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REVIEW

Metabolism of pluripotent stem cells

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BACKGROUND: Recently, growing attention has been directed toward stem cell metabolism, with the key observation that metabolism not only fuels the proper functioning of stem cells but also regulates the fate of these cells. There seems to be a clear link between the self-renewal of pluripotent stem cells (PSCs), in which cells proliferate indefinitely without differentiation, and the activity of specific metabolic pathways. The unique metabolism in PSCs plays an important role in maintaining pluripotency by regulating signaling pathways and resetting the epigenome.
OBJECTIVE: To review the most recent publications concerning the metabolism of pluripotent stem cells and the role of metabolism in PSC self-renewal and differentiation.
METHODS: A systematic literature search related to the metabolism of PSCs was conducted in databases including Medline, Embase, and Web of Science. The search was performed without language restrictions on all papers published before May 2016. The following keywords were used: "metabolism" combined with either "embryonic stem cell" or "epiblast stem cell."
RESULTS: Hundreds of papers focusing specifically on the metabolism of pluripotent stem cells were uncovered and summarized.
CONCLUSION: Identifying the specific metabolic pathways involved in pluripotency maintenance is crucial for progress in the field of developmental biology and regenerative medicine. Additionally, better understanding of the metabolism in PSCs will facilitate the derivation and maintenance of authentic PSCs from species other than mouse, rat, and human.

Keywords metabolism, pluripotent stem cells, pluripotency, epigenetics

Introduction

Metabolism is the sum of the chemical reactions taking place within each cell. It lays the material and energetic foundation for all living cells. Metabolism provides the ingredients to synthesize nearly any functional component in cells, such as the cellular membrane, cytoskeleton, and nucleus. Additionally, metabolism generates adenosine triphosphates (ATPs), which fuel energy consuming processes such as concentration gradients, cytoskeletal dynamics, and gene expression. A clear link between a defined metabolic pathway and an increased proliferative rate has been well characterized in cancer cells (Hanahan and Weinberg, 2011). This phenomenon, known as the Warburg effect, is employed by the majority of the highly proliferative and malignant cancer cells that consume large amounts of glucose by anaerobic glycolysis rather than oxidative phosphorylation in order to meet the large energy demands of this high proliferative rate. Fast, unlimited cell proliferation is a common characteristic of both cancer cells and pluripotent stem cells (PSCs), at least under *in vitro* culture conditions.

PSCs, including embryonic stem cells (ESCs), epiblast stem cells (EpiSCs), and the recently developed induced pluripotent stem cells (iPSCs), are able to self-renew indefinitely while retaining the ability to differentiate into any cell type in the body (Evans and Kaufman, 1981; Takahashi and Yamanaka, 2006;Brons et al., 2007; De Los Angeles et al., 2015). Understanding how pluripotency is maintained holds tremendous promise for understanding early embryonic development, and for developing regenerative medicine and cell therapies. ESCs are derived from the inner cell mass (ICM) of preimplantation embryos (Evans and Kaufman, 1981; Thomson and Odorico, 2000), whereas EpiSCs are isolated from the post-implantation epiblasts, a later stage in development (Brons et al., 2007; De Los Angeles et al., 2015). After the blastocyst implants in the uterine wall, the cells in it become "primed": one of X-chromosomes becomes silenced and the cells cannot contribute to a chimera when injected into another blastocyst

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(De Los Angeles et al., 2015). ESCs and EpiSCs are pluripotent, yet display distinct features in terms of gene expression, epigenetic modifications, and developmental capacity following blastocyst injection (De Los Angeles et al., 2015). Though isolated from the ICM, in vitro cultured human ESCs (hESCs) are more similar to mouse EpiSCs, based on their transcriptional profiles and protein expression profiles as well as their epigenetic states, than to naïve mESCs (Kim et al., 2013).

Compared with differentiated somatic cells, PSCs have many unique metabolic characteristics, with prominent differences in carbohydrate and amino acid metabolism (Sperber et al., 2015; Wang et al., 2011). Furthermore, the unique metabolism in PSCs plays an important role in maintaining the pluripotency of PSCs. In this review, we summarize the metabolic profiles of mouse and human PSCs and the metabolism's contribution to the pluripotency of PSCs. Next, we discuss how the metabolism contributes to the pluripotency of PSCs through signaling pathways. Finally, the epigenetic link between metabolism and pluripotency in PSCs is presented.

Glucose metabolism in PSCs and its regulation

A major source of new cell mass and cellular energy is glucose. It is first metabolized by glycolysis in the cytoplasm to pyruvate, which can be further oxidized into CO_2 in the mitochondrial tricarboxylic acid (TCA) cycle to generate large amounts of ATP through the process of oxidative phosphorylation (OXPHOS) (Agathocleous and Harris, 2013). Glycolysis does not require oxygen, but it is far less efficient than the TCA cycle coupled to OXPHOS at generating ATP. All mammalian cells produce ATP through differing proportions of glycolysis and oxidative phosphorylation depending on the requirement of bioenergetics, availability of glucose, and the maturity of the mitochondria.

PSCs are highly proliferative cells with a short cell cycle and a need to consume large amounts of glucose. The cells use glucose to fuel the biosynthesis of macromolecules and to synthesize ATP (De Los Angeles et al., 2015). To meet such requirements, PSCs highly express glucose transporter 1 (GLUT1) and GLUT3 to absorb adequate glucose from the environment (Segev et al., 2012). Interestingly, even at a normal oxygen level, ESCs show high rates of glycolysis with the secretion of a large amount of lactate, known as the Warburg effect (Wang et al., 2011; Ito and Suda, 2014). Similarly, iPSCs use anaerobic glycolysis to generate adequate energy for its propagation. A switch from OXPHOS back to glycolysis is also seen during the conversion of differentiated somatic cells into iPSCs, which reflects metabolic reprogramming (Zhang et al., 2012).

Past studies have shown that anaerobic glycolysis can generate more ATP than OXPHOS by producing ATP at a faster rate, despite its low ATP yield per mole of glucose (Ito and Suda, 2014). If the supply of glucose is abundant, this inefficient yet fast pathway is preferred to meet the energy demand of proliferating PSCs. In addition, the intermediates generated from glycolytic metabolism provide the precursors to the structural constituents used for building the macromolecules essential for highly proliferative PSCs. For example, these intermediates feed into glycolytic shunts to the pentose phosphate pathway for nucleotide synthesis, shunts to amino acid synthesis by glyceraldehyde 3-phosphate and pyruvate, and shunts into fatty acid synthesis via dihydroxyacetone phosphate (Fig. 1) (Ito and Suda, 2014; Shyh-Chang and Daley, 2015; Sperber et al., 2015). Therefore, a main function of upregulated glycolysis may be to support anabolic metabolism in proliferating PSCs. Moreover, compared with OXPHOS, glycolysis decreases the release of reactive oxygen species (ROS), which are potentially harmful to genomic stability, and provides reductive nicotinamide adenine dinucleotide phosphate (NADPH) via the oxidative reactions of the pentose phosphate pathway (Wang et al., 2011; Ryu et al., 2015). In fact, NADPH is a necessary reducing agent for nucleotide, amino acid, and lipid biosynthesis. In conclusion, the above benefits provided by anaerobic glycolysis highlight its importance and necessity in PSCs.

Some studies suggest that PSCs have reduced mitochondrial mass as well as limited mitochondrial oxidative capacity which could necessitate the use of glycolysis (Cho et al., 2006; Prigione et al., 2010). However, a recent study suggests that hESCs and their differentiated counterparts have similar mitochondrial mass and mitochondrial DNA copy number and that the mitochondria in hESCs retain maximal ability to consume oxygen (Zhang et al., 2011). Despite this, hESCs express higher levels of uncoupling protein 2 (UCP2), which leads to inhibition of glucose oxidation and facilitation of glycolysis, through transportation of four carbon TCA cycle intermediates out of the mitochondria (Vozza et al., 2014). In this case, ATP in hESCs is mainly generated by glycolysis and consumed by the F(1)F(0) ATP synthase to partially maintain mitochondrial inner membrane electrochemical potential and contribute to viability by preventing the release of proapoptotic intermembrane space proteins, such as cytochrome c.

In addition, another key player that predisposes PSCs, especially hESCs and EpiSCs, to undergo glycolysis is hypoxia-inducible factor 1 (HIF1), a transcription factor indispensable for cellular and systemic responses to low oxygen availability. HIF1 is a heterodimeric protein consisting of two subunits: HIF1a and HIF1b. The oxygen level and prolyl hydroxylase, a von Hippel-Lindau tumor suppressor protein (pVHL), regulate the degradation of HIF1a (Mathieu et al., 2014). It has been well documented that a culture condition with low oxygen tension reduces the differentiation of hESCs whereas high oxygen impairs the maintenance of hESCs (Forristal et al., 2013). Similarly, reduced oxygen

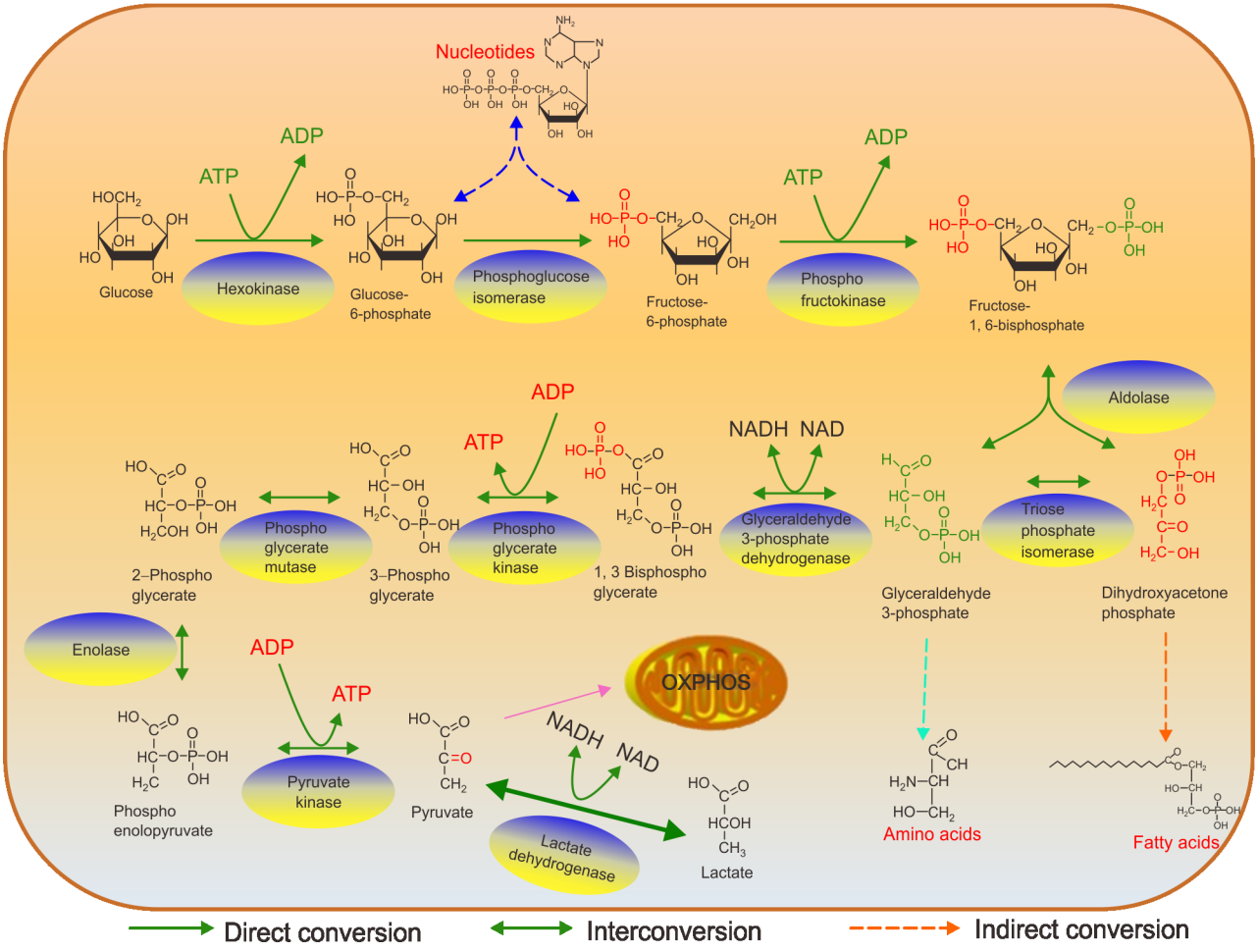

Figure 1 The metabolism of glucose in pluripotent stem cells (PSCs). In PSCs, glucose is sequentially catalyzed by multiple enzymes in the cytoplasm to become pyruvate via glycolysis, which can be further oxidized into CO_2 in the mitochondrial tricarboxylic acid (TCA) cycle to generate large amounts of ATP through the process of oxidative phosphorylation (OXPHOS) . Pyruvate can also be reductively metabolized to lactate, which predominates in PSCs even under normal oxygen levels. In addition, the metabolism of glucose provides ample intermediates for macromolecule synthesis. For instance, glucose-6-phosphate, glyceraldehyde-3-phosphate, and dihydroxyacetone produced during glucose metabolism can be used to synthesize nucleotides, amino acids, and fatty acids, respectively. NAD: Oxidized nicotinamide adenine dinucleotide; NADH: reduced NAD.

concentration stabilizes EpiSCs (Takehara et al., 2012). For PSCs under hypoxic conditions, HIF1a is stabilized and associates with HIF1b to form a functional HIF complex, which can then bind to the promoters of multiple glycolytic genes and activate these genes in PSCs. HIF1a can be stabilized by epigenetically repressing prolyl hydroxylase in EpiSCs and hESCs and induces the metabolic switch from utilizing both OXPHOS and glycolysis to solely utilizing glycolysis (Sperber et al., 2015) .

It was recently reported that ESCs highly express specific cell cycle regulating miRNA clusters, such as miR-290 and miR-371. These miRNA clusters inhibit Mbd2 (a methyl-CpG binding domain protein family member) post-transcriptionally, thus leading to the downregulation of the MBD2 protein and reactivation of its target gene c-Myc (Cao et al., 2015). c-Myc activates glycolysis by directly stimulating the transcription of glycolytic enzymes pyruvate kinase isoform 2 (Pkm2) and lactate dehydrogenase (Ldha). As a result, highly expressed miRNA clusters in ESCs promote the incidence of glycolysis. Additionally, core pluripotency factors, such as Oct4, can directly bind to promoter of glycolytic enzyme hexokinase 2 (Hk2) and Pkm2 to facilitate glycolysis in PSCs (Kim et al., 2015). To meet the biosynthetic and bioenergetic requirements of highly proliferating PSCs, it seems that both intrinsic signals and extrinsic stimuli are involved in regulating glycolysis in PSCs.

Anaerobic glycolysis not only fuels PSCs but also promotes PSC self-renewal and accelerates the reprogramming process. The direct evidence supporting the role of glycolysis in enhancing pluripotency is that PSCs artificially overexpressing glycolytic enzymes Hk2 and Pkm2 maintain the expression of pluripotency-related genes in the absence of LIF (Kim et al., 2015). Glucose-derived acetyl-coenzyme A (acetyl-CoA) is also important for maintaining histone

acetylation and thus pluripotency in PSCs (details will be discussed below) (Moussaieff et al., 2015). Indirect evidence comes from studies reprogramming somatic cells into iPSCs. Folmes and colleagues showed that the metabolic shift from a pro-oxidative state to glycolysis is a key step for iPSC reprogramming and is characterized by upregulated glycolytic enzymes (Folmes et al., 2011). They also showed that the metabolic switch precedes the expression of signature genes for PSCs. More importantly, reprogramming of somatic cells into iPSCs is enhanced by promoting glycolysis and suppressed by stimulating OXPHOS (Folmes et al., 2011; Yoshida et al., 2009). In line with this, hypoxia highly improves the reprogramming efficiency from mature cells into iPSCs (Prigione et al., 2014; Moussaieff et al., 2015). These results suggest that changes in glucose metabolism may not simply be a consequence of acquiring pluripotency.

Amino acid metabolism in PSCs: more than a source of carbon and nitrogen

More than six decades ago, it was revealed that amino acids are essential for culturing mammalian cells (Eagle et al., 1956; Eagle, 1959). Amino acids, including those that are glucogenic and ketogenic, are important energy sources PSCs with a high rate of proliferation. For example, pyruvate from alanine metabolism and fumarate from tyrosine metabolism can supplement and refuel the TCA cycle, providing large amounts of intermediates for synthesizing carbon-based macromolecules to build new cells. More importantly, amino acids are major donors of nitrogen, which can be used to synthesize nucleotides for daughter cells. Compared with somatic cells, PSCs have very different amino acid metabolic profiles (Panopoulos et al., 2012; Wang et al., 2009).

Glutamine, the most abundant amino acid in human plasma and culture media, is thought to be a major amino acid contributor to PSCs (Eagle et al., 1956; Windmueller and Spaeth, 1974). This is because Acetyl-CoA derived from glutamine is an ingredient for fatty acid synthesis and nitrogen derived from glutamine can be used to generate nucleotides, non-essential amino acids, and hexosamines (amino sugar precursors to glycosylated proteins and lipids) (Lunt and Vander Heiden, 2011). Furthermore, glutamine can be converted to glutamate, which is an important substrate for transamination reactions and folate metabolism (Fig. 2). In the absence of glutamine, mESCs and iPSCs can grow and proliferate under the "2i + LIF" condition (Carey et al., 2015), the simultaneous inhibition of GSK3 and MEK by their specific inhibitors and the activation of Stat3 by leukemia inhibitory factor (LIF), which robustly maintains the self-renewal of ESCs and iPSCs (Ying et al., 2008). Despite this, supplementation of glutamine or its precursor is still beneficial for the pluripotency of PSCs. Under the "2i + LIF" condition, glutamine is rewired to generate α-ketoglutarate (αKG) and thus more αKG-dependent demethylase, which contributes to the undifferentiated state via demethylation of repressive chromatin marks such as trimethylation of histone H3 at Lys9 (H3K9me3), H3K27me3, and H4K20me3.

Notably, threonine is a unique and crucial amino acid required for mESC growth and pluripotency maintenance (Wang et al., 2009). Mouse ESCs and iPSCs express extremely high levels of threonine dehydrogenase (Tdh), which converts threonine to glycine and acetyl-CoA, relative to more differentiated counterparts. Glycine is then used by the mitochondrial enzyme glycine decarboxylase (GLDC) to produce a one-carbon pool to promote nucleotide synthesis and rapid proliferation of mESCs; acetyl-coA joins the TCA cycle and feeds the bioenergetics and biosynthetics of mESCs (Lane and Fan, 2015). Furthermore, Tdh enhances the synthesis of S-adenosylmethionine (SAM), leading to a high ratio of SAM/S-adenosyl homocysteine (SAH) and high levels of H3K4me3, which is also thought to promote the pluripotency and proliferation of ESCs (Wang et al., 2009; Shyh-Chang et al., 2013). In addition, Han et al. reported that both threonine and Tdh are essential for successful reprogramming of mouse somatic cells into iPSCs (Wang et al., 2009; Shyh-Chang et al., 2013).

In contrast to mice, humans do not express functional Tdh enzyme due to the loss of the splice acceptor site preceding both exon 4 and 6 together with the generation of an in-frame premature stop codon in exon 6 (Edgar, 2002). It was believed that hESCs may still require threonine and primarily use the serine/threonine dehydratase pathway for threonine catabolism. However, Shiraki and colleagues revealed that human PSCs, including hESCs and iPSCs, consume high amounts of methionine instead of threonine (Shiraki et al., 2014). Short-term deprivation of methionine in culture medium decreases histone and DNA methylation, reduces Nanog expression, and thus impairs the pluripotency of human PSCs. Similar to threonine, methionine is an important source of SAM. Deprivation of methionine lowers SAM levels and further triggers the demethylation of H3K4me3, changing the epigenome and the transcriptome of human PSCs. Additionally, long-term starvation without methionine can activate the p53-p38 signaling pathway and induce apoptosis of human PSCs.

Moreover, other amino acids, such as proline, leucine, lysine, and tryptophan are also important for the maintenance of PSCs. Culturing mESCs at clonal density with L-proline causes a mesenchymal-like invasive phenotype while maintaining the expression of pluripotency genes (Comes et al., 2013). However, higher concentrations of proline specifies mESCs to differentiate into epiblast-like cells (Washington et al., 2010). As far as leucine and lysine are concerned, depletion of either one induces the apoptosis of human PSCs (Shiraki et al., 2014). This is because a shortage of leucine and lysine increases the expression of C/EBP homologous protein (CHOP) in human PSCs and further activates

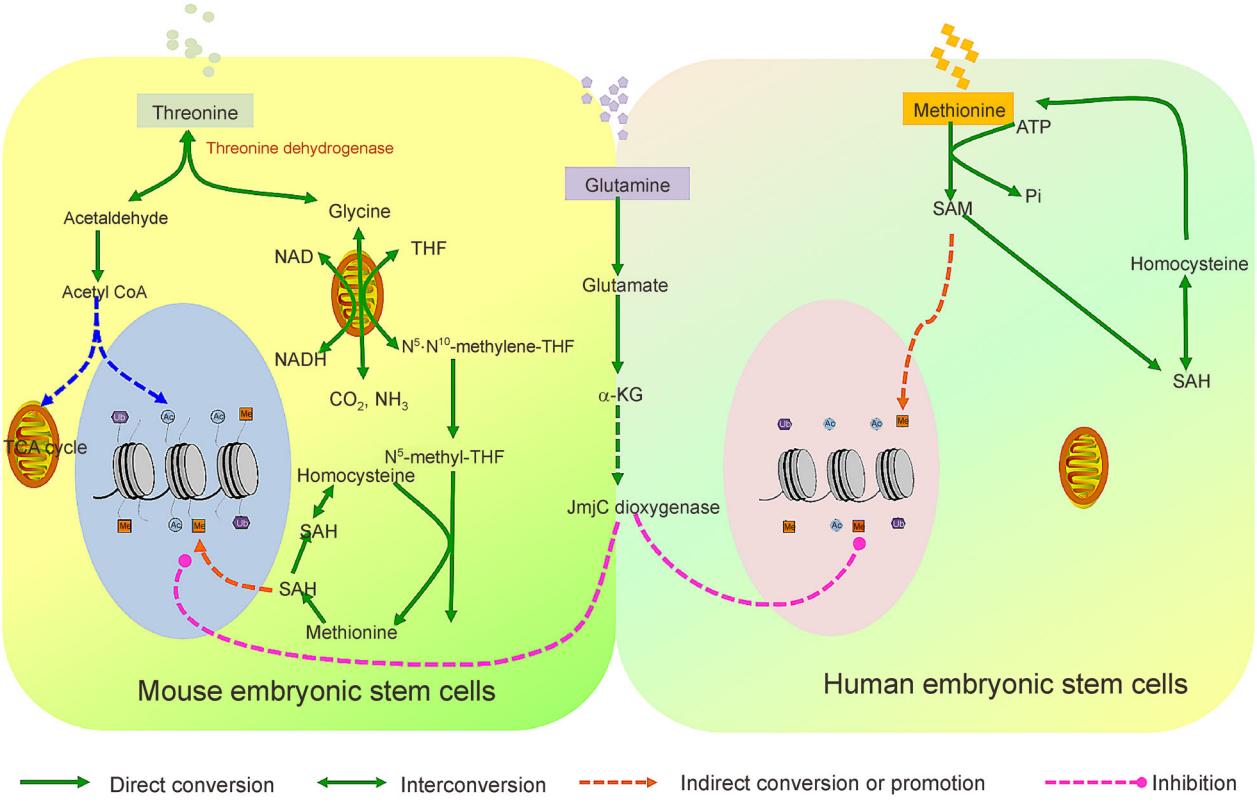

Figure 2 The characteristic amino acid metabolism of PSCs. Under the "2i + LIF" condition, PSCs can convert glutamine into glutamate, which is further rewired to generate α-ketoglutarate (αKG) and thus more αKG-dependent demethylase, such as JmjC dioxygenease. JmjC contributes to pluripotency maintainence through demethylation of the repressive chromatin landscape. In addition, mouse embryonic stem cells (mESCs) are characterized by a unique threonine metabolism. Threonine dehydrogenase (Tdh) converts threonine into glycine and acetyl-CoA. Glycine is then used to produce a one-carbon pool to promote nucleotide synthesis and rapid proliferation of mESCs; Acetyl-coA joins the TCA cycle and feeds the bioenergetics and biosynthetics of mESCs. Furthermore, Tdh enhances the synthesis of S-adenosylmethionine (SAM), leading to a high ratio of SAM/S-adenosyl homocysteine (SAH) and high levels of H3K4me3, an active transcriptional chromatin marker. In contrast, human embryonic stem cells (hESCs) rely on methionine rather than threonine since Tdh is a pseudogene in human. Similar to threonine, methionine is an important source of SAM. Methionine deprivation lowers SAM levels and induces the demethylation of H3K4me3. THF: tetrahydrofolate.

endoplasmic reticulum stress-mediated apoptosis (Averous et al., 2004). Additionally, tryptophan is critical for the survival and growth of human PSCs (Shiraki et al., 2014). In fact, primed human PSCs express high levels of the tryptophan-metabolizing enzyme IDO1 and are enriched with the tryptophan degradation product kynurenine, a ligand for the nuclear receptor AHR (Shin et al., 2013; Sperber et al., 2015). However, very little is known about the role of IDO1 and kynurenine in mouse PSCs.

The understanding of the amino acid regulation in PSCs remains very limited. As to the metabolism of threonine, increased Tdh expression is achieved by both transcriptional and post-transcriptional mechanisms in mESCs. Transcriptionally, Oct4, Nanog, Sox2, and c-Myc along with other transcription factors bind to TDH promoter and cooperatively activate Tdh transcription to a high level (Kim et al., 2008). Post-transcriptionally, microRNA-9 and PRM5 arginine methyltransferase were identified as Tdh regulators (Han et al., 2013). However, the mechanisms underlying the regulation of other amino acids, such as glutamine and methionine, are not so clear. It would be interesting to probe the role of non-essential amino acids in different types as well as stages of PSCs.

Lipid metabolism in PSCs and its contribution to pluripotency: a virgin land to uncover

Similar to the metabolism of glucose and amino acids, lipid metabolism supports both the biosynthetic and bioenergetic requirement of cell proliferation and survival. Lipids are essential components of plasma and organelle membranes and even participate in certain signal transduction pathways. In the mouse blastocyst, for example, fatty acid oxidation (FAO) supports ICM growth (Dunning et al., 2010).

Disruption of mESC carnitine O-palmitoyltransferase (CPT), the rate-limiting enzyme for FAO, leads to ATP depletion and decreased resistance to nutrient deprivation, suggesting a key role of lipids in enzyme production in mESCs (Zaugg et al., 2011). In addition, lipids as secondary messengers, including arachidonic acid, diacylglycerol, and prostaglandins, are among the most highly expressed metabolites in PSCs (Yanes et al., 2010). However, the specific role of lipids in stem cell biology is only beginning to be elucidated.

Theoretically, rapidly proliferating PSCs consume large amounts of fatty acids as the area of the membrane is greatly expanding and consuming a large amount of energy. However, undifferentiated hESCs consume relatively small amounts of fatty acid, which is corroborated by the quantity of ketone bodies generated (Moussaieff et al., 2015). In fact, hESCs accumulate unsaturated fatty acids in the cytoplasm and form lipid droplets (Yanes et al., 2010; Sperber et al., 2015). Interestingly, it is revealed that primed state mEpiSCs and hESCs, similar to the cells used in Moussaieff's research (Moussaieff et al., 2015), have a different lipid metabolism compared with naïve mESCs and hESCs. In primed PSCs, the fatty acid transporter carnitine acyltransferase 1A (CPT1A), which transfers acyl groups from long-chain fatty acid CoA to carnitine to help catabolism of fatty acids, is downregulated in contrast to naïve PSCs (Sperber et al., 2015). Meanwhile, SLC25A1, ACLY, and other key enzymes involved in fatty acid synthesis are upregulated in primed PSCs. Therefore, it is likely that the changes in enzymes responsible for fatty acid anabolism and catabolism result in this discrepancy in fatty acid metabolism of the different states of PSCs.

Conversely, evidence has been found linking fatty acids to the regulation of the pluripotent state of stem cells. Through fractionizing knockout serum replacement (KSR), Garcia-Gonzalo et al. found that albumin-associated lipids, including fatty acids, enhance the self-renewal of hESCs (Garcia-Gonzalo and Belmonte, 2008). Inhibiting the eicosanoid pathway, which is involved in fatty acid oxidation, promotes pluripotency in ESCs and maintains the levels of unsaturated fatty acids (Yanes et al., 2010). Linoleic acid-derived arachidonic acid and eicosapentaenoic acid bias the differentiation of PSCs to a specific lineage (Kang et al., 2014). As to the reprogramming of fibroblasts to iPSCs, butyrate, a naturally occurring fatty acid, dramatically improved the reprogramming efficiency without compromising the quality of iPSCs (Mali et al., 2010). Genome-wide analysis showed that butyrate, a histone deacetylase inhibitor, promoted histone H3 acetylation, promoter demethylation, and the expression of pluripotency-related genes (Mali et al., 2010; Zhang et al., 2014a).

Metabolites as signaling molecules directly regulate the fate of PSCs

Signaling pathways play an important role in the regulation of metabolic networks in PSCs. Conversely, the signaling pathways influencing metabolism can be regulated by the metabolic products themselves. As a result, it is possible that metabolites can regulate the pluripotent state of PSCs by manipulating signaling pathways.

One typical example is the mammalian target of rapamycin (mTOR) kinase. mTOR kinase plays a central role in the cellular sensing of oxygen, nutrients, and growth factors through the phosphatidylinositol 3-kinase (PI3K)/Akt pathway (Ryu and Han, 2011; Yoon and Chen, 2013). It exists in two different complexes, mTORC1 and mTORC2. Glucose and amino acids are potent mTORC1 stimulators. Upon activation, mTORC1 phosphorylates its downstream targets 4E-BP1 and S6K1 to promote mRNA translation, glycolysis, and lipid as well as nucleotide synthesis (Hay and Sonenberg, 2004). It seems that activation of mTOR is necessary for the long-term self-renewal of hESCs since inhibition of mTOR markedly reduces OCT-4, SOX2 and NANOG protein expression, predisposing hESCs to differentiate into mesoderm/endoderm layers (Zhou et al., 2009). However, the activity and the role of mTOR in mESCs and EpiSCs are unknown and warrant further investigation.

Furthermore, ROS may act as a signaling molecule to regulate the fate of PSCs. ROS directly react with numerous proteins, such as kinases, phosphatases, or transcription factors, to affect processes that regulate cell cycle, apoptosis, or differentiation (Ryu et al., 2015). ROS can also directly modify metabolic enzymes that participate in nutrient-sensing pathways to direct the metabolic flux (Bigarella et al., 2014). As to ESCs, increased ROS levels in vitro can induce transient G2/M cell cycle arrest. However, continuous ROS exposure induces apoptosis in ESCs. Additionally, forced activation of oxidative phosphorylation, an important source for ROS, led to the loss of stem cell properties and increased differentiation or apoptosis (Ito and Suda, 2014). Conversely, enhancing glycolysis through hypoxia-mediated HIF activation and inhibition of oxidative phosphorylation improved the proliferation and maintenance of ESCs, while repressing differentiation (Mandal et al., 2011; Zhou et al., 2012). This evidence suggests that ROS affect the pluripotency of PSCs. However, direct evidence linking the function of ROS to the maintenance of PSCs is still lacking.

Jang and colleagues reported that O-linked-N-acetylglucosamine (O-GlcNAc), synthesized from glucose via the hexosamine biosynthetic pathway, directly regulates pluripotency networks in PSCs through O-GlcNAcylation, which facilitates pluripotency (Jang et al., 2012). O-GlcNAcylation is abundant in cytoplasmic and nuclear proteins, which are modified with a single β-N-acetylglucosamine monosaccharide moiety through an O-β-glycosydic attachment to serine and/or threonine side chains of the polypeptide backbone (Hart et al., 2011). Similar to phosphorylation, O-GlcNAcylation quickly regulates protein function in response to environmental stimuli, including nutrients and growth factors (Hart, 2014). In PSCs, Oct4 and Sox2 undergo O-GlcNAcy-

lation to enhance their transcriptional activity and to maintain undifferentiated PSCs (Jang et al., 2012).

An epigenetic link between metabolism and pluripotency in PSCs

PSCs and their differentiated counterparts share the same genome but have distinct transcriptional profiles. The differences in transcription and translation between PSCs and somatic cells are closely related to the regulation of the structure and function of the genome, as well as the expression levels of genes. Binding of transcriptional regulators to their targets before, during, and after these fate changes relies on the dynamic alterations in DNA methylation, histone modification, non-coding RNAs, and chromosomal remodeling (Kobayashi and Kikyo, 2015). Cellular metabolism provides abundant substrates and co-factors important for the activity of enzymes involving histone acetylation and methylation, and DNA methylation as well. Therefore, metabolism in PSCs is considered the hub linking epigenetics and gene functions (Fig. 3).

Histone acetylation is a major regulator of gene expression and chromatin remodeling. Acetylation of the lysine residues at the N terminus of histone proteins removes positive charges, thereby reducing the affinity between histones and DNA. This gives RNA polymerase and transcription factors easier access to the promoters of target genes, thus enhancing transcription. Histone acetyltransferases (HATs) utilize acetyl CoA as an acetyl donor to perform acetylation with glucose-derived acetyl-CoA being crucial for histone acetylation in ESCs (Moussaieff et al., 2015). Using nuclear magnetic resonance (NMR), Moussaieff and colleagues showed that acetate, an acetyl CoA precursor, inhibited deacetylation and prevented the differentiation of ESCs. In line with this, inhibition of enzymes downstream of acetyl CoA suppressed differentiation, whereas inhibition of those upstream of acetyl CoA impaired self-renewal. These results highlighted the important contribution of glucose-derived acetyl CoA (another reason for committing to glycolysis in PSCs) to the maintenance of pluripotency in ESCs through regulation of H3K9/K27 acetylation.

Deacetylation of histones is also involved in regulating

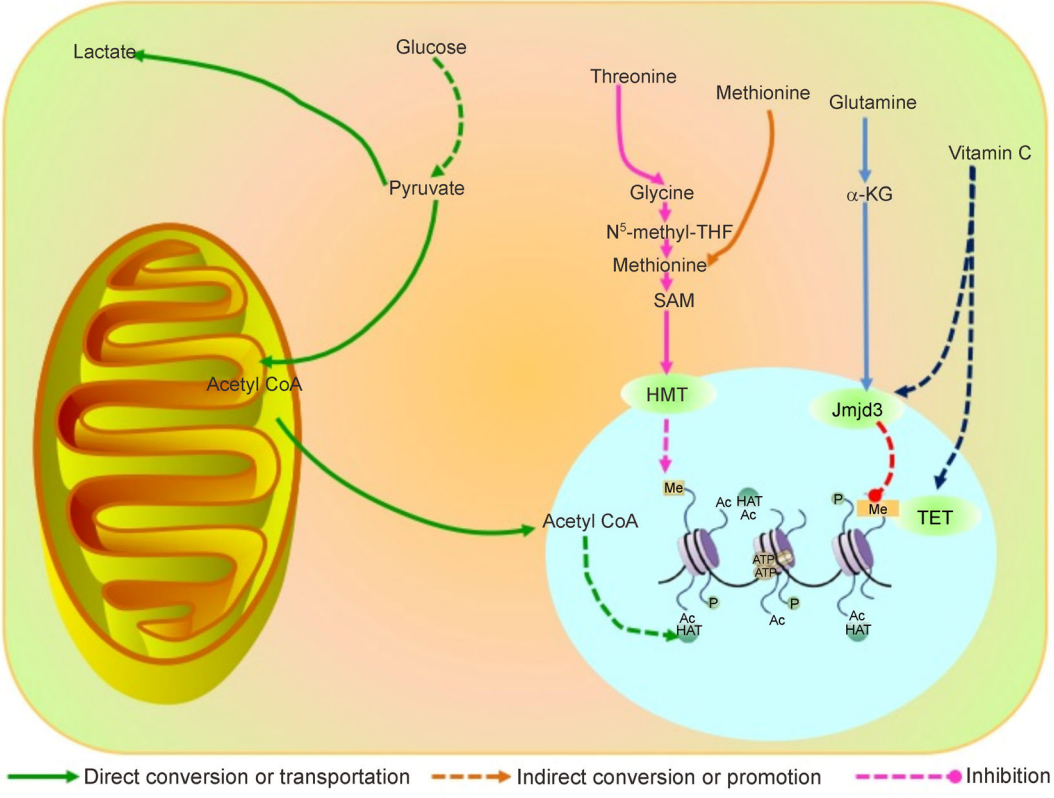

Figure 3 The epigenetic connection between metabolism and pluripotency of PSCs. Cellular metabolism provides ample substrates and co-factors for epigenetic regulation of the genome of PSCs and contributes to pluripotency maintenance. Glucose-derived acetyl-CoA is an important substrate for histone acetylation mediated by histone acetyltransferases (HAT). SAM converted from threonine and methionine is the essential methyl donor for histone methyltransferase (HMT) to maintain proper levels of H3K4me3. In addition, α-ketoglutarate (αKG) from glutamine metabolism activates Jmjd3, an αKG-dependent demethylase, and triggers the demythelyation of repressive chromatin markers. Also, Vitamin C promotes the demethylation of repressive histone markers by enhancing the activity of Tet and JmjC demethylases.

gene expression. Deacetylation is carried out by class I and II histone deacetylases (HDACs), as well as the class III deacetylases termed sirtuins (Haberland et al., 2009). Because the sirtuins are NAD-dependent deacetylases, a high NAD^+ / NADH ratio from glycolysis is expected to lower the activity of sirtuins (namely Sirt1 and Sirt6) in the nucleus and cytoplasm. (Zhang et al., 2014b). It is possible that low sirtuin activity regulated by glycolysis would increase the acetylation status of proteins in the cytosolic/nuclear compartment, directly or indirectly promoting pluripotency epigenetically. For example, Sirt1 is required for genomic stability and telomere elongation of iPSCs (De Bonis et al., 2014). Also, Sirt6 can enhance iPSC reprogramming efficiency from somatic cells derived from senior patients (Sharma et al., 2013).

Furthermore, methylation of DNA and histones plays a crucial role in regulating the epigenome of PSCs. The addition of methyl groups is carried out by a family of enzymes called methyltransferases that use SAM as a methyl donor. Interestingly, SAM is present in high concentrations in various types of PSCs, including iPSCs (Han et al., 2013; Shyh-Chang et al., 2013; Shiraki et al., 2014). In fact, during the reprograming process, there is an increasing level of SAM, suggesting its role in the shift to a pluripotent state, at least when resetting the methylation of DNA and histones. As discussed during the amino acid metabolism in PSCs, threonine and methionine are important sources for SAM in mouse PSCs and human PSCs, respectively. With a high level of SAM, PSCs are able to maintain proper levels of H3K4me3 and H3K4me2. Sperber et al. revealed there are different expressions of SAM in naïve PSCs and primed PSCs, which is related to nicotinamide N-methyltransferase (NNMT) (Sperber et al., 2015). In naïve PSCs (e.g. mESCs and iPSCs), highly expressed NNMT consumes SAM, making it unavailable for the histone methylation that represses Wnt and activates the HIF pathway in primed PSCs (e.g. hESCs). In addition, reduced availability of SAM impacts the epigenetic modification of genes involved in the metabolism of glucose, amino acids, and lipids, leading to differences in metabolic profiles between naïve and primed state PSCs.

Demethylation, the removal of a methyl group, is equally important as DNA and histone methylation in pluripotency regulation. The demethylation process is necessary for epigenetic reprogramming of genes and is also directly involved in regulating the fate of PSCs. Demethylation of DNA can be either passive or active, or a combination of both. Passive DNA demethylation usually takes place on newly synthesized DNA strands via DNA methyltransferase 1 during replication rounds. Active DNA demethylation mainly occurs by removal of the 5-methylcytosine via the sequential modification of cytosine bases that have been converted by Tet methylcytosine dioxygenases. In contrast, histone demethylation is carried out by two main classes of histone demethylases: a flavin adenine dinucleotide (FAD)-dependent amine oxidase and a JmjC domain-containing histone dioxygenase (Klose and Zhang, 2007). The activity of Tet and JmjC dioxygenase is dependent on vitamin C and α-ketoglutarate (αKG) as substrates and Fe(II) as a cofactor. Vitamin C enhances iPSC reprogramming, which is not related to its role as a reductive agent (Chen et al., 2013). Increased activity of Tet induced by Vitamin C can greatly increase the reprogramming efficiency (Blaschke et al., 2013). Conversely, the absence of Vitamin C impairs Tet-mediated iPSC reprogramming. Exposure to Vitamin C can reverse L-proline-induced H3K36me2 and H3K9me3, which is associated with a mesenchymal-like invasive phenotype in PSCs treated with L-proline (Comes et al., 2013). Furthermore, glutamine is an important source of αKG that promotes PSC self-renewal as discussed above. One possible mechanism is that more αKG from glutamine elevates the activity of JmjC dioxygenase, facilitating demethylation of repressive chromatin marks such as DNA methylation and H3K9me3, H3K27me3, and H4K20me3 (Carey et al., 2015). Additionally, lysine-specific demethylase 1 (LSD1) provides another link between cellular metabolism and histone methylation. The activity of LSD1 is dependent on FAD, which also serves as a coenzyme in many oxidative reactions including mitochondrial fatty acid oxidation and in the respiratory chain (Hino et al., 2012). Too much FAD used for fatty acid oxidation, for example, reduces the LSD1 activity and, consequently, the pluripotency of hESCs will be impaired, as LSD1 is necessary for the maintenance of hESC pluripotency (Adamo et al., 2011).

Concluding remarks

PSCs have unique metabolic patterns and shifts in metabolism are closely related to PSC fate regulation. However, the cause-and-effect between the metabolism and pluripotency remains elusive. We anticipate that the fate of PSCs can be manipulated by simply changing the nutrients in the medium. A good example of this is the great success of in vitro embryo culture and fertilization (Trounson et al., 1981). This achievement is closely related to the understanding of metabolism during zygote development (Wordinger and Kell, 1978; Brinster and Troike, 1979). Naïve ESCs and primed EpiSCs have distinct metabolic patterns, which may contribute to the differences in their requirements for the maintenance of pluripotency. A better understanding of metabolism will likely facilitate the establishment of PSCs from different species and the clinical translation of these PSCs.

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Compliance with ethics guidelines

Liang Hu, Edward Trope, and Qi-Long Ying declare that they have no conflict of interest. This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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