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Metabolic switching and cell fate decisions: implications for pluripotency, reprogramming and development

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Cell fate decisions are closely linked to changes in metabolic activity. Over recent years this connection has been implicated in mechanisms underpinning embryonic development, reprogramming and disease pathogenesis. In addition to being important for supporting the energy demands of different cell types, metabolic switching from aerobic glycolysis to oxidative phosphorylation plays a critical role in controlling biosynthetic processes, intracellular redox state, epigenetic status and reactive oxygen species levels. These processes extend beyond ATP synthesis by impacting cell proliferation, differentiation, enzymatic activity, ageing and genomic integrity. This review will focus on how metabolic switching impacts decisions made by multipotent cells and discusses mechanisms by which this occurs.

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Introduction

Energy generation through ATP synthesis is critical for driving biochemical processes. Different cell types, however, adopt alternate strategies for energy generation and biosynthesis with glucose, ketogenic amino acids and fatty acids being the major carbon sources that drive ATP-generating catabolic pathways. Glycolytic-dependent energy generation can occur in two general contexts. In many cell types glucose oxidation generates pyruvate and then acetyl coenzyme A (acetyl-CoA), which is fed into the tricarboxylic acid (TCA) cycle. Reducing equivalents generated by glycolysis and the TCA cycle then serve as an electron source to drive the electron transport chain (ETC) and protons for coupled ATP synthesis, known as oxidative phosphorylation (OxPhos) [1]. In some cells however, glycolysis proceeds at an elevated rate in the absence of OxPhos, producing lactate from pyruvate in preference to acetyl-CoA. This is seen in muscle cells, under anaerobic conditions when the electron transport chain is inactive [2]. This mode of metabolism is frequently seen in tumor cells under aerobic conditions and generally referred to as the 'Warburg effect' or, 'aerobic glycolysis' [3]. Glutamine-dependent energy generation involves its conversion to α-ketoglutarate, which then feeds into the TCA cycle to drive energy generation [4,5].

Energy-generating pathways are highly dynamic and metabolic fluxes vary dramatically across different cell types and tissues in response to developmental signals [6], nutritional status [7], environmental signals [8] and disease pathogenesis [9]. Metabolic flux is finely tuned to maximize function in different cell types and is linked to cell identity just as gene expression, epigenetics and morphology are. Whether to produce signaling molecules such as insulin in pancreatic β-cells or dopamine in neurons, packaging of lipids into vesicles in the liver, or to generate ATP for motor function in skeletal muscle; regulating metabolism is integral for maintenance of cell identity and function. This review will summarize recent developments linking metabolic activity and cell identity with a focus on multipotent stem cells.

Metabolic regulation in adult stem cells

Numerous populations of multipotent stem cells undergo aerobic glycolysis in the stem cell niche to sustain their energy demands [10]. Examples include hematopoietic stem cells in the bone marrow [11], intestinal crypt stem cells [12] and hair follicle stem cells [13]. Muscle satellite stem cells (MuSCs) illustrate how dynamic metabolic regulation can be under different physiological conditions. After postnatal growth muscle MuSCs undergo a metabolic switch from aerobic glycolysis to OxPhos coinciding with exit from the cell cycle [14,15••]. Upon injury cues quiescent MuSCs then re-enter the cell cycle to proliferate for muscle repair/regeneration. As part of this mechanism, key rate-limiting enzymes associated with aerobic glycolysis such as lactate dehydrogenase A (*LDHA)* and pyruvate kinase muscle splice variant 2 (*PKM2)* are induced during MuSC activation [16]. Curiously, while establishment of elevated glycolytic flux is a requirement of MuSC activation, OxPhos is not reduced, implying that the induction of glycolysis is not related to increased energy production. Ryall *et al.* [15••] showed that this metabolic switch functions by adjusting the epigenetic status of stem cells via modulation of the

redox state. Induced aerobic glycolysis during MuSC activation lowers the intracellular NAD^+:NADH ratio leading to reduction in NAD^+-dependent SIRT1 histone deacetylase activity. This causes an increase in global H4K16 acetylation, localized decondensation of chromatin and activation of myogenic genes *MYOD*, *MYOG*, *MYKL9*, and *H19* [15••]. Knockdown of *SIRT1* under quiescent conditions is sufficient to activate MuSCs without metabolic switching, suggesting that the role of metabolic regulation is solely to regulate SIRT1 activity. This study provides a clear link between metabolic switching, redox status, epigenetic regulation and cell fate decisions.

Mesenchymal stem cells (MSCs) are another multipotent cell type where metabolic activity impacts biological function beyond energy generation. MSCs are isolated from numerous anatomical locations including the bone marrow, skeletal muscle, white adipose tissue and the placenta [17]. Under most conditions, MSCs utilize aerobic glycolysis for energy production [18,19] through a mechanism regulated by *HIF1α* [20,21]. During both osteogenic and adipogenic differentiations of MSCs, *HIF1α* is down-regulated, resulting in a loss of aerobic glycolysis accompanied by increased mitogenesis and elevated OxPhos [20,22]. Increased levels of reactive oxygen species, predominately produced by the ETC, induce adipogenic differentiation within MSCs which can be blocked by antioxidant treatment [10,23]. These observations provide a link between differentiation status and metabolic activity. ROS scavengers such as catalase and superoxide dismutase are down-regulated as MSCs transition to an adipose cell fate [10,22]. However, ROS generation inhibits osteogenic differentiations through the canonical Wnt signaling pathway [17,24]. This provides interesting connections between metabolic products such as ROS, cell signaling pathways and cell fate decisions.

Metabolic switching also plays a key role in directing cell fate in the central nervous system. Neural stem cells (NSCs) in adult brain tissue that differentiate into neuronal and glial lineages are an interesting example. Here, glycolysis is the predominant form of metabolism [25] but, as angiogenesis proceeds within the cerebral cortex, NSCs transition to specialized cell fates due to changes in oxygen tension and oxidative metabolism [26]. Lange and colleagues have shown that increased oxygen tension in the NSC niche inactivates HIF1α, resulting in differentiation to neuronal and glial fates [26]. Not surprisingly, embryonic neural progenitor cells (NPCs) utilize aerobic glycolysis and switch to OxPhos during neuronal differentiation [27,28]. This correlates with up-regulation of PGC1α which induces mitochondrial biogenesis and the establishment of OxPhos [28]. As adult neural stem cells differentiate *in vitro* to neurons they also decrease aerobic glycolysis though a HIF1α-independent mechanism [25]. Interestingly pre-neural cells that retain an elevated number of mitochondria have a neuronal stem cell differentiation defect [29], emphasizing the developmental link between metabolic flux and cell fate decisions.

Pluripotent stem cells and metabolic remodeling during cell fate specification

Elevated rates of aerobic glycolysis and absence of OxPhos are defining features of human embryonic stem cells (hESCs) and is required for maintenance of their pluripotency. In support of this, reduced aerobic glycolysis arising from reduced lactate transporter [30] or hexokinase activity [31] results in the spontaneous differentiation of hESCs. Regulation of epigenetic modifying enzymes [32•] or the availability of metabolites for use as epigenetic modification substrates [31] has been investigated, and for a detailed review of epigenetic regulation of pluripotent stem cell fate see Ryall *et al.* [16] and Harvey *et al.* [33]

Pluripotent stem cells however, can exist in two distinct states. First, the 'primed' pluripotent state in which cells are developmentally equivalent to the primitive ectoderm of embryonic epiblasts [34]. The second class of pluripotent cells are referred to as 'naïve' PSCs and they represent cells in the inner cell mass (ICM) of preimplantation stage embryos [34]. Human embryonic stem cells (hESCs) and mouse epiblast-like stem cells (mEpiSCs) exhibit characteristics of the primed state and utilize elevated aerobic glycolysis for energy generation [31,35,36]. In contrast, naïve murine PSCs utilize OxPhos and lower glycolytic flux [37••]. Although little is known about metabolic switching during the transition between different PSC states, a role for the RNA-binding protein LIN28 has recently been proposed [37••]. In this report, up-regulation of LIN28 during the naïve to primed PSC transition was shown to be required for establishing aerobic glycolysis by increasing expression of glycolytic genes and repressing OxPhos through inhibition of ETC complex 1 transcripts *NDUFB3* and *NDUFB10* [37••]. Recently, methods to convert primed hESCs to a naïve state have been established [34,38–41] and naïve hESCs have been directly derived from the ICM of human embryos [42]. However initial reports contradict whether naïve hESCs utilize aerobic glycolysis, just as their human primed counterparts [30], or switch to OxPhos [41] like murine naïve and primed PSCs [35,37••]. Determining whether this contradiction marks a distinct developmental property in humans, or other primates, or is a consequence of cell culture will be of great interest.

As pluripotent cells pass through the primed state and commit to one of the three embryonic germ layers, glycolytic flux decreases and OxPhos becomes important for energy generation [30,36]. This also corresponds to increased production of ROS, which is generally linked to DNA damage and compromised genomic integrity. This