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Original article

Significance of circulating matrix metalloproteinase-9 to tissue inhibitor of metalloproteinases-2 ratio as a predictor of disease progression in patients with metastatic renal cell carcinoma receiving sunitinib[1]

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Abstract

Objectives: To assess the significance of circulating matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) as predictors of disease progression in patients with metastatic renal cell carcinoma (mRCC) receiving sunitinib.

Materials and methods: Circulating levels of MMP-2, MMP-9, TIMP-1, and TIMP-2 in sera from 52 patients with mRCC treated with sunitinib were measured at the baseline and on the first day of each treatment cycle until progression using enzyme-linked immunosorbent assays.

Results: The baseline level of MMP-9 in nonresponders to sunitinib was significantly higher than that in responders, whereas the baseline level of TIMP-2 in nonresponders was significantly lower than that in responders. However, there were no significant differences in the serum levels of MMP-2 and TIMP-1 between responders and nonresponders. The serum MMP-9/TIMP-2 ratio at the baseline in nonresponders was also significantly higher than that in responders. Univariate analysis showed that the MMP-9/TIMP-2 ratio, but not MMP-9 and TIMP-2 levels, was significantly correlated with progression-free survival, and the MMP-9/TIMP-2 ratio, in addition to the Memorial Sloan-Kettering Cancer Center classification and C-reactive protein level, appeared to be independently associated with progression-free survival on multivariate analysis. Furthermore, despite the lack of significant differences in the serum levels of MMP-9 and TIMP-2 between the baseline and the time of progression, the MMP-9/TIMP-2 ratio at the time of progression was significantly elevated compared with the baseline ratio.

Conclusions: An imbalance between the serum MMP-9 and TIMP-2 levels could be a novel biomarker to predict disease progression in patients with mRCC under treatment with sunitinib.

Keywords: Renal cell carcinoma; Sunitinib; Resistance; Matrix metalloproteinase; Tissue inhibitor of metalloproteinases

1. Introduction

Renal cell carcinoma (RCC) is the most common malignancy of the adult kidney, and annual incidences of newly diagnosed cases have been steadily increasing. A high incidence of metastatic spread is regarded as one of the most unique characteristics of RCC, that is, approximately 30% of patients with RCC demonstrate visceral metastasis at diagnosis, and up to half of the remaining patients eventually develop distant metastases [1]. Because of a phenotype of RCC that is highly resistant to conventional chemotherapeutic agents, cytokine therapies had been the only approaches available for patients with metastatic RCC (mRCC) until recent years; however, limited efficacy could be achieved by this treatment with a median overall survival of about 1 year [2]. In recent years, novel molecular-targeted agents have been developed based on the precise understanding of molecular mechanisms involved in the progression of RCC, and the introduction of these new drugs into clinical practice has resulted in a dramatic paradigm shift in the therapeutic strategy for mRCC [3].

Of several molecular-targeted agents, sunitinib is characterized by one of the most powerful antitumor activities against mRCC [4]. In preclinical experiments, sunitinib has

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been demonstrated to exhibit inhibitory effects on tumor cell proliferation as well as angiogenesis through the inactivation of multiple receptor tyrosine kinases, such as vascular endothelial growth factor (VEGF) receptors and platelet-derived growth factor receptors [5]. In a clinical setting as well, Motzer et al. [6] reported the excellent cytotoxic effect of sunitinib on mRCC, demonstrating significantly favorable prognostic outcomes compared with those of patients treated with interferon-α in a phase III randomized clinical trial. However, several limitations associated with the treatment of mRCC using sunitinib have been reported, including the low proportion of patients achieving a complete response and a short interval of durable response [7]; therefore, it would be of marked clinical significance to identify novel variables associated with susceptibility to this agent so as to provide individualized risk-directed therapy for patients with mRCC.

To date, several model systems for predicting the prognosis of patients with mRCC have been reported [8,9]; however, these prognostic profiles were developed based on data from patients who mainly participated in clinical trials using cytokine therapies. Furthermore, RCC has been shown to be characterized by unique biological features, as well as heterogeneous genetic backgrounds [10], suggesting limitations for predicting the clinical course of patients with mRCC using conventional clinicopathological parameters alone. Therefore, in this study we obtained serum samples from patients with mRCC treated with sunitinib and measured circulating levels of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs), extracellular proteases and their inhibitors that are involved in tumor cell invasion and metastasis through the degradation of the basement membrane [11,12] to identify potential biomarkers associated with the efficacy of sunitinib in this cohort of patients.

2. Materials and methods

This study included 52 patients undergoing radical nephrectomy for clear cell RCC who were diagnosed with metastatic diseases and subsequently received sunitinib as a first-line agent between January 2011 and March 2013. Informed consent was obtained from each patient before participating in this study, and the study design was approved by the research ethics committee at our institution. Before entry, all the patients were examined by computed tomography of the brain, chest, and abdomen as well as radionucleotide bone scan. The therapeutic effects of sunitinib were generally evaluated by computed tomography before the introduction of treatment with sunitinib and every 6 weeks after. Response and progression were assessed by the treating physician according to the Response Evaluation Criteria in Solid Tumors.

In this study, all patients initially received 50 mg of sunitinib once daily in repeated 6-week cycles consisting of 4 weeks on therapy, followed by 2 weeks off therapy; however, dose modification of sunitinib was permitted based on adverse events in accordance with the manufacturer's recommendations. Clinicopathological examinations, performance status, and risk classification were assessed according to the Union for International Cancer Control (UICC) TNM classification system, Karnofsky performance status scale, and Memorial Sloan-Kettering Cancer Center (MSKCC) prognostic factor model [9], respectively.

Blood samples were obtained from 30 healthy age-matched volunteers as well as from all the included patients before the initiation of treatment with sunitinib and then on the first day of each treatment cycle until progression. These samples were allowed to separate at room temperature for 30 minutes before being centrifuged at 1,000*g* for 15 minutes. The sera thus obtained were immediately frozen and stored at −80°C until subsequent assessment. Serum concentration levels of MMP-2, MMP-9, TIMP-1, and TIMP-2 were measured using human MMP-2, MMP-9, TIMP-1, and TIMP-2 enzyme-linked immunosorbent assay kits (R&D Systems Inc, Minneapolis, MN) according to the manufacturer's instructions. All analyses and calibrations were performed in duplicate. The optical density was determined with a microculture plate reader (Becton Dickinson Labware, Lincoln Park, NJ) at 450 nm. The blank value was subtracted from the duplicate reading for each standard and sample, and a standard curve was created using Statview 5.0 software (Abacus Concepts, Inc, Berkely, CA) by plotting the logarithm of the mean absorbance of each sample against the sample concentration.

Differences between both the groups were compared using the unpaired *t* test and the chi-square test. Progression-free survival (PFS) rates were calculated by the Kaplan-Meier method, and differences were determined by the log-rank test. The prognostic significance of certain factors was assessed by the Cox proportional hazards regression model. All statistical calculations were performed using Statview 5.0 software, and $P < 0.05$ was considered significant.

3. Results

Clinicopathological characteristics of the 52 patients included in this study are summarized in Table 1. All of these patients received sunitinib as a first-line agent and were evaluable for the best response to this agent. In this series, 19 patients achieved partial response, whereas the remaining 21 and 12 patients were judged to have stable disease and progressive disease, respectively. Therefore, the overall response rate to sunitinib in these patients was 36.5%.

The baseline serum levels of MMPs and TIMPs are presented in Table 2. Compared with the levels in healthy controls, serum levels of MMP-2, MMP-9, TIMP-1, and