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Metabolic Switches Linked to Pluripotency and Embryonic Stem Cell Differentiation

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Embryonic stem cells (ESCs) manifest a unique metabolism that is intimately linked to their pluripotent state. In this issue, Moussaieff et al. (2015) find that ESCs utilize glycolysis to fuel high rates of cytosolic acetyl-CoA synthesis to maintain the histone acetylation required for pluripotency.

One of the great mysteries of biology is how myriad cells derived from the same embryo, sharing the same genome, can engender vastly different cell types with distinct transcriptomic and metabolomic states. Pluripotent embryonic stem cells (ESCs), the master progenitors of all cells in an organism, possess immature mitochondria with poorly developed cristae, which mature into a densely tubular network only upon differentiation. Consistent with these observations, ESCs prefer high rates of glycolysis even when cultured outside the hypoxic blastocyst in atmospheric oxygen. Upon differentiation, ESCs downregulate glycolysis and oxidize most of the glycolysis-derived pyruvate in mitochondria via oxidative phosphorylation (OXPHOS). However, understanding exactly why this metabolic switch occurs and how metabolism is linked to pluripotency has become a research topic of considerable recent interest. Some have suggested that the high glycolytic flux in ESCs is strongly reminiscent of the Warburg effect in cancer (Varum et al., 2011). But rapid proliferation is not unique to pluripotent ESCs.

Recent studies have implicated distinct aspects of ESC metabolism in regulating histone modifications and pluripotency, e.g., S-adenosylmethionine for histone methylation, and ascorbate and α-ketoglutarate for histone demethylation (Wang et al., 2011; Shyh-Chang et al., 2013; Carey et al., 2014). Now, the paper by Moussaieff et al. (2015) shows that glycolysis-derived acetyl-CoA is also important for maintaining histone acetylation in ESCs, further extending the connection between metabolic intermediates and the regulation of open chromatin that is essential to the unique capacities of pluripotent stem cells.

Using nuclear magnetic resonance (NMR), Moussaieff et al. identified 44 metabolites and a rapid decrease in glycolysis-derived acetyl-CoA during the early differentiation of ESCs. Acetate, an acetyl-CoA precursor, prevented histone deacetylation and blocked the differentiation of ESCs. Inhibitors upstream of acetyl-CoA caused ESC differentiation, whereas inhibitors downstream of acetyl-CoA delayed differentiation. These results highlight the important role that glycolysis-derived acetyl-CoA plays in the balance between pluripotency and differentiation.

Here, a distinction should be drawn between glycolysis and the famed Warburg effect. Unlike anaerobic glycolysis, the Warburg effect is a more specific mode of glycolytic metabolism, defined as unusually rapid glycolysis followed by lactate synthesis even under aerobic conditions. Originally described in cancer cells, it is now widely accepted that the Warburg effect primarily operates in highly proliferative cells to rapidly build up anabolic intermediates for proliferation while minimizing ROS-induced damage. These intermediates feed into, but are not limited to, glycolytic shunts into the pentose phosphate pathway for nucleotide and NADPH synthesis, shunts into amino acid synthesis via 3-phosphoglycerate and pyruvate, and shunts into lipid synthesis via dihydroxyacetone phosphate. The pyruvate-lactate step in the Warburg effect is necessary to recycle the rate-limiting NAD^+ coenzyme and keep the redox reactions in glycolysis running rapidly.

However, Moussaieff et al. showed that it is the pyruvate-acetyl-CoA step that is important for pluripotency, not the pyruvate-lactate step. This is a critical distinction to make, because it suggests that the Warburg effect is not rate-limiting for pluripotency. Their data suggest that instead of the classic glycolysis-versus-mitochondrial OXPHOS (Warburg) dichotomy, it is through a third dimension, cytosolic acetyl-CoA, that glycolysis makes its most significant contribution to the regulation of pluripotency (Figure 1).

Previous work on the Warburg dichotomy in ESCs had suggested that the reduced OXPHOS in ESCs was due to inactivation of the pyruvate-acetyl-CoA step in mitochondria, via pyruvate dehydrogenase kinase (PDK) (Varum et al., 2011) or uncoupling protein 2 (UCP2) (Zhang et al., 2011). In contrast, Moussaieff et al. suggested that the changes in UCP2 and the PDK-PDH (pyruvate dehydrogenase) pathway were surprisingly mild. Furthermore, the anti-Warburg effect drug dichloroacetate (DCA), which inhibits PDK to de-repress PDH, promoted the pluripotency of ESCs. Although earlier studies had shown that DCA inhibits iPSC reprogramming (Folmes et al., 2011), the process of reprogramming and the maintenance of pluripotency ought not to be conflated, as a clear

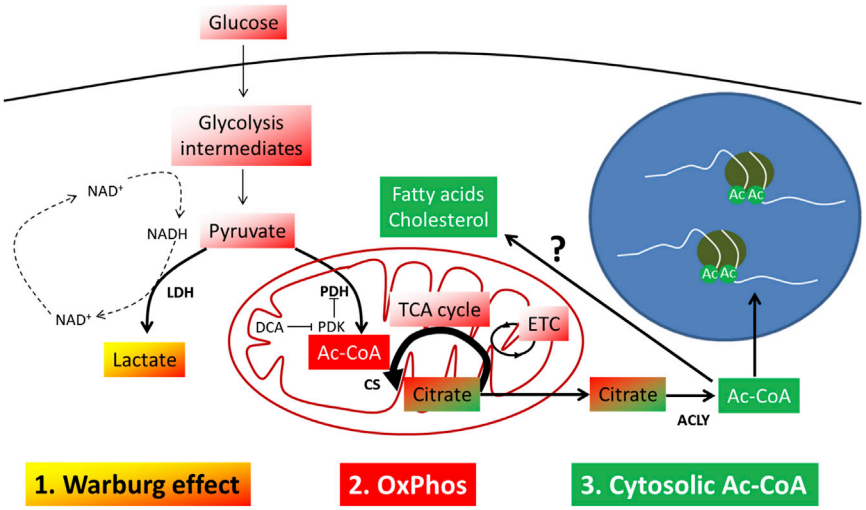

Figure 1. Carbon Metabolism in ESCs

After glycolysis, pyruvate can take three alternative routes of metabolism in ESCs: (1) Pyruvate can feed into lactate synthesis via LDH to facilitate the rapid recycling of NAD^+ necessary for the Warburg effect. (2) Pyruvate can feed into mitochondrial Ac-CoA synthesis via PDH and CS to fuel the TCA cycle and ETC for OXPHOS. (3) Pyruvate can feed into cytosolic Ac-CoA synthesis via PDH, CS, and ACLY. Moussaieff et al. show that this third route is critical to fuel histone acetylation and maintain the ESC chromatin in a pluripotent state. Cytosolic Ac-CoA can also be used to fuel lipid synthesis in the cytosol. Ac, acetylation marks on histones; Ac-CoA, acetyl-CoA; ACLY, ATP-citrate lyase; CS, citrate synthase; DCA, dichloroacetate; ETC, electron transport chain; LDH, lactate dehydrogenase; NAD, nicotinamide adenine dinucleotide; PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase.

distinction can be drawn between them. Indeed, the upregulation of glycolysis occurs early during iPSC reprogramming, well before a small fraction of cells acquires pluripotency (Folmes et al., 2011; Shyh-Chang et al., 2013). The epigenetic changes that occur during somatic cell reprogramming are also distinct from the epigenetic changes required to maintain pluripotency. Whereas somatic cell reprogramming involves complex global remodeling of histone modifications and DNA methylation, pluripotency maintenance requires an enrichment of histone acetylation to maintain chromatin plasticity, relative to differentiated cells (Mattout and Meshorer, 2010). A paper by Craig Thompson and colleagues was the first to show that glycolysis-derived acetyl-CoA, through ATP citrate lyase (ACLY), fuels the histone acetylation needs of adipogenesis (Wellen et al., 2009). Although histone acetylation increased with adipogenic differentiation, suggesting that cytosolic acetyl-CoA does not always correlate with "stemness," it suggests that a general glycolysis-acetyl-CoA switch might exist to modulate histone acetylation and chromatin plasticity during cellular differentiation.

More questions remain to be answered, of course. For example, what regulates this glycolysis-acetyl-CoA switch during ESC differentiation? Why did citrate fail to mimic acetate supplementation? Exactly how does cholesterol and fatty acid metabolism modulate pluripotency? Finally, could the importance of pyruvate-acetyl-CoA in ESC histone acetylation also explain the importance of pyruvate as a carbon source for oocytes and IVF embryos (Shyh-Chang et al., 2013)? Answers to these questions will not only enable us to optimize the precise metabolic conditions required for stem cell cultures in vitro, but also provide druggable metabolic targets to modulate stem cells and their potential therapeutic applications.

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