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Signalling networks in focus

miRNA and piRNA mediated Akt pathway in heart: Antisense expands to survive

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ABSTRACT

The Akt signalling pathway is a crucial network of proteins, which plays a role in neonatal cellular proliferation, hypertrophy and cellular survival mechanism in the heart through a multifaceted system including, small non-coding RNAs (sncRNAs). Despite numerous reports on the distorted expression of these proteins in various cardiovascular diseases, this review focuses on the role of miRNA and piRNA in altering Akt signalling. Nevertheless the role of these sncRNAs in the Akt pathway needs to be studied in detail, there are evidence indicating that they can play a vital function in Akt-mediated cardiac survival. Recent reports indicate that, modification of such miRNA/piRNA causes alteration in the Akt pathway during both physiology and pathology. Therefore, understanding the antisense mediated molecular mechanisms of Akt pathway can devise a new vision towards biomarkers and therapeutic approaches to various cardiovascular diseases.

1. Introduction

The heart is considered as the first organ to form during mammalian embryonic development. The development and function of the heart require a precise cardiac gene expression for its myogenesis, morphogenesis and contractility. Pathological cardiac development is associated with activation of a fetal gene program, interstitial fibrosis and myocyte apoptosis. In contrast, physiological cardiac development does not display any of these features. While most studies have focused on explicating the mechanism of pathological heart growth, the molecular mechanism behind the inhibition of the normal survival pathway in cardiac growth is less understood.

The signalling downstream of serine/threonine kinase (Akt) is known to be vital in cell survival. Inappropriate activation of Akt is well proven in several cardiovascular disorders (Sussman et al., 2011). As a prominent pathway involved in cell survival, researchers have intensively focused on its regulatory network. However the discovery of microRNA (miRNA), PIWI (P-element-induced wimpy testis) – interacting RNA (piRNA) and their associated proteins add another layer of complex connections on Akt-mediated signalling. Though several recent review articles described the role of Akt in heart (Sussman et al., 2011) and also the role of miRNA in Akt signaling (Xu and Mo, 2012), there aren't review focusing the importance of miRNA/piRNA in cardiac survival. This review predominantly focuses on the impact of miRNA and piRNA in Akt-mediated survival pathway during various pathophysiological conditions of the heart.

2. Functions and cascades

The Akt (also known as protein kinase B (PKB)) protein is a key downstream target of the signalling pathway mediated by phosphoinositide-3 kinase (PI3K). It plays a vital role in the regulation of diverse cellular processes including neonatal cellular proliferation, hypertrophy and cellular survival (Sussman et al., 2011). In the adult heart, *Akt1* is abundantly expressed, while *Akt3* is abundantly expressed in the embryonic heart. Akt activation is a defensive effect on post ischemic cardiac injury by stimulating cellular proliferation, survival mechanism and by neutralizing the apoptosis. It is known that cardiomyocytes are enormously defiant to mitotic activity and oncogenic transformation. Active Akt proteins can modulate the function of numerous substrates related to the regulation of cell proliferation such as GSK3, mTOR, GLUT4,

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TSC2 and cyclin-dependent kinase inhibitors, P21/Waf1/Cip1 and P27/Kip2 (Sussman et al., 2011).

The Akt regulates cell survival through phosphorylation of downstream substrates that control the apoptotic machinery. Akt inhibits transcription of pro-apoptotic genes such as *FasL*, *IGFBP-1*, *Bim* and *Bad* and exerts indirect control on apoptosis through regulation of the forkhead family of transcription factors. Intriguingly, Akt phosphorylates the MDM2 and indirectly regulates the tumour suppressor p53 and activates the cyclic AMP-response element-binding protein which increases the transcription of anti-apoptotic genes such as *Bcl-2*, *Mcl-1* and *Akt* itself (Sussman et al., 2007). In this review, we focus on miRNA/piRNA mediated regulation of Akt. We have also included, FOXO3 as an example for the regulation of transcription factor by miRNAs.

3. Key molecules

The discovery of small non-coding RNAs has fundamentally changed our understanding of how genes and transposable elements are regulated. Recent studies have shown that eukaryotic cells express a large number of different small non-coding RNAs (sncRNAs), especially 18 to 32-nucleotide (nt) long, which trigger RNA interference (RNAi) of Akt signalling. Among these sncRNAs, miRNAs are the richly studied and piRNAs are the most abundantly expressed with the least number of reports (Ross et al., 2014; Rajan and Ramasamy, 2014). While investigating miRNA profiling by next generation sequencing, we also found active and differential expression of PIWI-piRNA in the heart during hypertrophy in both *in vivo* and *in vitro* models (unpublished data).

3.1. miRNA mediated Akt signaling

miRNAs are sncRNAs of ~18–23 nt length, that has emerged as master regulators of transcriptome of all biological processes, including cell differentiation, proliferation and growth. They regulate the transcriptome either at the transcriptional or post transcriptional by binding to the 3′-untranslated region.

In Akt signalling, miRNAs can either be positive or negative regulators (Table 1). It has been shown that miR-1 is highly expressed in the heart by the activation of active Akt, which in turn directly targets the *NCX1* (Kumarswamy et al., 2012). miR-1 and miR-133, belongs to the same transcriptional unit and are expressed at low levels in mouse and human models of cardiac hypertrophy. miR-133 targets a variety of mRNA, including *RhoA*, *Cdc42*, *HERG*, *HCN2*, *Cyclin D2*, *Caspase-9*, *CTGT*, *SRF* and *RUNX2*. Reduced expression of miR-133 was sufficient to induce apoptosis and fetal gene program in the heart (Abdellatif, 2010). Akt can also regulate miR-210, thereby exerts cytoprotective effects potentially by reducing mitochondrial reactive oxygen species production by targeting *PTPN2*. Consequently, it has been reported that miR-210 induces phosphorylation and activation of Akt and ERK proteins respectively, followed by nuclear translocation of *HIF-1α* (Kim et al., 2013).

During cardiac hypertrophy, the induced miR-208a expression targets myostatin (Callis et al., 2009) which is known to regulate cardiomyocyte growth through modulation of Akt signalling. The overexpression of miR-26 inhibits *GSK3β* and myocardial hypertrophy. Reports from non-cardiac models indicate that the expression of PI3K and Akt phosphorylation was increased after miR-26a overexpression (Jiang et al., 2014). Higher levels of cardiac-enriched miR-486 lead to reduced level of *PTEN*, *FOXO1a* and *DOCK3*, which are known to augment the Akt phosphorylation (Alexander et al., 2014). The activation of miR-126 in cardiac myocytes may be cardioprotective by phosphorylation of Akt upon VEGF stimulation and targeting *HDAC* (Shi et al., 2013).miR-21 inhibits *FasL*, that is positively regulated *via* Akt-dependent pathway and also targets the *PTEN*, subsequently elevating MMP-2 expression which is implicated in cardiac remodelling post-myocardial infarction (Tu et al., 2013). Similarly, miR-22 directly targets *PTEN* and is also up regulated by Akt to effectively protect cardiomyocytes from hypertrophy (Xu et al., 2012). Recently, it has been shown that the increased miR-494 level leads to enhancement of *HIF-1α* through *PTEN* curtailment (Sun et al., 2013). miR-214 also targets *PTEN* and protects cardiac myocytes against H_2O_2-induced injury (Lv et al., 2014). The miR-106b~25 cluster consists of miR-106b, miR-93, miR-25 and is a paralogue of the miR-17-92 cluster. miR-25 targets *PTEN* and together with increased calcineurin/Nfat signalling, the decreased miR-25 expression results in the diseased human and mouse myocardium (Dirkx et al., 2013). The miR-17-92 cluster consisting of miR-17, miR-18a, miR-19a, miR-20a, miR-19b, and miR-92a promote resistance to apoptosis by directly inhibiting pro-apoptotic protein and by activating PI3K/AKT pathways (Zhou et al., 2013).

Table 1
miRNAs mediating Akt pathway reported in cardiac system.

miRNA	Target genes	Akt signaling activation	Reference
miR-133	*RhoA*, *Cdc42*, *HERG*, *HCN2*, *Cyclin D2*, *Caspase-9*, *CTGT*, *SRF* and *RUNX2*	+	Abdellatif (2010)
miR-210	*PTPN2*	+	Kim et al. (2013)
miR-208a	*TRAP1*,*Myostatin*	+	Callis et al. (2009)
miR-26a	*GSK3β*	+	Jiang et al. (2014)
miR-486	*PTEN*, *FOXO1a*, *DOCK3*	+	Alexander et al. (2014)
miR-126	*HDAC*	+	Shi et al. (2013)
miR-21	*FasL*, *PTEN*	+	Tu et al. (2013)
miR-22	*PTEN*	+	Xu et al. (2012)
miR-494	*PTEN*	+	Sun et al. (2013)
miR-214	*PTEN*	+	Lv et al. (2014)
miR-25	*PTEN*	+	Dirkx et al. (2013)
miR-17-92 cluster	Pro-apoptotic proteins	+	Zhou et al. (2013)
let-7c	*Oct4*, *Sox2*	–	Tolonen et al. (2014)
miR-15	*Bcl2*, *Arl2*	–	Hullinger et al. (2012)
miR-199a-3p	*IGF-1*, *mTOR* and *RPS6KA6*	–	Jia et al. (2013)
miR-378	*IGF1R*	–	Knezevic et al. (2012)
miR-29	*Mcl-2*	–	Ye et al. (2010)
miR-128a	*INSR*, *IRS1*	–	Motohashi et al. (2013)
miR-143	*Elk-1*,*versican*	–	Rangrez et al. (2011)
miR-145	*Cofilin*	–	Rangrez et al. (2011)
miR-143/145	*Myocardin*, *Klf4*,*Klf5*	–	Rangrez et al. (2011)

+ indicates the activation of Akt and – indicates the inactivation of Akt.

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