

## Review Article

# Molecular Targeting Therapy for Renal Cell Carcinoma: Current Status and Our Suggestion

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Renal cell carcinoma (RCC) is a common urological malignancy. Patients with low stage RCC have a good prognosis by radical operation. On the other hand, advanced RCC do not respond to most treatment and survival is poor in such patients. Some immuno-therapies were performed to patients with advanced RCC, but the patients who obtained clinically meaningful benefit were very limited. To identify promising targets for novel therapeutic agents, numerous investigations have been carried out regarding the molecular mechanisms of tumor growth and progression. Recently, some molecular-targeting therapies, including anti-vascular endothelial growth factor agent, have demonstrated to prolong survival in phase II and III trials. However, such treatments also showed limited anti-tumoral effects and various severe side effects beyond expectation. These results necessitate more extensive and profound knowledge of pathological features to discuss the treatment strategies for RCC. We have been investigating the molecular mechanism of cancer cell invasion and metastasis in patients with RCC for last few years, and we have obtained several new findings and information about them. In this article, we will describe clinical and pathological significance of several invasion-related and/or angiogenesis-related factors, such as matrix metalloproteinases, thrombospondin, and hepatocyte growth factor/c-Met. These factors are well known to regulate invasive function, angiogenesis, and cancer cell proliferation in various malignant tumors, and animal experiments demonstrated that some selective molecular inhibitors of them could inhibit tumorigenicity and tumor development. Our studies show that some molecular inhibitors may provide a novel therapeutic approach to RCC.

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## Introduction

Human renal cell carcinoma (RCC) is the most common malignant tumor of the kidney, and tumors in approximately 25% of the patients present invasion to the surrounding tissues and distant metastasis.<sup>1</sup> Patients with early, localized RCC have a good prognosis by radical operation, while in those with advanced RCC, most traditional treatment regimens including chemotherapy and radiation therapy were not effective.<sup>2</sup> To such advanced RCC patients, immunotherapy with interferon and/or interleukin-2 is generally considered as standard and effective treatments.<sup>3</sup> However, response rates with these agents were approximately 10%. Thus, with few exceptions, there is no useful or curative therapy in patients with high stage and metastatic RCC, and the median survival time is less than 1 year, and the 5-year survival rate is less than 10%.<sup>4</sup> These facts require new treatment strategies, and information on the mechanism of tumor pro-

gression of RCC is important for development of new novel targeted agents.

Angiogenesis is an important factor in the tumor development and progression of RCC. Vascular endothelial growth factor (VEGF) is one of the most representative and strongest regulators of angiogenesis, and increased levels of VEGF have been reported in RCC.<sup>5</sup> Therefore, therapeutic effects of Bevacizumab, a recombinant monoclonal antibody to VEGF, have been investigated in clinical trial (Table 1). In addition, several proteins and signaling cascade are known by pre-clinical studies to be useful targets for inhibiting cancer cell proliferation, and some agents of them have been investigated with advanced RCC patients in phase II and III studies (Table 1). Furthermore, phase I study of several new targets, including ABX-EGF and CI-1033 which target endothelial growth factor receptor, is now in progress in patients with advanced RCC.<sup>6</sup> Thus, some studies have investigated anti-tumoral effects of single agents in RCC

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**Table 1.** Current status of molecular target therapy for renal cell carcinoma

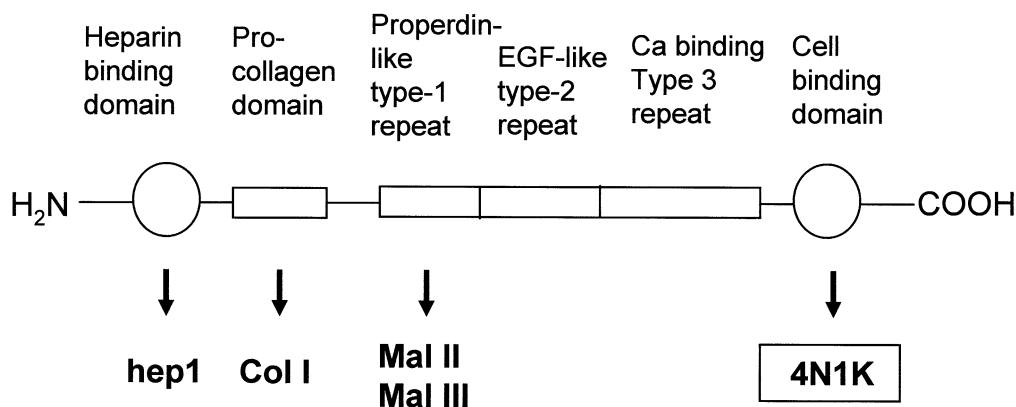
Agent	Target	Clinical development stage and results
Bevacizumab	VWGF	Phase III Prolonged time to progression of disease.
SU 011248	VEGFR PDGFR FLT3 Kit	Phase II Median time to progression was 8.7 months.
BAY 43-9006 (Sorafenib)	VEGFR PDGFR FLT3 Raf kinase Kit	Phase III Prolonged progression free survival time (5.5 months) compared with placebo (2.8 months).
AG 013736	VEGFR PDGFR	Phase II Partial response was obtained in 46%. However, significantly prolonged progression free survival was not shown. About half of patients had side effects.
CCI-779	Mammalian target of rapamycin (mTOR)	Phase II Prolonged time to tumor progression was 5.8 months. Over three quarters of patients had side effects.

patients. However, their clinical effects appear to be limited; improvement in survival time is less than 1 year compared with placebo-equivalent therapy. Based on these facts, to find the new therapeutic agent, numerous investigations have been carried out regarding anti-tumoral effects of various molecular targets.

Systemic dissemination of cancer cells influences prognosis of most malignancies. Although many factors are associated with this process, we paid special attention to mechanism of angiogenesis and cancer cell invasion. For last few years, we have reported the new knowledge on pathological significance of several proteins, which are associated with cancer cell invasion and metastasis, in patients with RCC. In this article, we will present the results and opinions obtained from our studies.

### TSP-1 and TSP-derived 4N1K peptide-containing protein

Thrombospondin (TSP)-1 is a well-known negative regulator of angiogenesis in physiological and pathological conditions, while it also acts as a positive regulator of angiogenesis under different conditions. Thus, TSP-1 is a multi-functional protein.<sup>7,8</sup> With respect to RCC tissues, there has been no report on the clinical significance of TSP-1. In various research fields, synthetic peptides derived from TSP have been used to examine the biological roles of TSP (Figure 1). For example, pathophysiological functions of peptides from Col I overlap (NGVQYRN), type I repeat (GSVTGG, KRFK, and WSHSPW), and NH2-terminal region (ELTGAARKGSGRRLVKGPD) were investigated, and several reports showed that these peptides possessed

**Figure 1.** Substructure of TSP-1 and its derived peptides.

specific angiogenic-related functions.<sup>9-12</sup> The 4N1K peptide was derived from the COOH-terminal cell binding domain of TSP-1, and is identified as a ligand for integrin-associated protein. Our previous report<sup>13</sup> demonstrated that 4N1K peptide (KRFYVVMWKK) exhibited anti-angiogenic activity in both in vitro and in vivo models. However, clinical significance of this peptide in human malignant tissues including RCC has not fully been understood. We therefore investigated the pathological and clinical significance of TSP-1 and 4N1Kpeptide-containing protein in patients with RCC.<sup>14</sup>

TSP-1 and 4N1K-containing protein are mainly detected in interstitial tissue of tumor area. When the relationship between TSP-1 expression and clinicopathological features in human RCC tissues was investigated, there was no significant correlation between TSP-1 expression and TNM staging or grade. On the other hand, expression of 4N1K peptide-containing proteins is significantly and negatively associated with T ( $p<0.001$ ), N ( $p=0.045$ ), and M classification ( $p=0.007$ ). In addition, its expression was also significantly associated with tumor size ( $p<0.001$ ), microvessel density ( $p<0.001$ ), and apoptotic index ( $p=0.023$ ). From these results, we speculated that 4N1K-containing peptide possessed anti-angiogenic activity, which would be a potential predictive factor for RCC progression.

## MMP-2 and COX-2

Proteolytic degradation of extracellular matrix (ECM) proteins by tumor cells is one of the early and critical steps. Matrix metalloproteinases (MMPs) play pivotal roles in the degradation of ECM of tissues surrounding tumors. Based on these features, many investigators have paid attention to the pathological significance of MMPs in cancer cells and it is conceivable that MMPs inhibitors could have anti-tumour effects in various malignancies.<sup>15-17</sup> However, the results of many pre-clinical studies and clinical trials of MMPs inhibitors have been disappointing with regard to improvement in survival.<sup>18</sup> In addition, the side effects of these agents are frequent and sometimes serious because of their broad-spectrum effects on various physiological functions.<sup>18</sup> Accordingly, research and development of specific MMP inhibitors for prevention and treatment of tumors are necessary. In addition, regulation mechanism of MMPs is important for planning treatment strategies to use these inhibitors.

Among MMPs, MMP-2 have been the most investigated and are detected in numerous malignancies, and their elevated expression significantly correlated with tumor invasion and poor prognosis in various carcinomas including RCC.<sup>19-21</sup> We also reported that MMP-2 played important roles for tumor invasion and prognosis in patients with RCC.<sup>22</sup> Furthermore, we found that MMP-2 expression was regulated by cyclooxygenase (COX)-2, and that COX-2 expression was significantly associated with TNM stage ( $p<0.001$ ) and grade ( $p<0.01$ ).<sup>22</sup> In addition, COX-2 expression was significantly associated with cancer cell proliferation ( $p<0.01$ ) and angiogenesis ( $p<0.01$ ), while the association with apoptosis was marginally significant ( $p=0.054$ ). This study first reported the clinical significance of COX-2 expression in human RCC tissues. Some non-steroidal anti-inflammatory

drugs (NSAIDs) are known as COX-2 inhibitor, and several epidemiological and animal studies have reported that NSAIDs can reduce the risk of carcinogenesis in a variety of cancers.<sup>23,24</sup> Based on our study, we speculate that COX-2 plays important roles in biological and pathological activities of human RCC, and may be a useful target for preventing carcinogenesis and cancer cell progression in RCC.

## MMP-7

In addition to MMP-2, MMP-1, -3, -7, -9, -11, -12, and -14 are also reported to be overexpressed in human RCC tissues,<sup>25-27</sup> while only a few reports are available regarding the clinical significance of other types of MMPs. We paid special attention to the relationship of MMPs with cell proliferation and angiogenesis; some MMPs are secreted by endothelial cells and can modulate angiogenesis by regulating endothelial cell proliferation and migration, and are associated with cancer cell proliferation.<sup>28-30</sup>

Among MMPs, we focused on the roles of MMP-7 (matrilysin) in human RCC tissues because clinical significance of MMP-7 in human RCC cells was not well understood. Furthermore, although MMP-7 is secreted by endothelial cells and can affect their activity, its expression or its independent role on endothelial cells in human RCC tissues with regard to tumor progression or survival was not investigated. We therefore determined the correlation of MMP-7 expression in cancer cells and blood vessels with tumor invasion, metastasis and survival using logistic regression model and Cox regression model.<sup>31</sup>

Our results<sup>31</sup> showed that MMP-7 expression was weak-to-moderate in most normal tubules, and strongly-stained normal tubular cells were relatively rare (frequency  $\pm$  standard deviation =  $5.3 \pm 4.1\%$ ). On the other hand, the proportion of MMP-7-positive cells was significantly higher in cancer cells ( $21.4 \pm 8.9\%$ ) than in normal cells ( $p<0.001$ ). In addition, cancer cells at the invasive front and margin showed strong MMP-7 immunostaining. However, such strong expressions were not observed in other stromal cells including fibroblasts, lymphocytes and macrophages. Another interesting finding was that MMP-7-positive vessels were considered blood vessels based on staining for CD34.

With regard to clinical significance, the proportion of MMP-7-positive cancer cells was significantly ( $p<0.001$ ) higher in patients with high pT stage, presence of metastasis and high grade compared to those with low pT stage, no metastasis and low grade.<sup>31</sup> Similar findings were also noted with regard to the density of MMP-7-positive vessels ( $p<0.001$ ). In addition, we found that elevated status of MMP-7 in cancer tissues (cancer cells and/or blood vessels) was a strong predictor of poor prognosis (hazard ratio = 8.61; 95% confidential interval (CI) = 1.10-67.28;  $p=0.040$ ) in multivariate Cox regression model. Therefore, targeting of MMP-7 may be an additional tool for prevention of tumor development and improvement of survival.

## MMP-10

MMP-10 degrades multiple components of the ECM or stromal connective tissue and is reported to be overexpressed in various cancers and to affect their malignant aggressiveness.<sup>32-35</sup> However, to our knowledge, no report is available regarding the clinical significance and pathological roles, including cell proliferation and angiogenesis, of MMP-10 expression in human RCC tissues. We therefore examined MMP-10 expression in RCC patients and analyzed its clinical significance and the relationship with cancer cell proliferation and microvessel density.<sup>36</sup> The proportion of MMP-10-positive tumors was significantly ( $p < 0.001$ ) higher in high pT stage (63.1%) than in low pT stage (33.8%). However, the association of MMP-10 expression with presence of metastasis was marginally significant ( $p = 0.058$ ). The analysis based on multivariate logistic regression model including grade as one of the covariates indicated that MMP-10 expression was a significant factor for high pT stage (odds ratio = 3.39; 95% CI = 1.14-10.09;  $p = 0.029$ ). With regard to biological mechanism of MMP-10, we found that its expression was significantly associated with angiogenesis ( $p = 0.022$ ), but not with cancer cell proliferation ( $p = 0.583$ ). In univariate Cox regression model, MMP-10 expression was identified as a significant predictor for cause-specific survival (hazard ratio = 2.87; 95% CI = 1.20-6.86;  $p = 0.018$ ), while not significant in multivariate Cox regression model including other factors as well. (hazard ratio = 1.92; 95% CI = 0.75-4.87;  $p = 0.173$ ). Our results suggest that MMP-10 could affect at least in part the invasive steps and angiogenesis. However, we speculate that the malignant activity of MMP-10 in human RCC does not influence profoundly the metastatic potential and survival.

## HGFR/c-Met and phosphorylated HGFR/c-Met

Hepatocyte growth factor receptor (HGFR)/c-Met belongs to the family of receptors of tyrosine kinases, and signals via HGFR/c-Met are implicated in a variety of normal cellular processes, oncogenic processes, and malignant aggressiveness, such as tumor growth, invasion, and metastasis.<sup>37-40</sup> Although mutation and overexpression of HGFR/c-Met have extensively been studied in human malignant tumors, clinical significance of phosphorylated HGFR/c-Met is examined little. We therefore examined the possible role of two tyrosine residues (pY 1234/1235 and pY 1349) in HGFR/c-Met (Figure 2) in patients with RCC.<sup>41</sup> By immunohistochemical staining, pY1349 HGFR/c-Met showed a positive staining rate from 0-64.3% with the median of 14.3%, while the immuno-positive rate of pY1234/1235 HGFR/c-Met was remarkably low (ranged from 0-5.2% with the median of 0%). With regard to clinical significance, expression of pY1349 HGFR/c-Met was significantly associated with T ( $p < 0.001$ ), N ( $p = 0.014$ ) and M ( $p = 0.021$ ) stage. In addition, tumor size and proliferation index were significantly higher in pY1349 HGFR/c-Met-positive tumor than in negative tumor;  $p = 0.017$  and  $p < 0.001$ , respectively. Furthermore, high expression of pY1349/c-Met was

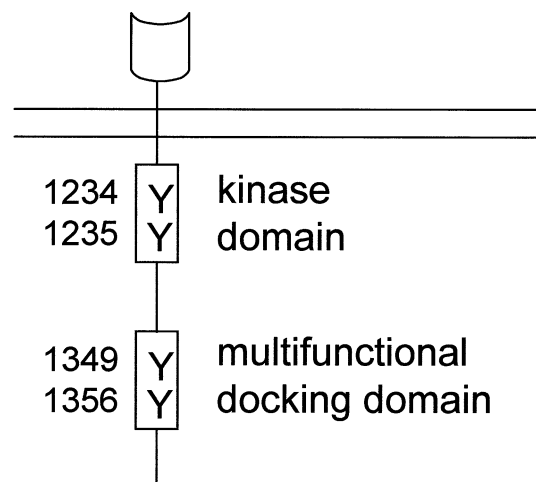


Figure 2. Major phosphorylation site of HGFR/c-Met.

recognized as a strong predictive factor for cause-specific survival (hazard ratio = 2.94; 95% CI = 1.12-7.72;  $p = 0.028$ ) in multivariate Cox regression model.

Finally, we speculate that detection of pY 1349 HGFR/c-Met expression is an excellent predictor for the prognosis of patients with sporadic conventional RCC patients.

## Conclusion

Our findings indicate that targeting TSP-derived 4N1K peptide-containing protein, such as COX-2, MMP-2, -7, and -10, and pY1349 HGFR/c-Met, could be a potential powerful tool to predict and control progression of RCC. However, before studying the clinical effects of these targeting therapies in patients with RCC, more detailed studies on animal model are needed to determine the exact role of these proteins.

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