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METABOLIC SWITCHING AND CELL FATE DECISIONS: IMPLICATIONS FOR PLURIPOTENCY, REPROGRAMMING AND DEVELOPMENT

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Abstract

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.

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

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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,

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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 pre-implantation 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

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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 is a critical issue for stem cells that must minimize ROS production to limit the transmission of damaged DNA to progeny cells. Glutamine-dependent ROS activity is also important for modulating the oxidation status of OCT4, a transcription factor required for maintaining the pluripotent state [43]. Elevated ROS levels following glutamine withdrawal or chemical treatment induces the oxidation of cysteine bridges within OCT4 resulting in decreased transcriptional activity and loss of pluripotency. This led to the hypothesis that ROS and glutathione levels modulate pluripotency by regulating the post-translational modification of pluripotency factors. Although hESCs are dependent on glutamine for maintenance of pluripotency, naïve mESCs cultured with leukemia inhibitory factor and inhibitors CHIR-99021 and PD0325901, can self-renew in the absence of glutamine [32]. This underscores the concept that distinct metabolic strategies are used by primed and naïve PSCs and raises the possibility of cross-species differences.

Establishment of aerobic glycolysis during reprogramming

Reports describing aerobic glycolysis in primed PSCs suggested that some form of metabolic remodeling may be required for establishment and maintenance of induced pluripotent stem cells (iPSCs). This was first indicated by reports showing that glycolytic activators and OxPhos inhibitors increase reprograming efficiencies [44–46]. Further analysis showed that glycolytic activation and decreased OxPhos occurs at an early stage of reprogramming and prior to the expression of endogenous pluripotency factors [44,46–49]. The implication that metabolic remodeling precedes expression of pluripotency markers suggests a ‘cause’ rather than ‘effect’ mechanism and has generated considerable interest. This led to work showing that HIF1α and HIF2α are required to induce early metabolic remodeling required for iPSC formation under normoxic conditions [50], however HIF2α must be down-regulated later in reprograming to allow for caspase 3 activity. Hawkins et. al [51] provided a mechanistic basis for this by showing that an OxPhos-driven metabolic burst, in response to Yamanaka factor expression, increased ROS levels leading to HIF stabilization. Elevated ROS activity signals through proteins such as KEAP1 and NRF2 and ultimately leads to increased HIF1α levels [51–53]. Transient ROS signaling during early reprograming demethylates NANOG’s promoter and induces its expression [54]. This is in contrast to elevated ROS generation during MSC differentiation, as increased ROS production during early reprogramming promotes the establishment of aerobic glycolysis and iPSC generation.

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Kida and colleagues also observed an oxidative burst during the early phase of reprogramming and went on to define an additional pathway, upstream of the one utilizing HIF1α, that is required for establishment of aerobic glycolysis during reprogramming [55]. This report showed that the OxPhos burst is initiated through the temporal expression of *EERα* and *PGC1α/β*, which are induced by reprogramming factors SOX2, KLF4 and MYC [55]. Besides performing a critical role in cell proliferation, MYC's role in reprogramming is also tied to its induction of aerobic glycolysis. LIN28 can substitute for MYC during reprogramming and functions by promoting glycolytic gene expression [56] and repression of ETC genes [37]. Furthermore, MYCs role in reprogramming can be entirely replaced by ectopic expression of *LDHA* or *PKM2*; two genes that encode rate-limiting enzymes for glycolysis [49]. Taken together, these reports show that a key role of reprogramming factors during iPSC formation is to establish aerobic glycolysis. The transition from an OxPhos mode of metabolism to aerobic glycolysis signifies a metabolic switch that is important for maintenance and establishment of the pluripotent state.

CONCLUSIONS

Aerobic glycolysis is required for maintenance of the primed pluripotent state and its establishment is an early event during reprogramming. Moreover, many adult stem cell populations utilize aerobic glycolysis while actively dividing. Regulation of aerobic glycolysis and OxPhos can occur though multiple mechanisms in a context-dependent manner. HIF1α signaling in NSCs and MSCs, growth factor regulation of epigenetic pathways in pluripotent cells, redox regulation of transcription factor and epigenetic networks in muscle and pluripotent stem cells and modulation of reactive oxygen species during reprogramming are all examples where metabolic regulation is coupled to cell identity and/or fate (see Figure). Research into metabolic regulation of mammalian cells is undergoing a renaissance because it is now clear that it impacts cellular process far beyond its known functions in biosynthesis and energy generation. This renaissance is sure to continue over the next ten years and beyond because of the growing interest in the link between metabolism and cellular decisions in development and disease.

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HIGHLIGHTS

- metabolic regulation is highly-dynamic, cell-type specific and involved in cell fate determination
- aerobic glycolysis is a feature of ‘primed’ pluripotent cells and widely seen in adult stem cells
- elevated rates of aerobic glycolysis are required for maintenance of the pluripotent state
- metabolic switching is required for cellular reprogramming and establishment of pluripotency
- aerobic glycolysis in stem cells is dependent on epigenetic control, redox regulation and signal regulated transcription0factor networks

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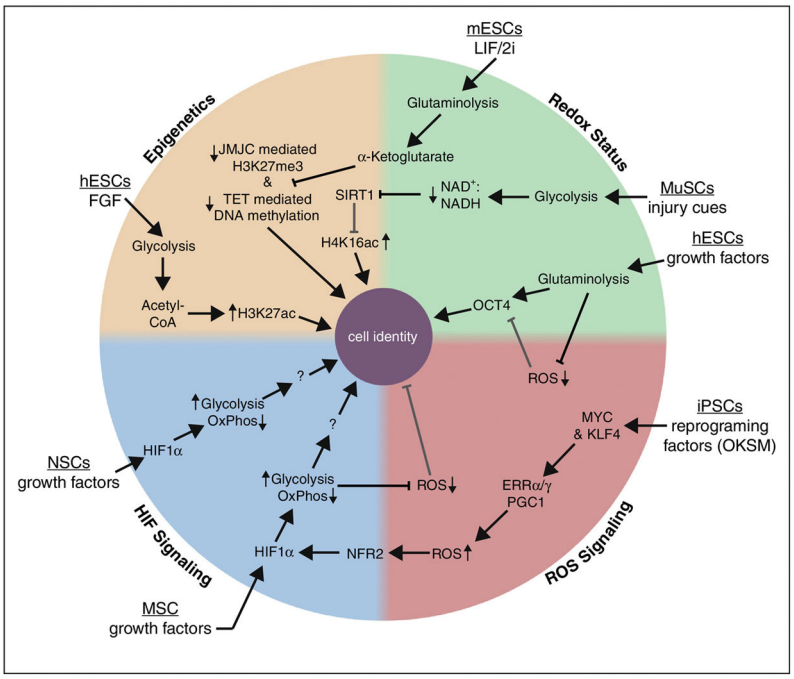

Figure. Mechanisms for the metabolic regulation of cell identity in multipotent cells

Extracellular signaling pathways, generated in human and mouse embryonic stem cells (hPSCs and mPSCs, respectively), muscle satellite stem cells (MuSCs), induced pluripotent stem cells (iPSCs), mesenchymal stem cells (MSCs), and neural stem cells (NSCs) modulate intracellular metabolic pathways. Metabolic flux then regulates cell identity through one or more of four main pathways: epigenetics [orange], redox status [green], reactive oxygen signaling (ROS) signaling [red], hypoxia induced factor (HIF) signaling [blue]. Large arrows with open arrowheads represent activating signals while repressive signals are marked by barred lines. Grey lines represent pathways that are repressed by aerobic glycolysis or glutaminolysis. Small arrows with closed arrowheads indicate whether that adjacent pathway, metabolite, signaling molecule, epigenetic mark, or metabolite ratio is increased or decreased. Abbreviations: mouse embryonic stem cells (mESCs), leukemia inhibitory factor (LIF), PD0325901 and CHIR99021 inhibitors (2i), Jumonji C domain-containing proteins (JMJC), ten eleven translocation enzymes (TET), human embryonic stem cells (hESCs), fibroblast growth factor (FGF), muscle satellite stem cells (MuSC), ratio of oxidized to reduced nicotinamide adenine dinucleotide (NAD^+:NADH), NAD-dependent deacetylase sirtuin 1 (SIRT1), octamer-binding transcription factor 4 (OCT4), Kruppel like factor 4 (KLF4), reprograming factors: OCT4, KLF4, Sex determining region Y-box 2, MYC (OKSM), estrogen-related receptor α and γ (ERRα/γ), nuclear factor, erythroid 2 like 2 (NFR2), oxidative phosphorylation (OxPhos), mesenchymal stem cells (MSCs), neural stem cells (NSCs)