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Molecular imaging of the extracellular matrix in the context of atherosclerosis☆

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ABSTRACT

This review summarizes the current status of molecular imaging of the extracellular matrix (ECM) in the context of atherosclerosis. Apart from cellular components, the ECM of the atherosclerotic plaque plays a relevant role during the initiation of atherosclerosis and its' subsequent progression. Important structural and signaling components of the ECM include elastin, collagen and fibrin. However, the ECM not only plays a structural role in the arterial wall but also interacts with different cell types and has important biological signaling functions. Molecular imaging of the ECM has emerged as a new diagnostic tool to characterize biological aspects of atherosclerotic plaques, which cannot be characterized by current clinically established imaging techniques, such as X-ray angiography. Different types of molecular probes can be detected *in vivo* by imaging modalities such as magnetic resonance imaging (MRI), positron emission tomography (PET) and single photon emission computed tomography (SPECT). The modality specific signaling component of the molecular probe provides information about its spatial location and local concentration. The successful introduction of molecular imaging into clinical practice and guidelines could open new pathways for an earlier detection of disease processes and a better understanding of the disease state on a biological level. Quantitative *in vivo* molecular parameters could also contribute to the development and evaluation of novel cardiovascular therapeutic interventions and the assessment of response to treatment.

Contents

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1. Introduction

Atherosclerosis is a major contributor to the group of cardiovascular diseases (CVD). Clinical manifestations of atherosclerosis, such as acute myocardial infarction (MI) and stroke, remain major causes of morbidity and mortality worldwide, even in spite of the growing awareness of the disease in recent years [1]. A substantial rise in the incidence of cardiovascular diseases is expected over the next decades due to an aging western population and an increase in risk factors such as obesity, diabetes and hypercholesterolemia [2,3]. The last decades of research in the field of atherosclerosis revealed novel molecular pathways and new mechanisms causing complications in human atherosclerotic plaques [4–7]. The growing understanding of the pathogenesis of atherosclerosis has opened up new perspectives for molecular imaging. This review summarizes the state of the art of molecular imaging with a specific focus on the extracellular matrix (ECM) in atherosclerotic vessel wall disease. The ECM is highly abundant in almost all biological tissues. It mainly consists of different kinds of collagen and elastin fibers, which represent the highest expressed extracellular matrixproteins [8], interspersed with glycosaminoglycans and proteoglycans [9]. The ECM is also the most abundant component of the normal arterial wall and the atherosclerotic plaque, including its fibrous cap. A dynamic balance between the synthesis and breakdown of ECM proteins controls its available amount and influences the progression of atherosclerotic disease. Within the matrix of atherosclerotic plaques, smooth muscle cells (SMCs), fibroblasts and to a lesser extent macrophages synthesize and excrete different kinds of ECM proteins. In contrast, ECM-degrading enzymes such as metalloproteinases, cathepsins, serine proteases, chymase and tryptase are expressed by macrophages, SMCs, mast cells and T-lymphocytes [10]. These enzymes have a strong proteolytic activity, especially in vulnerable or unstable atherosclerotic lesions [11,12]. The great ECM components which leads to a fragmentation of the ECM and a decrease of the available amount of these extracellular proteins. This leads to a thinning of the plaque's fibrous cap. Therefore, ECM degradation can make plaque regions more prone to disruption and predispose instability [13]. From a biological standpoint, the transition from a stable to an unstable or vulnerable atherosclerotic plaque is the consequence of complex interactions between various different molecular components of the plaque.

Molecular imaging is emerging as a non-invasive method for the characterization of these pathological processes at the molecular and cellular level. It enables the direct *in vivo* visualization of biological processes and aims to elucidate the interactions between these processes during the initiation and progression of disease by applying different imaging techniques in combination with molecular probes [14–16]. In contrast to conventional clinical imaging methods such as X-ray angiography, molecular imaging aims at visualizing and quantifying underlying pathological molecular mechanisms, rather than imaging the resulting anatomical/morphological consequences, such as the degree of stenosis [17,18].

2. The role of extracellular matrix components during the development of atherosclerosis

2.1. Role of elastin

Elastin is one of the dominant proteins of the ECM. It is found mainly in the media of the “healthy” arterial wall [19]. It contributes to up to 50% of the dry weight of arteries [20]. In veins it is expressed in substantially lower concentrations. Elastin plays a key structural role in maintaining the integrity of the arterial wall. It significantly contributes to the tensile strength of large and small arteries, enabling them to sustain permanent mechanical stress from arterial pulsation and intravascular pressure. Elastin is primarily expressed by SMCs. The production starts with the synthesis and secretion of the soluble precursor tropoelastin [8]. In the next step, tropoelastin is cross-linked and aligned into long elastin polymers that organize into rings of elastic lamellae in the arterial wall. The degree of elastin cross-linking is directly related to the tensile strength of the extracellular matrix in the arterial wall [21]. Elastin's abundance and its high expression in the matrix of atherosclerotic plaque make this protein a promising molecular target [22,23]. These properties make elastin especially useful as a biomarker for magnetic resonance imaging (MRI), which is associated with a lower sensitivity for probe detection compared to *e.g.* positron emission tomography (PET). However MRI enables imaging with a higher spatial resolution and soft tissue contrast. Imaging was high spatial resolution is important for the visualization of morphological and biological changes within the thin atherosclerotic arterial wall. Apart from its structural role, elastin has important biological signaling and regulatory functions during arterial development. It controls proliferation of for example proinflammatory cells in the vascular wall [24]. Different biological and biophysical triggers initiate the increased elastogenesis in the matrix of plaques. [25,26]. This leads to an increase in the relative amount of elastin during plaque development [8,19,27]. The relative composition of the matrix is relevant for plaque progression and the differentiation from stable to unstable/vulnerable plaques. Different factors lead to a degradation and fragmentation of elastin. Elastic fibers are targeted and degraded by various matrix metalloproteinases (MMPs), especially subtypes MMP 2 and MMP 9 [28]. Under physiologic conditions MMPs are already expressed in latent forms. Their activation is triggered by an injury of the arterial wall or by proinflammatory processes [29,30]. The reduction of elasticity of the tunica media is a result of reduced elastin expression and alterations in elastin cross-linking and elastolysis. These processes lead to further vascular damage and therefore represent a predispositional factor for the progression of atherosclerosis [31,32]. The visualization of quantitative changes in elastin expression in the matrix enables an improved *in vivo* characterization of plaques. This is highly relevant as human stable and unstable/vulnerable plaque types can be discriminated based on their relative elastic fiber composition [8].

2.2. Role of collagen

Collagen describes a family of proteins with at least 19 genetically distinct subtypes [33]. Six of these collagen subtypes (subtypes I, III, IV, V, VI and VIII) are expressed in the arterial system [34]. Collagen subtypes I and III are highly expressed and are found mainly in the ECM of plaques [35]. Approximately two-thirds of the total amount of collagen is made up of subtype I [36]. In advanced atherosclerotic plaques, subtype V collagen is also highly expressed in the ECM [37]. In the fibrous cap regions of plaques, pronounced subtype IV collagen depositions can be measured [35,37,38]. Following vascular injury and during the progression of atherosclerosis, subtype VIII collagen, a short chain collagen, is highly expressed. The accumulation of collagen is characteristic for atherosclerotic plaques and it is estimated that the different forms of collagens can comprise up to 60% of the overall protein