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The relationship of plasma decoy receptor 3 and coronary collateral circulation in patients with coronary artery disease

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ABSTRACT

Objective: Previously, decoy receptor 3 (DcR3) was found to be a potential angiogenetic factor, while the relationship of DcR3 with coronary collateral circulation formation has not been investigated. In this study, we aimed to investigate whether plasma decoy receptor 3 levels was associated with CCC formation and evaluate its predictive power for CCC status in patients with coronary artery disease.

Methods: Among patients who underwent coronary angiography with coronary artery disease and had a stenosis of ≥ 90% were included in our study. Collateral degree was graded according to Rentrope Cohen classification. Patients with grade 2 or 3 collateral degree were enrolled in good CCC group and patients with grade 0 or 1 collateral degree were enrolled in poor CCC group.

Results: Plasma DcR3 level was significantly higher in good CCC group (328.00 ± 230.82 vs 194.84 ± 130.63 ng/l, $p < 0.01$) and positively correlated with Rentrope grade ($p < 0.01$). In addition, plasma DcR3 was also positively correlated with VEGF-A. Both ROC (receiver operating characteristic curve) and multinomial logistical regression analysis showed that plasma DcR3 displayed potent predictive power for CCC status.

Conclusions: Higher plasma DcR3 level was related to better CCC formation and displayed potent predictive power for CCC status.

1. Introduction

Coronary collateral circulation (CCC) functions as a vital natural bypass to supply blood to the myocardium in patients with coronary artery disease (CAD) [1]. Although angiogenesis plays an important role in CCC formation and there are many determinants of coronary angiogenesis including chronic inflammation, certain growth factors such as vascular endothelial growth factor (VEGF), they cannot fully explain the mechanism of CCC formation or to form a potent biomarker for CCC status [2]. Therefore, there is of great clinical significance to identify the novel molecules associated with angiogenesis or CCC.

Inflammation plays an important role in angiogenesis or CCC formation and many inflammatory molecules have been found to promote angiogenesis [3]. CCC formation is always associated with activation of vascular endothelial cells [4]. Activated leukocytes bind to endothelial cells and result in shedding of adhesion molecules, including vascular cell adhesion molecule-1 (VCAM-1), which in turn facilitates binding of circulating monocytes to the blood vessel walls, which further promotes endothelial activation [5,6].

Decoy receptor 3 (DcR3) is an inflammation molecule and found to promote angiogenesis [7]. DcR3 could enhance endothelial cell differentiation into cord vascular-like structures in vitro and promote neovascularization in vivo [8]. The pro-angiogenesis ability of DcR3 was associated with up-regulation of VCAM-1 and VEGF-A expression [8,9]. It was found that DcR3 could up-regulate the expression of VCAM-1 in endothelial cells, while VCAM-1 plays potent angiogenesis-promoting effect [9,10]. In addition to VCAM-1, DcR3 could up-regulate the expression of VEGF-A, a well- known angiogenesis factor, by neutralizing vascular endothelial growth inhibitor (VEGI) including TNF-like cytokine 1A (TL1A) in endothelial cells [8]. Clinically, DcR3 was associated with lymphatic microvessel density and could predict the severity of CAD in patients with multivessel CAD [11,12]. These findings indicate that DcR3 might be involved in the formation of CCC and could serve as potent biomarker for CCC. However, the correlation between DcR3 and the CCC has not been substantially investigated in CAD patients.

In this study, we tested plasma levels of DcR3 and assayed its correlation with CCC status as well as its predictive power for CCC formation. In addition, we also measured the relationship of DcR3 with

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VCAM-1 or VEGF-A to explain the mechanism of DcR3 in regulation of CCC.

2. Patients & methods

2.1. Study population

In this study, we enrolled the patients who underwent coronary angiography at the Second Hospital of Jilin University for Cardiovascular Diseases, Changchun, China, from March 2015 to December 2016. Quantitative coronary angiography was performed using standard Judkins method via transfemoral route. The inclusion criteria were the presence of 90%, or greater, degree of diameter stenosis in at least one coronary artery [18,19]. Collateral degree was graded according to Rentrope Cohen classification. Patients with grade 2 or 3 collateral degree were included in good collateral group and patients with grade 0 or 1 collateral degree were included in poor collateral group. 48 consecutive patients with good CCC and 44 subjects with poor CCC were retrospectively investigated. Patients were selected for the present study according to the following exclusion criteria: patients with coronary artery lumen diameter stenosis < 90%; patients with a recent history (history of < 1 month) of acute coronary syndrome, congestive heart failure, concomitant inflammatory diseases, and neoplastic diseases; and patients taking steroids, immunosuppressive drugs, or nonsteroidal anti-inflammatory drugs except for low-dose aspirin. A detailed physical examination and electrocardiographic and echocardiographic evaluations were performed on all patients. Written consent was obtained from all subjects, and the study protocol was approved by the ethics committee of Jilin University.

2.2. Definitions

Hypertension was defined as blood pressure ≥ 140/90 mm Hg or current use of antihypertensive medication. Diabetes was defined as fasting blood glucose ≥ 7.0 mmol/l or taking oral anti-diabetic drug or insulin. Dyslipidemia was defined as serum TC ≥ 6.22 mmol/l, LDL cholesterol ≥ 4.14 mmol/l, HDL cholesterol ≥ 1.04 mmol/l or TG ≥ 1.70 mmol/l or taking any lipid-lowering medication. Smoking status means smoking history was over one month within the past year [17].

2.3. ELISA assay for plasma DcR3, VCAM-1 and VEGF-A levels

All blood samples (5 ml per patient) were collected via a direct venous puncture and placed into tubes containing sodium citrate, centrifuged at 1000 × *g* for 5 min and 3000 × *g* for 10 min, the layer of the supernatant (plasma) was carefully transferred into other tubes. We used the human DcR3 ELISA kit from Kamiya Biomedical and the human VCAM-1 or VEGF-A ELISA kit from Elabscience to measure plasma levels of DcR3, VCAM-1 or VEGF-A, respectively, following the manufacturer's instructions. Briefly, 100 μl each sample was incubated for 2 h. Following incubation, biotin-conjugate anti-DcR3, anti-VCAM-1, or anti-VEGF antibody was added and incubated as a primary antibody for 2 h, respectively. After the microplate had been washed, streptavidin-horseradish peroxidase (HRP) was added and incubated for 1 h. After washing, tetramethylbenzidine was added as a substrate, and the absorbance was measured. Absorbance of each sample was plotted against a standard curve produced by serial dilutions of recombinant human DcR3, VCAM-1or VEGF-A, run in duplicate. Absorbance was measured at 450 nm (primary wave length).

2.4. Statistical analyses

SPSS 24.0 for Windows v. was used to perform the statistical analyses. Data are presented as the mean ± SD and median for the general characteristics of the subjects. Differences among the different groups were assessed using the one-way ANOVA comparison method. Values with a $p < 0.05$ were considered to indicate statistical significance. The relationship between plasma DcR3 and collateral grade, VCAM-1 or VEGF-A was assessed with the Spearman correlation test. Receiver operating characteristic (ROC) and multinomial logistical regression analysis were also used to compare the predictive powers of plasma DcR3, VCAM-1 or VEGF-A for CCC status. The area under the ROC curve (AUC) was used to assess the predictive power. The sensitivity and specificity were calculated according to the standard formulas.

Table 1
Clinical characteristics and biochemical parameters of the patients.

Variable	Good CCC (n = 48)	Poor CCC (n = 44)	P
Age (y)	63.56 ± 9.14	61.34 ± 7.529	0.209
Heart rates (t/m)	77.52 ± 12.96	73.93 ± 8.35	0.121
Male/female (%)	16(33.3)	15(34.1)	0.939
Diabetes n (%)	17(35.41)	11(25.0)	0.278
Hypertension, n (%)	25(14.1)	16(26.9)	0.130
Smoking n (%)	13(27.1)	19(43.2)	0.105
TG (mmol/l)	1.86 ± 1.45	1.47 ± 0.87	0.125
TC (mmol/l)	4.58 ± 1.43	4.81 ± 1.56	0.463
HDL-C (mmol/l)	1.08 ± 0.43	1.19 ± 0.26	0.146
LDL-C (mmol/l)	2.58 ± 0.95	2.79 ± 1.09	0.326
AMI history	6(12.5)	5(11.4)	0.867
Statins, n (%)	28 (58.3)	29 (65.9)	0.470
ACEIs n (%)	15(31.3)	19(43.2)	0.236
BMI (kg/m^2)	26.8 ± 2.75	27.6 ± 3.62	0.241
Beta-blocker n (%)	15(14.1)	9(26.9)	0.130
Aspirin, n (%)	14 (29.1)	11 (25.0)	0.842
Clopidogrel, n (%)	32 (66.7)	28 (63.6)	0.940
Calcium antagonists, n (%)	14 (29.1)	10 (22.7)	0.658
Oral antidiabetic drug	8 (16.7)	5 (11.4)	0.471

Data are presented as mean ± SD, or n (%). CCC, coronary collateral circulation; ACEIs, angiotensin converting enzyme inhibitors; TG, Triglyceride; TC, Total cholesterol.; BMI, body mass index.

3. Results

3.1. Baseline characteristics

There were 44 patients with poor CCC and 48 patients with good CCC. Their clinical characteristics and biochemical parameters are listed in Table 1. Age; heart rate; sex; rates of hypertension and diabetes mellitus; smoking history; lipid profiles including LDL and HDL cholesterol, triglycerides, and total cholesterol; history of AMI; and the number of patients who were taking medications such as statins or ACEIs were not different between the good CCC and poor CCC groups.

3.2. Plasma DcR3 levels and its correlation with Rentrop grade

DcR3 could promote angiogenesis in vivo or in vitro [8]. To investigate whether DcR3 is associated with CCC formation, we tested plasma levels of DcR3 and assayed the correlation of DcR3 with Rentrop grade. The results showed that the plasma DcR3 level was significantly higher in the good CCC group compared with poor CCC group (328.00 ± 230.82 vs 194.84 ± 130.63 ng/l, $p < 0.01$) (Fig. 1A). Furthermore, there was a positive correlation of DcR3 with Rentrop grade ($R = 0.292$, $p < 0.01$) (Fig. 1B).

The plasma levels of VCAM-1 and VEGF-A and their correlations with DcR3.

It was reported that the pro-angiogenesis effect of DcR3 was associated with productions of VCAM-1 and VEGF-A [8,9]. To investigate the mechanism of DcR3 in regulation of CCC formation, we tested the plasma levels of VCAM-1 and VEGF-A and assayed their correlations with DcR3, respectively. The results showed that plasma levels of VCAM-1 and VEGF-A were higher in good CCC group than that in poor