Journal of Molecular and Cellular Cardiology 101 (2016) 44–49

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

Regulation of cardiac hypertrophy and remodeling through the dual-specificity MAPK phosphatases (DUSPs)

Ruijie Liu [a,b], Jeffery D. Molkentin [b,c,*]

[a] Department of Biomedical Sciences, Grand Valley State University, Allendale, MI 49401, USA
[b] Division of Molecular Cardiovascular Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA
[c] Howard Hughes Medical Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA

ARTICLE INFO

Article history:
Received 28 July 2016
Received in revised form 24 August 2016
Accepted 25 August 2016
Available online 27 August 2016

Keywords:
Cardiac hypertrophy
Concentric remodeling
Dual specificity phosphatases
Mitogen-activated protein kinases

ABSTRACT

Mitogen-activated protein kinases (MAPKs) play a critical role in regulating cardiac hypertrophy and remodeling in response to increased workload or pathological insults. The spatiotemporal activities and inactivation of MAPKs are tightly controlled by a family of dual-specificity MAPK phosphatases (DUSPs). Over the past 2 decades, we and others have determined the critical role for selected DUSP family members in controlling MAPK activity in the heart and the ensuing effects on ventricular growth and remodeling. More specifically, studies from mice deficient for individual *Dusp* genes as well as heart-specific inducible transgene-mediated overexpression have implicated select DUSPs as essential signaling effectors in the heart that function by dynamically regulating the level, subcellular and temporal activities of the extracellular signal-regulated kinases (ERKs), c-Jun N-terminal kinases (JNKs) and p38 MAPKs. This review summarizes recent literature on the physiological and pathological roles of MAPK-specific DUSPs in regulating MAPK signaling in the heart and the effect on cardiac growth and remodeling.

1. Introduction and overview

The heart can hypertrophy in response to increased workload caused by physiological or pathological stimulation [1–3]. Cardiac hypertrophy is an adaptive response that reduces wall stress in an attempt to augment or preserve cardiac function [4]. Sustained cardiac hypertrophy in response to pathologic insults is maladaptive and can eventually lead to systolic insufficiency and heart failure characterized by increased rates of myocyte apoptosis, interstitial fibrosis and ventricular dilation [4]. Over the last two decades a great deal of progress has been made in annotating the intracellular signaling pathways that are associated with cardiac remodeling and hypertrophy. Neuroendocrine receptor initiated mitogen-activated protein kinase (MAPK) signaling has been demonstrated to play a pivotal role in controlling both physiological and pathological responsiveness of the heart.

The MAPKs are central signaling intermediates that coordinate diverse physiological and pathological events such as cancer, inflammation, diabetes, memory and cardiac remodeling [5]. MAPK activation is typically achieved by membrane receptors or other ill-defined stress-sensing effectors, which then leads to activation of a highly organized sequence of successively acting protein kinases that both amplify the signal and mediate phosphorylation of diverse cytoplasmic and nuclear regulatory proteins (Fig. 1). In its broadest terms the MAPK signaling cascade is divided into 3 major pathways that culminate in the activation of either the p38 MAPKs, the c-Jun N-terminal kinases (JNK), or the extracellular signal-regulated kinases (ERK) [6,7]. Phosphorylation within their activation loop (TxY) of these three terminal MAPKs is mediated by the upstream dual-specificity MAPK kinases (MAPKKs, also named MEKs or MKKs) including MEK1/2 for ERK1/2, MKK3/MKK6 for p38, and MKK4/MKK7 for JNK1/2 [7]. Upstream of MAPKKs, multiple MAPKKKs are either directly activated by environmental stress, reactive oxygen species, G proteins (Ras, Rac, Rho, and others), G protein-coupled receptors, receptor tyrosine kinases or receptor serine/threonine kinases [8–11] (Fig. 1). Once activated, MAPKs phosphorylate a variety of downstream substrates to regulate events such as cell proliferation, differentiation, apoptosis, growth, cellular re-organization and metabolism [12]. In end-stage heart failure or pathological cardiac hypertrophy, almost all MAPK signaling components are activated where they phosphorylate diverse effectors that drive or modify the disease process [13,14].

MAPKs are phosphorylated at both threonine and tyrosine residues within their activation loop (TxY motif), and dephosphorylation is achieved by either serine/threonine phosphatases such as protein phosphatase 2A (PP2A) and protein phosphatase 2C (PP2C) [15], tyrosine

Abbreviations: DUSP, dual-specificity phosphatase; ERK, extracellular signal-regulated kinase; I/R, ischemia reperfusion; JNK, c-Jun N-terminal kinase; KO, knockout; MAPK, mitogen-activated protein kinase; MAPKKs, MAPK kinases; MKPs, MAPK phosphatases; TAC, transverse aortic constriction; Tg, transgenic.

* Corresponding author at: Cincinnati Children's Hospital Medical Center, Howard Hughes Medical Institute, Molecular Cardiovascular Biology, 240 Albert Sabin Way, MLC 7020, Cincinnati, OH 45229, USA.
E-mail address: jeff.molkentin@cchmc.org (J.D. Molkentin).

http://dx.doi.org/10.1016/j.yjmcc.2016.08.018
0022-2828/

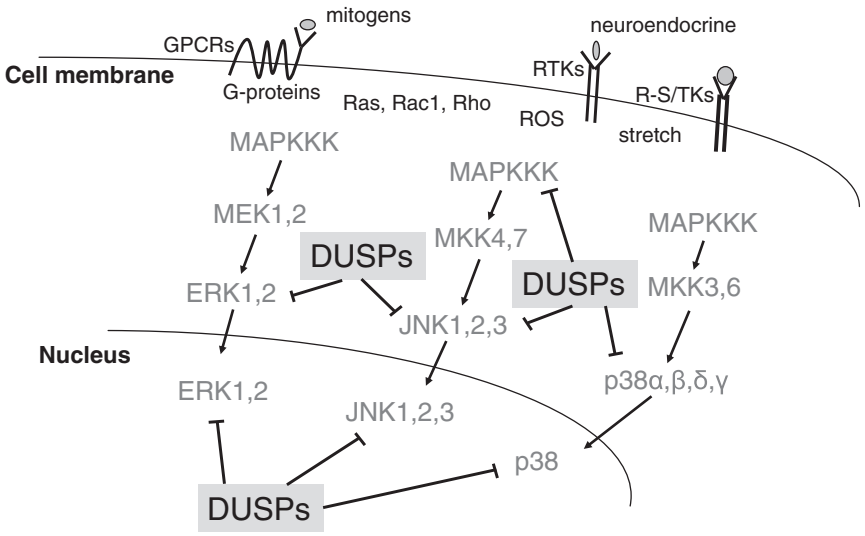

Fig. 1. Overview of MAPK signaling and DUSP regulation. Simplified diagram of the MAPK signaling cascade showing each step in the cascade culminating in ERK1/2, JNK1/2/3 or p38α, β, δ, γ activation, which are inactivated by specific DUSP proteins in either the cytoplasm or nucleus. Other MAPK branches not shown include MEK5-ERK5 and ERK3/4, which are less well characterized for DUSPs counter-regulation.

phosphatases [16,17] or more specifically by dedicated dual-specificity phosphatases (DUSPs) that can dephosphorylate both residues concurrently, previously called MAPK phosphatases (MKPs) [18,19]. There are 25 human *DUSPs* genes that are roughly divided into those specifically dedicated to MAPK signaling inactivation (approximately 11 genes) and those that regulate the dephosphorylation of diverse and often unknown targets, although all 25 genes are expressed in unique patterns across cell-types and tissues [20]. For example, DUSP2 is enriched in hematopoietic cells [21] while DUSP10 is abundantly expressed in cerebellum, skeletal muscle and bone marrow [22]. The majority of the 11 MAPK-specific DUSP genes are transcriptionally induced in response to the same mitogen and stress stimuli that activate selected MAPKs, and thereafter translated leading to protein appearance within 10–30 min where they then inactivate the MAPKs to permit their recycling [23,24] (Table 1).

The 11 MAPK-specific DUSPs are further classified into 3 subfamilies based on domain structure, sequence homology and subcellular localization [25,26]. The first subfamily is composed of nuclear localized DUSPs: DUSP1/MKP1, DUSP2/PAC-1, DUSP4/MKP2, and DUSP5/HVH3, which more or less dephosphorylate all three MAPKs within the nucleus (Table 1). The second and cytoplasmic-localized group includes DUSP6/MKP-3, DUSP7 and DUSP9/MKP4, which are largely specific for ERK1/2 dephosphorylation [25]. The final group includes the cytoplasmic and nuclear localized DUSPs, including DUSP8 (M3/6), DUSP10/MKP5, DUSP14/MKP6, and DUSP16/MKP7, which are thought to have greater specificity for JNK and p38, although DUSP14 is also involved in inactivation of transforming growth factor beta-activated kinase 1 (TAK1) and DUSP8 appears to be more specific for ERK1/2 [27,28]. The exact specificity of a given DUSP protein for selected MAPKs is often difficult to determine as it can vary between cell-types and depend on the type of assay employed. For example, DUSP8 was originally shown to be JNK-specific based on work in cultured cells [29] but in knock-out (KO) mice lacking *Dusp8* we have shown a greater preference for ERK1/2 inactivation within the heart [28].

Table 1
MAPK-DUSPs: names and subcellular localization.

Gene	MKP name	Other names	Localization
DUSP1	MKP-1	CL100, HVH1, ERP, PTPN10	Nuclear
DUSP2		PAC-1	Nuclear
DUSP4	MKP-2	NKP2, HVH2, TYP	Nuclear
DUSP5		HVH3	Nuclear
DUSP6	MKP-3	PYST1	Cytosolic
DUSP7	MKP-X	PYST2	Cytosolic
DUSP8		M3/6, HVH5	Cytosolic/nuclear
DUSP9	MKP-4	PYST3	Cytosolic
DUSP10	MKP-5		Cytosolic/nuclear
DUSP14	MKP-6		Cytosolic/nuclear
DUSP16	MKP-7		Cytosolic/nuclear

Abbreviations: DUSP, dual-specificity phosphatase; MKP, MAPK phosphatase.

All three subfamilies of DUSPs have a common phosphatase domain as well as CDC25 homology domains and a kinase interacting motif (KIM) within the N-terminal region [20]. The DUSPs also demonstrate different subcellular localizations due to divergent N or C-terminal sequences that can contain a nuclear localization sequence [30,31] or a nuclear export sequence [32]. DUSP8 and DUSP16 also contain PEST sequences (proline [P], glutamic acid [E], serine [S], and threonine [T] rich) within their C-terminal region that controls protein stability and turnover [20,33]. An elegant study performed by Tanoue et al. demonstrated that the interaction of MAPKs with MAPKKs or DUSPs is achieved through a domain within the MAPKs that contains two negatively charged aspartic acids and a cluster of positively charged amino acids [34]. Moreover, DUSPs are also found within signaling complexes of the cognate MAPKs and their selected scaffold proteins, which achieves efficient regulation of the MAPKs. For example, scaffold protein JNK-interacting protein-1(JIP-1) recruits MKK7, JNK, and DUSP16 to form a signaling complex for rapid proximity regulation of signaling [29,35].

As briefly mentioned above, the DUSPs are transiently induced in response to growth factors or cellular stresses, although some are more constitutively expressed and have basal effects on MAPK activity [28,36]. The induction and transcription of DUSPs are dependent on the activation of MAPKs, which creates a "self-limiting" feed-back loop. For example, DUSP1 was originally identified as an immediate early gene in cultured cells that is induced in response to mitogen stimulation [37]. Overexpression of calcineurin in neonatal rat cardiomyocytes led to increased level of DUSP1 through a specific regulatory site in the *Dusp1* promoter, which then reduced p38 activity [23]. Analysis of the *Dusp5* promoter showed two CArG boxes that bound to the transcription factor, serum response factor (SRF), which was part of a regulatory mechanism whereby the serum response factor interacting factor Elk-1 was phosphorylated by ERK1/2 to induce gene transcription [38]. DUSP6 expression was also induced in response to elevated ERK1/2 signaling as a feedback regulator [39]. Further analysis of *Dusp6* promoter discovered two binding sites for Ets2 protein, a known target of ERK2 [40].