

REVIEW

Extracellular signal-Regulated Kinases1/2 and their role in cardiac diseases

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Mitogen-activated protein (MAP) kinases are members of a well-studied family of serine/threonine protein kinases involved in signal transduction pathways, which control multiple cellular processes. The extracellular signal-regulated kinase (ERK1/2) cascade is a MAP kinase pathway that transmits signals from the cell surface to substrates either in the nucleus or in the cytoplasm. The transmission of the signal through the ERK1/2 cascade is mediated by serial phosphorylations and activations of protein kinases. Abnormal regulation of the ERK1/2 signals has been linked to diseases and recent work clearly implicated ERK1/2 signaling in the development of cardiac pathologies. Understanding the underlying mechanism and the consequences of the aberrant modulation of ERK1/2 cascade will lead to the development of pharmacologic inhibitors for the treatment of these cardiac disorders.

Keywords: Mitogen-activated protein (MAP) kinases; Lamin; cardiomyopathy; Extracellular signal-regulated kinases 1/2

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Introduction

A proportion of inherited cardiomyopathies display deregulated MAP kinase signaling. As these diseases are a major cause of cardiac disease in human, it is therefore not surprising that the MAP kinase signaling continues to be the subject of intense researches for future pharmacological intervention. The development of molecules targeting this pathway has focused mostly on the development of small-molecule inhibitors. This review will gather information on MAP kinase signaling, focusing on the ERK1/2 branch and will discuss important research progresses in the field of inherited cardiomyopathies. The recent years provided some clues to explain the pathogenesis

of such disease, involving ERK1/2, which might open novel and promising perspectives for future clinical trials.

MAP kinases

Protein kinases are important players to achieve the integrated function of cells. Protein kinases are able to transfer phosphoryl groups onto target proteins, altering their activity^[1]. This mechanism participates in the transmission of intra- and extracellular signals throughout the cell and to the nucleus. Thus, protein kinases play a pivotal role in signaling pathways that could regulate cell growth, differentiation, development, and death^[2]. Hence, any disruption of the phosphorylation could alter cell functions and may cause diseases^[3].

The kinases are classified by the amino acids they phosphorylate^[4]. The two main classes of kinases are tyrosine kinases, which phosphorylate tyrosine, and serine-threonine kinases, which phosphorylate serine or threonine^[5]. Manning *et al.* have defined the human «*kinome space*» consisting of more than 500 protein kinase genes^[6]. A growing number of diseases are caused by deregulation of the phosphorylation, and thus protein kinases are regarded as a promising therapeutic target for diseases.

There is a family of protein kinase cascades known as mitogen-activated protein (MAP) kinases^[7, 8], which belongs to serine-threonine kinases. It comprises MAP kinases, MAP kinase kinases (MKKs) and MAP kinase kinase kinases (MKKKs)^[9]. MKKKs phosphorylate and activate MKKs, which in turn phosphorylate and activate MAP kinases. In all currently known MAP kinase cascades, the kinase immediately upstream of the MAP kinase is a member of the MAP/ERK kinase (MEK) family^[10-13], which are dual specificity enzymes that can phosphorylate serine/threonine and tyrosine residues. In spite of their ability to phosphorylate MAP kinases proteins, the substrate specificity of the known MEKs is very narrow: each MEK phosphorylates only one or a few of the MAP kinases. Much of the review highlights knowledge on extracellular signal-regulated kinases 1 and 2 (ERK1 and ERK2), two of the known MAP kinases.

ERK1/2

ERK1 and ERK2 are ubiquitously expressed proteins of 44 and 42 kDa, which are nearly 85% identical in their amino-acid sequences^[14-17]. Stimulation of different receptors can activate ERK1/2, e.g. receptors with intrinsic tyrosine kinase activity, cytokine receptors and G-protein-coupled receptors. Hence, ERK1/2 are stimulated by many extracellular ligands and cellular perturbations (e.g., mechanical stress, osmotic shock), with some cell type specificity^[7]. ERK1 and ERK2 are activated by closely related MEKs, MEK1 and MEK2^[10, 18-21]. It has been assumed, from lack of evidence to the contrary, that MEK1/2 have no other substrates. This specificity of MEK1/2 has been used by several pharmacological companies to design potent inhibitors of ERK1/2 signaling^[22]. A vast majority of the known MEK1/2 inhibitors are ATP non-competitive (i.e., they do not directly compete for the ATP binding site). These MEK1/2 inhibitors bind to a unique allosteric site adjacent to the ATP site, which explains their high specificity.

Of all the known MEKKs, Raf isoforms are the only ones known to date, which phosphorylate MEK1 and MEK2^[23-27]. Stimulation of extracellular receptors by ligands results in the activation of the protein Ras, which can interact with several

effectors, including Raf isoforms. ERK1/2 signaling requires the spatial and temporal organization of three different kinases, Raf, MEK1/2 and ERK1/2. This is controlled by scaffold molecules^[28], among which some have been identified in cardiac tissue (e.g., β -arrestin, FHL1, paxillin)^[29]. These scaffold molecules have important functions: i/ they organize ERK1/2 for the efficient activation in the cascade; and ii/ they determine the output signal by localizing ERK1/2 to selected sites of action, (e.g., the transcription machinery, the actin cytoskeleton). Phosphorylation by ERK1/2 induces conformational change of the substrate, which thereby, either induces their activation, or regulates their association with other molecules^[30]. To date, more than 150 substrates of ERK1/2 have been reported^[31], which can be categorized into several categories including: i/ transcription factors, ii/ protein kinases and phosphatases, iii/ cytoskeletal proteins, iv/ receptors, and vi/ others. ERK1/2 can translocate to the nucleus where they are able to phosphorylate transcription factors to regulate their activities (e.g., c-Jun, c-Fos and ATF-2, Elk-1). Although many ERK1/2's substrates are localized in the nucleus and are phosphorylated after ERKs' nuclear translocation, the number of cytosolic ERK substrates is not much smaller. It has been described that several cytoskeletal element are targets for ERK1/2 (e.g., tubulin^[32], vimentin^[33] or cortactin^[34]).

ERK1/2 and inherited cardiomyopathies

Studies point to ERK1/2 as a maladaptive signaling pathway in cardiomyopathy. Cardiomyopathy is an anatomic and pathologic condition associated with muscle and electrical dysfunction of the heart, which may be confined to the heart or may be part of a generalized systemic disorder, often leading to heart failure-related disability. A well-known negative effect of ERK1/2 signaling in human heart function is highlighted by the fact that different mutations able to increase ERK1/2 pathway activation lead to cardiac pathologies in patients with Noonan and related syndromes, such as Costello, LEOPARD and cardio-facio-cutaneous syndromes^[35-38]. Knock-in mice expressing a Noonan syndrome-associated mutation exhibit enhanced ERK1/2 signaling and an accelerated transition toward heart failure in response to pressure overload. Interestingly, postnatal treatment with MEK1/2 inhibition normalizes cardiac defects^[39]. Based on these results, Novartis recently launched a clinical trial on Noonan syndrome using a MEK1/2 inhibitor (ClinicalTrials.gov Identifier: NCT01556568).

A further example is represented by mutations in the A-type lamin gene and causing dilated cardiomyopathy. Since 1999, scientists have unraveled the role of the nuclear lamina in the development of cardiac disease^[40]. *LMNA* encodes

nuclear A-type lamins via alternative splicing^[41]. Lamins are intermediate filament proteins that polymerize to form the nuclear lamina, a fibrous meshwork underlining the inner nuclear membrane of most eukaryotic cells. We recently demonstrated an aberrant increase in ERK1/2 activity in hearts from a mouse model of the disease^[42]. These results provide proof of principle for ERK1/2 inhibition as a therapeutic option to prevent or delay the onset of heart failure in *LMNA* cardiomyopathy. Pharmacological or genetic blockade of signaling in the ERK1/2 cascade in these mice improves left ventricular dilatation and deterioration in cardiac contractility^[43-46].

Conclusion

Less than a decade ago the kinases constituting MAP kinase pathways were identified through intense efforts to understand the molecular events underlying cellular responses to extracellular signals. The kinases constituting ERK1/2 pathways appear to be key cellular signal transducers and thus attractive targets for drug development. These efforts are now beginning to bear fruit with the initiation of clinical trials in human cardiac diseases. Their positive outcome would be a triumph of translating basic scientific understanding of cellular function into successful human therapies.

Conflict of Interest

Dr. Muchir is inventor on a pending United States patent application on methods for treating and/or preventing cardiomyopathies by ERK inhibition filed by the Trustees of Columbia University in the City of New York.

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