

Role of “osteogenic” cardiac fibroblasts in pathological heart calcification

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Calcification of mammalian soft tissues as induced by age and injury is a cell mediated process that resembles bone formation in the skeletal system with calcification of the extracellular matrix by cells capable of mineralization (1). The abnormal accumulation of calcium salts occurs in the pathologic calcification of any tissue (2). Two basic forms of calcification are recognized; dystrophic and metastatic (3). Ischemic, traumatic, infectious and/or inflammatory, and neoplastic processes among others are significant causes of dystrophic myocardial calcification. Any abnormality of calcium metabolism can lead to metastatic myocardial calcification, including chronic renal failure, bone destruction or increased bone turnover, hyperparathyroidism, and vitamin D-related disorders. Calcification of the cardiovascular system, such as aortic valve and vascular calcification, has important clinical significance (4). Myocardial calcification is an unusual form of soft tissue calcification characterized by abnormal calcific accumulation within the heart muscle which can occur in the absence of calcification of vessels, valves, or other organs (5). Calcification within the myocardium is one of the most common underlying causes of heart block where calcification and fibrosis of the conduction system interrupts smooth propagation of electrical impulses (6). The occurrence of heart muscle calcification is observed in senile heart diseases and in varieties of disease conditions,

such as chronic renal disease, diabetes, and myocardial injury secondary to inflammation or ischemia (7). However, the identity of cells and the mechanism contributing to pathological myocardial calcification has remained elusive.

The heart is composed of cardiomyocytes (CMs) as well as interstitial fibroblasts in addition to other cells. The uninjured adult murine myocardium is comprised of roughly 56% myocytes, 27% fibroblasts, 7% endothelial cells and 10% vascular smooth muscle cells as well as other immune cells (8). Studies on the heart have historically focused in main on CMs, but the role of cardiac fibroblasts (CFs) has received increasing recent attention as pivotal functions in the maintenance of cardiac function, physiological cardiac remodeling after heart stress and pathological remodeling (9). CFs can exhibit plasticity after heart injury and adopt alternate cell fates (10). We have recently demonstrated that ataxia telangiectasia mutated (ATM)-regulated effects within CFs are pivotal in doxorubicin-induced cardiotoxicity, and antagonism of ATM and its functions may have potential therapeutic implications (11). Ectopic calcification of soft tissues usually occurs in areas of injury associated with fibrosis (12). CFs adopt osteogenic cell fate which are thought to contribute to pathological cardiac calcification, as illustrated in the paper by Pallai *et al.* in the recent issue of *Cell Stem Cell* (Figure 1) (13).

These authors started from the observation that

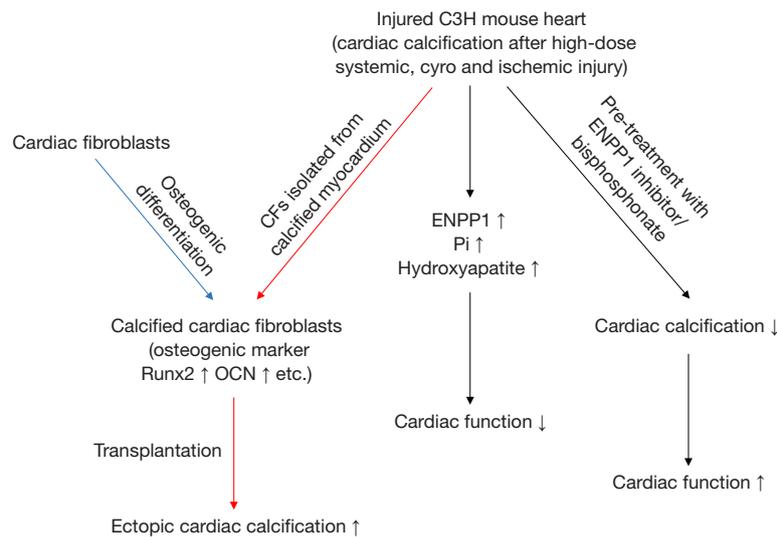


Figure 1 Cardiac fibroblasts adopt osteogenic cell-like fates, and the CF and the ENPP1-PPi-Pi axis can be targeted to decrease pathological cardiac calcification and improve cardiac function. Induction of expression of stable canonical osteoblast markers (i.e., Runx2, osteocalcin) were confirmed in cardiac fibroblasts following osteogenic differentiation (blue arrow) or cardiac fibroblasts (CFs) isolated from multiple models of myocardial calcification induced after a variety of injuries (high-dose systemic steroids, cyro and ischemic injury) (red arrow). Cell transplantation experiments where CFs harvested following explant culture of calcified or uninjured myocardium of C3H mice were injected into subcutaneous pockets of another host showed a direct role of CFs isolated from calcific heart lesions in mediating ectopic soft tissue calcification (red arrow). Expression of ENPP1 and Pi, and formation of calcium hydroxyapatite were markedly increased in calcified regions of injured C3H mouse hearts. Pre-treatment with small molecules (SYL-001 or ARL67156) that inhibit ENPP1, or inhibitors of bone mineralization, bisphosphonates (e.g., etidronate), that inhibit the function of ENPP1-PPi-Pi axis followed by injury, led to significantly decreased cardiac calcification and improved post-injury cardiac function.

deposition of calcium hydroxyapatite (visualized with Alizarin Red staining) and induction of expression of stable canonical osteoblast markers (i.e., Runx2, osteocalcin quantified by qPCR) were confirmed in CFs following osteogenic differentiation or CFs isolated from multiple models of myocardial calcification induced after a variety of injuries (i.e., high-dose systemic steroids, cyro and ischemic injury), suggesting that CFs can adopt an osteoblast cell-like fate *in vitro* and *in vivo*. Next, they observed that CFs adjacent to calcified myocardium induced by injury not only expressed abundant osteogenic markers (i.e., Runx2, osteocalcin) and extracellular matrix protein (i.e., osteopontin), but also CFs could directly influence mineralization of extracellular matrix. Moreover, cell transplantation experiments showed that CFs harvested following explant culture of calcified or uninjured myocardium of C3H mice when injected into subcutaneous pockets of another host showed a direct role of CFs from calcific heart lesions in mediating ectopic soft tissue calcification.

They then addressed mechanisms of osteogenesis or mineralization with RNA-seq on uninjured and injured cardiac regions of C3H strains (calcification after injury) and B6 strains (no calcification after injury) and identified that 11 osteogenic genes (ENPP1, Runx2, Col1a1, and Fibronectin etc.) were markedly higher in injured C3H hearts compared to control uninjured C3H hearts or injured B6 hearts. None of the B6 mice after injury developed cardiac calcification, therefore the disparate injury response in C3H and B6 mouse strains demonstrated a mechanism that is differentially regulated in calcific versus non-calcific hearts.

ENPP1 is expressed in differentiated osteoblasts, is thought to be an established mediator of osteoblast maturation and tissue mineralization by increasing extracellular pyrophosphate, and animals deficient in ENPP1 have decreased mineralization of long bones (14). What is the role of ENPP1 in heart calcification? To demonstrate its causal involvement, Pallai *et al.* first showed abundant expression of ENPP1 in CFs and significantly

higher phosphate (Pi) levels in calcified regions of the heart after injury. They also used Raman spectroscopy (15) to show that extracellular pyrophosphate (PPi) generated by ENPP1 can induce myocardial calcific deposits by serving as a substrate for Pi generation and formation of calcium hydroxyapatite. Mechanistically, they provide evidence that CFs harvested from calcific myocardium are sufficient to induce mineralization of the matrix via upregulation of ENPP1.

They next investigated involvement of the ENPP1-PPi-Pi axis as a target to decrease ectopic cardiac calcification. They provide evidence that post-injury cardiac calcification was decreased by pre-treatment with the ENPP1 inhibitors SYL-001 or ARL67156, respectively. Moreover, pre-treatment with inhibitors of bone mineralization, bisphosphonates (e.g., etidronate) which serve as functional antagonists of the ENPP1-PPi-Pi axis, followed by injury could significantly attenuate calcification and improve cardiac function. However, etidronate administered after the development of calcification did not reverse or decrease the amount of deposited calcium. These findings highlight the aberrant plasticity of CFs in contributing to ectopic calcification and identify novel pharmacological targets (CF and the ENPP1-PPi-Pi axis) for therapeutic implications.

The cell-matrix mechanics described in this study are similar to that of underlying vascular and valvular calcification. Vascular cells, like bone cells, interact with their matrix substrate through molecular signals and biomechanical signals to control pathological calcification of the artery wall and cardiac valves (4). The present study by Pallai *et al.* also showed involvement of cell-matrix mechanics in pathological heart calcification—that CFs express ENPP1, and ENPP1-generated PPi in heart increases mineralization via hydrolysis of PPi to generate Pi by tissue non-specific alkaline phosphatase (TANP) and subsequent calcium hydroxyapatite formation in extracellular matrix to develop post-injury cardiac calcification. Calcification of the extracellular matrix is critically regulated by the balance of extracellular Pi and PPi via regulation of ENPP1. The interaction of CFs with matrix may contribute to control of pathological cardiac calcification. The authors add to our understanding of CF-matrix mechanics in pathological heart calcification, but it is necessary to further investigate the interaction of CM-matrix or CF-CM.

The study by Pallai *et al.* is the first to focus on the identity of cells contributing to pathological heart calcification. Their results showed that, surprisingly, CFs and not

CMs, as would be expected *a priori*, adopt osteogenic fates and that the ENPP1-PPi-Pi axis is a potential cellular and pharmacological target for developing therapies for pathological heart calcification. Per-administration of small molecules (SYL-001 or ARL67156) that inhibit ENPP1, or inhibitors of bone mineralization, bisphosphonates (e.g., etidronate), that inhibit the function of the ENPP1-PPi-Pi axis followed by injury, led to significantly decreased cardiac calcification and preservation of post-injury cardiac function in the present study, and might serve as a potential anti-calcification drug. Cell-type specific targeted therapy (e.g., fibroblast-specific ENPP1 antagonism), possibly in combination with those targeting other pathways involved in injury-induced cardiac calcification might pose further therapeutic possibilities which need to be addressed in translational studies targeting lineage-restricted modulation of cell functions.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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