

Review

Molecular Control of Cardiac Fetal/Neonatal Remodeling

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Abstract: Immediately following birth, the mammalian heart switches from generating ATP via glycolysis to β -oxidation of lipid. Coincident with this metabolic remodeling, cardiomyocyte mitosis ceases and regenerative capacity is lost. Recently, our understanding of the molecular pathways linking physiological stimuli with gene expression and phenotype changes around birth has increased, although fundamental gaps remain. This review discusses recent work that sheds light on this important area of mammalian cardiovascular development.

Keywords: cardiac; neonatal; metabolism; HIF; PPAR

1. Introduction

In the week following birth in the mouse, the predominant mode of cardiac energy generation switches from glycolysis to β -lipid oxidation, which persists through life [1]. Over the same seven day timescale in the mouse, cardiomyocyte mitosis virtually ceases and cardiac growth occurs via cardiomyocyte hypertrophy [2]. Recently, it was reported that the murine heart maintains regenerative capacity after surgical injury for the first seven postnatal days, after which it is lost [3]. Surprisingly little is known about this fundamental transition in mammalian development, or the molecular mechanisms and physiological stimuli underlying them.

It is worth noting that fetal myocardium is adapted to function in extremely low ambient levels of oxygen *in utero*, and that this adaptation is permanently lost soon after birth. Neither the mechanism of fetal hypoxia tolerance nor its loss is fully understood. This information may be useful for developing future treatments to protect vulnerable adult myocardium from hypoxia, for example during

myocardial infarction. The focus of this short review is the molecular control of the neonatal cardiac transition. For a full description of the metabolic changes occurring at birth in the heart, the reader is directed to excellent recent reviews [4,5].

At birth, cardiac output increases dramatically [6]. To support the resultant increased cardiac energy requirement, the heart shifts from energy generation via glycolysis in the fetal heart to predominant oxidative-phosphorylation of lipid in the postnatal heart [7–9], accompanied by a decrease in the amount of stored glycogen in the heart after birth [10]. A number of parallel physiological events are thought to underlie this process. Tissue oxygen tension increases immediately following birth. Following birth, a period of starvation results in formation of ketone bodies [11], followed by the first meal, which leads to an increase in blood lipid content [12]. All of these physiological events could potentially regulate maturational processes in the heart. Our understanding of this process, and its physiological triggers, is very rudimentary, however.

2. Molecular Control of Myocardial Metabolism

The molecular pathways mediating the metabolic shift in the fetal neonatal heart and their physiological stimuli are still incompletely understood. For obvious reasons, our knowledge of these mechanisms in the healthy human heart is extremely limited. Recent work in mouse models has increased our understanding of changes in the myocardial activity of several signaling axes connecting metabolic function in the cardiomyocyte with changes in the extracellular *milieu* in the immediate postnatal period.

2.1. HIF Signaling

At birth, the ambient oxygen concentration encountered by the neonate increases dramatically, with concomitant downregulation of Hypoxia Inducible Factor (HIF) signaling at birth [13]. Oxygen tension in adult mammalian arteries at sea level is approximately 100 mmHg, whereas in the fetal placental vein it is in the range of 20–30 mmHg in humans and other mammals [14–16]. HIF signaling is likely to be of prime importance in this context, given its well-established role in controlling metabolism in the adult heart [17]. HIF1 α is necessary for embryonic heart development, as evidenced by the early death of HIF1 α ^(-/-) embryos [18]. Several studies have now analysed the effects of experimental perturbation of the HIF signaling axis in the heart immediately after birth. Possible roles for HIF1 β or HIF2 have yet to be defined. The list of transcription factors shown to be under the control of HIF signaling is small (but growing), and will be outlined below.

Regulation of HIF1 α is thought to occur principally at the level of protein degradation. HIF1 α is targeted for destruction by the E3 ubiquitin ligase VHL/Vhl (von Hippel-Lindau factor) [19]. Deletion of VHL either by inherited recessive alleles in humans or targeted mutation in genetically modified mice results in decreased destruction of HIF1 α protein and elevated nuclear translocation and genomic binding of HIF1 α . Elevated cardiac HIF signaling in the neonatal heart of MLCvCre::VHL^(fl/fl) neonatal mice results in a striking phenotype of heart failure and cardiac tumours in early adult life [20]. Using the α MHC-Cre line to delete VHL results in a more severe phenotype; failure of the cardiac conduction system to mature and arrhythmogenic death two weeks after birth [21]. We have recently shown that expression of the transcription factor *Hand1* is under control of HIF signaling, by direct stimulation of *Hand1* transcription by HIF1 α protein. In turn, *Hand1* controls cellular lipid metabolism by direct

transcriptional regulation of a number of genes [22]. The postnatal decrease in cardiac HIF signaling therefore leads to downregulation of cardiac *Hand1* expression, in turn leading to upregulation of lipid metabolism and lipid oxidation after birth.

Several potential metabolic control targets of HIF signaling have been found in skeletal muscle [23], and the expression of some genes encoding glycolytic enzymes in the heart are directly HIF-regulated [24]. It therefore seems likely that HIF signaling has a pivotal (yet incompletely defined) role in the neonatal cardiac metabolic shift. A full list of cardiac HIF targets is awaited, however.

2.2. AMPK Signaling

The onset of suckling results in changes in circulating energy substrate and consequent alterations in levels of hormones such as insulin and glucagon. It is recognised that this event is potentially an important event in cardiac maturation [1]. The effects on cardiac metabolism of starting suckling and the first lipid meal are not fully understood, however. One important candidate pathway linking cardiac metabolism with circulating nutrients is 5'-AMP-activated protein kinase (AMPK), although the physiological events controlling AMPK activity in the heart around birth are not fully understood. AMPK enzymatic activity increases following birth in the neonatal mouse, rabbit [1] and human heart [25]. Insulin has the effect of inhibiting AMPK activity, and as circulating levels of insulin drop following birth, increased AMPK activity has the effect of increasing lipid entry into cardiac mitochondria [25,26]. The rise in AMPK activity in the postnatal heart also stimulates the TCA cycle by inhibiting the activity of Acetyl coA Carboxylase leading to decreased production of malonyl coA, which inhibits lipid entry into the mitochondrion [1].

2.3. PPAR Signaling

The peroxisome proliferator-activated receptors family (PPARs α , β , γ) function as ligand-activated transcription factors. Control of myocardial metabolism by the PPAR axis is well described, in response to circulating lipid [27] and hypoxia [28], and direct control of expression of PPAR isoforms by HIF signaling has been described [29,30]. As cardiac transcription factors under control of hypoxia, it is therefore likely that PPARs are important in controlling postnatal cardiac metabolic remodeling, although explicit confirmation of this is awaited. Certainly pharmacological manipulation of PPAR β signaling alters neonatal cardiomyocyte metabolism and hypertrophy *in vitro* [31,32], and expression of PPARs α , β and γ increase in the heart in the days after birth [33], albeit that the physiological stimulus underlying this is unknown.

3. Control of Postnatal Mitochondrial Biogenesis

One of the most striking changes occurring in the neonatal cardiomyocyte following birth is a large increase in mitochondrial capacity and alterations in mitochondrial morphology [34–36]. The significance of the relatively low mitochondrial number in fetal hearts is currently unknown, as is whether this contributes to fetal cardiac function in hypoxic conditions. The peroxisome proliferator-activated receptor co-activators 1 α and β (*Pgc1 α/β*), transcriptional co-activators of PPAR signaling, have a key role in controlling neonatal cardiac mitochondrial biogenesis [37,38]. *Pgc1 α* and *β* seem to have

redundant roles in embryonic development. The neonatal cardiac phenotypes of *Pgc1 α* [39,40] and *Pgc1 β* [39,41,42] null mice are minimal. However, the double *Pgc1 α/β* cardiac null mouse exhibits defective cardiac mitochondrial biogenesis, which is first observed in the late stages of embryonic development rather than following birth, resulting in a neonatal cardiomyopathy phenotype [35]. This raises the interesting question as to the nature of the stimulus underlying the action of *Pgc1 α/β* on mitochondrial biogenesis in late fetal development. However, our understanding of the cardiac maturational events occurring during the third trimester of fetal gestation is currently extremely limited.

4. Neonatal Cardiac Mitosis and Regeneration

The control mechanisms mediating the cessation of cardiomyocyte mitosis and the loss of regenerative potential during the mammalian neonatal period are a focus of current research. The dogma that the mammalian heart is “post mitotic” shortly after birth [2] has been challenged by work showing significant detectable cardiomyocyte mitosis in the adolescent human heart [43].

Sengupta *et al.* showed that AMPK signaling regulates neonatal rat cardiomyocyte mitosis thereby describing a putative mechanism linking the extracellular *milieu* with mitotic rates in neonatal mouse cardiomyocytes [44]. Their model proposes that regulation by AMPK signaling of competing stimulatory (FoxM1) and inhibitory (FoxO1) transcription factors controls cell-cycle withdrawal through expression of Insulin-like Growth factor I (IGF-1). In prenatal cardiomyocytes, where AMPK signaling is depressed, FoxO1 nuclear import is inhibited and FoxM1 import results in high rates of cardiomyocyte mitosis. Elevated AMPK signaling postnatally results in nuclear import of the transcription factor FoxO1, with the effect of inhibiting IGF-1 transcription and withdrawal from mitosis.

Regeneration of the myocardium after surgical injury has long been recognised in axolotl [45] and zebrafish [46]. Porrello and colleagues recently described a window of the seven days following birth during which a mouse heart had the capacity to regenerate following surgical injury [3] and myocardial infarction [47]. This finding is of great interest, as efforts to find mechanisms to regenerate failing and infarcted adult myocardium continue.

miRNAs are prime candidates for mediators this regenerative ability. Inhibition of the miRNA-15 family prolonged the regenerative window in neonatal mice [47]. Profiling of miRNA expression in the mouse heart following birth has suggested a role for the miRNA-15 family as mediators of mitotic withdrawal [48]. Furthermore, a screen of miRNAs in neonatal cardiomyocytes identified two (miRNAs 590 and 199a) that conferred the ability for cardiomyocytes to continue proliferating in the neonatal heart and for the myocardium to regenerate following infarction in the adult [49].

Another clue as to a possible molecular control of postnatal cardiac mitotic arrest came from the recent demonstration by Mahmoud *et al.* that deletion of the homeobox-containing transcription factor *Meis1* in mouse neonatal cardiomyocytes prolonged postnatal mitosis. Although the mechanism for this remains unclear, the authors were able to show that *Meis1* activated function of the CDK inhibitors p15, p16 and p21 [50]. Whether AMPK, *Meis1* and miRNA15 interact, or are part of the same pathway is an interesting question.

A key question in this field is how the loss of cardiomyocyte regenerative capacity is related to the other events occurring in the first postnatal week in the mouse. For example, there is evidence that alterations in energy generation over this period may be related to loss of “stemness” in cardiomyocytes [8,34].

5. Future Challenges

It is clear that there are significant gaps in our understanding of late fetal and neonatal mammalian cardiac development. However, our understanding of how the physiological changes in the heart around birth influence cellular signaling and gene expression is increasing. It will be interesting to see how the molecular control of neonatal cardiac remodeling relates to the response of the adult heart to metabolic perturbations encountered in disease states, such as diabetes mellitus and cardiac ischaemia. A more complete understanding of the fetal/neonatal transition is therefore likely to be important in several areas of medicine.

Conflicts of Interest

The author declares no conflict of interest.

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