MEK1/2 Inhibitors to Treat Dilated Cardiomyopathy Caused by LMNA Mutations

Antoine Muchir1,2
1Department of Medicine, 2Department of Pathology and Cell Biology, College of Physicians and Surgeons, Columbia University, New York, NY USA

1. Introduction

Inherited dilated cardiomyopathies are a major cause of heart disease in human, often with an onset in adolescence or early adult life. Despite technological advances that foster early diagnosis and alleviation of some symptoms, inherited dilated cardiomyopathies remains a critical unsolved problem for public health. The recent years provided some clues to explain the pathogenesis of inherited dilated cardiomyopathies, which might open new and encouraging perspectives for clinical trials.

2. LMNA dilated cardiomyopathy

Cardiomyopathy is an anatomic and pathologic condition associated with muscle dysfunction of the heart. Dilated cardiomyopathy, the most common form, is characterized by an increase in both myocardial mass and volume, which compromises cardiac contractility and ultimately results in reduced left ventricular function (Luk et al. 2008). Dilated cardiomyopathy is the third leading cause of heart failure in the United States behind coronary artery disease and hypertension. Genetically inherited forms of dilated cardiomyopathy have been identified in 30% of patients presenting with this disease (Michels et al., 1992). Many other acquired conditions may result in an identical clinical presentation and pathological function, which include alcohol-induced cardiomyopathy, hypertension, chronic anemia, ischemic cardiomyopathy, valvular diseases and viral myocarditis (Maron et al., 2006).

Inherited dilated cardiomyopathies are caused by mutations in genes that encode components of a wide variety of cellular components and pathways, including the nuclear envelope, contractile apparatus and the force transduction apparatus (Morita et al., 2005). The generation of contractile force by the sarcomere and its transmission to the extracellular matrix are the fundamental functions of cardiac cells. Inadequate performance in either components of this structural cellular network leads to cardiac remodeling and ultimately to dilated cardiomyopathy. Defects in generating force are typically due to a loss of integrity of the sarcomere unit. Mutations in the loci coding for β-cardiac myosin heavy chain, actin (Olson et al., 1998) and cardiac troponin T (Li et al., 2001) have been identified to disrupt
force generation (Kamisago et al, 2000). Defects in the force transmission occur when there is impairment in the propagation of force from the sarcomere to the sarcolemma (Schonberger et al. 2001). Mutations in α-tropomyosin (Olson et al. 2001), dystrophin (Muntoni et al., 1993), desmin (Li et al 1999) and δ-sarcoglycan (Tsubata et al. 2000) have been identified to disrupt force transmission.

Among the causing genes, LMNA mutations encoding proteins of the inner nuclear membrane, have also been found associated to dilated cardiomyopathy (Fatkin et al. 1999). This finding raises the possibility that the nuclear envelope may play an important function as mechanosensor in cardiomyocyte (Nikolova et al. 2004, Lammerding et al. 2004). LMNA mutations appear to be responsible for approximately 8% of cases of inherited cardiomyopathy (Taylor et al., 2003), which strongly suggest that LMNA may be the most prevalent dilated cardiomyopathy gene. LMNA dilated cardiomyopathy is characterized by cardiac dilatation and impaired systolic function. In addition, affected patients exhibit early conduction defects before the left ventricular dysfunction and dilatation stages. The onset of symptoms in LMNA dilated cardiomyopathy is variable, ranging from the first to sixth decade of life and occurring most frequently in the third decade (mean age = 38 years) (Ben Yaou et al., 2006). There are high rates of life-threatening arrhythmias (abnormal electrical conduction), gradually worsening and leading to sudden death (Sanna et al. 2003). LMNA dilated cardiomyopathy has a more aggressive course than other inherited dilated cardiomyopathies. While sudden death from arrhythmias may be prevented by implantation of a pacemaker and/or implantable defibrillator, the progressive heart failure eventually becomes resistant to treatment (Golzio et al. 2007, van Berlo et al. 2005, Meune et al., 2006). No drugs are curative and heart transplantation is frequently necessary.

3. A-type nuclear laminas

LMNA, located on human chromosome 1q21.2-21.3, encodes A-type lamins. Lamin A and lamin C are the major A-type lamins expressed in somatic cells. They arise via alternative splicing of pre-mRNA encoded by exon 10 (Lin & Worman 1993). Lamin A is synthesized as a precursor, prelamin A, which has a unique C-terminal amino acid tail that triggers a series of enzymatic reactions to yield lamin A. Two other genes in the mammalian genome, LMNB1 and LMNB2, respectively encode lamins B1 and B2. Lamins A and C are widely expressed in most differentiated somatic cells but lacking from early embryos and some undifferentiated cells whereas lamins B1 and B2 are expressed in all or most somatic cells. However, there are little data and no systemic studies on the differences in the relative amounts of lamins A, C, B1 and B2 expression. Lamins are intermediate filaments proteins that polymerize to form the nuclear lamina, a fibrous meshwork underlining the inner nuclear membrane of most eukaryotic cells (Fisher et al. 1986, McKeon et al. 1986, Aebi et al. 1986). The nuclear lamina is attached to the inner nuclear membrane via interactions with integral proteins and to the chromatin. More recently, it has been demonstrated that lamin A/C also have interactions with the cytoskeleton, through a multi-protein complex called “LINC” (Linker of Nucleoskeleton and Cytoskeleton) (Stewart et al. 2007). One function of the lamina is to provide structural support to the nucleus. Nuclear lamins have also been implicated in processes such as chromatin organization, gene regulation, DNA replication and RNA splicing (Dechat et al. 2008). However, the specific mechanistic roles of lamins in these processes, particularly in a cell or tissue type-specific context, remain obscure.
4. Pathogenesis of LMNA dilated cardiomyopathy

The pathogenesis of LMNA dilated cardiomyopathy remains a puzzle in medical genetics. Mouse models have been extremely helpful in deciphering critical mechanisms, which could partially explain the pathogenesis of the disease as well as for proposing potential innovative pharmacological therapies. Using a murine model of LMNA dilated cardiomyopathy, we recently brought some insights into the molecular pathogenesis of this disease, which have paved the way to potential therapies. To approach the issue of understanding the pathogenesis of LMNA dilated cardiomyopathy, we studied the transcriptome from hearts of Lmna H222P mice (a mouse model of LMNA dilated cardiomyopathy), using the Affymetrix® array technology. Male Lmna H222P mice develop cardiac chamber dilation, decreased left ventricle ejection fraction and hypokinesis detectable by echocardiography at 8 to 10 weeks of age (Arimura et al. 2005). To avoid interference caused by fibrotic cells and nonspecific tissue damage in hearts from older Lmna H222P mice, we initially analyzed samples from mice at 10 weeks of age where there were no detectable cardiac histological abnormalities. We analyzed gene ontology terms applied to genes, to identify functional classes of genes differentially expressed in hearts of Lmna H222P mice compared with those expressed in controls. Analysis using functional class scoring improves sensitivity by statistically evaluating genes in biologically meaningful groups. Genes encoding proteins in mitogen-activated protein kinase (MAPK) signaling pathway demonstrated significantly altered expression in hearts of Lmna H222P mice (Muchir et al. 2007) (Figure 1). Because enhanced activity of the Extracellular signal-regulated kinase1/2 (ERK1/2), a branch of MAPK signaling pathways, has been formerly shown to be causing cardiomyopathy, we focused subsequent experiments on analyzing this signaling in tissues form Lmna H222P mice and in cultured cells. We then demonstrated an aberrant activation of ERK1/2 signaling in hearts from Lmna H222P mice, as early as 4 weeks of age (Muchir et al. 2007). Our work proved that the activation of ERK1/2 signaling pathway preceded the cardiac dysfunction of Lmna H222P mice and that it is a consequence of alterations in A-type lamins and not secondary to non-specific affects.

5. MEK-ERK signaling pathway

MAPK signaling pathways are major information highways from extracellular mitogens, growth factors and cytokines at the cell surface to the nucleus to control gene expression (Davis, 1993). These signaling pathways control complex cellular programs, such as embryogenesis, differentiation, proliferation and cell death, in addition to short-term changes required for mechanical stress response and acute hormonal responses. The output of these pathways is transduced via MAPK family members that phosphorylate and regulate a wide array of substrates including transcription factors, cytoskeletal elements and other protein kinases (Seger & Krebs, 1995). Stimulation of many receptor classes can activate ERK1/2 including receptors with intrinsic tyrosine kinase activity, cytokine receptors and G-protein-coupled receptors (GPCR), including those coupling via G-proteins of the $G_{q/11}$, $G_{i/o}$ and $G_s$ family. In the heart, ERK1/2 stimulation has been shown by fibroblast growth factor, insulin-like growth factor-1, estrogen, neuregulin-1, atrial natriuretic peptide, $\alpha_1$- and $\beta$-adrenoceptor agonists. Moreover, cardiac ERK1/2 can be activated independently than GPCR receptors, via mechanical stress, osmotic shock.
The MAPKs are activated by protein kinase cascades comprising at least three enzymes acting in series. ERK are activated directly by ERK kinases (MEK), which are dual specificity protein kinases that generally recognize only certain MAPKs as substrates. MEK are activated by MEK kinases, a structurally diverse group of kinases with less predictable specificities. MEK1/2, which activate ERK1/2, have very narrow substrate specificity. It is assumed, from lack of evidence to the contrary, that ERK1/2 are the only substrates of MEK1/2. Activated ERK1/2 kinases phosphorylate and activate a variety of substrates. All these substrates can be categorized into several groups including: transcription factors (Atf2, Elk1, c-Fos...), protein kinases and phosphatases (FAK1, MLCK, PAK1,...), cytoskeletal and scaffold proteins (dystrophin, Tau, Synaptin...), receptors and signaling molecules (EGFR, PLCg,...) and apoptosis-related proteins (Bad, Calpain, caspase 9, ...). Some of the substrates can be found in the cytosol (paxilin, calnexin...), in agreement with the role of ERK1/2 in the regulation of both cytosolic and nuclear processes.

6. Pharmacological therapy

Because we found abnormal activation of ERK1/2 signaling pathway in hearts of Lmna H222P mice, we hypothesized that pharmacological inhibition of this signaling pathway would prevent the cardiac deterioration. We treated Lmna H222P mice with PD098059, a tool compound that inhibits MEK1/2. We administered PD098059 or placebo (dimethylsulfoxide; DMSO) (daily, intraperitoneal injection) to Lmna H222P mice. We first treated male Lmna H222P 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). Pathological dilatation of the cardiac left ventricle is often associated with fibrosis, and reactivation of a fetal gene expression program characterized by increased levels of atrial natriuretic peptide, brain natriuretic peptide, and β-myosin light and heavy chains. Accordingly, in hearts from untreated Lmna H222P mice and those treated with vehicle (DMSO), expression of mRNAs encoding natriuretic peptide precursors as well as mRNAs encoding myosin light chains were significantly increased. We showed that after treatment with PD098059, the cardiac expression of these mRNAs was significantly lowered compared to vehicle-treated Lmna H222P mice (Muchir et al. 2009). Similarly, we also demonstrated that Lmna H222P mice treated with PD98059 had a lower degree of cardiac fibrosis than the Lmna H222P mice treated with the vehicle. After 8 weeks of treatment with DMSO or PD98059 Lmna H222P mice were anesthetized and the cardiac dimensions and function measured by cardiac ultrasound. M-mode transthoracic echocardiography showed increased left ventricle end-diastolic diameter and left ventricle end-systolic diameter in Lmna H222P mice treated with DMSO compared with control mice. Lmna H222P mice treated with PD98059 had significantly smaller left ventricle end-systolic diameters compared to the DMSO-treated mice (Table 1). Cardiac fractional shortening and ejection fraction were reduced in Lmna H222P mice compared to control mice but increased in the Lmna H222P mice treated with PD98059.

As treatment of cardiomyopathy in human subjects may more likely be administered after the onset of symptoms or detectable cardiac abnormalities, we next treated mice with PD98059 starting at 16 weeks of age, when male Lmna H222P mice have left ventricular dilatation and an ejection fraction approximately 70 percent that of wild type mice, and
analyzed the mice at 20 weeks (Wu et al. 2011). Treatment with PD98059 prevented left ventricular end-systolic dilatation, increased ejection fraction (Table 1), blocked increased cardiac expression of RNAs encoding natriuretic peptide precursors and reversed the induction of elements of the “fetal gene program” compared to placebo-treated mice. As significant cardiac fibrosis occurs in end-stage dilated cardiomyopathy, particularly LMNA dilated cardiomyopathy, we also examined cardiac fibrosis after treatment. Lmna H222P mice treated with PD98059 had a lower degree of cardiac fibrosis than the Lmna H222P mice treated with placebo. Overall, this work showed that inhibiting ERK1/2 signaling had positive effects on cardiac biochemistry and physiology in a mouse model of LMNA dilated cardiomyopathy, (Figure 1).

<table>
<thead>
<tr>
<th>Genotype (Treatment)</th>
<th>8-16 weeks</th>
<th>16-20 weeks</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>LVEDD (mm)</td>
</tr>
<tr>
<td>Lmna+/+</td>
<td>12</td>
<td>3.3 ± 0.1</td>
</tr>
<tr>
<td>LmnaH222P/H222P (DMSO)</td>
<td>15</td>
<td>3.6 ± 0.1 *</td>
</tr>
<tr>
<td>LmnaH222P/H222P (PD98059)</td>
<td>7</td>
<td>3.1 ± 0.2</td>
</tr>
</tbody>
</table>

LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; EF, ejection fraction.
Values are means ± standard errors.
Comparison between DMSO-treated LmnaH222P/H222P and Lmna+/+ mice was performed using Student unpaired t-test, *P<0.05, ***P<0.0005.
Comparison between PD98059-treated LmnaH222P/H222P and DMSO-treated LmnaH222P/H222P mice was performed using Student unpaired t-test, **P<0.005, ***P<0.0005.

Table 1. Echocardiographic data for Lmna+/+ mice and LmnaH222P/H222P mice treated with vehicle (DMSO) or MEK1/2 inhibitor (PD98059) between 8-16 weeks of age and 16-20 weeks of age.

7. MEK1/2 inhibitors

In the field of target identification there has been a great deal of enthusiasm for identifying novel drug targets based on knowledge of key signal transduction components and their link to human disease. As signaling disorders represent a major cause for the pathological states and as most of the recently validated target molecules of drug research are signal transduction kinases, signal transduction therapy has become one of the most important areas of drug research (Keri et al. 2006, Levitzki 1996). Approximately 25% of the druggable genome consists of kinases involved in signal transduction. However, only a handful of kinases inhibitors are being used in clinical practice (Margutti & Laufer 2007). This remains then a wide perspective for drug discovery. The common feature conserved throughout the entire protein kinase family is the catalytic domain. The chemical activity of a kinase involves removing a phosphate group from ATP and covalently attaching it to a free hydroxyl group. Most kinases act on both serine and threonine, others act on tyrosine, and a number act on all three (dual-specificity kinases), like MEK1/2. The fact that kinases share a highly homologous catalytic domain, and the common co-substrate ATP, initially led to the assumption that protein kinases constitute a non-druggable family of protein kinases. A
Fig. 1. Study from Lmna<sup>H222P/H222P</sup> mice suggests that activation of ERK1/2 underlies the development of LMNA dilated cardiomyopathy. Lmna<sup>H222P/H222P</sup> mice develop cardiac chamber dilation and decreased left ventricle ejection fraction. Affymetrix approach showed that ERK1/2 signaling pathways is abnormally activated in the heart of Lmna<sup>H222P/H222P</sup> mice compared to Lmna<sup>+/+</sup> mice, before any detectable sign of cardiac deterioration. Pharmacological intervention using PD98059, an inhibitor of MEK1/2, the kinase that activate ERK1/2, improves the cardiac function of Lmna<sup>H222P/H222P</sup> mice.

Type of inhibitor, allosteric (non-ATP competitive), could potentially solve the selectivity issues related to protein kinase inhibition. PD098059 (Dudley et al. 1995) and U0126 (Favata et al. 1998) were among the first specific MEK1/2 allosteric inhibitors described. Although they have been extremely useful in the in vitro study of MAPK signaling, they have not been pursued in clinical development because of poor pharmacologic characteristics.
Several allosteric MEK1/2 inhibitors are in clinical and pre-clinical development. Some of the MEK1/2 inhibitors that have been used in human subjects include:

Cl-1040 (PD184352), a benzhydroxamate from Pfizer, was the first small-molecule MEK1/2 inhibitor that proceeded to clinical testing. It was developed based on compounds and structures identified during the screening that led to the identification of PD098059, but had improved potency and selectivity (Barrett et al. 2008). Cl-1040 is an oral MEK1/2 inhibitor with promising pre-clinical activity that led to its clinical development (Sebolt-Leopold et al. 1999). It underwent phase I testing in 77 patients with advanced solids tumors (LoRusso et al. 2005). Cl-1040 was well tolerated with no grade IV toxicities and only a limited number of grade III toxicities. The majorities of toxicities (98%) were grade I/II and included diarrhea (43%), fatigue (30%), rash (18%) and nausea (16%). Antitumor activity was seen in 1 pancreatic cancer patient who had a partial response lasting 12 months. Nineteen (25%) subjects had stable disease for 3 months, and this observation was commonly associated with symptomatic benefit. On the basis of these results, a multicenter, parallel arm phase II study of Cl-1040 was performed in patients with advanced breast, colon, pancreatic, and non-small cell lung cancer. Cl-1040 was relatively well tolerated, with 19% experiencing grade III toxicities and no patients having grade IV toxicities. The toxicities included diarrhea, nausea, fatigue, rash, edema, abdominal pain, anorexia, and facial edema. However, no patients had a partial or complete response and the trial was closed. It appears that Cl-1040 will not be further developed in these tumor types.

PD0325901 is a second-generation oral MEK1/2 inhibitor subsequently developed by Pfizer. Relatively minor changes distinguish the chemical structure of PD0325901 from that of Cl-1040. The cyclopropylmethoxy group of Cl-1040 was replaced with a R-dihydroxy-propoxy group and the 2-chloro substituent of Cl-1040 was replaced with a 2-fluoro group on the second aromatic ring. Nevertheless, these minor structural changes imparted significant increases in potency with PD0325901 (Brown et al. 2007). Pre-clinical findings of significantly improved pharmacologic and pharmaceutical properties of PD0325901 were determined to hold promise for the use of the compound as a therapeutic agent. The first-in-human trial of PD0325901 employed an open-label, dose-escalating design in 41 patients with advanced colon, melanoma, and non-small cell lung cancer. Adverse events were observed, including rash (49%), diarrhea (49%), fatigue (34%), visual disturbance (34%), nausea (29%), edema (29%), pruritus (14%), anemia (11%) and dyspepsia (11%). The compound underwent a phase II testing in patients with advanced colon, melanoma and non-small cell lung cancer. More concerns have been focused on neurologic adverse event (confusion and hallucination) in three patients observed in the first trial, leading to putting a halt on the human trials using PD0325901. It is unclear if the drug caused these adverse events.

ARRY-142886/AZD6244/selumetinib (Array Biopharma/AstraZeneca) is a potent, highly specific MEK1/2 inhibitor. ARRY-142886/AZD6244 has undergone phase I testing in a trial of 57 patients with solid tumors. Hypoxia, rash, diarrhea, nausea, fatigue and blurred vision have been documented as the most common treatment-related toxicities in this study (grade I to III). Thirty-nine patients completed the study and 19 of them had stabilization of their disease after the treatment, 9 remained stable for five or more months. These promising results triggered a phase II study, which is currently under investigation.
ARRY-162, ARRY-300 and other Array MEK inhibitors (Array Biopharma/Novartis) are potent, highly specific MEK1/2 inhibitors. ARRY-162 is currently in phase I development for cancer. Previously, it failed to meet efficacy endpoints in phase II studies in rheumatoid arthritis. ARRY-162 is an orally active, potent, selective, non-ATP-competitive inhibitor of MEK 1/2.

RDEA119 (Ardea Biosciences/Bayer) is another highly selective MEK1/2 inhibitor. Preclinical and clinical results suggest that RDEA119 has favorable properties, including oral dosing, excellent selectivity and limited retention in the brain, which, in turn, may result in a reduced risk of central nervous system side effects. In preclinical studies, RDEA119 has demonstrated synergistic activity when used in combination with multiple anti-cancer agents in a wide range of tumor cell lines. Ardea Biosciences initiated a phase I clinical of RDEA119 trial in 60 patients with advanced cancer.

8. Discussion

Less than a decade ago the kinases constituting mammalian MAPK pathways were identified through intense efforts to understand the molecular events underlying cellular responses to extracellular signals. During this decade the kinases constituting ERK1/2 signaling pathways have come to be appreciated as key cellular signal transducers and thus attractive targets for drug development. Successful drug development has required the demonstration that the difficulties presented by a large gene family with a highly conserved catalytic core could successfully be targeted with specific and potent small-molecule inhibitors. These efforts are now beginning to bear fruit with the initiation of clinical trials in multiple human diseases. It is currently unclear whether it will be efficacious in LMNA dilated cardiomyopathy. The relevance of pre-clinical to basic research to human clinical protocols is still relatively unclear. Nevertheless, the outcome of clinical trials of compounds inhibiting ERK1/2 signaling pathways is of significant interest to both the basic and the clinical scientific communities focusing on LMNA dilated cardiomyopathy. Their positive outcome would be a triumph of translating basic scientific understanding of cellular function into successful human therapies.

9. References

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Proteins are the work horses of the cell. As regulators of protein function, protein kinases are involved in the control of cellular functions via intricate signalling pathways, allowing for fine tuning of physiological functions. This book is a collaborative effort, with contribution from experts in their respective fields, reflecting the spirit of collaboration - across disciplines and borders - that exists in modern science. Here, we review the existing literature and, on occasions, provide novel data on the function of protein kinases in various systems. We also discuss the implications of these findings in the context of disease, treatment, and drug development.

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