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Original Article

Correlation between mimecan expression and coronary artery stenosis in patients with coronary heart disease

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Abstract: Objective: This study aimed to investigate the correlation between coronary artery stenosis and Mimecan expression in patients with coronary heart disease (CHD). Methods: Seventy eight patients with CHD and 80 controls without vascular lesions were recruited into present study. CHD patients were divided into one-vessel CHD subgroup, 2-vessel CHD subgroup and multivessel CHD subgroup. ELISA was performed to detect the expressions of serum Mimecan and nuclear factor kappaB (NF-κB). Results: When compared with control group, the expressions of serum mimecan gene and NF-κB significantly increased in CHD groups ($P < 0.05$); When compared with one-vessel and two-vessel CHD subgroups, the expressions of serum mimecan and NF-κB significantly increased in multivessel CHD subgroup ($P < 0.05$), significant difference was observed among three subgroups ($P < 0.05$). The expressions of serum mimecan and NF-κB were positively related to the severity of coronary lesions ($r_{mimecan}=0.79$, $r_{NF-\kappa B}=0.83$, $P < 0.05$). Conclusion: Increased expressions of serum mimecan and NF-κB in CHD patients are related to cardiac insufficiency, which may be ascribed to the binding of NF-κB to mimecan gene.

Keywords: Mimecan gene, nuclear factor κB, coronary heart disease, coronary artery stenosis

Introduction

Coronary atherosclerosis (CA) is the most common cause of coronary heart disease (CHD) and accounts for 95-99% of causes of CHD [1]. CA is a chronic disease that advances over time by accumulation of atheromatous plaque within the vessel wall in response to arterial injury and systemic risk factors. Multiple factors contribute to the pathogenesis of atherosclerosis, including endothelial dysfunction, hyperlipidemia, hypertension, diabetes, smoking, immunological and inflammatory factors [2]. In recent years, numerous studies on CHD mainly focus on the pathogenesis of CA. In the pathogenesis of AS, inflammation plays an important role [3, 4].

NF-κB is an important participant in the inflammation [5] and closely related to the pathogenesis of CA [6].

Mimecan, also known as osteoglycin, is a secretary protein encoded by the osteoglycin gene on human chromosome 9q22 [7]. The protein product isolated from demineralized bone is a corneal keratan sulfate proteoglycan and is present in many noncorneal tissues without keratan sulfate chains, such as the aorta and myocardium [8]. In normal adult rat carotid artery, osteoglycin is expressed in both the media and adventitia. Osteoglycin has been identified as a new marker of differentiated vascular smooth muscle cells (VSMCs) and may be an essential component of normal vascular matrix [9]. Increasing evidence has demonstrated that mimecan has a close relationship with the risk of cardiovascular (CV) disease (CVD). In atherosclerotic lesions, osteoglycin mRNA was up-regulated in the activated endothelium and thickened neointima [10]. Further research has shown that down-regulation of osteoglycin is required for arteriogenesis [11]. Currently, clinical investigation of mimecan expression has shown that it is decreased in patients with calcified abdominal aortic aneurysms [12]. However, there are no studies that investigate the relationship between severity of coronary lesions and mimecan.

Table 1. Baseline characteristics of patients in both groups

Group	n	Age (yrs)	Gender		Smoking	Hyperlipidemia	BMI
			M	F			
Control	80	58.75±6.51	42	38	25	20	22.02±3.01
CHD	78	56.38±3.31	40	38	23	18	23.21±2.53

Table 2. Mimecan and NF-κB expressions in CHD group and control group

Group	Mimecan	NF-κB
CHD	13.28±0.83	31.29±2.61
Control	4.62±0.51	18.12±2.04
t	40.36	19.46
P	< 0.001	< 0.001

Thus, In recent years, basic studies reveal that mimecan play an important role in the pathogenesis of CA [13]. The NF-κB binding site at the first intron of mimecan gene binds to NF-κB to involve the occurrence and development of CA. Thus, to investigate the correlation between mimecan expression and severity of coronary lesions is of great clinical importance.

Materials and methods

General information

A total of 158 patients were recruited between July 2010 and March 2013 from our department. There were 82 males and 76 females and the age ranged from 55 years to 85 years. Female patients were postmenopausal. On the basis of findings from coronary angiography, patients were divided into CHD group (n=78) and control group (n=80). Diagnostic criteria for CHD: ≥ 50% stenosis of left main coronary artery (LMCA), left anterior descending artery, left circumflex artery and/or right coronary artery under coronary angiography. Exclusion criteria: patients had a history of coronary intervention, coronary artery bypass grafting, myocardial infarction, left ventricular dysfunction, valvular heart disease, diabetes, stroke, hypertension, infectious diseases, cancer, autoimmune disease, connective tissue disease, renal dysfunction (Cr > 110 mmol/L), liver dysfunction (AST or ALT > 80 mmol/L). There were no marked differences in the baseline characteristics between two groups (**Table 1**).

Calculation of lesioned coronary arteries

According to the coronary lesions, CHD patients were divided into one-vessel CHD subgroup, two-vessel CHD subgroup and multivessel CHD subgroup. The involvement of LMCA was regarded two-vessel lesion. Gensini score was employed to evaluate the severity of coronary stenosis: 25% stenosis, 1; 50% stenosis, 2; 75% stenosis, 4; 90% stenosis, 8, 99% stenosis, 16 and 100% stenosis, 32. The final score was the sum of different scores. Results showed score 0-10 in 18 patients, score 11-23 in 22 patients, score 24-34 in 19 patients and score > 34 in 19 patients.

Methods

The expressions of serum mimecan and NF-κB were compared between CHD patients and control. The correlation of expressions of serum mimecan and NF-κB with the severity of coronary lesions was also evaluated.

Measurement of Mimecan and NF-κB: ELISA was performed for the detection of Mimecan and NF-κB in the serum with a microplate reader (BIO-RAD, USA) according to manufacturer's instructions. Reagents were purchased from Diagnostic Systems Laboratories INC.

Measurement of blood lipids: On the second day of admission, fasting blood was collected (3 ml) and blood lipids were measured with an automatic biochemical analyzer (OLYMPUS AU2700, Japan).

Statistical analysis

Statistical analysis was performed with SPSS version 13.0. All the quantitative data are expressed as mean ± standard deviation (SD) ($\bar{x}$±s), and comparisons were performed with one way analysis of variance among groups. Linear correlation analysis was employed to evaluate the relationship between Mimecan and NF-κB expressions and severity of stenosis. A value of $P < 0.05$ was considered statistically significant.

Results

The expressions of Mimecan and NF-κB in CHD patients were significantly higher than in controls (P < 0.05) (**Table 2**).

Expressions of Mimecan and NF-κB in different CHD subgroups: When compared with one-vessel CHD subgroup and two-vessel CHD sub-

Table 3. Expressions of Mimecan and NF-κB in different CHD subgroups

Group	n	Mimecan	NF-κB
1-vessel CHD	30	11.09±2.72	26.81±6.03
2-vessel CHD	29	17.93±3.18	33.76±8.41
Multivessel CHD	19	25.17±5.29	40.93±10.68
F		745.67	461.53
P		< 0.001	< 0.001

Table 4. Expressions of Mimecan and NF-κB in patients with different Gensini scores

Gensini score	n	Mimecan	NF-κB
0-10	18	9.21±1.21	20.13±7.32
11-23	22	16.32±2.81	31.13±6.13
24-34	19	24.35±6.22	39.56±12.81
> 34	19	36.16±9.32	52.35±13.13
F		5963.54	2087.32
P		< 0.001	< 0.001

group, the expressions of Mimecan and NF-κB increased significantly in multivessel CHD subgroup ($P < 0.05$), and marked differences were observed in the expressions of Mimecan and NF-κB among three subgroups ($P < 0.05$; **Table 3**).

Expressions of Mimecan and NF-κB in patients with different Gensini scores: When compared with 0-10 group, 11-23 group and 24-34 group, the Mimecan and NF-κB expressions in > 34 group increased significantly ($P < 0.05$), and significant differences were also observed between any two groups ($P < 0.05$) (**Table 4**).

Correlation of mimecan and NF-κB expressions with Gensini score

Results showed the Mimecan and NF-κB expressions were positively related to Gensini score (severity of coronary lesions) ($r2=0.79$ and 0.83, respectively; $P < 0.001$). The Mimecan and NF-κB expressions increased with the increase in the severity of coronary lesions. That is, NF-κB may act on the NF-κB binding site in the first intron of mimecan gene to exert its effect. Thus, the expressions of Mimecan and NF-κB are beneficial for the determination of severity of coronary lesions.

Discussion

Study has shown that mimecan play an important role in the formation of atherosclerotic plaques [14], but the specific mechanism is poorly understood. There is a NF-κB binding site in the first intron of mimecan gene, and the relationship between NF-κB and mimecan has been a focus in recent studies.

Mimecan, a member of the small leucine-rich proteoglycans gene family, was initially isolated in a truncated form from bovine bone and subsequently characterized as one of the three major keratan sulfate-containing proteoglycans, along with lumican and keratocan. Encoded by a single copy gene that is located on chromosome 9q22 in humans, mimecan is transcribed into at least 8 mRNAs, all of which produce an identical protein that is conserved in mice, bovine, and man, an indication of its functional importance [15, 16]. The protein is located in the extracellular matrix and is important for the regulation of the structure of the matrix, but also in the regulation of cell cycle and growth factor actions [17-19].

Mimecan, differentially expressed in aortae of SAD rats, is originally called osteoinductive factor and later renamed as mimecan. Mimecan in aorta was mainly produced by VSMCs and perivascular fibroblasts. It was confirmed a downregulation after the onset of arteriogenesis [11]. Its expression was also regulated during atherosclerosis in patients and animals. Differential expression of mimecan was found in VSMCs during neointima formation and in atherosclerosis plaques [9, 10]. And it was observed that absence of mimecan caused medial damages including medial degeneration, inflammation, and leukocyte extravasation through the media, associated with atherosclerotic lesions in apoE-deficient mice [20], which indicated that mimecan in aorta, might be one beneficial factor in the regulation of vascular function.

Mimecan is a molecule with 12-34 kD and belongs to the small leuc inerich proteog lycan (SLRP) family. The members of SLRP family share a central structure with 6-12 tandem leucine-rich repeats and a core protein composed of specific cysteine cluster at N and C terminals. Human mimecan gene is mapped to chromosome 9q22 which encodes a propeptide of secreted protein with 298 amino acids. There is a NF-κB binding site in the first intron of mimecan gene [21]. Mimecan promote is a nucleoprotein factor with pleiotropic transcriptional regulation. Overactivation of mimecan

may cause the formation of atherosclerotic plaques [22].

Rel or nuclear factor-kappa κ (NF-κB) proteins are composed of a group of structurally-related eukaryotic transcription factors, they include five NF-κB proteins in mammals: RelA/NF-κB-p65, RelB, c-Rel, NF-B1/NF-κB-p105, and NF-B2/NF-κB-p100. These factors regulate normal cellular and organismal processes, and some abnormal conditions. After activation by various stimuli, NF-κB translocates into the nucleus and stimulates the expression of genes involved in several biological functions. Inappropriate activation of NF-κB has been associated with many inflammatory diseases while persistent inhibition of NF-κB leads to inappropriate immune cell development or delayed cell growth. NF-κB plays a pivotal role in atherosclerotic plaques and peripheral blood mononuclear cells in patients with carotid atherosclerotic stenosis [23]. The expression of NF-κB is not detectable in normal carotid artery of animals, but can be detected after balloon-induced injury of the carotid artery [24]. More plaques with large atheroma and heavy plaque calcifications have developed gradually as the patients with risk factors of atherosclerosis get aged.

Studies have confirmed that NF-κB is an initiator in the formation of atherosclerotic plaques [25]. At resting status, NF-κB binds to its inhibitor unit (IKB) in the cytoplasm. The NF-κB activation is dependent on the degradation of IKB. Some risk factors (such as hyperlipidemia, hyperglycemia, diabetes and pathogens) may activate the IKB kinase on cell membrane, leading to the IKB Phosphorylation. Thus, IKB binds to several ubiquitins, and protein kinase degrades IKB. Then, NF-κB in the cytoplasm translocates into nucleus and binds to the NF-κB binding site of minecan gene, which is able to regulate the expressions of different inflamamtory factors [26] such as Tumor necrosis factor (TNF), interferon (IFN), IL-1, IL-6, vascular cell adhesion molecule (VCAM-1), intercellular adhesion molecule (ICAM-1), E-selectin and MCP-1. These inflammatory cytokines may promote the occurrence and development of atherosclerosis via similar or different pathways [27-29].

Our results showed the expressions of Mimecan and NF-κB in CHD patients were significantly higher than in controls, and they increased with the increase in severity of coronary lesions and Gensini score. These suggest that serum Mimecan and NF-κB expressions are positively relatyed to the severity of coronary lesions. Under physiological conditions, the Mimecan and NF-κB expressions remain stable and on a dynamic balance. When risk factors (such as endothelial damage or other factors causing atherosclerotic plaque formation) are present, their expressions become imbalanced, the binding of NF-κB to Mimecan is compromised, and Mimecan expression increases as a compensation to adapt to the environment.

Gensini score was positively related to the serum Mimecan and NF-κB expression. The expressions of Mimecan and NF-κB increased with the elevation of Gensini score. Thus, both Mimecan and NF-κB may be associated with the coronary stenosis.

Although numerous studies have conducted to investigate the mechanisms underlying the atherosclerotic plaque formation, the specific mechanism is still poorly understood. Our study indicates that Mimecan and NF-κB plays important roles in the atherosclerotic plaque formation, which provide evidence for the investigation of atherosclerotic plaque formation.

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Disclosure of conflict of interest

None.

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