From furless to heartless—unraveling the diverse functions of cathepsin L.

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Cysteine proteases are enzymes that use a cysteine residue, activated by a histidine residue, to serve the role of the nucleophile that attacks the peptide bond. The prototype cysteine protease is papain, an enzyme purified from the fruit of the papaya. Why does the papaya need papain? It turns out that cysteine proteinases are involved in virtually every aspect of plant physiology and development. They play a role in development, senescence, programmed cell death, storage, and mobilization of germinal proteins and enable the plant to deal with various types of environmental stress. Mammalian proteases homologous to papain have been discovered, most notably the *cathepsins*, cysteine, or aspartyl proteases, which have a role in immune responses, cell survival, and death, and numerous other cell systems and processes. The cathepsin cysteine-based active site apparently arose independently at least twice in the course of evolution. For instance, the caspases are enzymes that play a major role in apoptosis and have active sites similar to papain. However, their structure is otherwise unrelated to cathepsins. Cysteine cathepsins are active in pericellular environments as soluble enzymes or are bound to cell surface receptors at the plasma membrane and possibly even within secretory vesicles, lysosomes, the cytosol, mitochondria, and within the nuclei of eukaryotic cells. The proteolytic actions performed by cysteine cathepsins are essential in the maintenance of homeostasis and depend heavily upon their correct sorting and trafficking within cells.

Lysosomal cysteine proteinases are involved in lysosomal bulk proteolysis, major histocompatibility complex class

II-mediated antigen presentation, prohormone processing, and extracellular matrix remodeling. Cathepsin L (CTSL) is an ubiquitously expressed major representative of the papain-like family of cysteine proteinases. Roth et al. [1] inactivated the CTSL gene in mice. CTSL-gene-deficient mice developed periodic hair loss and epidermal hyperplasia, acanthosis, and hyperkeratosis. The hair loss was due to alterations of hair follicle morphogenesis and cycling, dilatation of hair follicle canals, and disturbed club hair formation. The primary characteristics underlying the mutant phenotype were hyperproliferation of hair follicle epithelial cells and basal epidermal keratinocytes, cell types that are both of ectodermal origin. The authors found that the CTSL-gene-deficient phenotype resembles the spontaneous mouse mutant, termed *furless* (fs). Roth et al. analyzed the *Ctsl* gene of fs mice and found a G149R mutation inactivating the enzyme's proteinase activity. They suggested that CTSL is essential for epidermal homeostasis and regular hair follicle morphogenesis and cycling.

How did we get from furless to heartless? Stypmann et al. [2] found that 1-year-old CTSL gene-deficient mice developed ventricular and atrial enlargement (dilatative cardiomyopathy) that was associated with only a modest increase in relative heart weight. Interstitial fibrosis and pleomorphic nuclei were found in the myocardium of the CTSL-gene-deficient mice but not in the control mice. CTSL-gene-deficient cardiomyocytes contained multiple large and apparently fused lysosomes characterized by storage of electron-dense heterogeneous material, when studied with electron microscopy. Myocardial contraction in the CTSL-gene-deficient mice was markedly impaired. Furthermore, the CTSL gene-deficient mice exhibited valvular insufficiency that developed in the face of dilatation and remodeling. Finally, the CTSL-gene-deficient mice developed supraventricular tachycardia, ventricular extrasystoles, and first-degree atrioventricular block.

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Petermann et al. [3] elaborated on these findings. They observed that the myocardium of CTSL-gene-deficient and control mice revealed no differences in incidence of cell death, proliferation, and capillary density during cardiomyopathy development and progression. However, they did find that natriuretic peptide messenger RNA (mRNA) expression was increased in young adult CTSL-gene-deficient mice, indicating the activation of the adaptive “fetal” gene program. Proteome analysis revealed decreased levels of the sarcomere-associated proteins alpha-tropomyosin, desmin, and caldesmon 1, as well as considerable changes in metabolic enzymes. Their data suggested an essential role for CTSL in maintaining the structure of the endosomal/lysosomal compartment in cardiomyocytes.

Spira et al. [4] next performed a rescue experiment. They used an alpha-myosin heavy chain promoter-CTSL transgene and found that cardiomyocyte-specific CTSL expression in CTSL-gene-deficient mice results in improved cardiac contraction, normal mRNA expression of atrionatriuretic peptide, normal heart weight, and regular ultrastructure of cardiomyocytes. Furthermore, epithelial CTSL expression, using a K14 promoter-CTSL2 transgene, resulted in rescue of the furless phenotype. In these mice, cardiac atrionatriuretic peptide expression and end-systolic cardiac dimensions were also significantly attenuated. However, cardiac contraction was not improved and increased heart weight, as well as the typical changes in lysosomal ultrastructure of CTSL gene-deficient hearts, persisted. The authors concluded that the dilatative cardiomyopathy of CTSL gene-deficient mice is specifically caused by the absence of the protease in cardiomyocytes.

Tang et al. [5], in this issue, pursue variations on this theme further. They overexpressed human CTSL in the mouse heart. An alpha-myosin heavy chain-CTSL construct was employed in this transgenic experiment. The transgenic and control mice were subjected to aortic banding. In contrast to control mice, the CTSL transgenic mice were resistant to cardiac fibrosis, had a decreased hypertrophic response, and reduced cardiomyocyte apoptosis. These findings were accompanied by a reduction and attenuation of the protein kinase B (also known as Akt)/glycogen synthase kinase 3-beta (GSK3 β) signaling cascade. The authors indicate that their findings are the first to show a relationship between CTSL and Akt/GSK3 β signaling. Sugden et al. [6] recently reviewed this pathway, from whom I borrowed the schematic shown in the Fig. 1. Tang et al. [5] presented evidence that the mechanism could be related to nuclear factor kappa B (NF- κ B) inhibition by CTSL overexpression.

There are two GSK3 isoforms: GSK3 α and GSK3 β [6]. The enzyme was originally characterized in the context of glycogen metabolism regulation, although GSK3 is now known to regulate many other cellular processes. GSK3 α

Fig. 1 Readers are referred to Sugden et al. [6]. Receptor protein tyrosine kinase (RPTK) agonists such as insulin (left) or epidermal growth factor (right) can stimulate phosphoinositide 3 kinase (PI3K), protein kinase B (PKB) also known as Akt, mTOR, and eventually subunit 6 kinases (S6K) to inhibit the glycogen synthase kinase 3 (GSK3) isoforms. Recall that GSK3 inhibits cardiac hypertrophy. Thus, the inhibitory responses promote cardiac hypertrophy. The guanine nucleotide exchange factor system (Grb2/Sos) serves to activate the small nucleotide binding protein Ras. Gq protein receptor agonists (center) such as endothelin can activate protein kinase C (PKC). Conversion of Ras to RasGTP leads to activation of mitogen-activated protein kinase kinases 1/2 (MKK1/2), which then phosphorylate and activate extracellular-related kinase (ERK1/2). This activation in turn activates the p90-ribosomal subunit S6 kinases (RSKs), which phosphorylate and inhibit GSK3. Not shown on the figure are the exciting downstream targets of GSK3

(Ser21) and GSK3 β (Ser9) phosphorylation inhibits enzyme activity. GSK3 β appears particularly important in the heart. Catalytically active GSK3 generally restrains gene expression, and in the heart, active GSK3 has been implicated in anti-hypertrophic signaling. GSK3 inhibition results in transcription and translation activation and promotes hypertrophic responses. Considerable evidence suggests that signal transduction from hypertrophic stimuli to GSK3 passes primarily through protein kinase B/Akt. Protein kinase B/Akt is fully activated when phosphorylated by the mammalian target of rapamycin (mTOR) complex 2. Protein kinase B/Akt phosphorylates the two GSK3 isoforms and thereby inhibits their activities. However, as Tang et al. [5] point out, the situation is far more complicated. Numerous additional signaling pathways potentially regulate GSK3 activity. Depending on the stimulus, phosphorylation of GSK3 can be independent of PKB/Akt. Potential GSK3 substrates studied in relation to myocardial hypertrophy include nuclear factors of activated T cells, beta-catenin, transcription factors characterized by their ability to bind to the sequence “GATA” (GATA4), myocardin, cAMP response element-binding protein, and

eukaryotic initiation factors. These and other transcription factor substrates are also important in the heart.

The CTSL-related effects shown by Tang et al. [5] remain imperfectly defined. NF- κ B could be involved; however, this hypothesis needs to be tested further. Akt can mediate NF- κ B activation and increase cell survival. Bhattacharya et al. [7] showed that Akt-mediated NF- κ B activation in an intestinal epithelial cell line was independent of GSK3 β activity. NF- κ B super-repressor I κ B α Δ N constructs exist that could be expressed in the heart to terminate NF- κ B activation [8]. Perhaps, such mice could be used in a transgenic experiment to test whether or not the CTSL-related effects are indeed related to NF- κ B.

Respectfully,
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