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Talin and vinculin are downregulated in atherosclerotic plaque; Tampere Vascular Study

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ABSTRACT

Background and aims: Focal adhesions (FA) play an important role in the tissue remodeling and in the maintenance of tissue integrity and homeostasis. Talin and vinculin proteins are among the major constituents of FAs contributing to cellular well-being and intercellular communication.
Methods: Microarray analysis (MA) and qRT-PCR low-density array were implemented to analyze talin-1, talin-2, meta-vinculin and vinculin gene expression in circulating blood and arterial plaque.
Results: All analyzed genes were significantly and consistently downregulated in plaques (carotid, abdominal aortic and femoral regions) compared to left internal thoracic artery (LITA) control. The use of LITA samples as controls for arterial plaque samples was validated using immunohistochemistry by comparing LITA samples with healthy arterial samples from a cadaver. Even though the differences in expression levels between stable and unstable plaques were not statistically significant, we observed further negative tendency in the expression in unstable atherosclerotic plaques. The confocal tissue imaging revealed gradient of talin-1 expression in plaque with reduction close to the vessel lumen. Similar gradient was observed for talin-2 expression in LITA controls but was not detected in plaques. This suggests that impaired tissue mechanostability affects the tissue remodeling and healing capabilities leading to development of unstable plaques.
Conclusions: The central role of talin and vinculin in cell adhesions suggests that the disintegration of the tissue in atherosclerosis could be partially driven by downregulation of these genes, leading to loosening of cell-ECM interactions and remodeling of the tissue.

1. Introduction

Atherosclerosis is a disease of the vasculature with a complex etiology. Risk factors include age, sex, family history, dyslipidemia, high blood pressure and high body mass index (BMI), stress and dietary factors. The disease develops over a long time period and may remain asymptomatic over decades. It is characterized by

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Abbreviations

BMI	body mass index
CAD	coronary artery disease
ECM	extracellular matter
FA	focal adhesion
FC	focal complex
fc	fold change
HUVEC	human umbilical vein endothelial cell
ICAM	intercellular adhesion molecule
LDA	low density array
LITA	left internal thoracic artery
MA	microarray analysis
MΦ	macrophage
PECAM	platelet endothelial cell adhesion molecule
SMC	smooth muscle cell
TVS	Tampere Vascular Study
VBS	vinculin binding site
VCAM	vascular cell adhesion molecule
FFPE	formalin-fixed, paraffin-embedded
HE	hematoxylin-eosin

chronic inflammation of the arterial wall, by infiltration of macrophages (MΦ) and accumulation of oxidized low-density lipoproteins leading to MΦ conversion to foam cells [1].

The vasculature is continuously exposed to cyclical fluctuations of blood flow, pressure and fluid shear stress and also exhibits diurnal variation. The blood mechanical impacts of varying magnitudes exert significant influences on physiological and pathophysiological processes [2–4]. For illustration, veins and arteries are composed of several tissue layers with different cell and extracellular matter (ECM) content. This cell and ECM composition determines the tissue characteristics in terms of physicochemical properties [5,6]. Hence, each vessel layer possesses different ability to withstand, produce or transduce mechanical forces [5]. The mechanical pressure sensed by the endothelial cells is transferred from the extracellular space through the actin cytoskeletal network towards the nucleus [7,8].

To date, a number of genes implicated in cellular mechanostability and their altered expression has been associated with the progress of atherosclerosis. For example, ADAM metalloprotease disintegrins have been linked with cell-cell/surface adhesion and inflammation progression in the atherosclerotic plaque [9]. Moreover, the expression levels of integrin and kindlin family proteins were found to be altered in progressing atherosclerotic plaques [10]. Intergrin and kindlin proteins support leukocyte adhesion, transendothelial migration, platelet aggregation and thrombosis. Furthermore, integrins and kindlins are together with talin and vinculin among the major components of focal adhesions (FA). FAs are key attachments between cells and ECM and play an important role in cell morphology, differentiation, locomotion and intercellular communication. FAs are crucial for the tissue remodeling, integrity and homeostasis through the maintenance of intercellular gaps and cell adhesion supervision.

Talin is a large flexible protein [11] binding to transmembrane integrins (N-terminal FERM domain) [12] and to cytoskeletal actin (C-terminal rod) [13] providing a vital link between the intra- and extracellular space and allowing the communication between the ECM and nucleus [8]. Talin plays a significant role in the actin filament assembly and in spreading and migration of various cell types. During the adhesion maturation, talin recruits vinculin to crosslink with F-actin filaments and stabilize the adhesion complex. For this purpose talin rod contains several binding sites for vinculin [14]. Vinculin binding sites (VBSs) are buried inside the structural bundles and require a major conformational change in the bundle organization prior to vinculin binding [15]. Mechanical force has been suspected to mediate such domain reorganization and talin-vinculin binding [16,17]. Talin interacts with several ligands making it a vital component of numerous mechanosensor and chemical signaling pathways [18–21].

Vinculin is a cytoskeletal protein crosslinking talin and F-actin. Vinculin is ubiquitously expressed with high expression in skeletal, cardiac and smooth muscle. Vinculin head at the N-terminal end binds to talin's VBSs [22]. Vinculin tail at the C-terminal end binds F-actin [23]. Also other important interactions of vinculin have been recognized, for example with paxilin [24] and α-actinin [25]. These ligands make vinculin an important contributor to focal adhesion complex, as well as to the cytoskeletal assembly and stability.

The progress and the causatives of atherosclerosis have been intensively investigated during the past decades. Still, the mechanisms behind the disease development are not fully understood. In more detail, the mechanical impact of shear stress on the cell and tissue integrity has risen to attention only recently. We hypothesize that the cellular mechanostability and maintenance of tissue integrity through focal adhesions is an important factor in all stages of atherosclerotic plaque development. We speculate that the function of focal adhesions is compromised by altered expression of cell adhesion proteins talin and vinculin in atherosclerotic plaque as compared to non-atherosclerotic vessel wall.

In this work, we followed talin and vinculin expression in atherosclerotic plaque samples collected in ongoing Tampere Vascular Study (TVS) series. Gene expression in carotid, abdominal aortic and femoral plaque samples was compared to expression values in left internal thoracic artery (LITA) controls. Expression levels were determined by microarray analysis and low-density qRT-PCR-array. Results are supported by smooth muscle cell (SMC) and macrophage (MΦ) marker co-expression analysis. The tissue localization of talin and vinculin was investigated by confocal immunofluorescence study.

2. Materials and methods

2.1. Vascular samples

Arterial sample series from Tampere Vascular Study (TVS) [9,10,26], including samples from femoral, carotid and abdominal aortic regions, were obtained during open vascular procedures between 2005 and 2015. The patients fulfilled the following inclusion criteria: (1) carotid endarterectomy performed because of asymptomatic or symptomatic and hemodynamically significant carotid stenosis (>70%); (2) femoral or (3) aortic endarterectomy with aortoiliac or aortobifemoral bypass based on symptomatic peripheral arterial disease. The left internal thoracic artery (LITA) controls were obtained during coronary artery bypass surgery due to coronary artery disease (CAD). The samples were collected from patients subjected to open vascular surgery in the Division of Vascular Surgery and Heart Center, Tampere University Hospital. The patient's denial to participate in the study was used as a measure of exclusion. The vascular samples were classified according to American Heart Association recommendation [27]. The type V and VI atherosclerotic lesions were further histologically classified as stable and unstable according to the presence of fissure, rupture, hemorrhage or thrombosis. Gene expression was analyzed from carotid ($n = 29$), abdominal aortic ($n = 15$), and femoral ($n = 24$) plaques (cases) and compared to atherosclerosis-