ELSEVIER

Urologic Oncology: Seminars and Original Investigations ▮ (2017) ▮▮▮–▮▮▮

UROLOGIC ONCOLOGY

Original article

Serum level of ANGPTL4 as a potential biomarker in renal cell carcinoma

Dong Dong, Ph.D., M.D.[a,1], Li Jia, M.M.[a,1], Yunli Zhou, Ph.D., M.D.[a], Li Ren, Ph.D., M.D.[a], Juan Li, Ph.D., M.D.[b,*], Jun Zhang, Ph.D., M.D.[c,**]

[a] *Department of Laboratory, Tianjin Medical University Cancer Institute and Hospital, Tianjin's Clinical Research Center for Cancer and Key Laboratory of Cancer Prevention and Therapy, National Clinical Research Center for Cancer, Tianjin, PR China*

[b] *Department of Immunology, Tianjin Key Laboratory of Cellular and Molecular Immunology, Key Laboratory of Educational Ministry, Tianjin Medical University, Tianjin, PR China*

[c] *Department of Immunology, School of Basic Medical Sciences, Key Laboratory of Medical Immunology, National Health and Family Planning Commision of the People's Republic of China, Peking University Health Science Center, Beijing, PR China*

Received 8 October 2016; received in revised form 18 December 2016; accepted 20 December 2016

Abstract

Objectives: This study aimed to determine the serum levels of angiopoietin-like 4 (ANGPTL4) in patients with renal cell carcinoma (RCC) and explore its potential as a biomarker.

Materials and methods: Blood samples were taken from 110 patients with RCC, 66 healthy controls, and patients with other solid tumors. Serum ANGPTL4 levels were measured using the enzyme-linked immunosorbent assay, and their correlation with clinical characteristics was further analyzed. Received operating characteristic (ROC) curves, Kaplan-Meier curves, and log-rank analyses were used to evaluate diagnostic and prognostic significance.

Results: Serum ANGPTL4 levels were significantly higher in patients with RCC compared with healthy controls and patients with other types of cancers ($P < 0.0001$) and associated with sex, Fuhrman grades, metastasis states, and tumor node metastasis stages ($P < 0.05$), but not with age, tumor size, and histological types ($P > 0.05$). The ROCs/area under the ROC curve analysis indicated an area under the ROC curve of 0.844 (sensitivity = 0.691; specificity = 0.939) and 0.725 (sensitivity = 0.909; specificity = 0.568), respectively, to distinguish patients with RCC from healthy controls and those with metastasis from those without metastasis. The survival analysis revealed that patients with low serum ANGPTL4 had longer progression-free survival compared with those with high serum ANGPTL4 ($P = 0.033$).

Conclusion: The present study suggested that the elevated serum ANGPTL4 level might be a novel diagnostic and prognostic biomarker for patients with RCC.

Keywords: ANGPTL4; Biomarker; Prognosis; Renal cell carcinoma; Serum

1. Introduction

Renal cell carcinoma (RCC), the most common type of kidney cancer, accounts for approximately 2% to 3% of all malignancies [1]. It is the leading secondary cause of death among all types of urologic tumors [1]. Although the number of patients diagnosed with RCC is increasing over the years, many patients (25%–30%) nevertheless have distant metastases at diagnosis. In approximately 30% of the remaining patients, the disease will progress with metastasis [2]. To date, surgery is still the most effective

This work was supported by research grants from the National Natural Science Foundation of China (Nos. 81502519 and 81201653), and the Natural Science Foundation of Tianjin City, China (16JCYBJC26000).

[1]Dong Dong and Li Jia contributed equally to this work.

* Corresponding author. Tel.: +86-22-8333-6817.

** Corresponding author. Tel.: +86-10-8280-5649.

E-mail addresses: juanzi0223@163.com (J. Li), junzhang@bjmu.edu.cn (J. Zhang).

http://dx.doi.org/10.1016/j.urolonc.2016.12.017

treatment for local RCC. However, prognosis remains poor for advanced and metastatic RCCs because of low sensitivity to chemotherapy and radiotherapy [3,4]. It is, therefore, of great importance to find the biomarkers for early diagnosis and to develop more effective systemic therapies for progressive disease.

Angiopoietin-like protein 4 (ANGPTL4), also known as hepatic fibrinogen/angiopoietin-related protein, fasting-induced adipose factor, or peroxisome proliferator-activated receptor-c angiopoietin-related gene, is a circulating glycoprotein that structurally belongs to the angiopoietin/ANGPTL family [5]. Human ANGPTL4 protein consists of 406 amino acids with a signal peptide directing secretion, an amino-terminal coiled-coil domain, a linker, and a carboxy-terminal fibrinogen-like domain [6]. ANGPTL4 has been reported to exhibit distinct biological functions, and the most studied function of ANGPTL4 is its effects in regulating lipid metabolism, particularly as an inhibitor of lipoprotein lipase activity [7–10]. Recent studies have concentrated on the roles of ANGPTL4 in tumor progression in various cancers; however, the effects of ANGPTL4 in human cancers remain controversial. The overexpression of ANGPTL4 can promote tumorigenesis, angiogenesis, tumor invasion, and metastasis [11–15]. On the contrary, ANGPTL4 has antimetastatic effects on tumor cells through inhibiting vascular permeability, motility, and invasiveness [5] or through attenuating endothelial cell adhesion, migration, and sprouting [16].

Increased levels of ANGPTL4 mRNA have been demonstrated in RCC compared with the nontumor tissues [14,15]. However, no study has investigated the clinical significance of serum ANGPTL4 levels in RCC. In this study, the serum levels of ANGPTL4 were measured in patients with RCC, healthy controls, and patients with other types of solid tumor using an enzyme-linked immunosorbent assay (ELISA), and the association between the clinical characteristics of RCC and serum ANGPTL4 levels was analyzed.

2. Materials and methods

2.1. Patients and specimens

Blood samples were taken from 110 patients with RCC before surgery and 66 healthy controls. Patients with other cancers were evaluated regarding marker specificity, including 34 with bladder cancer, 30 with breast cancer, 21 with gastrointestinal cancer, and 21 with lung adenocarcinoma. All of the serum samples were harvested before surgery or treatment from Tianjin Medical University Cancer Institute and Hospital between July 2010 and August 2014. All samples were collected, aliquoted, and snap frozen at –80°C till use. Tumor staging was determined according to the 2009 tumor node metastasis (TNM) staging classification system [17]. Pathological data, including age, sex, tumor size, histological type, and Fuhrman grade, were collected. Blood samples were postoperatively collected 2 weeks after resection from 28 patients with RCC, including 22 patients without metastasis and 6 patients with metastasis at surgery.

2.2. Quantification of ANGPTL4 using ELISA

Serum levels of ANGPTL4 in patients with RCC, healthy volunteers, and patients with other types of solid tumors were measured by the ELISA method using a commercial kit (R&D System Inc., MN), according to the manufacturer's protocol. Briefly, 96-well ELISA microplates were coated overnight with 100 µl of ANGPTL4 antibody at a final concentration of 0.8 µg/ml in phosphate-buffered saline (PBS). After washing with PBS/0.05% (w/v) tween-20 (PBST, pH = 7.4), the wells were blocked with 300 µl of blocking buffer at room temperature for 1 hour. Then, 100 µl of serum samples were added and incubated at room temperature for 2 hours. Similarly, 100 µl of PBST-lacking antibody was used as a negative control. Following 3 washes with PBST, 100 µl of detection antibody diluted to a concentration of 0.4 µg/ml was added. After incubation at room temperature for 2 hours, 100 µl of avidin-horseradish peroxidase–conjugated secondary antibody (at 1:200 dilution) was added, and the plates were incubated at room temperature for 20 minutes. The excess conjugate was removed by washing the plates 3 times with PBST. The amount of bound conjugate was determined by adding 3,3′,5,5′-tetramethylbenzidine substrate solution to each well, and the plates were incubated at room temperature for color development. The absorbance was measured at 405 nm using a Model 680 microplate reader. All samples were tested in duplicate.

2.3. Statistical analysis

Statistical significance was determined with the nonparametric Mann-Whitney U test (differences between 2 groups) and paired Student's t-test (difference between the preoperative and the postoperative levels of ANGPTL4 in patients with RCC). Nonparametric received operating characteristic (ROC) curves were generated to assess diagnostic efficiency. Disease-free survival was analyzed using the Kaplan-Meier product limit method and log-rank test. All of these statistical analyses were performed with SPSS 16.0 software (Chicago, IL). Values of $P < 0.05$ were considered statistically significant.

3. Result

3.1. Serum ANGPTL4 was up-regulated in patients with RCC

Serum ANGPTL4 levels were detected in patients with RCC and healthy controls. A total of 176 participants were enrolled in this study. The serum levels of ANGPTL4 in patients with RCC ($n = 110$) and healthy controls ($n = 66$) were assessed using ELISA. The serum levels of ANGPTL4 in the 2 groups are shown in Fig. 1. Compared with the control patients, ANGPTL4 was highly expressed