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Review

The dark and bright side of atherosclerotic calcification

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ABSTRACT

Vascular calcification is an unfavorable event in the natural history of atherosclerosis that predicts cardiovascular morbidity and mortality. However, increasing evidence suggests that different calcification patterns are associated with different or even opposite histopathological and clinical features, reflecting the dual relationship between inflammation and calcification. In fact, initial calcium deposition in response to pro-inflammatory stimuli results in the formation of spotty or granular calcification ("microcalcification"), which induces further inflammation. This vicious cycle favors plaque rupture, unless an adaptive response prevails, with blunting of inflammation and survival of vascular smooth muscle cells (VSMCs). VSMCs promote fibrosis and also undergo osteogenic transdifferentiation, with formation of homogeneous or sheet-like calcification ("macrocalcification"), that stabilizes the plaque by serving as a barrier towards inflammation. Unfortunately, little is known about the molecular mechanisms regulating this adaptive response. The advanced glycation/lipoxidation endproducts (AGEs/ALEs) have been shown to promote vascular calcification and atherosclerosis. Recent evidence suggests that two AGE/ALE receptors, RAGE and galectin-3, modulate in divergent ways, not only inflammation, but also vascular osteogenesis, by favoring "microcalcification" and "macrocalcification", respectively. Galectin-3 seems essential for VSMC transdifferentiation into osteoblast-like cells via direct modulation of the WNT-β-catenin signaling, thus driving formation of "macrocalcification", whereas RAGE favors deposition of "microcalcification" by promoting and perpetuating inflammation and by counteracting the osteoblastogenic effect of galectin-3. Further studies are required to understand the molecular mechanisms regulating transition from "microcalcification" to "macrocalcification", thus allowing to design therapeutic strategies which favor this adaptive process, in order to limit the adverse effects of established atherosclerotic calcification.

List of abbreviations: CVD, cardiovascular disease; VSMC, vascular smooth muscle cell; CAC, coronary artery calcification; AU, Agatston units; CHD, coronary heart disease; CT, computed tomography; ApoE, apolipoprotein E; MV, matrix vesicle; Th, T helper; M, macrophage; PET, positron emission tomography; AMI, acute myocardial infarction; IVUS, intravascular ultrasound; ACS, acute coronary syndrome; HR, hazard ratio; CI, confidence interval; SD, standard deviation; OPN, osteopontin; Gla, γ-carboxyglutamic acid; MMP, metalloprotease; ALP, alkaline phosphatase; Cbfα1, core binding factor α1; Runx2, Runt-related transcription factor 2; α-SMA, α-smooth muscle actin; IL, interleukin; M_{reg}, regulatory macrophages; T_{reg}, regulatory T lymphocytes; RELMα, resistin-like molecule-α; AGEs, advanced glycation endproducts; ALEs, advanced lipoxidation endproducts; RANK, receptor activator of nuclear factor-κB; RANKL, RANK ligand; OPG, osteoprotegerin; miRs, microRNAs; RAGE, receptor for AGEs; sNCX1, sodium–calcium exchanger 1; PMCA1, plasma membrane Ca^{2+} ATPase 1; NCKX4, Na^{+}/Ca^{2+}–K^{+} exchanger 4; Enpp1, ectonucleotide phosphodiesterase 1.

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Contents

1. Introduction

Atherosclerotic calcification is an ancient phenomenon, since it was documented in an autopsy of the mummy of an elderly Egyptian woman [1] and, more recently, in radiographs of a 5300-year-old, naturally mummified "ice-man" discovered in 1991 in the Tyrolean Alps at the Austrian-Italian border [2]. In the 19th century, the pathologist Rudolph Virchow first recognized the role of vascular calcification in the pathobiology of cardiovascular disease (CVD), though he considered it as a passive, degenerative phenomenon [3]. In contrast, over the last two decades, vascular calcification has been increasingly recognized as an active, tightly regulated process [4].

Four non-mutually exclusive mechanisms have been claimed to explain the occurrence of calcification within the vessel wall, i.e. (1) macrophage and vascular smooth muscle cell (VSMC) death leading to release of apoptotic bodies and necrotic debris that may serve to nucleate apatite at sites of injury; (2) circulating nucleational complexes released from actively remodeling bone or matrix vesicles released locally by VSMCs and macrophages; (3) reduction of constitutively expressed circulating and tissue-derived mineralization inhibitors leading to default apatite deposition; and (4) induction of osteogenic transdifferentiation of VSMCs possibly resulting in bone formation [5].

A large body of evidence indicates that vascular calcification is strongly associated with morbidity and mortality from CVD. In a population-based sample of 6722 men and women from 4 racial or ethnic groups, coronary artery calcification (CAC) between 101 and 300 increased the adjusted risk of a coronary event by a factor of 7.73 and CAC >300 by a factor of 9.67 [6]. In addition, in a representative sample of Dallas County residents aged 30–65 years, CAC was shown to be strongly associated with conditions conferring a high CVD risk, such as diabetes and chronic kidney disease [7]. CAC was also found to be a predictor of future CVD events beyond traditional risk factors [6] and over and above Framingham risk score [8]. In particular, adding CAC score ($\geq$400 Agatston units, AU) to traditional coronary heart disease (CHD) scoring systems improved prediction of coronary death and nonfatal AMI in asymptomatic subjects at intermediate risk (i.e. Framingham risk score of 10–20% and Adult Treatment Panel score of 6–20%), with reclassification to the high-risk category [9]. As a consequence, the American College of Cardiology Foundation/American Heart Association guidelines recommend to include measurement of CAC by multidetector or electron beam computer tomography (CT) in CVD risk assessment in these individuals [10].

These findings imply that vascular calcification is a common and unfavorable event in the natural history of atherosclerosis that predicts the occurrence of major acute CVD events such as nonfatal and fatal myocardial infarction and stroke. However, increasing evidence supports the concept that, once established within a plaque, vascular calcification may either promote plaque progression toward formation of unstable, rupture-prone lesions or favor its stabilization, depending on the type and pattern of calcium deposition.

2. Dual relation between calcification and inflammation

Though Inflammation may trigger vascular calcification via all the mechanisms listed above, including the stimulation of VSMC osteogenic transdifferentiation, the relation between the two phenomena is dual [11]. This has been demonstrated *in vivo*, in an elegant molecular imaging study showing that calcification associates with inflammation in apolipoprotein E (ApoE) null mice [12]. In the very early stages of atherosclerosis, vascular inflammation and osteogenesis evolved in close proximity, overlapped at border regions, and increased concomitantly with plaque progression, suggesting an intimate relationship between them. Moreover, microcalcifications and apatite nanocrystals co-localized with cholesterol crystals and were found in membrane-bound vesicles in the size range of apoptotic bodies and matrix vesicles. In contrast, advanced lesions were characterized by an inverse relation between inflammation and calcification, which were spatially distinct, with little or no inflammation in extensively calcific plaques.

These findings support the concept that initial calcium deposition within apoptotic bodies and matrix vesicles (MVs) released from macrophages and VSMCs in response to pro-inflammatory stimuli results in the formation of microcalcification nuclei, which induce further cycles of inflammation and triggering of calcium deposition, causing propagation of damage and frustrated attempts at tissue repair [11] (Fig. 1). Ultimately, this vicious circuit favors plaque rupture due to the progressive thinning of the fibrous cap and the unfavorable mechanical effect of microcalcification, which increases local stress on the thinned cap and leads to interfacial debonding [13]. However, if an adaptive response prevails, inflammation is blunted and VSMCs survive, with promotion of fibrosis and stabilization of the atherosclerotic lesion. In addition, if pro-osteogenic conditions persist, VSMCs continue to transdifferentiate with acquisition of a mature osteoblast-like phenotype. These cells are able to orchestrate a properly regulated mineralization process leading to formation of macrocalcification, which further stabilizes the plaque also by acting as a barrier toward the spread of inflammation [11] (Fig. 1). Yet, calcified plates may fracture, thus resulting in the formation of nodular calcification that is accompanied by fibrin deposition. Nodules may