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Original article

Monocarboxylate transporters MCT1 and MCT4 are independent prognostic biomarkers for the survival of patients with clear cell renal cell carcinoma and those receiving therapy targeting angiogenesis

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Abstract

Background: Prognostic biomarkers for patients with clear cell renal cell carcinoma (ccRCC), particularly those receiving therapy targeting angiogenesis, are not well established. In this study, we examined the correlations of monocarboxylate transporter 1 (MCT1) and MCT4, 2 critical transporters for glycolytic metabolism, with various clinicopathological parameters as well as survival of patients with ccRCC and those treated with vascular endothelial growth factor receptor (VEGFR) inhibitors.

Methods: A cohort of 150 ccRCC patients were recruited into this study. All patients underwent radical or partial nephrectomy as the first-line treatment, and 38 received targeted therapy (sorafenib or sunitinib) after the surgery. Expression levels of MCT1, MCT4, and CD34 were examined by immunohistochemistry. Correlations between MCT1 or MCT4 expression and different clinicopathological parameters or patient survival were analyzed among all as well as patients receiving targeted therapy.

Results: MCT1 or MCT4 expression did not significantly correlate with sex, age, tumor diameter, microvascular density, tumor staging, pathological Furmann grade, or MSKCC ($P > 0.05$). High expression of either MCT1 or MCT4 significantly correlated with reduced overall survival (OS) and progression-free survival (PFS) among the total cohort of ccRCC patients. For patients receiving targeted therapy, high expression of either MCT1 or MCT4 significantly correlated with reduced PFS, but not OS. Both conditions were independent prognostic biomarkers for reduced PFS among all patients or those receiving targeted therapy.

Conclusion: MCT1 and MCT4 are prognostic biomarkers for patients with ccRCC or those receiving targeted therapy. High expression of these 2 proteins predicts reduced PFS in these patients.

Keywords: Prognostic biomarker; Monocarboxylate transporter; Clear cell renal cell carcinoma; Overall survival; Progression-free survival; Targeted therapy

1. Introduction

Renal cell carcinoma (RCC) is the most common malignancy in the kidney, contributing to approximately 2.4% of the total cancer burden and 1.7% of cancer-related deaths worldwide [1]. Of more than 10 histological types of RCC, clear cell renal cell carcinoma (ccRCC) is the predominant subtype and responsible for most of the deaths resulting from RCC [2]. Although advances in early detection and the development of targeted therapies have slightly reduced the mortality of the disease over the last decade, the overall incidence rates of RCC and that of metastatic RCC (mRCC) at the time of diagnosis are still on the rise [3]. The median survival for patients with mRCC is 10 to 12 months and the 5-year survival of these patients, if not treated, is less than 18% [4].

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The pathology of RCC is characterized by a high level of vascularization, with variations in vascularity likely contributing to differences in the intrinsic aggressiveness of the disease. Accordingly, therapies specifically targeting the angiogenesis signaling, including the vascular endothelial growth factor receptor (VEGFR) inhibitor sunitinib [5] and the multi-kinase inhibitor sorafenib [6], have been developed and with present therapeutic benefits [7]. However, based on assessments of objective response rate (ORR), progression-free survival (PFS), and overall survival (OS), the effects of most targeted therapies are below expectations [8]. Currently, no biomarkers have been validated to guide treatment and predict the prognosis of mRCC patients, and thus great efforts are dedicated to identifying potential biomarkers [8].

According to the "Warburg Effect" and "Reverse Warburg Effect" [9–11], enhanced glycolysis is closely associated with development of primary as well as metastatic cancers [12]. Monocarboxylate transporters (MCTs) are a family of proteins responsible for moving lactate in and out of cells and thus are critical to the regulation of glycolysis [13]. Among the various members of the MCT family, MCT1 and MCT4 are the key controllers of lactate concentrations under physiological as well as pathological conditions [14]. In different types of cancers, studies have revealed the significant associations of up-regulated MCT1 and/or MCT4 expression with increased malignancy of tumors and poor prognosis of patients [9,15–18]. Consistently, targeting MCT4 expression in gastric cancer cells reduced the proliferation of and lactate uptake by gastric cancer cells [13].

In this study, we explored the associations of MCT1 and MCT4 expression with various clinicopathological parameters in ccRCC patients. Particularly, we assessed the potential of MCT1 and MCT4 expression as a prognostic biomarker for ccRCC patients in general and specifically for those receiving targeted therapy.

2. Methods

2.1. Specimens and patient information

A total of 150 patients diagnosed with ccRCC and undergoing radical or partial nephrectomy in the Affiliated Hospital of Medical College, Qingdao University (Shandong, China), from June 2009 to March 2014 were recruited into this study. Of these patients, 38 received oral sorafenib or sunitinib as the targeted therapy after nephrectomy. The TT patients were selected if they met all the following criteria: >18 years old; with advanced ccRCC as confirmed by pathological examinations; developing distant metastasis before or after the operation; and had not received prior systemic treatment or molecular targeted therapy [19]. Sorafenib was prescribed at 400 mg twice a day for 4 weeks (the 4-week schedule). Sunitinib was administered at 50 mg once per day on a 6-week schedule of 4 weeks on followed by 2 weeks off. Renal tissue samples obtained during nephrectomy were retrieved from the archive of the Department of Pathology, the Affiliated Hospital of Qingdao University. General clinicopathological information, including age, sex, Fuhrman grade (I/II/III), tumor size (diameter), tumor stage (TNM), Memorial Sloan-Kettering Cancer Center (MSKCC) prognostic risk factors, the status of tumor recurrence or disease progression, and cause of death, is summarized in Table 1. All patients enrolled in the study were followed up for 3 to 60 months. This study was designed according to Ethics Guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Affiliated Hospital of Qingdao University.

2.2. Immunohistochemical analysis on tissue microarray

Fresh renal tissue specimens were fixed in 4% paraformaldehyde overnight, embedded in paraffin, and prepared into donor blocks. Then RCC tissues from donor blocks were used to prepare the recipient tissue microarray blocks as described previously [20]. After deparaffinization in xylene and rehydration through a series of decreasing concentrations of alcohol solutions, the tissue sections in microarray slides were treated with 3% hydrogen peroxide to block endogenous peroxidase activity and microwaved in citrate buffer for 10 minutes for antigen retrieval. Upon blocking for nonspecific staining in phosphate-buffered saline (PBS) containing 10% goat serum for 30 minutes, the sections were incubated with a monoclonal mouse antibody against human MCT1 (1:100), MCT4 (1:200), or CD34 (1:500) (Abcam, Cambridge, UK) at room temperature for 10 minutes. The slides were then washed with PBS and incubated with horseradish peroxidase (HRP)-labeled anti-mouse immunoglobulin G (IgG) at room temperature for 2 hours. The target protein was detected using diaminobenzidine (DAB) substrate, and the slides counter-stained with hematoxylin. Dual staining of MCT 1 and CD34 or MCT4 and CD34 was performed by incubating the tissue sections with anti-MCT1 (1:100) or -MCT4 (1:200) as the first antibody for 10 minutes at room temperature, followed by 3 PBS washes and a further 2-hour incubation with HRP-labeled anti-mouse immunoglobulin G (IgG) at room temperature. Monoclonal rabbit anti-CD34 (1:500) was used as the second antibody, which was detected using HRP-labeled anti-rabbit IgM.

After immunohistochemical staining, each slide was examined by 3 independent pathologists, who quantified the staining score for MCT1 or MCT4 as follows: 0 ($<5\%$ positively stained cells), 1 ($<25\%$ positively stained cells), 2 (25%–50% positively stained cells), and 3 ($>50\%$ positively stained cells). Furthermore, a staining intensity of "–" was assigned if the staining grade was 0 or 1, and "+" was assigned to samples with the staining score of 2 or 3 [21]. The microvascular density (MVD) was quantified