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KLF15 and Cardiac Fibrosis: The Heart Thickens

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Cardiac fibrosis is a pathognomonic feature of pathological cardiac hypertrophy and contributes to the development of heart failure, a growing epidemic in developed countries. The traditional model for the development of heart failure has postulated that cardiac myocytes subjected to pathological loading conditions initially adapt by undergoing cellular hypertrophy, eventually become injured by prolonged stress and subsequently undergo apoptosis, leading to heart failure [1]. Implicit in this model is the subordinate role of other cell types such as inflammatory cells and cardiac fibroblasts, which are activated only in response to the cries of injured and dying myocytes. A number of interesting studies, however, highlight the important role of cardiac fibroblasts in the pathogenesis of cardiac fibrosis and underscore the dynamic nature of the extracellular matrix, leading to reexamination of this paradigm (reviewed in [2]).

Fibrosis has long been studied in the context of wound healing and in systemic rheumatologic disorders characterized by excessive fibrosis, such as scleroderma and progressive systemic sclerosis. Tissue fibrosis is generally considered to reflect excessive production and deposition of extracellular matrix (ECM), and is hypothesized to arise under conditions where the normal wound healing response fails to terminate. Normal wound healing requires a complex interaction between injured cells, circulating cells and resident mesenchymal fibroblasts that communicate via a complex network of profibrotic and antifibrotic signals.

Numerous studies have implicated Transforming Growth Factor B (TGF- β) as the major profibrotic factor in multiple contexts (reviewed in [3]). TGF- β isoforms are normally stored in latent form in the extracellular matrix as a complex with latent TGF- β binding protein and are activated by proteolytic cleavage of the carboxyl-terminal region which releases the active form from the latent complex. Once released, TGF- β isoforms will bind to the heterodimeric TGF- β receptors expressed on a wide variety of cells, activate their receptor tyrosine kinase activity and signal through the Smad family of transcriptional activators. Typically, Smad3 will be phosphorylated directly by the receptor tyrosine kinase, heterodimerize with Smad4, translocate to the nucleus, bind to the DNA and activate transcription of target, profibrotic genes. Connective Tissue Growth Factor (CTGF), is one of the primary target genes of TGF- β and is considered a downstream effector of TGF- β signaling in both cardiac fibroblasts and cardiac myocytes [4]. CTGF is known to promote fibroblast proliferation, collagen and fibronectin production [5] and potentiate the effects of TGF- β [6,7], in part through suppression of the inhibitor Smad7 [8]. After stimulation with TGF- β , fibroblasts are also stimulated to

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proliferate and undergo conversion to myofibroblasts, smooth muscle-like cells that are able to migrate to injured areas and continue to contribute to extracellular matrix deposition [9].

In the heart, studies have shown that TGF- β 1 is increased in the myocardium of patients with LVH [10], is expressed during the transition from hypertrophy to heart failure [11] and can induce the production of collagen types I and III, fibronectin and proteoglycans by cardiac fibroblasts (reviewed in [12]). It has also been shown that transgenic overexpression of TGF- β 1 leads to cardiac hypertrophy characterized by hypertrophy of myocytes and interstitial fibrosis [13].

Ongoing studies on cardiac hypertrophy have implicated a large number of proteins in the development of hypertrophy (reviewed in [14]). The relationship between cardiac myocyte hypertrophy and cardiac fibrosis, though long observed, has remained incompletely understood. Multiple studies have questioned the primacy of cardiac myocytes in the development of cardiac fibrosis, and have suggested that increased turnover of ECM molecules can lead to altered cellular connection, deposition of an altered matrix and release of matrikines that affect cell proliferation, migration and matrix metalloprotease production [15–17]. While transcriptional control of cardiac hypertrophy has been studied extensively, the transcription factors that regulate the process of cardiac fibrosis have not been well characterized. In this issue of the Journal of Molecular and Cellular Cardiology, Wang et al. have identified an important transcriptional pathway that controls cardiac fibrosis. Specifically, they have found that the Kruppel like factor 15 is an important repressor of TGF- β 1 activation of CTGF expression in cardiac fibroblasts [18].

Kruppel like factors, named for the Kruppel protein in drosophila, have been implicated in a wide variety of important cellular processes, such as erythropoiesis, lung development, glucose homeostasis and cardiac hypertrophy (for review see [19]), through binding to GC-rich motifs. KLF5 (aka BTEB2) was first reported to have an important role in cardiovascular remodeling through a positive transcriptional effect on PDGF-A and TGF- β [20]. KLF15 has previously been implicated in hepatic gluconeogenesis [21] and cardiac myocyte hypertrophy [22].

KLF15 is expressed in both adult cardiac myocytes and cardiac fibroblasts. A previous study has shown that overexpression of KLF15 in cardiac myocytes can suppress cardiac hypertrophy and that mice globally lacking KLF15 develop severe eccentric hypertrophy in response to pressure overload [22]. Mechanistically, KLF15 was found to inhibit the binding of MEF2 and GATA4 to target promoters, thereby suppressing activation of prohypertrophic genes.

In this issue, Wang et al. have extended the previous findings by analyzing the role of KLF15 in cardiac fibroblasts [18]. Additional analysis of the previously described KLF15 null mice demonstrated increased fibrosis after ascending aortic constriction when compared to wild type mice, associated with increased expression of CTGF. In isolated neonatal rat ventricular fibroblasts, KLF15 expression was decreased with TGF- β 1 stimulation, while CTGF expression was simultaneously increased. Reporter assays demonstrated a direct repressive effect of KLF15 on both basal and TGF- β 1 stimulated activity of the CTGF promoter. Interestingly, KLF15 had no effect on binding of Smad3 to the CTGF promoter. Since Smads and KLF factors are known to interact with the coactivator P/CAF and coactivators are often present in rate limiting amounts, the authors examined whether KLF15 may compete with Smad3 for P/CAF binding and reduce transcriptional activity. They were able to demonstrate that KLF15 does compete for P/CAF activity, thereby preventing association of P/CAF with the Smad3 transcription complex on the CTGF promoter.

These findings have important implications and raise a number of interesting questions. For one, the current study and the previous study together demonstrate that KLF15 is acting concurrently in two different cell types to suppress pathological cardiac myocyte hypertrophy

and prevent cardiac fibrosis under conditions of pressure overload. The presence of KLF15 in both cell types in conjunction with the ubiquitous presence of TGF- β receptors allows for augmentation of both hypertrophic and fibrotic signals in each of these cell types through parallel stimulation. Myocytes express TGF- β receptors, and it is likely that the downregulation of KLF15 by TGF- β 1 observed in fibroblasts also occurs in cardiac myocytes, thereby enhancing the development of pathological cardiac hypertrophy through activation of GATA4 and MEF2. Similarly, cardiac myocytes express CTGF in response to TGF- β [4] and presumably this induction works through downregulation of KLF15 as well. Myocyte expression of CTGF would further augment the fibrotic signal generated by cardiac fibroblasts. These dual, reinforcing points of control amplify the effect of KLF15 and underscore its importance in cardiovascular homeostasis. The major question that arises is whether the effects of KLF15 in cardiac myocytes predominate or whether the fibroblast effects contribute more to the development of fibrosis and heart failure. Either scenario is plausible, in that defects in cardiac myocytes could exacerbate strain on the extracellular matrix and promote activation of fibroblasts. Similarly, inappropriate conversion of fibroblasts to ECM secreting myofibroblasts may promote ECM remodeling and fibrosis prior to the onset of myocyte hypertrophy. Resolution of this question will require the study of animals in which KLF15 is specifically deleted in cardiac myocytes or through the use of a myocardially expressed KLF15 transgene to rescue the global null phenotype.

Another important question involves the general characterization of the cardiac fibroblast in the KLF15 null animals. Does KLF15 play a more general role in determining the fibroblast phenotype in the heart, as well as other tissues? Experiments on cultured cardiac fibroblasts from KLF15 null mice would be useful in determining whether these cells are defective in the TGF- β 1-dependent transformation to myofibroblasts, as measured by increased expression of smooth muscle α -actin, extra domain A fibronectin, collagen I, collagen III, tissue inhibitor of metalloproteinases (TIMPs), plasminogen activator inhibitor 1 (PAI-1) and osteopontin. A similar study examining the role of osteopontin in myofibroblast transformation has recently been reported [23]. In addition, it will be interesting to determine whether KLF15 null animals are more susceptible to other types of fibrosis, such as pulmonary, hepatic or renal fibrosis.

Another question relates to the significance of increased CTGF expression and other markers in the development of fibrosis and progression to heart failure. Theoretically, increased expression of ECM proteins early on in pressure overload would be expected to strengthen the matrix and perhaps prevent the development of eccentric hypertrophy, a result opposite to that seen in KLF15 null mice. The net effect of fibrosis is generally to inhibit diastolic function, rather than inhibit systolic function. One possible way to reconcile these paradoxical findings is based on previous observations in a hypertensive rat model. In this model, the transition from hypertrophy to heart failure has been associated with increased ECM degradation that presumably develops in response to increased ECM synthesis [24]. Increased ECM turnover in the setting of high ventricular filling pressures may facilitate pathological remodeling consisting of both ventricular enlargement and increased fibrosis. Another plausible mechanism would be that perhaps KLF15 plays an essential role in matrix homeostasis such that loss of KLF15 affects the intrinsic composition of the matrix, resulting in increased compliance of the ventricle and subsequent enlargement during pressure overload. Whether KLF15 affects the intrinsic composition of the extracellular matrix through its effects on cardiac fibroblasts or affects turnover of the matrix during pressure overload remains to be determined.

The study by Wang et al. implicating KLF15 in the regulation of CTGF is an important study that links KLF15 to cardiac fibrosis, through an effect on induction of CTGF by TGF- β 1. This novel role of KLF15 in cardiovascular ECM homeostasis in conjunction with its previously reported role in cardiac hypertrophy demonstrates it to be an essential regulator of pathological

cardiac hypertrophy and heart failure. Future studies to further define the role of KLF15 in cardiac hypertrophy and tissue fibrosis and to explore the interdependence of these two processes will be eagerly anticipated.

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