Original article

Significance of HER2 expression in patients with upper tract urothelial carcinoma: A meta-analysis

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Keywords:
edermal growth factor receptor family expression assessment in UTUC patients were reviewed and a systemic review of intravesical recurrence, progression, and overall survival (OS) from survival analyses.

Results: The pooled results showed that HER2 expression is significantly associated with higher stage, but not with tumor grade in patients with UTUC. Thirty-five articles from 679 articles related to the epidermal growth factor receptor family expression assessment in UTUC patients were reviewed and seven papers were found to be fit for analyses. The estimates included the odds ratio (OR), distribution related to stage and grade, hazard ratios (HRs), and 95% confidence intervals (CIs) from survival analyses of intravesical recurrence, progression, and overall survival (OS).

Conclusion: Existing studies on UTUC are heterogeneous and limited. Our analysis suggests that HER2 expression plays an important role in cancer recurrence in the urinary bladder after the primary treatment of UTUC.

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1. Introduction

Urothelial carcinomas (UCs) are composed of tumors arising from the uroepithelia of the urinary tract, including the renal pelvis, ureter, and urinary bladder. Upper tract urothelial carcinoma (UTUC) refers to UCs of the renal pelvis or ureter. Several environmental factors may contribute to the formation of UTUC, including exposure to tobacco or aromatic amines, and consumption of phenacetin or aristolochic acid-containing food or Chinese herbs. Some factors are more specific for UTUC, whereas others are similar to those associated with bladder cancer. In Taiwan, an unusual higher incidence of UTUC had been reported in the population on the southwest coast, and recent research has suggested that aristolochic acid-related Chinese medicine is responsible for this phenomenon.

Although only a few studies have focused on UTUC owing to its rarity all over the world, whether or not UTUCs and bladder cancer are disparate twins should be identified. Therefore, more evidence is required to understand the real characteristics of this tumor at the clinical and molecular levels.

The epidermal growth factor receptor (EGFR) receptor family (also known as the ErbB family) is a major class of receptor tyrosine kinase proto-oncogenes. They play important roles in several cellular processes, such as cell proliferation, migration, adhesion, and, potentially, cellular transformation, including urothelial carcinogenesis. The EGFR family consists of four members, including HER1 (EGFR and ErbB1), HER2 (erbB2 and HER2/neu), HER3 (ErbB3), and HER4 (ErbB4). Through homodimerization or heterodimerization by binding two monomers, the EGFR family signaling regulates biological processes important for the pathogenesis of human malignancies, including lung cancer, breast cancer, and prostate cancer. Despite this, the clinical relevance of EGFR
signaling reveals that it has tissue-specific characteristics. For example, the EGFR/HER2-MAPK axis is important in human breast cancer, whereas the kinase activity of the HER2/HER3 axis plays a major role in DNA binding and androgen receptor stability in prostate cancer. In terms of bladder cancer, a previous meta-analysis reported that the overall risks of disease progression for patients with EGFR or HER2 overexpression were 4.5 [95% confidence interval (CI): 2.5–8.4] and 1.1 (95% CI: 0.6–1.9), and the risks of mortality were 3.0 (95% CI: 1.6–5.9) and 1.1 (95% CI: 1.0–1.2), respectively. In addition, a recent meta-analysis showed that HER2 expression was significantly associated with tumor grade (high grade vs. low grade: odds ratio (OR) 4.08; 95% CI 1.29–12.93) and lymph node metastasis (positive vs. negative: OR 1.71; 95% CI 1.07–2.75).

To investigate the clinical relevance of HER2 expression in UTUC patients, we systematically reviewed papers published in the past 3 decades on EGFR family expression and their impact on patient prognosis. Our objectives were to confirm the significance of HER2 expression in predicting subsequent bladder cancer recurrence, progression, and mortality in UTUC patients by conducting a meta-analysis of available estimates.

2. Materials and methods

2.1. Search strategy and selection criteria

Original articles published between January 1990 and January 2015 and showing prognostic significance or clinical relevance of expression or amplification of the EGFR family in patients with bladder cancer or UTUC were systematically reviewed. Using the keywords “EGF,” “ECF-R,” “HER2,” “HER3,” “HER4,” “c-erb-B1,” “c-erb-B2,” “c-erb-B3,” “c-erb-B4,” “neu,” “epidermal growth factor,” “bladder neoplasms,” “urothelial carcinoma,” “upper tract urothelial carcinoma,” “or transitional cell carcinomas-in-humans,” we identified 679 relevant articles in the PubMed database. The number of studies was reduced to 35 by limiting the search to “renal pelvis,” “ureter,” or “upper tract.” Duplicate data, identified in the same cohort by reviewing the interstudy similarities of investigators, source of patients, recruitment period, or inclusion criteria, were excluded from this analysis.

2.2. Data extraction, handling, and analyses

The database was created by including relevant data based on study design, patient outcomes, tumor characteristics, statistical analyses, biological samples, analytical methods, and the status of expression or gene amplification of each EGFR family member. The data were extracted using the following methods. Because some articles only provided the p values or statements of whether results were significant, the analyses of subsequent bladder cancer recurrence, progression, and death were based on definitions in the original reports. In brief, “recurrence” was defined as a tumor that was found in the urinary bladder after definite surgery, and “disease progression” was defined as any tumor with a higher T stage in the local tumor, node, or metastasis. The p values (or statements of significance) were extracted from association analyses (such as χ² test, Student t test, Mann–Whitney U test, and logistic regression), and the risk estimates and 95% CIs were extracted from univariate (Kaplan–Meier curves and the log-rank test) and multivariate survival (Cox regression) analyses.

2.3. Statistical analysis

For recurrence-free survival (RFS), progression-free survival (PFS), and overall survival (OS), hazard ratios (HRs) and their corresponding 95% CIs were pooled for the meta-analysis. The association between HER2 overexpression and histological grade and tumor stage were pooled and calculated using ORs and their CIs. The Wolf method was used to combine the risk estimates by applying the inverse of variance as the weighting factor. Potential sources of heterogeneity were investigated using graphical methods, such as the funnel plot. A fixed-effects model was used when P tests showed <50% heterogeneity. In cases of substantial heterogeneity, random-effect models were used. Sensitivity analysis was conducted by sequentially omitting individual studies to validate the stability of the outcomes, and publication bias was visually evaluated using funnel plot. The extent to which the combined risk estimate was affected by individual study was examined by consecutively omitting every study from the meta-analysis. Meta-regression was used to explain the potential heterogeneity arising from the same characteristics included in the p value analysis. The publication bias was investigated using Egger’s and Begg’s graphical methods. The analyses were done using Comprehensive Meta Analysis Version 2 (Biostat, Inc., Englewood, NJ, USA). Significance was set at p < 0.05 (two sided).

3. Results

3.1. Study selection and characteristics

A total of 679 publications were initially identified through database searching, and 35 potential relevant reports remained after removing irrelevant or duplicate publications based on title and abstract screening. Among 28 full-text articles that were available for eligibility assessment, 21 articles were further excluded because they lacked key information for estimating OR, or HR and seven eligible articles were finally identified (Table 1). The flow diagram shows the article selection process (Fig. 1). The main characteristics of the included studies are summarized in Table 1. Among the seven studies, a total of 593 UTUC patients (ranging

Table 1

<table>
<thead>
<tr>
<th>References</th>
<th>No. of patients</th>
<th>Stage</th>
<th>IHC Method or Ab</th>
<th>Cutoff</th>
<th>Expression (%)</th>
<th>Follow up (mo), median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imai et al (1995)</td>
<td>30</td>
<td>Ta-3</td>
<td>Rabbit c-erbB2 pAb (Nichirei, Tokyo, Japan)</td>
<td>&gt;25%</td>
<td>43.3</td>
<td>22.2 (3.9–78.2)</td>
</tr>
<tr>
<td>Langner et al (2005)</td>
<td>53</td>
<td>T1-3</td>
<td>HercepTest (Dako, Hamburg, Germany)</td>
<td>&gt;=2</td>
<td>52.8</td>
<td>26 (1–148)</td>
</tr>
<tr>
<td>Tsai et al (2005)</td>
<td>94</td>
<td>Ta-4</td>
<td>Mouse anti-erbB2 mAb (CB11; BioGenex, San Ramon, CA)</td>
<td>&gt;5%</td>
<td>13.8</td>
<td>40 (1–177)</td>
</tr>
<tr>
<td>Izquierdo et al (2010)</td>
<td>100</td>
<td>Ta-4</td>
<td>Rabbit c-erbB2 pAb (A0485; Dako, Denmark)</td>
<td>NA</td>
<td>10.0</td>
<td>33.03 (0.3–182.7)</td>
</tr>
<tr>
<td>Galanakis et al (2013)</td>
<td>99</td>
<td>Ta-4</td>
<td>Mouse anti-erbB2 mAb (CB11; Biocare Medical, Concord, CA, USA)</td>
<td>&gt;5%</td>
<td>64.8</td>
<td>52.5 (1–127)</td>
</tr>
<tr>
<td>Elsons et al (2014)</td>
<td>46</td>
<td>Ta-4</td>
<td>Mouse anti-HER2 mAb (Dako, Carpinteria, CA, USA)</td>
<td>&gt;5%</td>
<td>73.9</td>
<td>NA</td>
</tr>
<tr>
<td>Sasaki et al (2014)</td>
<td>171</td>
<td>Ta-4</td>
<td>Anti-HER-2/neu mAb (485, Roche, Tokyo, Japan)</td>
<td>&gt;2, &gt;10%</td>
<td>18.1</td>
<td>NA</td>
</tr>
</tbody>
</table>

Ab = antibody; IHC = immunohistochemistry; mAb = monoclonal antibody; NA = not available; pAb = polyclonal antibody.

a The used antibody or tested method.

b % positive cells or scoring.

c Ventana Medical Systems Inc.
from 30 patients/study to 171 patients/study) were reported. All included studies were retrospective cohort studies.

### 3.2. Study findings and meta-analysis

#### 3.2.1. Correlation of HER2 with tumor stage and histological grade

The HER2-positive rates across the seven studies ranged from 13.8% to 73.9%, with a pooled positive rate of 32.5% (193/593). Five studies reported the association of HER2 expression with tumor stage and four studies involved the histological grade. Moderate heterogeneity ($I^2 = 38\%$; $p = 0.168$) and high heterogeneity ($I^2 = 57\%$; $p = 0.070$) regarding tumor stage and histological-grade status, respectively, were determined among the studies. Thus, the fixed-effects and random-effects models were, respectively, used to pool the data for the tumor stage and histological grade results. Meta-analysis showed that the HER2 expression was significantly associated with tumor stage (pooled OR, 2.05; 95% CI, 1.15–3.68; $p = 0.016$; Fig. 2A) and histological grade (pooled OR, 4.73; 95% CI, 0.80–27.8; $p = 0.086$; Fig. 2B).

#### 3.2.2. Correlation of HER2 with survival

For each survival analysis (RFS, PFS, and OS), three individual studies reported each own outcome. High heterogeneity ($I^2$ of 69% and 63%; $p$ values, 0.038 and 0.067, respectively) and low heterogeneity ($I^2 = 0\%$; $p = 0.792$) regarding PFS, OS, and RFS outcomes,
respectively, were determined among the studies. Thus, the random-effects and fixed-effects models were, respectively, used to pool the data for the PFS, OS, and RFS results. Meta-analysis showed that the HER2 expression was significantly associated with decreased RFS (pooled HR, 4.32; 95% CI, 2.17–8.60; \( p < 0.0001 \); Fig. 3A). However, there is lack of statistical significance in terms of PFS and OS (HR, 2.08; 95% CI, 0.46–9.32; \( p = 0.339 \) and HR, 1.06; 95% CI, 0.48–2.37; \( p = 0.879 \), respectively; Figs. 3B and 3C).

### 3.2.3. Sensitivity analysis and publication bias

Sensitivity analysis showed that the study result of Imai et al\(^{12} \) is influencing the meta-analysis result. After excluding this study, the pooled correlation with histological grade became significant (pooled OR, 10.6; 95% CI, 3.2–35.4; \( p < 0.0001 \)). For publication bias analysis, funnel plots for the included studies showed no obvious sign of asymmetry.

### 4. Discussion

This meta-analysis revealed that estimates of the significance of HER2 expression vary substantially between studies in patients with UTUC, which is somewhat unlike bladder cancer.\(^{10,11} \) HER2 expression is predictive of intravesical recurrence in UTUC patients following nephroureterectomy and is significantly associated with

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**Fig. 3.** Forest plots of prognostic significance of HER2 expression on (A) intravesical recurrence, (B) disease progression, and (C) overall survival. Hazard ratios and 95% confidence intervals (CIs) for upper tract urothelial carcinoma patients according to HER2 expression.

<table>
<thead>
<tr>
<th>Study name</th>
<th>Statistics for each study</th>
<th>Hazard ratio and 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A - HER2 and intravesical recurrence</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hazard ratio</td>
<td>Lower limit</td>
<td>Upper limit</td>
</tr>
<tr>
<td>Tsai et al (2005)(^{31} )</td>
<td>4.600</td>
<td>1.058</td>
</tr>
<tr>
<td>Galanakis et al (2013)(^{32} )</td>
<td>7.180</td>
<td>1.300</td>
</tr>
<tr>
<td>Sasaki et al (2014)(^{36} )</td>
<td>3.700</td>
<td>1.542</td>
</tr>
<tr>
<td>4.322</td>
<td>2.171</td>
<td>8.603</td>
</tr>
</tbody>
</table>

Fixed model
Heterogeneity: \( \tau^2=0; Q=0.467, df (Q)=2 (p=0.792); I^2=0\% 

| **B - HER2 and progression** |                     |                         |
| Study name               | Statistics for each study | Hazard ratio and 95% CI |
| Hazard ratio | Lower limit | Upper limit | Z value | \( p \) value |
| Langner et al (2005)\(^{30} \) | 12.800 | 1.425 | 114.997 | 2.276 | 0.023 |
| Tsai et al (2005)\(^{31} \) | 2.400 | 1.226 | 4.699 | 2.554 | 0.011 |
| Izquierdo et al (2010)\(^{32} \) | 0.424 | 0.085 | 2.115 | -1.048 | 0.295 |
| 2.079 | 0.464 | 9.315 | 0.956 | 0.339 | |

Random model
Heterogeneity: \( \tau^2=1.193; Q=6.559, df (Q)=2 (p=0.038); I^2=69\% 

| **C - HER2 and overall survival** |                     |                         |
| Study name               | Statistics for each study | Hazard ratio and 95% CI |
| Hazard ratio | Lower limit | Upper limit | Z value | \( p \) value |
| Tsai et al (2005)\(^{31} \) | 1.900 | 1.100 | 3.282 | 2.302 | 0.021 |
| Galanakis et al (2013)\(^{33} \) | 0.650 | 0.420 | 1.720 | -0.452 | 0.651 |
| Izquierdo et al (2010)\(^{32} \) | 0.360 | 0.065 | 1.994 | -1.170 | 0.242 |
| 1.064 | 0.477 | 2.372 | 0.152 | 0.679 | |

Random model
Heterogeneity: \( \tau^2=0.295 Q=5.409, df (Q)=2 (p=0.067); I^2=63\% 

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higher tumor stage. Nonetheless, HER2 expression did not show any significant value in predicting UTUC patients’ OS and progression. By contrast, HER2 expression is weakly predictive of cancer mortality and significantly associated with nodal metastasis in bladder cancer patients.19,10,11 This might be one of the reasons why only few studies have examined the implications of HER2 expression in UTUCs. Therefore, these findings should be interpreted carefully because relatively few studies were eligible for analysis.

In 1992, HER2 gene amplification was first reported to exist in UTUC and urinary bladder cancer.14 Along with the investigation of EGFR, EGF, and HER2 expressions in bladder cancer;15,16 Imai et al12 reported 2-decades-ago that the expressions of EGF and c-erbB2 can predict subsequent bladder cancer tumor recurrence following primary treatment. Since then, only limited studies have discussed the clinical significance or biological role of HER2 protein in UTUCs. Therefore, our findings can provide more strengthening information about the significance of HER2 expression in UTUC.

Although several studies have reported that overexpressed EGFR family members drive the development of human cancers, such as breast cancer,17 little is known about the molecular mechanism of EGFR family signaling in UTUC. Recently, aristolochic acid has been shown to be an important chemical carcinogen for UTUC in Taiwan and the Balkan areas.15,19 It is well documented that aristolochic acid can induce renal fibrosis-related nephropathy in rats and humans20; however, it is still unclear how this substance induces the formation of this malignancy, or how it influences the molecular signal transduction through aristolochic acid–DNA adduct. Nevertheless, EGF/EGFR signaling is responsible for the promotion of tumor growth factor–β-dependent renal fibrosis after acute kidney injury through an unpredicted signaling activation such as extracellular signal-regulated kinase.21,22 Therefore, it is worth investigating the biological role of EGFR family signaling such as EGFR or HER2 expression in the formation of aristolochic acid-related UTUC.

There were several discouraging results of clinical trials focusing on blocking EGFR or HER2 signaling in treating UC of urinary bladder or UTUCs,23,24 despite evidence of the significance of EGFR or HER2 signaling in bladder cancer. Little progress is noted in treating advanced UC compared with other types of malignancies, even with chemotherapy,25,26 although some small series showed a promising sensitivity to everolimus in bladder cancer with tuberous sclerosis complex 1 mutation.27 Recent genomic studies demonstrated that diverse genomic aberrations existed in bladder UC,28,29 and that bladder cancers split into three pan-cancer subtypes. However, the question of whether UTUCs exhibit similar biological characteristics as those of bladder cancer remains unanswered. The answer to this question will confirm whether or not UTUC and bladder cancer are disparate twins. Therefore, it is worth further investigating the difference between these twins or the influence of aristolochic acid.

There were some limitations or inconsistencies in this meta-analysis. For example, there was an inconsistency regarding the efficacy of HER2 expression in predicting the risk of progression and mortality in UTUC patients except for intravesical recurrence. Such incongruity is somewhat consistent with the discrepancies in this meta-analysis. The factors include a wide spectrum of disease statuses (Ta-4) in limited cases, variability in the immunostaining assays used, biases from scoring, cutoff points, and interpretation. There are additional contributing factors, namely, methods used for detecting events of interest, treatment methods, the length of follow up, and inconsistency in the inclusion of clinical and pathological factors for multivariate analysis. Finally, the risks calculated in our meta-analysis may be overestimated by reporting biases because HRs and 95% CIs were not described when associations were not significant or published in the local journal.

5. Conclusion

In conclusion, this meta-analysis has revealed a significant association between HER2 expression, and bladder cancer recurrence and tumor stage in UTUC patients. Considering current molecular information, assessing HER2 expression provided only limited prognostic information for UTUC patients. Although being considered a twin with bladder cancer, little attention has been paid to studying UTUC. Limited information is available on the molecular carcinogenesis of the aristolochic acid-related UTUCs, especially about its relationship with EGFR or HER2 signaling. Therefore, there is a need for more additional investigations in this regard.

Conflicts of interest

All contributing authors declare no conflicts of interest.

References


