi-square with Yates' correction, P = 0.002) (Supplementary Table 2, http://links.lww.com/URSC/A65). HER3 expression was significantly associated with deteriorated renal function (P = 0.035) (Supplementary Table 3, http://links.lww.com/URSC/A65). The analysis comparing cancer-specific survival between negative and positive expression subgroups revealed a significantly shorter survival in the RON-positive group than RON-negative group (P = 0.0051; hazard ratio, 2.34; 95% confidence interval, 1.27–4.33), while no significant association between EGFR, HER2, and HER3 expression status and clinical outcome was observed (all P values >0.05) (Fig. 2A).

3.3. Cox regression analyses for prognostic factors associated with survival

Univariate Cox regression analyses showed that histologic grade, tumor staging, and RON expression were associated with poorer cancer-specific survival, and multivariate analyses revealed that both tumor stage (P = 0.014; hazard ratio, 1.85; 95% confidence interval, 1.13–3.00) and RON expression (P = 0.038; hazard ratio, 1.95; 95% confidence interval, 1.04–3.66) were independent predictors of poorer cancer-specific survival (Table 2).

Among 45 RON-positive tumors, 8 (17.8%), 5 (15.2%), and 6 (18.2%) had EGFR, HER2, and HER3 coexpression, respectively (Supplementary Table 4, http://links.lww.com/URSC/A65), in which those either HER2 (+),EGFR (–), or HER3 (–) expression exhibited worse outcome, with a median survival of 23 (versus not reached), 51 (versus 54 months), and 40 (versus not reached) months, respectively (Fig. 2B–D). The subgroup with RON and HER2 coexpression had the poorest survival compared with the remaining participants, with a hazard ratio for death of 9.26 (95% confidence interval, 1.59–53.9; P = 0.014).

3.4. Metastatic T24 cell lines

Using data mining from the Cancer Cell Line Encyclopedia (Novartis/Broad, Nature 2012), urothelial cancer cell lines showed various distributions of RON mRNA expression (Fig. 3A). Our previous studies showed that metastatic T24 sublines T24L (derived from T24 lung metastasis) and T24B (derived from T24 bone metastasis) exhibited more EMT marker expressions than nonmetastatic T24 parental cells. Using RT-PCR assays, both metastatic T24 sublines (T24-L and T24-B) exhibit more RON mRNA expression, as well we more HER2/neu expression, compared with T24-P cells (Fig. 3B).

3.5. Gene set enrichment analysis

To explore the biological significance of RON expression in UTUC tumors, we applied GESA to RNA-seq data of UTUC tumors from the published dataset of Cornell/Baylor/MD Anderson Cancer Center[31]. There were 32 UTUC tumors

Table 2

Cox proportional hazard regression analyses for disease-specific overall survival.

Parameters	HR	95% CI	*P*
Univariate analysis			
Age (continuous)	1.00	0.97–1.03	0.937
Gender (male vs female)	0.67	0.37–1.24	0.206
History of bladder UC (yes vs no)	0.77	0.35–1.69	0.519
Prenephrectomy renal function			
eGFR ≥ 60 mL/min/1.73 m^2	(ref)		
eGFR < 60 mL/min/1.73 m^2	0.54	0.24–1.21	0.14
Dialysis	0.63	0.22–1.82	0.40
Stage (<pT2 vs ≥pT2)	1.87	1.43–2.45	<0.001
Grade (high vs low)	3.56	1.73–7.34	0.001
Gross morphology (nonpapillary vs papillary)	1.93	0.59–6.29	0.277
Multifocality (multifocal vs unifocal)	1.30	0.63–2.65	0.477
Location (ureter vs renal pelvis)	1.11	0.59–2.08	0.741
RON (positive vs negative)	2.34	1.27–4.33	0.005
EGFR (positive vs negative)	0.97	0.41–2.33	0.953
HER2/neu (positive vs negative)	1.87	0.82–4.29	0.138
HER3 (positive vs negative)	0.90	0.42–1.92	0.781
Multivariate analysis			
Stage (<pT2 vs ≥pT2)	1.85	1.13–3.00	0.014
Grade (high vs low)	1.64	0.67–4.02	0.275
RON (positive vs negative)	1.95	1.04–3.66	0.038

Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate; EGFR, epidermal growth factor receptor; HER2/neu, human epidermal growth factor receptor 2; HR, hazard ratio; RON, recepteur d'origine nantais; UC, urothelial carcinoma.

Values are expressed as the number (%), mean ± standard deviation, or median (range). The case numbers of variables with missing data were specifically annotated.

analyzed, including 20 males and 12 females, T1 (n = 13) and T2 at least (n = 19). Gene set enrichment analysis (GSEA) results revealed that high RON-expressing tumors exhibit more expressions of metastasis-related genes in the gene sets of both the Rickman metastasis model and Cromer metastasis model (NES, 1.90 and 1.89; $P = 0.008$ and < 0.0001, respectively) (Fig. 4A, B). Also, there were more bladder cancer-reference gene enrichments of the Kyoto Encyclopedia of Genes and Genomes pathway in the high RON-expressing UTUC tumors (NES, 1.55; $P = 0.021$) (Fig. 4C). Heat map analysis showed higher expression of Harvey Rat sarcoma virus gene, HER2/neu, and TP53 in high RON-expressing UTUC (Fig. 4D).

4. Discussion

In the current study, we demonstrated that RON expression exhibits the borderline association of distant metastasis and is an independent prognostic factor for disease-specific overall survival in UTUC patients, particularly in those coexpressed with HER2/neu. In vitro UC T24 cells and its metastatic sublines study showed that there were higher expressions of EMT markers (fibronectin, vimentin, E-, and N-cadherin), oxidative stress markers (Nrf-1 and Nrf-2), and HER2/neu. GSEA analysis demonstrated gene enrichments of the metastasis-related pathway in higher RON-expressing UTUCs. Taken together, RON expression in collaboration with HER2/neu expression can provide prognostic information in UTUC patients, which also hints at the therapeutic direction of RON- or HER2/neu-targeted therapy in the future.

Our previous study has demonstrated in a large cohort of 183 human bladder cancer patients that RON expression was overexpressed in 32.8% of primary bladder tumors, in which RON expression correlated positively with histological grading, tumor size, nonpapillary morphology, and tumor staging[21]. RON activation can activate JNK/HIF2α signaling pathway that results in an increase of cell migration and invasion of bladder cancer cells through modulation of inhibition of HIF-2α ubiquitination and MMP12 expression[32]. Besides, RON expression can be downregulated by the binding of hsa-miR-659-3p[32]. Also, tumor hypoxia can trigger RON nuclear translocation, which serves as a transcriptional factor to bind with HIF-1α and c-Jun promoter, resulting in cell proliferation, survival adaptation, and migration[33]. On the other hand, blockade of RON expression can induce cell cycle arrest at the G_1/S boundary, increased expression of cyclin-dependent kinase (CDK) inhibitor 1A and 1B, and decreased expression of cyclin D1, cyclin D3, and CDK4[34]. In contrast, Comperat et al[23] reported a small cohort of 40 UTUC patients, in which 83% (33% weak, and 50% strong) of UTUCs exhibited RON expression and there was lack of any statistical significance. Sagalowsky commented that the small number of UTUC compared with the bladder UC restricted the ability to perform studies on upper tract tumors. It requires not only extrapolation from studies of bladder tumors but also enough number of UTUC patients to reappraise the significance of any potential molecules investigated in human bladder cancer. In the current study of 124 UTUC patients, we reported that 36.3% of patients exhibited RON expression and lack of any statistical significance with tumor grading, staging, or size. Despite this, RON expression correlates borderlinely with distant metastases and is an independent factor for disease-specific survival in UTUC patients. These results highlight the clinical significance of RON expression in UTUC patients following nephroureterectomy.

Both the EGFR and RON are members of the receptor tyrosine kinase family and the collaboration of each two members has been proven to play pivotal roles in regulating cell proliferation, migration, and the cell cycle progress through stabling cell membrane[35] and transcriptional regulation[36]. In human bladder cancer, there were 33.3% detected with coexpression of RON/EGFR that was significantly associated with tumor invasion the risk of local recurrence, and decreased patient survival. Both coexpression of RON/EGFR and tumor staging were independent worse prognostic factors[22] Nuclear translocation of RON/EGFR under hypoxia contributes chemoresistance to anticancer agents target for DNA double-strand break[37]. Such internalized complex of RON/EGFR can bind at least 134 target genes involved in 3 known stress-responsive networks, including p53, c-JNK, and PI3K/Akt signaling pathways. These findings pinpointed the importance of RON/EGFR coexpression in response to acute stress (starvation or hypoxia)[33,36]. Our previous studies showed that HER2/neu expression predicts disease progression and disease-related survival in UTUC patients, rather than EGFR, HER3[24]. Similarly, tumor staging, HER2/neu, EGFR/Her2/neu, and HER2/neu/HER3 were associated with short survival in human bladder cancer[25]. These findings highlight the prognostic significance of any 2-member coexpression, particularly collaboration with HER2/neu expression. Our recent study also showed that patients with HER2/neu-positive UTUC with micropapillary variant (MPUC) had a significantly higher risk of metastasis compared with HER2/neu-negative MPUC. MPUC is an aggressive variant of UTUC and usually presents as a small locally advanced disease. HER2 immunohistochemistry may identify the subset of patients with micropapillary UTUC that are candidates for targeted therapy[38]. In the current study, not only as an independent prognostic factor for disease-specific survival but also RON coexpressed with HER2/neu predicts the worst outcome in UTUC patients. These findings provide the need for RON-/HER/neu-targeted therapy.

Currently, several ADC targeted for RON- or HER2/neu-expressing tumors have been investigated in the various phase clinical trials. For example, anti-RON Zt/g4-MMAE ADCs have the potential as a novel modality for triple-negative breast cancer and pancreatic cancer[39,40]. As for HER2/neu-targeted ADC, RC-48 has been conducted in a phase II clinical trial for treating HER2 expressing metastatic UC[41]. Although there were several

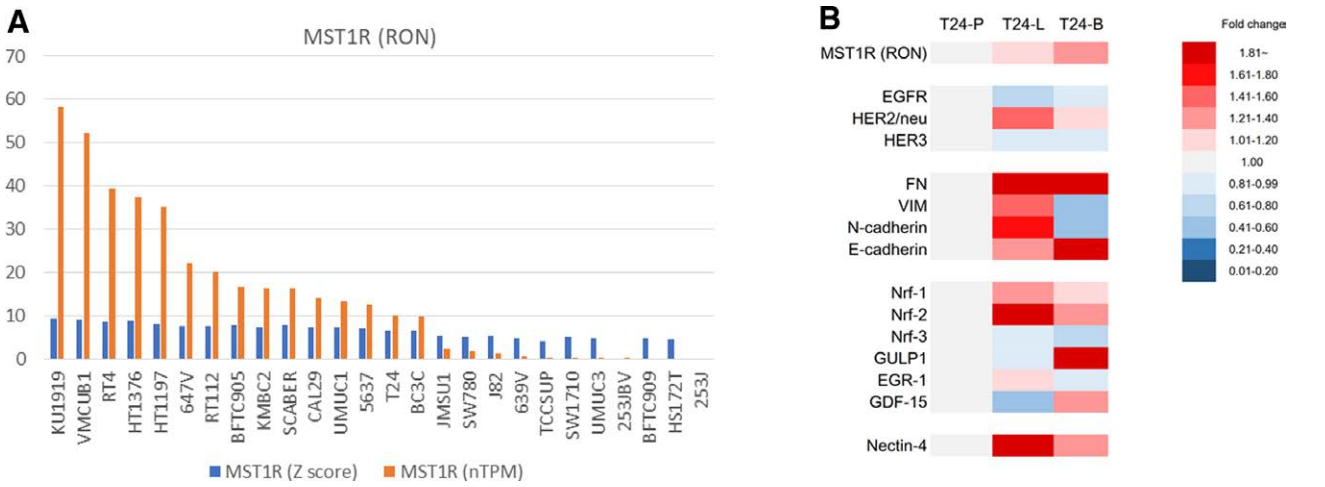

Figure 3. RON mRNA expression (A) MST1R (RON) mRNA expression in urothelial cell lines from Cancer Cell Line Encyclopedia of the Broad Institute and Novartis. (B) Heat map of RT-PCR assays for mRNA expression as indicated from parental T24 cells and its sublines. MST1R, macrophage stimulating 1 receptor; RON, recepteur d'origine nantais; RT-PCR, reverse transcription polymerase chain reaction; T24-B, to the bone; T24-L, derived from T24 cells metastasis to the lung; T24-P, parental T24 cells.

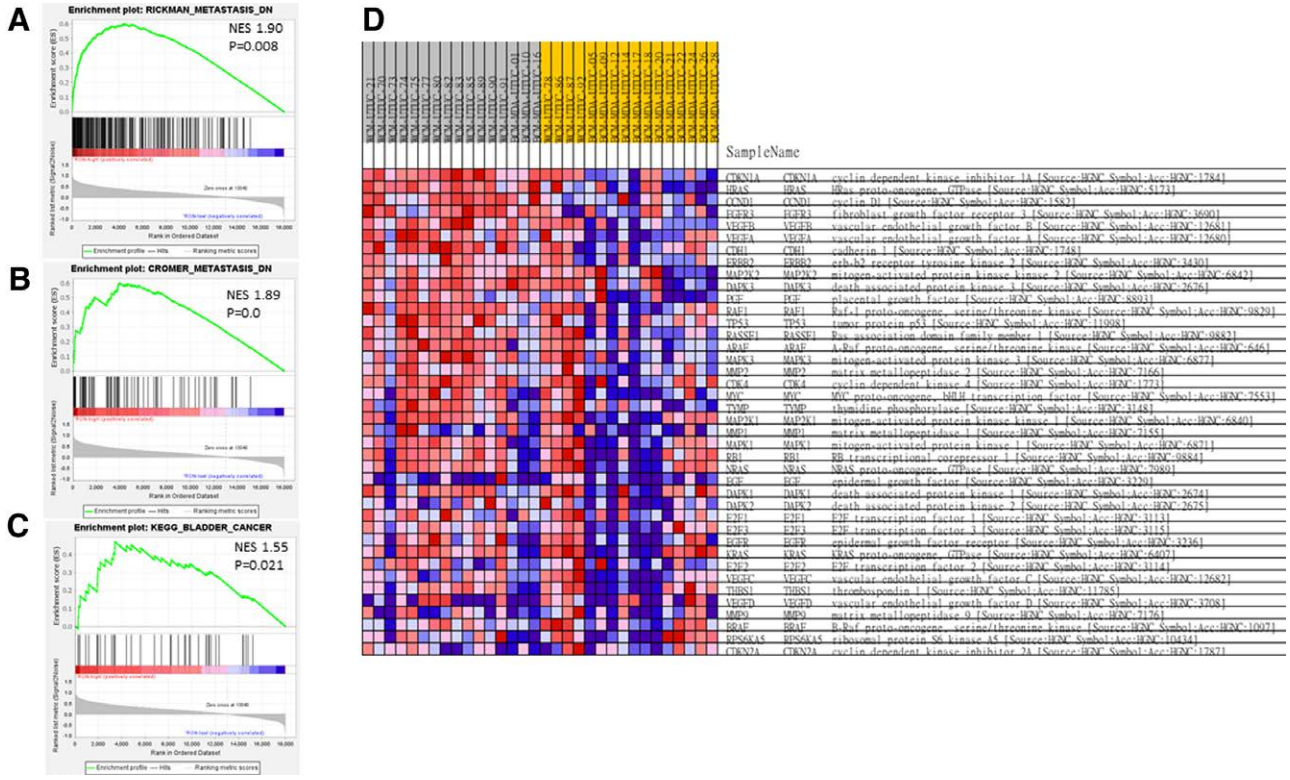

Figure 4. Gene set enrichment analysis (GSEA) in UTUC tumors (Cornell/Baylor/MDACC[28]). (A) Rickman metastasis model, (B) Cromer metastasis model, and (C) KEGG bladder cancer model. (D) Heat map analysis based on high- or low-RON expression. KEGG, Kyoto Encyclopedia of Genes and Genomes; MDACC, MD Anderson Cancer Center; RON, recepteur d'origine nantais; UTUC, upper tract urothelial carcinoma.

obstacles to be overcome, anti-RON targeted with or without the collaboration of anti-HER2/neu targeting therapy (Dual ADC) may be the direction for precise therapy in UTUC patients[11].

There were 3 limitations in the current study. First, the current study is retrospective, it is difficult to draw strong conclusions alone. Despite this, our finding can be supported with the data mining analysis from the Cornel group and in vitro study of T24 cell sublines. Second, T24 cell is a cancer cell line derived from the human bladder, which cannot totally represent the upper tract tumors. Third, coexpression of RON with any EGFR family member detected in IHC may not represent RON/EGFR, RON/HER2/neu, or RON/HER3 complex. The real molecular interplay still requires further investigation.

5. Conclusion

In the current study, we explored the clinicopathological significance of RON expression in human UTUC patients, particularly those with coexpression of EGFR family members (EGFR, HER2, and HER3). Together with the in vitro T24 cell line study and the data mining analysis from the Cornel group, our result demonstrated that RON expression predicts metastatic

potential and worse survival in human UTUC, particularly coexpressed with HER2/neu.

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