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Circulating soluble form of LR11, a regulator of smooth muscle cell migration, is a novel marker for intima-media thickness of carotid arteries in type 2 diabetes

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ABSTRACT

Background: Smooth muscle cell (SMC) migration from the media to the intima, a process affecting plaque stability in advanced-stage atherosclerosis, is under the control of LR11. To delineate the clinical significance of the circulating soluble form of LR11 (sLR11) in patients with type 2 diabetes (T2D), we analyzed the correlation of sLR11 levels with intima-media thickness (IMT) of carotid arteries.
Methods: Plasma sLR11 levels were measured in 165 patients with T2D (mean age 56.2 ± 10.4 y, 58.2% males, and BMI 24.6 ± 3.6) by ELISA. Averaged IMT levels of common carotid arteries were determined by ultrasonography.
Results: Circulating sLR11 levels were 9.8 ± 3.5 ng/ml, and correlated positively with the classical atherosclerosis risk factors age, sex, systolic blood pressure, low-density lipoprotein-cholesterol (LDL-C), fasting plasma-glucose (FPG), and glycosylated hemoglobin. Multivariate linear regression analysis indicated that only FPG was associated with sLR11; sLR11 correlated positively with IMT, together with age and FPG, but less with LDL-C. Among the serum risk factors for IMT, multivariate linear regression analysis uncovered that sLR11 was independently associated with IMT. Subsequent logistic analysis revealed that FPG correlated best with IMT values at a cut-off of 0.80 mm and sLR11 at a cut-off of 0.90 mm, respectively, while LDL-C showed lower discriminatory power at any IMT cut-off values.
Conclusion: Increased sLR11 concentrations are highly associated with increased IMT as well as with FPG in middle-aged, non-obese patients with T2D. Circulating sLR11 may be a novel marker representing the pathophysiology of intimal SMCs in patients with T2D.

1. Introduction

The migration of vascular smooth muscle cells (SMCs) from the medial layer to the intimal layer is a key step in the development of advanced atherosclerosis. The initial steps are a functional disturbance of endothelial cells and the infiltration of activated monocytes/macrophages from the circulation [1,2]. Following migration, intimal SMCs, upon conversion in the media from a 'contractile' phenotype to a de-differentiated 'synthetic' phenotype, regulate various potential functions including matrix production, protease release and cytokine secretion [1–3]. Thus, migrated intimal SMCs are believed to be a player in forming atherosclerotic plaques and determining their fragility upon interaction with other vascular and inflammatory cells. Using animal models and cultured SMCs, we showed that disturbed interactions of SMCs with other vascular cells or in phenotype conversion of SMCs in dyslipidemia can lead to atherosclerosis progression and raised plaque fragility [4]. However, the clinical significance in diabetes mellitus of the intimal SMCs migrated under pathophysiological conditions has not yet been elucidated for the process of atherosclerosis.

LR11 (also called sorLA), an unusually complex and highly conserved member of the family of LDL receptor relatives (LRs), has been discovered and molecularly characterized by us and others [5–7]. The receptor mediates the plasma membrane localization of urokinase-type plasminogen activator receptor (uPAR), as the shed soluble form of the receptor (sLR11) binds to and colocalizes with uPAR on the cell surface [8]. LR11 is highly expressed in intimal SMCs at the intima-media border in the plaque area of experimental models of

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atherosclerosis [9,10]. Furthermore, overexpression of LR11 in SMCs enhances their migration via elevated concentrations of uPAR [10,11]. We have developed a sandwich enzyme-linked immunosorbent assay (ELISA) for the exact quantitation of circulating sLR11 using specific monoclonal antibodies against human LR11 [12,13]. With this method, it could be shown that the concentrations of sLR11 were increased in patients with familial hypercholesterolemia [14], coronary artery diseases [15–17], type 2 diabetes (T2D) [15,18,19], and in patients with hematological malignancies [13,20–23]. Notably, several independent studies have shown that the concentrations of sLR11 were increased in relation to markers of glycemic disturbances among the classical risk factors for atherosclerosis [11,14–16,18,19]. Thus, sLR11 has been suggested to reflect the conditions or degrees of migrated intimal SMCs, particularly in the vascular complications observed in diabetes.

Based on the knowledge generated by the above described broad spectrum of studies, we have now investigated in patients with T2D the relationship of sLR11 with the degrees of carotid IMT. Elevated IMT has been shown to predict vascular events, myocardial infarction, and stroke in asymptomatic adults [24].

2. Materials and methods

2.1. Subjects

We prospectively enrolled 165 patients with T2D who were treated by conventional pharmaceutical medications at the Department of Endocrinology, Yanbian University Hospital, China, between March 2012 and December 2013 (see Suppl. Table 1). Patients with unstable glycemic control, malignant diseases, inflammatory diseases, or under hemodialysis were excluded from the study. Diabetes mellitus (DM) was defined by either a value >6.5% of glycosylated haemoglobin (HbA1c) or being under medication with insulin or oral hypoglycemic drugs. Among the study subjects, 99 were on insulin therapy, 46 were on oral hypoglycemic agents, and 29 were on dietary therapy alone. Patients with systolic blood pressure (SBP) ≧ 140 mmHg or diastolic blood pressure (DBP) ≧ 90 mmHg, or under *anti*-hypertensive medication were considered hypertensive. Dyslipidemia was defined as low-density lipoprotein cholesterol (LDL-C) ≧ 140 mg/dl, high-density lipoprotein cholesterol (HDL-C) < 40 mg/dl, or triglyceride ≧ 150 mg/dl, or being under treatment with statins and/or lipid-lowering agents. We defined renal dysfunction as an estimated glomerular filtration rate (eGFR) of <60 mL/min/1.73 m^2, and calculated the eGFR using baseline serum creatinine. Five patients were treated with angiotensin- converting enzyme inhibitors or angiotensin receptor blockers, and none of the patients received statins or aspirin. The diagnosis of coronary artery diseases (CAD) was based on a history of myocardial infarction or angina pectoris, and that of cerebrovascular diseases (CVD) was based on a history of cerebral bleeding or infarction. Among the study subjects, eighteen and twenty-five presented with CAD and CVD, respectively. Obesity was defined as body mass index (BMI) ≧ 30 kg/m^2, and among the subjects, 14 displayed obesity. The study protocol was approved by the Human Investigation Review Committees of Yanbian University Hospital and Toho University Sakura Medical Center, and adhered to the principles of Declaration of Helsinki.

2.2. Blood samples

Venous blood was drawn after an overnight fast of 12–14 h for all laboratory measurements except postprandial plasma glucose (PPG). Plasma was separated from blood cells by centrifugation and used for the measurement of biochemical markers by routine laboratory methods at our institutions. Low-density lipoprotein-cholesterol (LDL-C) was estimated by the equation of Friedewald [11]. Seventeen of 165 patients were excluded for obtaining the LDL-C values, because the plasma triglyceride (TG) concentrations were >4.5 mmol/l. Plasma sLR11 concentrations were determined using a sandwich ELISA system (Sekisui) using samples frozen at −80 °C after collection as described [12,13]. Briefly, 12.5 μl of each plasma was used for the measurement of sLR11 by ELISA with specific monoclonal antibodies directed against human LR11.

2.3. IMT measurement

Examination of IMT was carried out with an ultrasound scanner (HD15; Philips) equipped with a linear 7.5-MHz transducer as previously described [11,25]. Briefly, IMT was defined as the distance from the leading edge of the lumen intima interface to the leading edge of the media-adventitia interface of the far wall. The measurement of IMT in the common carotid artery was performed along a 10-mm section just proximal to the carotid bulb, and the average IMT was calculated from the right and left IMTs of common carotid arteries. The patients with IMT values that differed between right and left arteries by >1.0 mm were excluded from the study.

2.4. Statistical analysis

Statistical analysis was performed with SPSS statistics ver 23 (IBM). Unless indicated otherwise, clinical results are shown as means ± SD or as proportions (%) for categorical data. Associations of sLR11 or IMT levels with various risk factors were examined by Pearson correlation analysis for continuous variables and by Mann-Whitney's *U* test for categorical variables between groups. Variables with $p < 0.05$ were considered statistically significant, and included in the subsequent multivariable models. In Tables 4 and 5, LDL-C was also included for the multivariate analysis for the comparison with 2 other plasma risk factors, i.e., fasting plasma glucose (FPG) and sLR11. We developed multivariable linear or logistic regression analysis models using sLR11 or IMT as dependent variables to assess independent relationships for clinical parameters, with $p < 0.05$ considered statistically significant. In logistic regression analyses, the IMT values were categorized by four different values, i.e., 0.8, 0.85, 0.9, and 0.95, for the comparison of the 3 plasma markers sLR11, FPG and LDL-C.

3. Results

3.1. Patients' characteristics

We studied 165 patients with T2D. The patients' backgrounds showed that the study population were mainly non-obese, T2D patients who have not been treated to reach the control concentrations of glucose, lipids, or blood pressure sufficient for the prevention of atherosclerosis (see Suppl. Table 1). In the study subjects, circulating sLR11 and carotid IMT levels were 9.79 ± 3.54 ng/ml and 0.79 ± 0.18 mm, respectively.

3.2. Relationships of sLR11 with classical risk factors for atherosclerosis

We first studied serum sLR11 concentrations in association with classical risk factors for atherosclerosis (Table 1). Univariate analysis showed that sLR11 positively correlated with age, SBP, LDL-C, FPG, and glycosylated haemoglobin among the classical atherogenic risks, and that sLR11 concentrations were significantly higher in females than in males (Table 1). There were no significant differences in sLR11 concentrations among patients with complications or on medications. Multivariate linear regression analysis (Table 2) indicated that sLR11 concentrations were significantly associated only with FPG, and not with any of the other 5 risk factors suggested to be associated with increased concentrations of sLR11 by univariate analyses. These results indicate that sLR11 concentrations were indeed increased in association with known risk factor(s) for atherosclerosis, and particularly, that FPG