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MINI-REVIEW

PAK1 is a novel cardiac protective signaling molecule

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Abstract **We review here the novel cardiac protective effects of the multifunctional enzyme, p21-activated kinase 1 (PAK1), a member of a serine/threonine protein kinase family. Despite the large body of evidence from studies in noncardiac tissue indicating that PAK1 activity is key in the regulation of a number of cellular functions, the role of PAK1 in the heart has only been revealed over the past few years. In this review, we assemble an overview of the recent findings on PAK1 signaling in the heart, particularly its cardiac protective effects. We present a model for PAK1 signaling that provides a mechanism for specifically affecting cardiac cellular processes in which regulation of protein phosphorylation states by protein phosphatase 2A (PP2A) predominates. We discuss the anti-adrenergic and antihypertrophic cardiac protective effects of PAK1, as well as its role in maintaining ventricular Ca^{2+} homeostasis and electrophysiological stability under physiological, β-adrenergic and hypertrophic stress conditions.**

Keywords p21-activated kinase 1 (PAK1); heart

Introduction

Protein kinases are versatile signaling molecules that are involved in the regulation of most physiological responses in biological systems. The p21-activated kinases (PAKs) are a group of serine/threonine protein kinases that are activated by Cdc42 and Rac1, and were first discovered in rat brain tissue two decades ago [1]. The structure, substrate specificity and functional role of PAKs are evolutionarily conserved from protozoa to mammals. Vertebrate PAKs are particularly important for cytoskeletal remodeling and focal adhesion assembly, thereby contributing to dynamic processes such as cell migration and synaptic plasticity [2–4]. Our work, along with the work of others over the past few years, has led to the identification of new roles of PAK1 in cardiac physiology, such as the regulation of cardiac excitability and contractility. More recent studies have revealed that PAK1-deficient mice were vulnerable to cardiac hypertrophy, ischemia/reperfusion injury and readily progress to failure under sustained pressure overload. The PAK1 activator FTY720 was able to prevent this pressure overload-induced hypertrophy and ischemia/reperfusion injury in wild-type mice without compromising their cardiac functions. Taken together, these studies suggest that PAK1 is more important in the heart than previously thought, particularly in terms of cardioprotection and therapeutic potential.

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Anti-adrenergic effects

In our previous study [5], we demonstrated the functional effects of PAK1 in sinoatrial node (SAN) pacemaker cells. In SAN expressing the constitutively active (CA) PAK1, responses to adrenergic stimulation by isoproterenol (ISO), assessed by the frequency of firing electrical impulses, were significantly lower compared with those of controls, indicating that PAK1 antagonises β-adrenergic stimulation. Whole cell patch clamping demonstrated that both L-type Ca^{2+} current ($I_{Ca,L}$) and the delayed rectifier K^+ current had a repressed response to ISO in cells expressing the CA PAK1. In the SAN cells, CA PAK1 redirects intracellular localization of protein phosphatase 2A (PP2A) [5]. Our further biochemical study indicated that the regulatory role of PAK1 on $I_{Ca,L}$ and delayed rectifier K^+ channel currents is attributable to increased activity of PP2A. Based on these findings, we suggested that signaling through PAK1 provides a mechanism for specifically affecting cardiac

processes. The dynamic balance of phosphorylation is brought about through dedicated regulation by cAMP-PKA signaling (a well-established pathway) and PAK1-PP2A signaling (an under-studied pathway).

Recent studies have also provided evidence for the regulatory role of PAK1 on Ca^{2+} handling and Ca^{2+} homeostasis [6–8]. Overexpression of CA-PAK1 altered Ca^{2+} transient decay constants (τ_{Ca}) [6], promoted anti-adrenergic signaling by attenuating the ISO-induced increase in activities of $I_{Ca,L}$ and other proteins regulating Ca^{2+} handling, likely through PP2A activation. In ventricular myocytes from mice with cardiomyocyte-specific knockout of PAK1 ($PAK1^{cko}$), abnormal Ca^{2+} homeostasis including increased diastolic $[Ca^{2+}]_i$, altered sarcoplasmic reticulum Ca^{2+} content and sarcoplasmic reticulum Ca^{2+} ATPase (SERCA) function was observed, particularly under β-adrenergic stress conditions [8]. Such altered Ca^{2+} homeostasis is associated with high incidences of ventricular arrhythmias and electrophysiological instability during either acute or chronic β-adrenergic challenge induced by ISO in $PAK1^{cko}$ hearts [8]. These results indicate that modulation of PAK1 activity can have a significant impact on Ca^{2+} handling under both physiological and β-adrenergic challenge conditions. PAK1 is likely to regulate Ca^{2+} handling in cardiac myocytes through either PP2A-mediated or PP2A-independent regulation of the cellular proteins that regulate Ca^{2+} fluxes, including L-type Ca^{2+} channels, ryanodine receptors and phospholamban. However, the data from Sheehan *et al.* and DeSantiago *et al.* [6,7] do not show changes in phospholamban or ryanodine receptor open probability, respectively with either overexpression or deletion of Pak1. Interestingly, the study by DeSantiago *et al.* [7] also provides evidence for a prominent role of PAK1 activity, not only in the functional regulation of ventricular myocyte excitation-contraction coupling, but also for the structural maintenance of the T-tubular system, whose remodeling is an integral feature of hypertrophic remodeling. Another recent study by DeSantiago *et al.* [9] also provided evidence that changes in the Ca^{2+} handling properties of Pak1 deficient ventricular myocytes depended on the increased levels of NOX2 dependent ROS production in these cells. Their results suggest that Pak1 is a critical negative regulator of NOX2 dependent ROS production and that a latent ROS dependent stimulation of NCX activity can predispose ventricular myocytes to Ca^{2+} overload under conditions where no significant changes in excitation-contraction coupling are yet evident [9].

Antihypertrophic effects

In response to acute or chronic insults the heart initially undergoes hypertrophic growth as an adaptive response to normalize ventricular wall stress. However, the capacity of this compensation is limited. Prolonged application of deleterious stimuli eventually causes decompensated remodeling, leading to chamber dilation, reduced contractile function, interstitial fibrosis and myocyte loss. These maladaptive effects subsequently lead to heart failure, a devastating condition affecting millions of people worldwide. Since hypertrophy is generally regarded as a determinant of heart failure, studies to discover the molecular and cellular mechanisms underlying hypertrophic remodeling, and to identify potential therapeutic approaches for treating heart failure, are of paramount importance.

Using both primary cardiomyocytes and $PAK1^{cko}$ mice, we discovered that PAK1 acts as a novel signaling hub relaying antihypertrophic and survival signals from small GTPases to the JNK cascade in the heart [10]. The Pak^{cko} mice showed a greater degree of cardiac hypertrophy controls. They also showed clearcut apoptosis following 2 weeks of pressure overload, and a rapid progression to heart failure after 5 weeks of load [10]. The $PAK1^{cko}$ mice also demonstrated enhanced hypertrophy in response to angiotensin II infusion. Furthermore, we observed that application of FTY720, a synthetic analog of sphingosine with an ability to activate PAK1 [10,11], prevented the development of cardiac hypertrophy in load-stressed wild-type mice. This antihypertrophic effect was likely due to the activation of PAK1 by FTY720. Mechanistically, the loss of PAK1 led to a relative inability to activate the JNK signaling cascade and to an enhancement in the transcriptional activity of NFAT (nuclear factor of activated T cell). Furthermore, application of FTY720 induced PAK1 activation and prevented the development of cardiac hypertrophy in pressure overload stressed-wild type mice, but not in $PAK1^{cko}$ mice, suggesting the antihypertrophic effect of FTY720 was likely due to its function to activate PAK1 [11]. Furthermore, a recent examination of PAK1 global knockout mice [12] revealed a similar phenotype as that in $PAK1^{cko}$ mice. Absence of PAK1 in the whole heart rendered mice more capable of hypertrophic growth under conditions of ISO stimulation. Overall, these data demonstrate for the first time that PAK1 activation exerts a beneficial effect by preventing stress-induced hypertrophic remodeling. PAK1 may thus be a potential therapeutic target for antihypertrophic treatment.

Anti-ischemia/reperfusion injury

Acute myocardial ischemia/reperfusion injury is the major pathophysiological manifestation of ischemic heart disease (i.e., coronary heart disease). Interestingly, the heart has the ability to render itself resistant to ischemia/reperfusion injury. This phenomenon was first discovered by Murry *et*

al. and was described as ischemic preconditioning (IPC) [13]. "Conditioning" the heart to tolerate the effects of acute ischemia/reperfusion injury can be initiated through inducing brief non-lethal episodes of ischemia and reperfusion to the heart either prior to, during, or even after an episode of sustained lethal myocardial ischemia—a phenomenon termed ischemic preconditioning (IPC), preconditioning or postconditioning, respectively. To date, at least three different cardioprotective protein kinase programs have been proposed to be initiated by different G-protein coupled receptors including the adenosine, bradykinin, opioid, protease activated receptor 2 (PAR2), and sphingosine-1-phosphate (S1P) receptors, etc. [14].

S1P has been shown to be an important mediator (through S1P receptor signaling) of cardiac ischemic pre- and postconditioning in both pharmacological and knockout animal studies [15,16]. FTY720 protected against organ ischemia/reperfusion (I/R) injury in animal models [17,18]. In our recent study, we demonstrated that prevention of arrhythmias by FTY720 in an I/R model was attributed to the activation of PAK1/AKT. FTY720 is able to stimulate the autophosphorylation of both PAK1 and AKT and their kinase activities in cardiomyocytes. Further experiments demonstrated that FTY720 triggered NO release from cardiac myocytes through pertussis toxin (PTX)-sensitive PI3K/Akt/eNOS signaling [19]. AKT is a well-established regulator of myocardial growth and survival, contractile function, and coronary angiogenesis [20]. Using both gain- and loss-of-function approaches *in vitro* and *in vivo*, Mao *et al.* demonstrated that PAK1 alone is sufficient to activate AKT [21]. The functional significance of this PAK1-AKT link is underscored by the observation that the pro-survival effect of PAK1 is diminished by AKT inhibition [21]. The PAK1-conferred protection was blocked by the AKT inhibitor X (2-chloro-N,N-diethyl-10H-phenoxazine-10-butanamine), suggesting that the protective effect of PAK1 is mediated, at least in part, by AKT signaling. Our more recent study also demonstrated that PAK1 improves cardiac contractile function in the I/R model of PAK1 global KO mice through regulation of troponin-T and myosin light chain 2 phosphorylation [22].

Clinical perspectives

Heart failure (HF) is a devastating disease with a lifetime risk of 1 in 5 among both men and women. Its incidence is still rising rapidly due to an increase in three major risk factors: population aging, obesity and diabetes. Despite advances in diagnostic and therapeutic technology during past decades, HF mortality rates still remain significantly high. Innovative, cost effective therapeutic approaches are urgently needed to provide the best quality of care when prevention fails. While a considerable amount of knowledge has been generated by biomedical research over the past few decades, the development of new and effective therapies for HF is stagnating. Modulating specific intracellular signaling pathways such as PAK1 signaling to offset/regulate myocardial remodeling, offers a cost effective therapeutic strategy for the management of HF, in particular, by preventing the progression of maladaptive hypertrophy and HF.

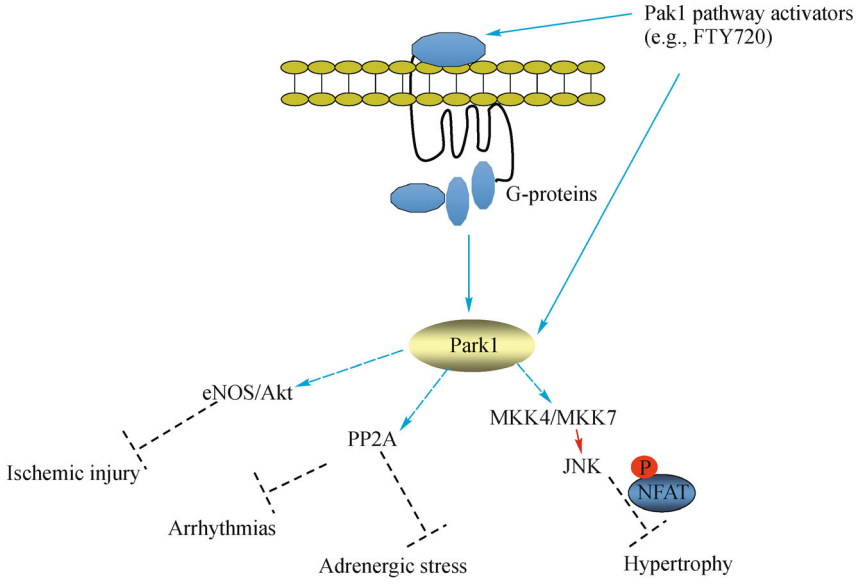

Fig. 1 PAK1 provides a novel target for developing target-based therapies for cardiac adrenergic and hypertrophic stress conditions through its multiple cardiac protective effects via transcriptional mechanisms and post-transcriptional mechanisms (regulating eNOS and PP2A).

In summary, as illustrated in Fig. 1, these novel PAK1 effects complement its previously established actions upon PP2A and the resulting balance between kinase and phosphatase activity controlling cardiac ion channel activity and rhythmic Ca^{2+} cycling. PAK1-PP2A signaling modulation exerts anti-adrenergic and antihypertrophic effects through MKK4/MKK7/JNK signaling in ventricular cells [23]. PAK1 offers a potential new target for developing novel therapy for cardiac disease conditions.

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Compliance with ethics guidelines

Yunbo Ke, Xin Wang, Xu Yu Jin, R. John Solaro, and Ming Lei declare that they have no conflict of interest. This manuscript is a mini-review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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