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Osteogenesis in calcified aortic valve disease: From histopathological observation towards molecular understanding

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ABSTRACT

Calcified aortic valve disease (CAVD) is the most common heart valve disease in aged patients, with a disease continuum that ranges from mild valve thickening to severe calcification. In the past, calcification in CAVD was considered degenerative because of the time-dependent wear-and-tear of the leaflets with passive calcium deposition. Now, insights into the histopathological features, clinical data, and molecular mechanisms of CAVD have been greatly highlighted by findings that valvular calcification is a tightly regulated process resembling the osteogenic process. In this review, we focus on osteogenesis (bone formation) during the progression of CAVD and highlight the recent advances in understanding the cellular and molecular mechanisms of osteogenesis in valvular calcification.

Contents

1. Introduction

Calcified aortic valve disease (CAVD) is the most common valvular disease in the developed world, and its prevalence will likely increase as life expectancy will continue to rise in the coming decades (Yutzey et al., 2014; Towler, 2013; Pawade et al., 2015). Despite this, there are no medical therapies to prevent or slow disease progression, and the only available treatment is surgical aortic valve replacement, which is not applicable for all patients (Asimakopoulos et al., 1997; Likosky et al., 2009; Une et al., 2015; Osnabrugge et al., 2013; Spaccarotella et al., 2011). Therefore, in order to develop a pharmacological strategy to delay disease

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progression, studies of valves from humans and experimental animals have begun to clarify mechanisms underlying CAVD.

CAVD is a slow but progressive disorder of the aortic valve characterized by calcification of the valve leaflets (Bonow et al., 2008; Otto, 2006). In the past, progressive valvular calcification was considered to be imminent, passive, and degenerative because of time-dependent wear- and-tear of the leaflets with passive calcium deposition. Now, work from laboratories worldwide has highlighted that it is a complex, regulated process and has numerous similarities with actively controlled processes occurring in the bone (Rajamannan et al., 2011; Mathieu et al., 2007; Yutzey et al., 2014). In this review, we focus on osteogenesis (bone formation) during the progression of CAVD, and highlight the recent advances in understanding the cellular and molecular mechanisms of osteogenesis in valvular calcification.

2. Normal aortic valve structure and cell types

In humans, the aortic valve has three thin and pliable leaflets. Each leaflet is composed of three layers: fibrosa, spongiosa, and ventricularis (Mendelson and Schoen, 2006; Donnelly, 2008). The fibrosa lines the aortic side of the leaflet and is rich in collagen fibers. The ventricularis, the extension of the ventricular endocardium, is the elastic lamina on the aortic side of the leaflet. The spongiosa lies between these two layers and consists of loose connective tissue, proteins, and glycosaminoglycans. These layers work together to provide tensile strength and pliability for decades of repetitive motion.

There are two major types of cells, valvular endothelial cells (VECs) and valvular interstitial cells (VICs), in the aortic valve. VECs form a continuous monolayer that completely lines both sides of the valve and is contiguous with the endothelial cell layer of adjacent regions of the endocardium and/or great vessels. They serve as a protective barrier for the underlying tissue by regulating permeability, mediating inflammatory cell adhesion, and preventing thrombosis (Butcher and Nerem, 2007). Furthermore, VECs have been shown to inhibit and regulate pathological proliferation and differentiation of VICs (Butcher and Nerem, 2006) and maintain valve homeostasis via paracrine signaling (Yip and Simmons, 2011; Gould et al., 2013).

VICs are the most abundant cells in the aortic valve and are dispersed throughout all three layers (Rabkin-Aikawa et al., 2004; Taylor et al., 2003; Yperman et al., 2004). They are critical for maintaining aortic valve homeostasis and function through many actions, such as proliferation, secretion of matrix metalloproteinases, and *de novo* extracellular matrix (ECM) molecules (Gould et al., 2014). In the healthy adult valve, VICs predominantly display a quiescent fibroblast phenotype, without synthetic or destructive activity in the ECM. The response of VICs to valve injury is attributable to pathological conditions and abnormal hemodynamic/mechanical forces. VICs can be activated to be a secretory myofibroblast phenotype. Activated VICs are more proliferative, show higher motility, and express specific α-smooth muscle actin (SMA) proteins, which may lead activated VICs to playing a key role in tissue repair and maintaining homeostasis (Liu and Gotlieb, 2007; Liu et al., 2007; Xu et al., 2012).

3. Osteogenesis during the progression of CAVD

In the context of CAVD progression, emerging evidence derived from human observations and animal studies has indicated that the disease spectrum of CAVD ranges from initial alterations in the cell biology of the leaflets to end-stage calcification. According to the findings of histological analyses and noninvasive imaging techniques (Freeman and Otto, 2005; Rajamannan et al., 2003; Sider et al., 2014), the progression of CAVD can be divided into three phases: an early initiation phase characterized by subendothelial plaque-like lesions, an advanced-stage lesion showing microcalcification, and an end-stage lesion dominated by serious calcium deposits. Of note, during disease progression, osteogenesis has been found to be involved in each phase of CAVD.

3.1. Early-stage lesions

Endothelial damage resulting from increased mechanical stress and reduced shear stress is believed to be the initial step of CAVD (Butcher and Nerem, 2007; Sucosky et al., 2009). Subsequently, lipids, such as lipoprotein and oxidized low-density lipoprotein cholesterol, infiltrate the valve. Consequently, focal subendothelial plaque-like lesions, including inflammatory cell infiltration, subendothelial lipid accumulation, ECM disorganization and subjacent elastic lamina fragmentation, can be seen (O'Brien et al., 1996; Olsson et al., 1999; Otto et al., 1994). These subendothelial plaque-like lesions represent the earliest pathological changes and preferentially occur on the aortic surface of the leaflet that extends to the adjacent fibrosa layer (Freeman and Otto, 2005; Towler, 2013).

The histological appearance of subendothelial plaque-like lesions supports the hypothesis that CAVD is the result of an active inflammatory disease process that has some similarities to atherosclerosis. Supporting this hypothesis, recent studies (Kaden et al., 2005) demonstrated that normal human aortic valves contain relatively few macrophages, whereas abundant leukocyte and macrophage infiltration were seen in excised calcified human aortic valves. Furthermore, inflammatory cell infiltration is typically seen at sites where VECs are activated, reflecting the importance of the VECs as the initial barrier to inflammatory insults (Aikawa et al., 2007, 2007a; Leopold, 2012). In addition, inflammatory cells within the subendothelium and fibrosa generate oxidative stress and release various growth factors and cytokines (Mohler et al., 2001), such as transforming growth factors (TGFs), tumor necrosis factor α, interleukin-1β, and receptor activator of nuclear factor κB ligand. These growth factors and cytokines are the leading causes of osteoblast differentiation through activation of multiple signaling pathways [e.g., Notch, bone morphogenetic protein (BMP), and Wnt/β-catenin] and creation of a milieu conducive to osteogenesis in valves.

3.2. Advanced-stage lesions

As CAVD progresses, microcalcification can be seen as fine stipples of amorphous calcium deposits under light microscopy (Leopold, 2012). This calcification-like structure is a result of cell death and the release of matrix vesicles in these areas. Early studies (Jian et al., 2003) have reported calcified nodules formed by apoptotic VICs in culture after treatment with TGF-β1, as the apoptotic bodies (300–1000 nm) from dying cells may provide favorable conditions and starting sites for calcification. Matrix vesicles (30–300 nm) can also serve as nucleation sites for calcium deposition and have been implicated in bone and cartilage mineralization (Kim, 1976; New et al., 2013 and New and Aikawa, 2013). During bone formation, matrix vesicles are synthesized and released from osteoblasts. They contain alkaline phosphatase (ALP) and other prerequisite components for calcium crystal deposition and facilitate the formation of needle-like crystals of hydroxyapatite. In this manner, the areas that are enriched in matrix vesicles may allow for calcium deposition, nodule formation, and expansion. In bone, as these hydroxyapatite crystals expand, they pierce the outer membrane of the vesicle and become exposed to the extracellular environment, thereby forming nucleation sites for