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Mitogen-Activated Protein Kinase Inhibitor Regulation of Heart Function and Fibrosis in Cardiomyopathy Caused by Lamin A/C Gene Mutation

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Abstract

Mutations in the lamin A/C gene (*LMNA*) encoding A-type nuclear lamins cause dilated cardiomyopathy. We have uncovered a novel connection between these mutations and hyper-activation of the extracellular signal-regulated kinase 1/2 and c-jun N-terminal kinase branches of the mitogen-activated protein kinase signaling pathway in a mouse model of the disease. This discovery has identified targets that can be inhibited by drugs that improve heart function and prevent fibrosis.

LMNA Cardiomyopathy

Dilated cardiomyopathy is characterized by increased myocardial mass and volume with thinning and stretching of the ventricular walls; this compromises cardiac contractility, ultimately resulting in poor left ventricular function (Luk et al. 2008). Genetic disorders are responsible for at least 20% of dilated cardiomyopathies (Michels et al., 1992). Causative mutations occur in genes that encode components of a wide variety of cellular structures, including the contractile apparatus, the force transduction apparatus and the nuclear envelope (Morita et al., 2005). One such gene is *LMNA* encoding A-type lamins of the nuclear envelope (Bonne et al. 1999, Fatkin et al. 1999).

LMNA may be the most prevalent dilated cardiomyopathy gene, as mutations appear to be responsible for approximately 8% of inherited cases (Taylor et al. 2003, Millat et al. 2011). Affected individuals sometimes have associated skeletal muscular dystrophy, most frequently the Emery-Deifuss phenotype (Bonne et al. 1999). The onset of symptoms in *LMNA* cardiomyopathy is variable, ranging from the first to sixth decade of life and occurring most frequently in the third decade (Ben Yaou et al. 2006). *LMNA*

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Potential Conflict of Interest

Drs. Worman and Muchir are inventors on a pending PCT patent application on methods for treating and/or preventing cardiomyopathies by ERK and JNK inhibition filed by the trustees of Columbia University in the City of New York.

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cardiomyopathy has a more aggressive course than most other inherited dilated cardiomyopathies (Taylor et al. 2003, van Berlo et al. 2005, Pasotti et al. 2008). In addition to left ventricular dilatation, affected individuals have early atrioventricular conduction block followed by ventricular arrhythmias. Arrhythmias gradually become more frequent with age, potentially leading to sudden death (Sanna et al. 2003). While sudden death from arrhythmias may be prevented by implantation of a pacemaker and defibrillator, progressive heart failure eventually becomes resistant to treatment (van Berlo et al. 2005, Meune et al., 2006, Golzio et al. 2007). No therapies are curative and heart transplantation is often necessary.

A-type Nuclear Lamins

LMNA is located on human chromosome 1q21.2–21.3 and encodes the A-type nuclear lamins, of which lamin A and lamin C are the major isoforms expressed in somatic cells (Lin and Worman 1993, Wydner et al. 1996). Lamins are intermediate filament proteins that polymerize to form the nuclear lamina, a fibrous meshwork underlining the inner nuclear membrane of most metazoan cells (Aebi et al. 1986, Fisher et al. 1986, McKeon et al. 1986). The nuclear lamina is attached to the inner nuclear membrane via interactions with integral proteins. The lamina also interacts with the cytoskeleton through a multi-protein complex called the linker of nucleoskeleton and cytoskeleton complex (Stewart et al. 2007b). A-type lamins appear to be essential for maintaining normal nuclear and cytoskeletal mechanics and stress-induced activation of transcription (Broers et al. 2004, Lammerding et al. 2004). These biomechanical functions may be particularly significant in contractile cells such as cardiomyocytes. Lamins are also believed to be involved in several cellular processes such as chromatin organization, gene regulation, DNA replication and RNA splicing (Dechat et al. 2008). The pleiotropic functions of A-type lamins are perhaps best appreciated by the fact that *LMNA* mutations different from (and rarer than) those causing cardiomyopathy cause phenotypically diverse diseases including partial lipodystrophy, peripheral neuropathy and Hutchinson-Gilford progeria syndrome (Worman et al. 2009).

Mitogen-activated Protein (MAP) Kinases in *LMNA* Cardiomyopathy

A-type lamins are expressed in most differentiated somatic cells in virtually all tissues, making it difficult to readily explain the tissue-selective defects that result from *LMNA* mutations. However, clues about the *in vivo* functions of A-type lamins have been gained from studies of mouse models in which their gene has been targeted by homologous recombination to generate either knockout or knock-in mutations (Stewart et al. 2007a). One of these mouse models has provided data that partially explain the pathogenesis of *LMNA* cardiomyopathy and give clues about potential therapies.

Male *Lmna*^{H222P/H222P} mice develop left ventricular dilatation and decreased ejection fraction at approximately 10 weeks of age that progressively worsen with subsequent cardiac fibrosis followed by death at a median age of approximately 26 weeks (Arimura et al. 2005). This mimics, albeit on a different timescale, what occurs in human subjects with *LMNA* cardiomyopathy. We therefore examined the transcriptome in hearts of *Lmna*^{H222P/H222P} mice using Affymetrix GeneChips (Muchir et al. 2007). We found a prominent increase in the expression of genes involved in fibrosis and in the “fetal gene program” that is induced in stressed and failing hearts (Schwartz et al. 1993). In addition, genes encoding proteins in the MAP kinase signaling pathway demonstrated significantly altered expression compared to controls. Further analysis revealed hyper-activation of extracellular signal-regulated kinase 1/2 (ERK1/2) and c-Jun N-terminal kinase (JNK) branches of the MAP kinase pathway and increased expression of genes stimulated by these enzymes. Activation of the same signaling cascades occurs in dilated hearts of human

subjects with late-stage cardiac failure of several different causes (Haq et al. 2001). However, in hearts of *Lmna*^{H222P/H222P} mice, there is increased ERK1/2 and JNK signaling as early as four weeks of age, before the onset of clinically detectable signs of cardiac dysfunction (Muchir et al. 2007).

MAP kinase signaling is activated by mitogens, growth factors and cytokines that bind to cell surface receptors as well as by mechanical and chemical stresses independent of receptors (Seger and Krebs 1995, Chang and Karin 2001). These stimuli induce the phosphorylation (activation) of enzymes that through a cascade of sequential reactions leads to phosphorylation a MAP kinase. For example, Raf phosphorylates MAP kinase/ERK kinase (MEK) 1/2, which directly phosphorylates ERK1/2. Similarly, MAP kinase kinase kinase 1 phosphorylates MEK4 and MEK7, which phosphorylate JNK. Activated ERK1/2 and JNK in turn phosphorylate a wide array of substrates including transcription factors, other protein kinases and cytoskeletal components. This affects gene expression, crosstalk with other signal transduction pathways and the control of processes such as cell proliferation, cell death and acute stress responses. Members of the dual-specificity phosphatase family dephosphorylate MAP kinases, leading to deactivation of signaling (Theodosiou and Ashworth 2002).

The simultaneous activation of multiple signaling pathways in dilated failing hearts has made deciphering the specific pathogenic roles of the different branches of the MAP kinase pathway difficult. The ERK1/2 branch has been classically associated with the hypertrophic response, during which cardiomyocytes increase in size without cell division (McKinsey and Olsen 1999, Molkentin 2004). While this may initially be compensatory in nature, a prolonged hypertrophic response can become detrimental, leading to dilated cardiomyopathy. JNK branch hyper-activation is thought to influence contractile function, extracellular matrix remodeling, intercellular communication and metabolic regulation (Petrich and Wang 2004). Examination of transcriptomes in hearts of transgenic mice with constitutive hyper-activation of ERK1/2 or JNK signaling has revealed distinct gene expression patterns with several clearly overlapping features that change over time (Mitchell et al. 2006). So while the specific contributions of increased ERK1/2 and JNK signaling to the development of dilated cardiomyopathy remain to be elucidated, there is little doubt that it plays an important pathogenic role. The fact that ERK1/2 and JNK are hyper-activated in a mouse model of *LMNA* cardiomyopathy prior to the onset of clinical disease lead us to hypothesize that inhibiting their activities to restore a more physiological balance would be beneficial.

Inhibition of MAP kinases as Treatment for *LMNA* Cardiomyopathy

To test our hypothesis, we treated male *Lmna*^{H222P/H222P} mice with PD098059, which inhibits MEK1/2 and blocks ERK1/2 phosphorylation. We also treated them with SP600125, which inhibits JNK activity. We first treated the mice starting at 8 weeks of age, prior to the onset of clinically detectable cardiac abnormalities, and analyzed them at 16 weeks (Muchir et al. 2009, Wu et al. 2010). Systemic administration of either of these compounds inhibited their respective targets in heart by approximately 50%. At 16 weeks of age, placebo-treated *Lmna*^{H222P/H222P} mice had left ventricular dilatation and decreased ejection fraction whereas these parameters were normal in mice treated with PD098059 or SP600125. Treatment with these inhibitors also blocked increased cardiac expression of RNAs encoding natriuretic peptide precursors and the induction of elements of the “fetal gene program” that occurred in placebo-treated mice.

As treatment for cardiomyopathy in human subjects may more likely be administered after the onset of symptoms or measurable cardiac abnormalities, we next treated male

Lmna^{H222P/H222P} mice with MEK1/2 or JNK inhibitors starting at 16 weeks of age (Wu et al. 2011). At this time, male *Lmna*^{H222P/H222P} mice have left ventricular dilatation and an ejection fraction approximately 70% that of wild type mice. We then analyzed the mice at 20 weeks. Treatment with PD98059 or SP600125 prevented further left ventricular end-systolic dilatation, increased ejection fraction, reduced cardiac expression of RNAs encoding natriuretic peptide precursors and reversed elements of the “fetal gene program” compared to placebo-treated mice. *Lmna*^{H222P/2PH222P} mice receiving either of these inhibitors also had significantly less cardiac fibrosis than those receiving placebo, indicating that blocking ERK1/2 or JNK signaling could prevent an irreversible late-stage feature of *LMNA* cardiomyopathy. Overall, this research showed that inhibiting ERK1/2 or JNK signaling has beneficial effects on heart function and fibrosis in a mouse model of *LMNA* cardiomyopathy (Figure 1).

Remaining Questions

An intriguing riddle is how certain alterations in A-type lamins, which are expressed in virtually all differentiated somatic cells, activate MAP kinase signaling pathways specifically in striated muscle (Muchir et al. 2007). Research suggests that *LMNA* mutations can be divided into two categories - those leading to functionally hypoactive A-type lamins and those leading to expression of “toxic” variants, with mutations causing cardiomyopathy falling into the former category (Davies et al. 2011). One study has shown that ERK1/2 interacts with A-type lamins at the nuclear periphery (Gonzales et al. 2008), suggesting that phosphorylated ERK1/2 may translocate to the nucleus where binding to A-type lamins inhibits accessibility to other nuclear substrates. This can lead to the hypothesis that functionally hypoactive A-type lamins reduce a nuclear envelope-mediated buffering of ERK1/2 activity on gene expression. Given their role in maintaining normal cellular mechanics, another hypothesis is that functionally hypoactive A-type lamins make contractile cells more susceptible to stress-induced damage, which activates ERK1/2 and JNK signaling. The crosstalk between these MAP kinases and other signal transduction pathways, as well as the deactivation of ERK1/2 and JNK, also require further study in *LMNA* cardiomyopathy.

A key remaining question is whether discoveries showing that inhibitors of MAP kinase signaling are beneficial in a mouse model of *LMNA* cardiomyopathy can be translated to humans. Drug development programs have produced highly specific, orally bioavailable inhibitors of MEK1/2 that are being studied in clinical trials for cancer (Adjei et al. 2008, Bekaii-Saab et al. 2011, O’Neil et al. 2011). Whether these drugs can be safely administered at appropriate doses over prolonged periods to effectively treat human subjects with *LMNA* cardiomyopathy needs to be determined. Nonetheless, the availability of such drugs makes clinical trials for this debilitating cardiovascular disease an imminent and exciting possibility.

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Figure 1.

Diagram of molecular and cellular events linking an *Lmna* point mutation in mice to MAP kinase activation and the development of cardiomyopathy. In *Lmna*^{+/+} mice (upper right), baseline ERK1/2 (ERK) and JNK signaling maintain normal cardiac function. In *Lmna*^{H222P/H222P} mice (upper left), there is hyper-activation of ERK1/2 and JNK signaling, leading to induction of a "fetal gene program" and myocardial fibrosis that over time results in cardiomyopathy. Treatment of *Lmna*^{H222P/H222P} mice with inhibitors of ERK1/2 and JNK signaling (bottom) reverse induced elements of the "fetal gene program" and prevent myocardial fibrosis, leading to improved cardiac function.