**High Levels of Thymidine Phosphorylase as an Independent Prognostic Factor in Renal Cell Carcinoma**

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**Background:** We investigated whether thymidine phosphorylase (TP) protein level in renal cell carcinoma (RCC) correlates with clinicopathological characteristics and clinical outcomes.

**Methods:** TP protein level was measured in 116 RCC specimens and in 90 non-neoplastic kidney tissues using a sandwich-type enzyme-linked immunosolvent assay.

**Results:** The median TP protein level in RCC tissues was 9.76-fold (range, 3.2–933.9) higher than those in non-neoplastic kidney tissues \((P < 0.0001)\). TP protein level was correlated with \(T\) classification, histological grade and mode of infiltration. TP as a prognostic variable was studied using a logistic regression model. TP at higher levels (128 U/mg protein or greater) would play a role as an independent prognostic factor (odds ratio, 13.73; 95% confidence interval, 2.09–90.41; \(P = 0.0064)\).

**Conclusion:** TP at high levels can be regarded as an unfavorable independent prognostic factor. These results may pave a way for a novel approach to effective treatment of RCC.

**Key words:** renal cell carcinoma – thymidine phosphorylase – prognostic factor

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**INTRODUCTION**

Tumor angiogenesis is a complicated multistep process that involves extracellular matrix remodeling, endothelial cell migration and proliferation, capillary differentiation and anastomosis.

Thymidine phosphorylase (TP), known as a platelet-derived endothelial cell growth factor (PD-ECDF) (1–4), may play an important role in both activation of 5-fluorouracil (5-FU) drugs and tumor angiogenesis (5). TP exerts angiogenic activity \(in vivo\) while its enzymatic activity is indispensable for its angiogenic effect (6). Many investigators have reported that TP levels are higher in various types of malignant tumor than in the adjacent non-neoplastic tissues, such as renal cell carcinoma (RCC) (7–10).

In this study, we determined TP protein levels in both RCC and non-neoplastic kidney tissues by using ELISA, which has been reported to correlate with the TP activity levels (8,11), and investigated the association of the TP protein levels with clinicopathological characteristics and clinical outcome in patients with RCC.

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**PATIENTS AND METHODS**

**Patients**

We investigated 116 patients (88 men and 28 women) with RCC. The patient characteristics are shown in Table 1. Tumors were removed surgically in the Department of Urology at Hamamatsu University Hospital and affiliated hospitals between December 1997 and July 2004. None of the patients had received prior chemotherapy, irradiation, or embolization. All investigations were performed after informed consent was obtained according to institutional ethical committee rules.

The mean age at surgery was 61.8 years with a range of 35–84. Among 116 patients with RCC, 98 had no metastasis and 18 had distant metastasis at the time of nephrectomy. Survival data were updated in September 2004. The median follow-up duration was 39 months (range, 1.2–97.9). Seventeen patients died of RCC and 5 died of other diseases. Ninety-four patients with RCC in this study remained alive throughout the follow-up period. The tumors were graded according to the criteria of the Japanese Urological Association (12) and were staged according to the TNM system (1997) (13). Moreover, they were also classified into histological subtypes. Tumor size was defined according to the largest diameter of the tumor. T stage was classified into two groups, pT1 + pT2 and pT3 + pT4. The node category was determined by intraoperative and preoperative findings such as ultrasonography.
computed tomography scans and magnetic resonance imaging. The metastasis category was based on the results of computed tomography and bone scans. C-reactive protein (CRP) was divided into two groups according to reference interval. Patients and tumor profiles are listed in Table 1.

**SPECIMENS AND TISSUE HOMOGENATE**

One hundred sixteen tumor specimens and 90 non-neoplastic kidney specimens in the farthest and separable region from the tumor were obtained at surgery from 116 patients with RCC. Non-neoplastic tissues were defined as macroscopically normal tissues with no tumor cells being observed histopathologically. Both tissue specimens were kept at −80°C before use.

An ELISA was used to measure TP levels in RCC and non-neoplastic tissues. Tissue specimens were first homogenized in 10-fold volume of 10 mM Tris–HCl buffer (pH 7.4) containing 15 mM NaCl, 1.5 mM MgCl₂ and 50 μM potassium phosphate, and then centrifuged at 10,000×g for 15 min. The supernatant was stored at −80°C until use. The protein concentration of the supernatant extracted from tumor tissues was determined by using a DC protein Assay Kit (Bio Rad Laboratories, Hercules, CA). The TP protein levels in RCC and non-neoplastic kidney tissues were measured by a sandwich ELISA with two monoclonal antibodies (MoAb) specific to human TP (11). The amount of TP was calibrated with the standard solution. TP protein levels in tumor tissues were expressed as U/mg protein, where 1 U is the amount equivalent to 1 μg of 5-FU produced in an hour as an enzyme activity.

**STATISTICAL ANALYSIS**

All data are presented as the mean (±SD). TP protein levels in tumors and non-neoplastic tissues were compared using the Wilcoxon single rank test. The Mann–Whitney U-test or Fisher’s exact test was used for the evaluation of the relationship between TP protein level in tumors and the clinicopathological features. Survival analysis was performed by Kaplan and Meier method. The differences between survival curves were tested by log-rank test. Statistical significance was then chosen for a logistic regression model to determine the prognostic value. All P-values presented are two-sided. Statistical significance was calculated by using package STATVIEW Ver. 5 (Abacus Concepts, CA, USA). P-values of <0.05 were considered to be statistically significant.

**RESULTS**

We examined the enzymatic activity of TP in 116 RCCs and 90 non-neoplastic kidney tissue specimens (Fig 1). The median value of TP protein level in RCCs (128.0 U/mg protein) was 10-fold higher than those in non-neoplastic kidney tissues (11.7 U/mg protein). The median activity ratio (carcinoma/non-neoplastic tissue) was 9.76.
Among patient factors, significant relation could not be evidenced. In contrast, however, TP protein level in tumor was found significantly higher in progressive T classification, histological grade and mode of infiltration among tumor factors (Table 1 and Fig. 2).

We investigated whether TP protein level would contribute as an independent prognostic variable. To evaluate cause-specific survival based on the TP protein level of RCC, 116 patients were divided into two groups at the median value (high TP, 128.0 U/mg protein ≤ TP value; low TP, TP value < 128 U/mg protein). Five patients with metastasis are included in low TP group, while 13 patients in high TP group. As depicted in Table 2, univariate analysis indicated that CRP, tumor status, lymph node metastasis, distant metastasis, cell type, histological grade, mode of infiltration, venous invasion and TP protein level in tumor would be significant prognostic factors. A logistic regression model was constructed using the aforesaid established prognostic factors. Multivariate analysis showed that both cell type ($P = 0.0033$) and TP activity ($P = 0.0099$) were independent significant factors, with the odds ratios being 8.552 and 11.180, respectively. As shown in Fig. 3, the 5-year cause specific survival rate of the patients with low TP was 93.6%, whereas that of patients with high TP was 68.6%. A significantly higher rate of cause-specific survival occurred in the patients with low TP than with high TP ($P = 0.0024$). In addition, as shown in Fig. 4, when all patients were divided into pT1 + pT2 and pT3 + pT4 or with and without metastasis, high level of TP was significantly unfavorable prognostic

![Figure 1](http://jjco.oxfordjournals.org/) TP level in tumor and non-neoplastic tissues. Boxes indicate the range of a half of data (between upper and lower quartile). Lines in boxes show median values. Bars at both sides of boxes denote the ranges of lower 10% and upper 90%; and open circles indicate values beyond these ranges.

![Figure 2](http://jjco.oxfordjournals.org/) TP protein level according to T classification (A), histological grade (B) and mode of infiltration (C) in renal cell carcinoma. Data are expressed as the mean ± standard deviation.
DISCUSSION

PD-ECGF/TP is a mitogenic and angiogenic factor derived from platelets, while the enzymatic activity of TP is required for angiogenesis (6). TP activity in several types of malignant tumors is higher compared with those in the adjacent non-neoplastic tissue (14–17). Interestingly, it is also evidenced that inhibitors of TP inhibit both invasion and metastasis in vivo model systems (18), suggesting that the angiogenic activity of TP might contribute to progression of some tumors.

Concerning RCC, Imazono et al. (7) and Kinsui et al. (8) reported that the median TP enzymatic activity in RCC was 9-fold higher than those in non-cancerous renal tissue. Our study also revealed that TP protein level in RCC was elevated to become 9.76-fold higher compared to that of paired non-neoplastic tissue.

Table 2. Prognostic factor for survival by univariate and multivariate analysis

<table>
<thead>
<tr>
<th>Factor</th>
<th>Categories</th>
<th>No. of patients</th>
<th>Univariate P-value</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>Multivariate P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP (U/mg protein)</td>
<td>&lt;128/&gt;128</td>
<td>58/58</td>
<td>0.0024</td>
<td>11.180</td>
<td>1.783–70.086</td>
<td>0.0099</td>
</tr>
<tr>
<td>T classification</td>
<td>1–2/3–4</td>
<td>88/28</td>
<td>&lt;0.0001</td>
<td>2.408</td>
<td>0.564–10.276</td>
<td>0.2353</td>
</tr>
<tr>
<td>N classification</td>
<td>0/1–2</td>
<td>113/3</td>
<td>&lt;0.0001</td>
<td>7.354</td>
<td>0.406–133.299</td>
<td>0.1771</td>
</tr>
<tr>
<td>M classification</td>
<td>0/1</td>
<td>98/18</td>
<td>&lt;0.0001</td>
<td>3.488</td>
<td>0.723–16.836</td>
<td>0.2955</td>
</tr>
<tr>
<td>Cell type</td>
<td>Clear cell/Others</td>
<td>100/15</td>
<td>0.0099</td>
<td>8.552</td>
<td>2.042–35.808</td>
<td>0.0033</td>
</tr>
<tr>
<td>Histological grade</td>
<td>1–2/3</td>
<td>102/13</td>
<td>0.0004</td>
<td>3.231</td>
<td>0.885–11.797</td>
<td>0.0759</td>
</tr>
<tr>
<td>Mode of infiltration</td>
<td>0–β/γ</td>
<td>106/4</td>
<td>0.0062</td>
<td>0.682</td>
<td>0.026–17.830</td>
<td>0.8183</td>
</tr>
<tr>
<td>Venous invasion</td>
<td>Absent/Present</td>
<td>64/41</td>
<td>0.0038</td>
<td>1.291</td>
<td>0.255–6.538</td>
<td>0.7580</td>
</tr>
<tr>
<td>CRP</td>
<td>Low/High</td>
<td>68/48</td>
<td>0.0194</td>
<td>0.549</td>
<td>0.132–2.282</td>
<td>0.4093</td>
</tr>
<tr>
<td>Sex</td>
<td>Male/Female</td>
<td>88/28</td>
<td>0.7463</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>&lt;62/&gt;62</td>
<td>57/59</td>
<td>0.3495</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Performance status</td>
<td>0/1–2</td>
<td>101/10</td>
<td>0.1790</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mode of discovery</td>
<td>Incidental/Symptomatic</td>
<td>73/38</td>
<td>0.0683</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Number of cases in unknown factors: Performance status, 5; Mode of discovery, 5; Cell type, 1; Histological grade, 1; Mode of infiltration, 6; Venous invasion, 11. CI, confidence interval.

Figure 3. Cause specific survival in the groups of low TP (<128.0 U/mg protein) and high TP (>128.0 U/mg protein) among all patients with renal cell carcinoma.

Viewed together, it is postulated that the value of TP expression might be related to prognosis. In fact, Imazono et al. (7) elucidated by multivariate analysis that TP overexpression was an independent prognostic factor in RCC. Suzuki et al. (20) also reported that there was a significant correlation between survival rates and TP expression, whereas angiogenesis did not correlate with TP expression or survival. On the other hand, Kinsui et al. (8) found no correlation between TP level and clinicopathological findings except for venous invasion and survival curves. The present study indicated that high TP protein level in tumor was an independent prognostic factor in RCC. Generally, high T stage or distant metastasis leads to the poor prognosis in various cancers. In order to more fully
investigate the possibility of TP as unfavorable prognostic factor, we divided pT and M classification into pT1 + pT2 and pT3 + pT4, and with or without metastasis, respectively, resulting in the high level of TP appeared to be a trend toward poor prognosis. We also indicated that not clear cell carcinoma but the other type of RCC was an independent prognostic factor. However, this result may reflect that the ratio of spindle cell carcinoma distinctly known as a poor prognostic factor in the other type of RCC is high.

Thinking about therapeutic strategy, we could find that the up-regulation of TP expression in RCC leads to doxifluoruridine (5'-DFUrd) because TP is also one of the key enzymes involved in the metabolism of fluorouracil-related drugs, and especially, 5'-DFUR is a prodrug of 5'-fluorouracil (5'-Fura) and TP converts it to 5'-Fura (21,22). Moreover, Morita et al. measured the expression of TP and dihydropyrimidine dehydrogenase (DPD), which is the first and rate-limiting enzyme catabolizing 5-FU, and suggested that a high TP/DPD ratio was the candidate for the possible role as indicator of responsiveness to fluoropyrimidines (17). Although this statement cannot be made from our study protocol, carcinoma cells with high TP activity may be more sensitive to fluorouracil (8). We reported that TP protein level in human RCC and normal kidney tissues exhibited a significant positive correlation with sensitivity to 5-FU and 5'-DFUrd using in vitro histoculture drugs response assay (23,24). Ikemoto et al. also reported that IFNα enhanced TP expression in high TP expression renal cell lines, and antitumor effects were enhanced by IFNα for 5-FU and 5'-DFUrd (25). In addition, ELISA method is much more convenient compared with conventional enzyme assay, and enough to measure TP levels even in samples weighting as little as 10 mg (11). On the other hand, a novel, specific TP inhibitor, TPI, inhibited TP-enhanced angiogenesis, tumor growth and metastasis (18). However, treatment of tumors with inhibitors of TP in combination with 5-FU and its products is impossible, because TP is one of the enzymes correlated with activation of 5-FU. To overcome these problems, there is a trend to inhibit the downstream mediators of TP function rather than to directly inhibit TP activity (26).

In conclusion, TP at high levels can be regarded as an unfavorable independent prognostic factor. These results may pave a way for a novel approach to effective treatment of RCC.

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