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Review

Metabolic remodeling in early development and cardiomyocyte maturation

Rebecca Ellen Kreipke[a,b,1], Yuliang Wang[b,d,e,1], Jason Wayne Miklas[b,c], Julie Mathieu[a,b], Hannele Ruohola-Baker[a,b,c,*]

[a] Department of Biochemistry, University of Washington, School of Medicine, Seattle, WA 98195, USA
[b] Institute for Stem Cell and Regenerative Medicine, University of Washington, School of Medicine, Seattle, WA 98109, USA
[c] Department of Bioengineering, University of Washington, Seattle, WA 98195, USA
[d] Department of Biomedical Engineering, Oregon Health & Science University, Portland, OR 97239, USA
[e] Computational Biology Program, Oregon Health & Science University, Portland, OR 97239, USA

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ABSTRACT

Aberrations in metabolism contribute to a large number of diseases, such as diabetes, obesity, cancer, and cardiovascular diseases, that have a substantial impact on the mortality rates and quality of life worldwide. However, the mechanisms leading to these changes in metabolic state – and whether they are conserved between diseases – is not well understood. Changes in metabolism similar to those seen in pathological conditions are observed during normal development in a number of different cell types. This provides hope that understanding the mechanism of these metabolic switches in normal development may provide useful insight in correcting them in pathological cases. Here, we focus on the metabolic remodeling observed both in early stage embryonic stem cells and during the maturation of cardiomyocytes.

Contents

1. Metabolism and cell fate

Metabolic signatures are highly characteristic for a cell and may contribute to the fate of the cell [1–8]. The recent demonstrations that mitochondria, redox state and metabolic intermediates can profoundly affect transcriptional programs and, thereby, cell fates show the regulatory function of cellular metabolism. Furthermore, some metabolites are shown to act as ligands in cellular signaling pathways [9]. The nematode *Caenorhabditis elegans* exhibits specialized intermediary metabolism in long-lived mutants and Dauer states, involving glycolytic, glyoxyate, branched chain amino acid, and fumarate metabolism [10–12]. In mammalian systems, redox state and hypoxia regulate self-renewal and mesoderm specification [13–16], while T lymphocyte differentiation requires the bioenergetic sensor pathway involving LKB1 and AMPK [17,18]. The signals arising from metabolic programs include redox and

* Corresponding author at: Institute for Stem Cell and Regenerative Medicine, University of Washington, School of Medicine, Seattle, WA 98109, USA.
E-mail address: hannele@uw.edu (H. Ruohola-Baker).
[1] Equal contribution.

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reactive oxygen species, as well as metabolic intermediates [19–22], allowing for a wide variety signaling mechanisms involved in the regulation and the effect of metabolic signatures in cell fate and maturation. While the alterations in metabolism underlie key developmental transitions in a number of different cell types, in this review, we will discuss the metabolic

changes observed in the naïve to primed transition in embryonic stem cells and the maturation of cardiomyocytes from fetal to adult stage [7,22,57].

2. Embryonic stem cell metabolic transitions

a Naïve-to-primed ESC transition

Pluripotent stem cells – mouse and human embryonic stem cells (mESCs and hESCs, respectively) or induced pluripotent stem cells (iPSCs) – demonstrate the remarkable capacity to remain unspecialized in culture. Depending on chemical cues received from their environment and their internal genetic programs, they can also differentiate into a number of different cellular lineages. The recently stabilized naïve and primed hESCs are biologically equivalent *in vitro* representations of pre- or post-implantation embryos, respectively, and, hence, form excellent models for studying early normal and pathological developmental processes. The naïve hESCs have more developmental potential than primed ESCs [24,25] and are marked by significantly lower H3K27me3 levels compared to primed hESCs. While, the molecular mechanisms that affect the transition between these two states are incompletely understood, recent studies have revealed that metabolic signatures of these stages play an important role in their fate.

Pluripotent stem cells are thought to acquire specific metabolic signatures important for stemness through common means. Since pluripotency does not represent a single defined state, subtle stages of pluripotency provide an experimental system for studying potential regulators of the developmental capacity of human and mouse ESCs [24–32]. Both naïve and primed stem cells have been derived in mouse as well as in humans, albeit with some heterogeneity among the populations. We and others have shown that the earlier developmental stage, naïve ESC has highly active mitochondria, but the naïve to primed ESC transition accompanies a dramatic metabolic switch from bivalent to highly glycolytic state both in mouse and in human [22,28,31]. Importantly, we have shown that a gene expression signature indicative of the metabolic switch is observed in mouse *in vivo* inner cell mass (ICM) to post-implantation embryonic cells [31]. The unique metabolic signature of each pluripotent stage led us to postulate that the down-regulation of the electron transport chain (ETC) in the epiblast stage must have a tremendous beneficial value for the pluripotent cell population. While reduction of complex IV (cytochrome c oxidase) activity was previously shown to associate with pathological cases, the developing pluripotent stem cell can harness this reduction to its benefit, possibly to protect its pluripotent state against oxidative stress [5].

Similar reduction of mitochondrial activity is observed in the context of cancer in the Warburg effect. The Warburg effect, increased glycolysis in cancer cells, leads to increased metabolic flux of glucose carbons into biosynthetic precursors. This is thought to be beneficial for fueling anabolic processes and control of redox potential and reactive oxygen species (ROS) that are required for rapid tumor cell growth and division. The developmental suppression of oxidative phosphorylation in post-implantation Epiblast stem cells (EpiSCs)/hESCs may serve a similar function in preparation for embryonic growth and the formation of germ cell layers. However, in normal development, this state of low mitochondrial activity is exceedingly transient, since the primed state of inert mitochondria rapidly changes to highly respiring mitochondria when cells begin to differentiate. It is not yet understood how and why the primed, post-implantation stage pluripotent cells enter the highly glycolytic, metabolically cancer-like stage and how a differentiating cell leaves this stage.

Recent data show that metabolites may play a more significant role in regulating embryonic stem cell fate than previously appreciated. In mouse embryonic stem cells, threonine and S-adenosyl methionine (SAM) metabolism are coupled resulting in regulation of histone methylation marks [33]. Methionine and SAM are also required for the self-renewal of hESCs, since depletion of SAM leads to reduced H3K4me3 marks and defects in maintenance of the hESC state [34]. SAM, therefore, is a key regulator for maintaining the ESC undifferentiated state and regulating their differentiation. We have shown that SAM levels, controlled by nicotinamide *N*-methyltransferase (NNMT), are also critical during the naïve-to-primed hESC transition, where the epigenetic landscape changes through increased H3K27me3 repressive marks [22]. NNMT consumes SAM in naïve cells, making it unavailable for histone methylation. Histone methylation (H3K27me3) further regulates the key signaling pathways important for the metabolic changes that are necessary for early human development. However, while NNMT regulates the substrate levels for Polycomb repressive complex, the regulators for positional methylation have not yet been identified. Differential metabolites between pluripotent stages may control epigenetic dynamics and signaling.

b Hypoxia and HIF in stem cell acquisition

The emerging role of hypoxia and the hypoxia-inducible factors (HIFs) in the acquisition of stemness is an example of metabolic context in cell fate and its impact on pathological conditions [13,14,31,33,34]. We have shown that hypoxia can induce the reversal of the early steps in human ESC differentiation [13]. In tumors, aggressive cancer cells display gene expression signatures characteristic of ESCs and are commonly exposed to hypoxic environments. These two processes may be mechanistically linked *via* HIFs. Hypoxia, through HIFs, can induce a human embryonic stem cell-like transcriptional program in cancer cells [36]. Furthermore, HIF is required for acquisition of pluripotency, early during reprogramming of somatic cells to induced pluripotent stem cells [14,35]. HIFs reprogram cellular metabolism, affecting substrate and energy utilization, redox state and mitochondrial TCA cycle flux. As metabolic states can be propagated once the initial conditions for their establishment change, the metabolic stability is associated with maintenance of stemness under normoxic conditions [31].

We have shown that stem cells acquire a common characteristic metabolic signature through exposure to stabilized HIF activity [14,31,36,37]. Further, this metabolic signature may be determinative for stemness. The dependency of stem cells on glycolysis to produce ATP could be an adaptation to low oxygen tensions *in vivo* since hypoxia is a key feature of the stem cell niche [38]. Further, low oxygen levels are beneficial for hESC, adult stem cells [39–43] and cancer cells [36,38]. HIF1α and HIF2α are stabilized in low oxygen, dimerize with HIF1β and control the transcription of multiple target genes, including genes involved in glucose metabolism [44,45]. Human iPSC are usually reprogrammed from somatic cells to a primed pluripotent stage and hence are very similar metabolically to hESC [2,46,47]. Therefore, a metabolic switch from oxidative to highly glycolytic needs to take place during iPSC formation. Supporting this idea, inhibition of glycolysis reduces the reprogramming efficiency while stimulation of glycolytic activity enhances iPSC generation [1,2,48]. This metabolic switch occurs

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