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A Salutary Role for Calcineurin in the Heart

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The intracellular serine/threonine phosphatase calcineurin (protein phosphatase 3) regulates several signal transduction pathways in eukaryotic cells [1]. Calcineurin is a dimeric protein consisting of a calmodulin-binding 59 kilodalton A subunit that contains the catalytic portion and a small 19 kilodalton regulatory calcium-binding B subunit [2]. The calcineurin Aβ subunit is the stress responsive form in cardiac tissue [3]. Calcineurin dephosphorylates a variety of substrates, including the nuclear factor of activated T cells (NFAT) family of transcription factors [1-3]. Dephosphorylation of NFAT proteins results in their translocation from the cytosol to the nucleus of cells where they promote gene transcription [1-3]. Over a decade ago, calcineurin was identified as an important factor in the development of cardiac hypertrophy in response to pressure overload via its ability to dephosphorylate and hence activate NFAT family members, especially NFAT4 (formerly called NFATc3) [4-6]. This finding implicated calcineurin as a potential target for pharmacological therapy to retard the development of pathological cardiac hypertrophy and thereby prevent the development of heart failure.

Muscle lim protein (MLP) is expressed in striated muscle, including heart, where it plays both a structural and a signaling role [7]. MLP accumulates in skeletal muscle at actin-containing complexes that are involved in the organization of the cytoarchitecture [7]. MLP contains two LIM double zinc fingers and it acts as a scaffolding protein for actin-based structures [7,8]. Mice lacking MLP were generated several years ago and they exhibit a complex phenotype [8]. In the initial characterization of these mice, about 30-40% of $MLP^{-/-}$ animals died of fatigue at postnatal days 5 to 10. All mice that survived the early postnatal period eventually developed a dilated cardiomyopathy during adulthood with prominent myofibrillar disorganization [8]. The cardiomyopathy that developed in $MLP^{-/-}$ adult mice appeared to be highly similar to human forms of dilated cardiomyopathy. Indeed, a mutation in the gene encoding MLP (W4R) was identified in patients with dilated cardiomyopathy [9]. In addition to its role in the development and maintenance of Z-disc structure in cardiomyocytes, MLP may also play a role at detecting cardiomyocyte wall stress. MLP was found to interact with telothonin (T-cap), a titin-binding protein, and the MLP/T-cap complex was proposed to be a key component of the stress sensor machinery [9]. Interestingly, MLP and calcineurin were found to co-localize at the Z-disc of cardiomyocytes, and calcineurin was displaced from the Z-disc in MLP haplo-insufficient mice [10]. Furthermore, $MLP^{+/-}$ mice were determined to have reduced cardiac NFAT activation in response to provocative stimulation such as experimental myocardial infarction [10]. Taken together, these results suggest that in response to cardiomyocyte wall stress, the MLP/T-cap complex directly activates the calcineurin-NFAT signal transduction cascade and thereby promotes cell growth.

In the current issue of JMCC, Heineke, Molkentin and colleagues evaluated the relationship between MLP and calcineurin by use of compound knockout mice [11]. Their initial observation was that the majority (73%) of MLP$^{-/-}$ calcineurin Aβ$^{-/-}$ double knockout mice died within 20 days of birth, but single knockout MLP$^{-/-}$ and calcineurin Aβ$^{-/-}$ mice exhibited no mortality within this time period. The uniform early post-natal survival of single knockout MLP$^{-/-}$ mice contrasts with the initial characterization of these animals [8]. Heineke et al. also observed that 5-day-old MLP$^{-/-}$ calcineurin Aβ$^{-/-}$ mice exhibited increased rates of cardiomyocyte apoptosis and necrosis by electron microscopy when compared to single knockout MLP$^{-/-}$ heart tissue [11]. Furthermore, 10-day-old MLP$^{-/-}$ calcineurin Aβ$^{-/-}$ mice developed increased cardiomegaly compared to single knockout MLP$^{-/-}$ mice as determined by measuring the heart weight-to-body weight ratio [11]. In the 27% of MLP$^{-/-}$ calcineurin Aβ$^{-/-}$ mice that survived until adulthood, left ventricular contractile function as determined by echocardiography was significantly reduced compared to single knockout MLP$^{-/-}$ mice. Heineke et al. found that adult MLP$^{-/-}$ calcineurin Aβ$^{-/-}$ double knockout mice exhibited prominent cardiomegaly with an increased heart weight-to-body weight ratio, increased cardiac fibrosis, and increased cardiomyocyte apoptosis and necrosis when compared to single knockout MLP$^{-/-}$ mice. The prominent cardiomegaly observed in MLP$^{-/-}$ calcineurin Aβ$^{-/-}$ mice at both postnatal day 10 and in adulthood shows that calcineurin Aβ is not universally required for cardiac growth. To attempt to rescue the phenotype MLP$^{-/-}$ mice, Heineke et al. developed an inducible transgenic mouse model system, with low level cardiac-specific overexpression of calcineurin. Indeed, inducible expression of calcineurin in heart abrogated the phenotype observed in MLP$^{-/-}$ mice with improved left ventricular contractile function and reduced cardiac fibrosis [11]. Taken together, these findings suggest that calcineurin plays a significant role in promoting cardiomyocyte survival and function and in antagonizing cardiac fibrosis in response to the absence of MLP.

The current work of Heineke et al. demonstrates that calcineurin has an important pro-survival effect in cardiac tissue [11]. These results complement previous work by Pu et al. who demonstrated that expression of VIVIT, a peptide antagonist that blocks calcineurin-mediated NFAT activation, sensitized cultured cardiomyocytes to phenylephrine-mediated apoptosis [12]. In addition, Molkentin and colleagues previously demonstrated that overexpression of an activated form of calcineurin in cultured cardiomyocytes protected against staurosporine and 2-deoxyglucose-induced apoptosis [13]. The same group previously reported that calcineurin Aβ$^{-/-}$ mice were sensitized to cardiac ischemia/reperfusion injury with increased cardiomyocyte apoptosis [14]. Furthermore, Molkentin and colleagues recently demonstrated that the deleterious effect of apoptosis signal-regulating kinase 1 (ASK1) overexpression on pathological cardiac remodeling and apoptosis in response to pressure overload was ameliorated in the calcineurin Aβ$^{-/-}$ genetic background [15]. Alternatively, calcineurin can also have a pro-apoptotic effect in cardiomyocytes via its ability to dephosphorylate and thereby inactivate apoptosis repressor with caspase recruitment domain (ARC) at threonine-149 [16].

Calcineurin is a phosphatase that has many targets in cardiomyocytes and it is possible that NFAT4 is not the only, or even the major, target responsible for its ability to promote cell survival [1,2]. However, assuming that NFAT4 dephosphorylation and transcriptional activation is the major anti-apoptotic target of calcineurin in cardiomyocytes, it still remains unclear how this leads to improved cell survival. In previous work, Molkentin and colleagues analyzed the calcineurin Aβ$^{-/-}$ mice by gene expression profiling and found that 383 genes (out of 6000) exhibited significantly downregulated expression and 54 genes showed upregulated expression [14]. Unfortunately, a specific explanation for the anti-apoptotic effect of calcineurin activity in cardiomyocytes did not emerge from this initial analysis of cardiac gene expression in calcineurin Aβ$^{-/-}$ mice. One interpretation of this study is that global gene downregulation leads to the increased sensitivity of calcineurin Aβ$^{-/-}$ heart tissue to pro-

apoptotic stimuli [14]. However, other investigators linked the expression of the antiapoptotic factors Bcl-2 and COX-2 to calcineurin-NFAT activity [17,18]. In particular, calcineurin $A\beta^{-/-}$ mice were found to exhibit increased spontaneous apoptosis of naïve T cells that was associated reduced Bcl-2 levels and that was rescued by overexpression of Bcl-x_L [19]. Therefore, it is possible that a simple mechanism for the antiapoptotic effect of calcineurin-NFAT signaling in cardiomyocytes will emerge from additional research.

At present, it remains controversial whether calcineurin is a suitable target for drug discovery to treat cardiac disease in humans. The calcineurin antagonist cyclosporine A, unlike VIVIT, does not promote cardiomyocyte death when tested in cultured cells, but this may be due to its ability to inhibit cyclophilin D and mitochondrial permeability transition [12,20]. Specific calcineurin or NFAT4 antagonists may prevent or reverse the development of pressure overload-induced cardiac hypertrophy while simultaneously promoting cardiomyocyte death leading to the development of dilated cardiomyopathy - a very unappealing outcome. On the other hand, agents that activate calcineurin or NFAT4 in heart may be useful adjuncts for the treatment of dilated cardiomyopathy. The paper by Heineke et al. in this issue of JMCC brings the issue of calcineurin as a target for pharmacotherapy full circle: promoting calcineurin activity, as opposed to inhibiting it, may be a useful approach to treat heart failure. Additional studies are required to test this novel therapeutic hypothesis.

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