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REVIEW

# Regulation of energy metabolism in human pluripotent stem cells

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## Abstract

All living organisms need energy to carry out their essential functions. The importance of energy metabolism is increasingly recognized in human pluripotent stem cells. Energy production is not only essential for cell survival and proliferation, but also critical for pluripotency and cell fate determination. Thus, energy metabolism is an important target in cellular regulation and stem cell applications. In this review, we will discuss key factors that influence energy metabolism and their association with stem cell functions.

**Keywords** Glycolysis · Oxidative phosphorylation · pH · Oxygen · Nutrient · Insulin

## Introduction

Cells are dynamic systems that rely on continuous production of cellular energy to carry out various biological processes. Energy metabolism involves diverse substrates and many interconnected pathways, which require coordinated regulation to maintain a balanced support for both energy production and cell type-specific functions under different circumstances. In recent years, numerous studies report that energy metabolism undergo dramatic changes during embryogenesis, inflammation, and cancer development [46, 102, 116, 118]. Besides the many studies on cell type-specific metabolic profiles and their functional impacts, the regulatory mechanisms that control cellular metabolic activities are gaining more and more interest. In this article, we will focus on how energy metabolism is regulated in human pluripotent stem cells (hPSCs).

hPSCs include human embryonic stem cells (hESCs) derived from the inner cell mass (ICM) of blastocyst and human induced pluripotent stem cells (hiPSCs) that are reprogrammed from somatic cells [96, 97, 112]. hPSCs can self-renew unlimitedly and have the potential to generate any cell types in the body, which have attracted enormous interest in regenerative medicine. hPSCs are also invaluable models to understand early embryogenesis. Depending on the culture conditions, hPSCs can be categorized into primed and naïve pluripotent stages that correspond to post-implant epiblast and preimplant ICM [41, 57, 97], respectively. Naïve and primed stem cells display key differences in colony morphology, developmental potential, epigenetic landscape and X-chromosome inactivation. Meanwhile, cells in each stage have distinct metabolic profiles [105]. Naïve cells rely on both glycolysis and oxidative phosphorylation for energy production, while cells in primed stage are mostly glycolytic [10, 40, 105]. A shift from bivalent to glycolytic metabolism takes place during the transition from naïve to primed state, and this process is regulated by hypoxia-inducible factor 1α (HIF-1α) [120]. The differences in glucose and glutamine metabolism contribute to distinct epigenetic modifications, which modulate gene expression to influence various cellular functions [116, 118]. For example, naïve cells display a higher α-KG/succinate ratio, which favors demethylation of repressive chromatin markers and enhances pluripotency [11]. Primed hPSCs are the most widely used in basic research and applications, so hPSC studies discussed in this review mainly refer to cells at primed stage unless otherwise mentioned.

hPSCs grow quickly in vitro, and can double every day in feeder-free conditions [58]. The fast proliferation puts great demand on both energy production and the supply of

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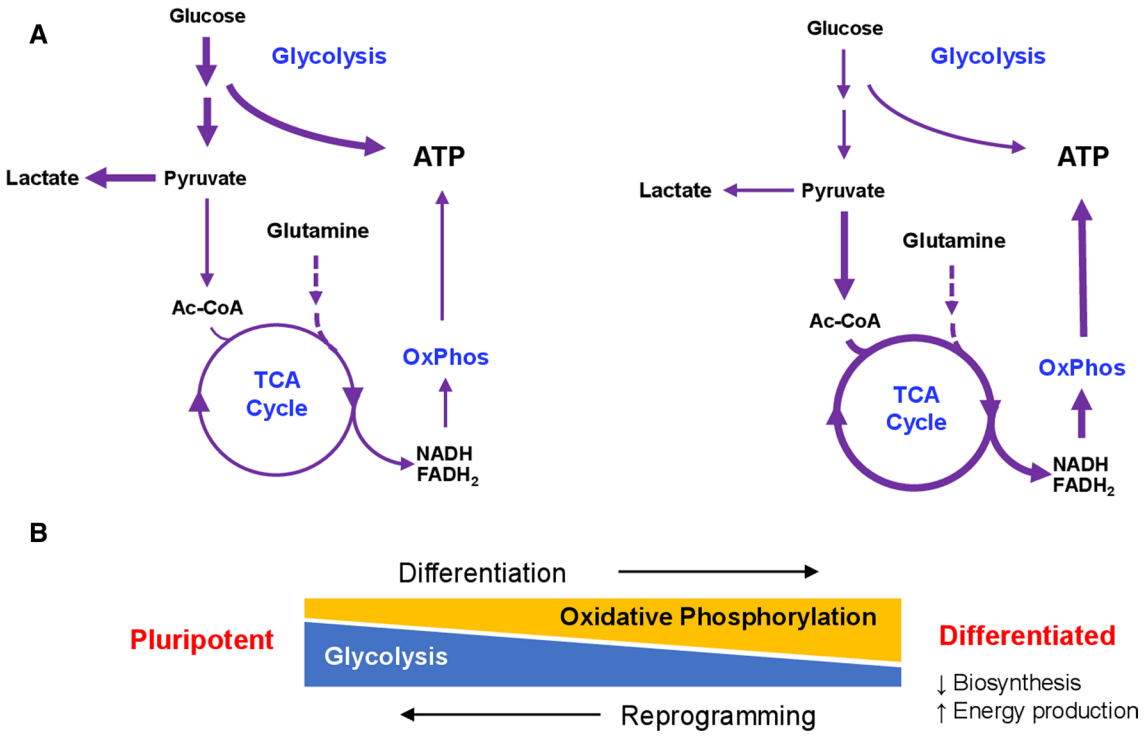


**Fig. 1** Energy metabolism in pluripotent and differentiated cells. **A** Main energy metabolism processes contributing to ATP production in hPSCs (left) and differentiated cells (right). **B** Metabolic changes associated with differentiation and reprogramming. OxPhos, oxidative phosphorylation

key building blocks from cellular metabolites. Glycolysis and oxidative phosphorylation are the two main energy metabolism processes in hPSCs (Fig. 1A). Through glycolysis in the cytosol, one glucose molecule can generate two net adenosine triphosphate (ATP), two reduced nicotinamide adenine dinucleotide (NADH) and two pyruvate molecules. ATP can be directly utilized by various biological processes. Meanwhile, pyruvate can either be converted to lactic acid by lactate dehydrogenase, or serve as the starting material for the TCA cycle, in which every two pyruvate molecules produce eight NADH, two $FADH_2$ and two GTP molecules. Energy stored in NADH and $FADH_2$ are then utilized for ATP production in oxidative phosphorylation at the electron transport chain. Electrons are transported from NADH to $O_2$ through a series of carriers while a proton gradient is formed across the mitochondrial inner membrane. Protons are then transported back into the mitochondrial matrix through complex V, forming ATP in the process. The net result is the generation of ~30 ATPs from each molecule of glucose. Even though glycolysis is less efficient in ATP generation compared to oxidative phosphorylation, high glycolysis flux is commonly seen in stem cells [40, 104]. Energy production through glycolysis is faster when the supply of glucose is abundant [100]. In addition, high levels of metabolism through glycolysis and the non-oxidative branch of the pentose pathway also provide macromolecular precursors to support the biosynthesis of nucleotides, amino acids and lipids, which is advantageous in highly proliferative cells like hPSCs [61, 101, 104].

Although glycolysis produces the majority of ATP in hESCs, oxidative phosphorylation is still indispensable. Mitochondrial respiration not only contributes to substantial energy production, but also plays essential roles in fundamental processes such as cell survival, proliferation and differentiation [6, 22, 55, 63, 83, 116, 118]. Suppression of mitochondrial function in mouse and human ESCs reduces ATP content, increases ROS production, and slows down cell proliferation [63]. Excessive mitochondrial respiration has been shown to cause DNA damage and epigenetic changes [113]. A change in redox status occurs during mESC differentiation, and metabolites involved in oxidative metabolism promote differentiation when added into the culture medium [108]. Inhibitors of the electron transport chain, such as complex III inhibitor antimycin A, have been shown to enhance pluripotency and suppress differentiation [76, 103]. These observations suggest that the balance between glycolysis and oxidative phosphorylation is essential for hPSC homeostasis.

When hPSCs exit the self-renewal cycle, the balance of energy metabolism is shifted toward oxidative phosphorylation in somatic cell types (Fig. 1B). In contrast, during

reprogramming, cellular metabolism undergoes remodeling toward a glycolytic phenotype [74, 115]. Upregulation of glycolysis was reported to enhance reprogramming, while inhibition of glycolysis reduced reprogramming efficiency. PS48, a chemical activator of pyruvate dehydrogenase kinase (PDK), can promote the expression of glycolytic enzymes, and enhance the efficiency of reprogramming from keratinocytes by about 15-fold [121]. On the other hand, suppression of glycolysis with 2-deoxyglucose (2DG), hexokinase 2 inhibitor 3-bromopyruvic acid (BrPA) or PDK inhibitor dichloroacetate (DCA) reduces the efficiency of nuclear reprogramming [32]. These observations demonstrate that the manipulation of hPSC metabolism can redirect cell fate or improve iPSC derivation, both of which are invaluable for clinical applications. With improved efficiency of reprogramming and differentiation, high-quality cells can be derived for disease modeling, drug screening, toxicity test, and cell therapy.

More and more studies demonstrate that energy metabolism is associated with gene expression and epigenetic changes, and modulation of metabolic activities can dictate stem cell pluripotency and cell fate determination through signal transduction and epigenetic mechanisms. Glycolysis and TCA cycle metabolites, such as acetyl-CoA, $NAD^+$ and α-KG, can serve as substrates or cofactors for epigenetic enzymes, and are involved in the regulation of pluripotency and differentiation [28]. For instance, acetyl-CoA generated from glycolysis is a substrate for histone acetylation, and manipulation of acetyl-CoA production can affect hESC differentiation [69]. Elevated intracellular α-KG serves as a cofactor for JHDMs and TET DNA demethylases, promotes histone and DNA demethylation, and enhances *Nanog* expression in mouse ESCs [11]. These interesting findings have been reviewed elsewhere [13, 24, 66, 92, 100]. Given the key roles of metabolic changes in stem cell function, a thorough understanding of factors that can regulate stem cell metabolism will enable us to develop powerful tools in stem cell technology.

During embryogenesis in vivo, pluripotent cells only exist transiently. hPSCs cultured in vitro are able to remain pluripotent for a prolonged period of time because they are maintained by artificial culture conditions. Factors in the culture system, either provided by the researcher or from the cells themselves, can change the microenvironment and profoundly influence hPSC metabolism. As discussed above, appropriate metabolic states and transitions between them are necessary for stem cell pluripotency, proliferation and differentiation, as well as the reprogramming from somatic cells. Factors that regulate hPSC metabolism are important targets through which stem cell functions can be controlled and improved. Here we will discuss a few main contributing factors that regulate hPSC energy metabolism, including (1) contribution by nutrients; (2) composition of culture components—oxygen and medium pH; and (3) regulation by signal transduction pathways (Fig. 2).

## Nutrients in energy metabolism

Numerous culture media have been successfully developed to sustain hPSC self-renewal, all of which provide glucose, glutamine and pyruvate as energy substrates [57]. Each energy substrate contributes to glycolysis and oxidative phosphorylation in a distinct fashion.

### Glucose

Glucose is the starting material for glycolysis, and generally is present in almost all the media for various cell types. Without glucose in the culture medium, there is no glycolysis and hPSCs cannot survive beyond a few days. The cell death is further accelerated when glutamine is simultaneously depleted [98]. Glycolysis is the primary pathway of glucose metabolism in hPSCs. Although glucose metabolism generates pyruvate which can be converted to acetyl-CoA and feed into the TCA cycle, pyruvate is generally not catabolized efficiently through the TCA cycle and oxidative phosphorylation in hPSCs, due to low levels of mitochondrial enzymes for converting citrate to α-KG in the TCA cycle [98]. Instead, pyruvate is mainly converted to lactic acid in hESCs [100]. Not only is glucose not a major contributor to oxidative phosphorylation, but it also suppresses basal oxidative phosphorylation and inhibits pyruvate- and glutamine-based respiration [80]. It suggests that glycolysis and oxidative phosphorylation are intrinsically linked in a dynamic balance in hPSCs.

The functional implications of glucose metabolism in hPSCs have been investigated in recent years. 2-Deoxyglucose (2-DG), a glucose analog, is often used to mimic glucose deprivation and manipulate glycolytic activity. 2-DG enters the cell via glucose transporters, and inhibits glycolysis by suppressing hexokinase [17]. 2-DG treatment at millimolar concentrations inhibits glycolysis by ~ 50% [2, 54]. In hPSCs, 2-DG was reported to lead to decreased expression of pluripotency genes, suggesting loss of pluripotency following manipulation of glycolytic activity. Treatment with BrPA, which inhibits glycolysis at glyceraldehyde 3-phosphate dehydrogenase (GAPDH), had similar effects. Inhibition of glycolysis by 2-DG or BrPA also decreased acetyl-CoA production and consequently impaired histone acetylation, implying the impact of metabolic regulation on epigenetics [69]. Based on the decreased pluripotency marker expression following glycolysis inhibition, glycolysis is considered to be essential

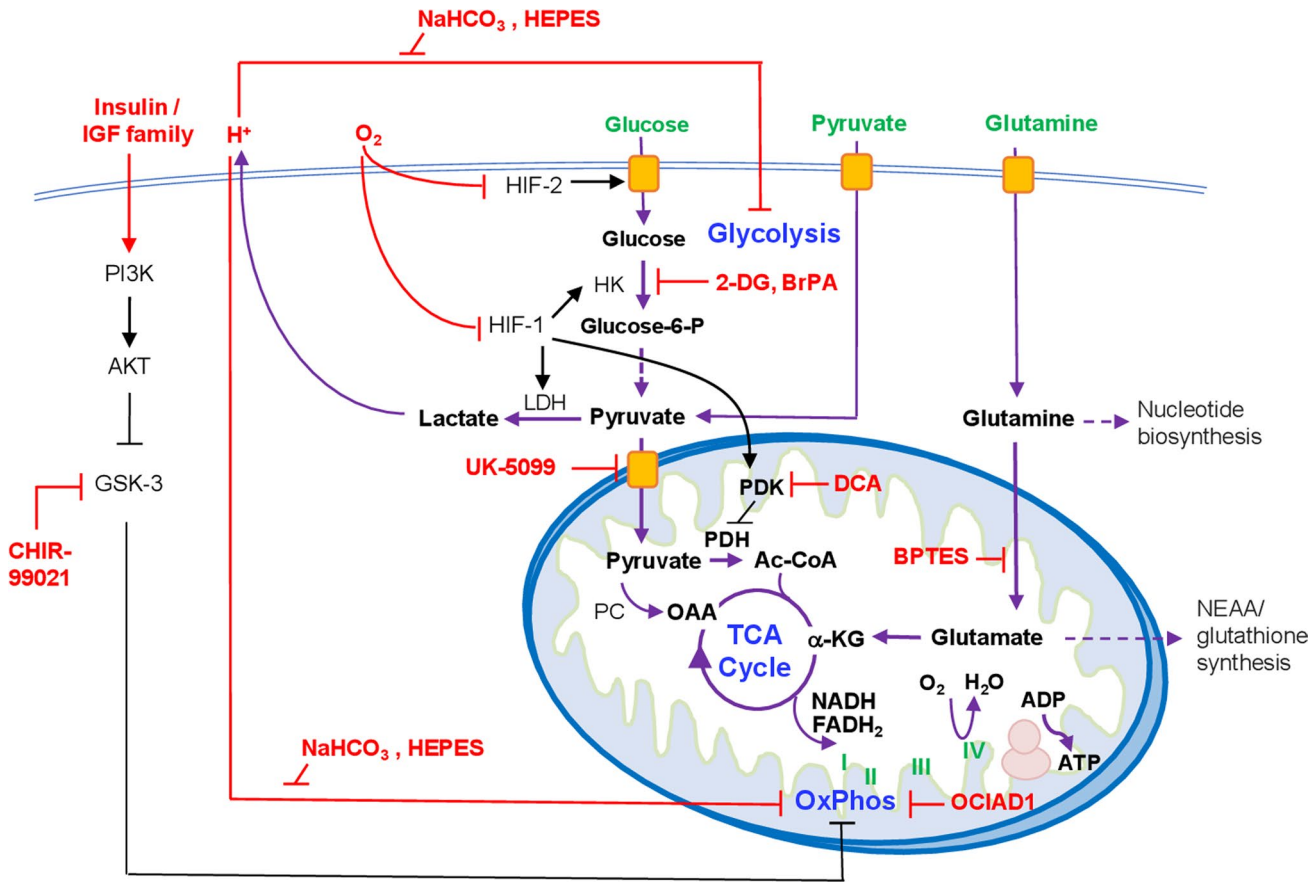


**Fig. 2** Regulation of hPSC metabolism. Major energy substrates in hPSC medium are labeled in green. Regulators of metabolic processes are labeled in red. OxPhos, oxidative phosphorylation. α-KG, α- ketoglutarate. *OAA* oxaloacetate, *PC* pyruvate carboxylase, *HK* hexokinase, *LDH* lactate dehydrogenase

for pluripotency. Because metabolites from glycolysis are involved in many other metabolic processes, the interactions of glucose metabolism with these pathways are still to be explored in hPSCs in the near future.

## Glutamine

Glutamine is the best studied substrate for oxidative phosphorylation in hPSCs, and it is indispensable for hPSC maintenance [98]. Glutamine contributes to nucleotide synthesis in the cytosol. When transported into the mitochondria, glutamine is converted to glutamate by glutaminase, and further into alpha-ketoglutarate (α-KG) which is the key intermediate metabolite for both lipid synthesis and for NADH/$FADH_2$ production in the TCA cycle. Glutamine-derived α-KG is converted to succinate, and subsequently generates two NADHs and one $FADH_2$ in the latter half of the TCA cycle, which are utilized in oxidative phosphorylation. Glutamate can be transported to the cytosol and contributes to glutathione and amino acid synthesis [110]. In the absence of glucose, the function of glutamine in hPSCs was rescued by dimethyl-α-KG, a cell-permeable form of α-KG, but not by pyruvate, fatty acid, nucleoside, or glutathione, highlighting the importance of glutamine-derived α-KG in hPSC survival [98]. Without glutamine, cellular oxidative phosphorylation is significantly decreased. The glutaminase inhibitor bis-2-(5-phenylacetamido-1,3,4-thiadiazol-2-yl)ethyl sulfide (BPTES) was shown to suppress glutamine-dependent oxidative phosphorylation in hESCs [80]. On the other hand, deprivation of glucose led to increased glutamine oxidation, suggesting the interaction between the metabolism of these two major energy substrates [98].

Other aspects of glutamine metabolism are also important for stem cell functions. In addition to feeding into the TCA cycle, glutamine also contributes to the biosynthesis of nucleotides [56]. Inhibition of glutaminase by 6-diazo-5-oxo-L-norleucine (DON) was reported to suppress the de novo synthesis of nucleotides in hematopoietic stem cells and affect erythroid commitment [71]. Glutamine is also required for maintaining intracellular glutathione

levels and suppressing reactive oxygen species. Depletion of glutamine led to the oxidation and degradation of pluripotency marker OCT4, and promoted hESC differentiation [64].

### Pyruvate

Pyruvate is the product of glycolysis, and it is also supplemented in most culture media. Pyruvate takes multiple potential routes in energy metabolism. In hPSCs, pyruvate produced from glucose is mainly converted to lactate in the cytosol at the expense of one NADH [13], and it does not have immediate impact on oxidative phosphorylation. In contrast, supplementation of pyruvate in the cell culture medium promoted oxygen consumption and suppressed glycolysis, suggesting exogenous pyruvate contributes significantly to oxidative phosphorylation [94]. In extracellular flux assays, the immediate impact of pyruvate supplementation on oxidative phosphorylation was shown to be stronger than glutamine [80]. Pyruvate can enter the TCA cycle through two routes, either oxidation to acetyl-CoA by pyruvate dehydrogenase, or conversion to oxaloacetate by pyruvate carboxylase to replenish TCA cycle intermediates [100]. The enzymes involved in these steps are likely targets for the manipulation of hPSC oxidative phosphorylation. Pyruvate-associated oxidative phosphorylation can be elevated by inhibition of pyruvate dehydrogenase kinase (PDK), which phosphorylates and inactivates pyruvate dehydrogenase [8, 89, 106]. Oxidative phosphorylation is suppressed by UK-5099 that inhibits pyruvate transportation into mitochondria [42, 67]. Not all pyruvate-derived acetyl-CoA are used in energy production. Instead, part of the acetyl-CoA contributes to lipid synthesis and protein acetylation [100]. These alternative uses are likely due to the compartmentation of pyruvate and associated enzymes in the cell. Pyruvate interacts with other substrates in their metabolism. Pyruvate and glutamine synergistically elevate oxidative phosphorylation, especially in the absence of glucose. Glucose suppresses pyruvate-dependent oxidative phosphorylation to a lower level [80]. The elevation of exogenous pyruvate significantly increases oxidative phosphorylation, while suppressing glycolysis at the same time [94]. It would be interesting to further investigate the interactions among the metabolism of major hPSC energy substrates.

### Lipids

Although lipid supplements are often used in hPSC culture, they are not essential for the maintenance of pluripotency [15, 57]. Cellular lipids can be generated by hPSCs through de novo lipogenesis using energy and metabolites from glycolysis and oxidative phosphorylation. As energy substrates, endogenous lipids contribute to a large portion of oxidative phosphorylation. Interestingly, exogenous lipids are not immediately utilized in oxidative phosphorylation [80, 95]. Depending on the specific lipid supplement, the metabolic landscape of hPSCs could be shifted greatly. For example, serum extract AlbuMAX contains diverse lipid species, and it significantly alters the balance of glycolysis and TCA cycle while relieving the metabolic burden of de novo lipogenesis [114]. It is proposed that lipid deprivation and the increased dependence on de novo lipogenesis could change the pluripotency state and induce naïve-like features in hPSCs [23]. Because of the diverse lipids involved, it is still unclear which lipid plays the key role and how it leads to significant metabolic, transcriptional, and epigenetic changes.

### Vitamins

When energy substrates are provided, hPSCs rely on specific enzymes to carry out the metabolic processes. Many of these enzymes require various vitamins as cofactors. Water-soluble B family vitamins play particularly important roles in energy metabolism. Vitamins B1 and B3 are involved in glycolysis, while B1, B2, B3, B5 and B7 are important for the TCA cycle and oxidative phosphorylation. Lipid synthesis and metabolism require the actions of B2, B3, B5 and B7; Vitamins B3, B6, B9 and B12 are involved in amino acid metabolism [37]. All of them are usually supplied in the base medium for hPSCs.

## Culture environmental components

In addition to all the energy substrates in the medium, hPSCs also require suitable environmental factors to maintain normal energy metabolism, such as pH and oxygen. Culture pH is normally regulated by $CO_2$ infusion and buffering reagents in the medium such as HEPES and sodium bicarbonate ($NaHCO_3$). Most cell cultures are maintained under normoxic conditions (~20% oxygen). The oxygen level can be adjusted to fit different purposes, either decreased to mimic the hypoxic physiological environment or elevated to improve expansion of suspension culture [65, 87]. Changes in pH and oxygen level have profound impacts on cellular metabolism.

### Biological control of pH and its impact on hESC metabolism

Environmental and intracellular pH has profound impacts on cellular functions, yet has received surprisingly little attention. The ionization status of acidic or basic amino acids in enzymes may change according to pH, leading to

conformational changes and resulting in pH sensitivity. Various cellular processes, including membrane transport, cell proliferation, membrane potential, energy metabolism, signal transduction, etc., have been shown to respond to pH variations [30, 36, 48, 62, 84].

It is well known that elevated pH promotes glycolysis and stimulates the production of lactate [44, 79]. Many enzymes in the glycolysis pathway and the TCA cycle have been shown to be pH sensitive [53, 60, 77, 99]. Under acidic pH conditions, both glycolysis and oxidative phosphorylation are suppressed [58]. In contrast, elevation of pH leads to higher energy production through glycolysis and oxidative phosphorylation. Because glycolysis is the main energy producing process in hESCs, more lactic acid is released into the medium as the cell density increases with proliferation. This phenomenon leads to a self-induced proliferation control for hESCs through metabolism. When cell density increases to near confluence, medium acidosis turns off glycolysis, leading to growth arrest or even cell death. When additional sodium bicarbonate is applied to increase medium pH and buffer capacity, it significantly promotes glucose uptake and its consumption through both glycolytic and oxidative metabolism, and increases ATP production, which allows hESC proliferation to higher densities [58]. In addition to the impact on glucose metabolism, medium acidosis also affects $CO_2$ fixation. Many cell culture systems utilize the $CO_2/HCO_3^-$ buffer system, where $HCO_3^-$ is included in the medium and $CO_2$ is supplied to the incubator. A drop in pH leads to the loss of $HCO_3^-$ from the medium, which normally serves as the carbon source for the synthesis of oxaloacetate, an essential intermediate in the TCA cycle utilized for biosynthetic pathways [38, 91].

Intracellular acid–base balance is maintained by an intricate system of buffers, membrane transporters and enzymes [12, 84]. Biological buffers, including phosphate groups, amino acid side chains, and $HCO_3^-$ groups, allow cells to respond rapidly to acute changes in pH. Cells are also equipped with multiple transporters in the plasma membrane, including ATP-driven proton pumps (ATPases), monocarboxylate transporters (MCTs), $Na^+$/Bicarbonate cotransporters (NBCs), sodium-dependent chloride/bicarbonate exchangers (NCBE), $Na^+/H^+$ exchangers (NHEs), and $Cl^-/HCO_3^-$ anion exchangers (AEs). V-type $H^+$ ATPases, NHEs, NBCs and NCBEs drive $H^+$ efflux or $HCO_3^-$ influx to protect cells against acidification, while AEs and NBCs are capable of transporting $HCO_3^-$ out of the cell to prevent alkalinization. In cell types with high levels of glycolysis, outward transport of lactate along with $H^+$ by MCTs also comprises an important pH regulatory mechanism [43]. During early chicken embryo development, a pH gradient exists in parallel with graded MCT1 expression in the tail bud, and plays important roles in cell fate specification [73].

Cellular pH and metabolism can be manipulated by targeting pH regulators. Knockout of $Na^+$/bicarbonate cotransporter NBCn1 in mice resulted in reduced intracellular pH in endothelial cells [7]. In human pluripotent stem cells, MCT1 is expressed at high levels, and inhibition of MCT1 was shown to reduce glycolytic flux [40]. In cell culture systems, control and regulation of environmental pH are achieved with buffering agents. The most frequently used buffer in cell culture media is the physiological $CO_2/HCO_3^-$ buffer system, which can be used in combination with non-volatile buffers such as HEPES [26, 68]. Given the close relationship between pH control, hypoxia, and energy metabolism, it would be interesting to further investigate the impact of environmental and cellular pH changes on hESC function.

## Metabolic regulation by oxygen tension

Oxygen is the terminal electron acceptor in metabolic pathways, and serves as the substrate/cofactor for many metabolic enzymes. Environmental oxygen level is a key regulator of hESC metabolism.

Oxygen homeostasis in hESCs is maintained by hypoxia-inducible factors (HIFs), global regulators of hypoxic responses [20, 47, 78]. HIFs are heterodimeric transcription factors consisting of the inducible HIF-α subunit and the constitutively expressed HIF-β (ARNT) subunit. hESCs express three different HIF-α isoforms (HIF-1α, HIF-2α and HIF-3α). Among these, HIF-3α lacks the binding domain for coactivators, and is proposed to work by regulating the expression of other isoforms. HIF-1α is transiently expressed for 48 h under hypoxia, suggesting a role in the initial adaptation to hypoxic conditions. In contrast, upregulation and nuclear translocation of HIF-2α and HIF-3α occur after long-term culture under hypoxia [34].

HIF is one of the central regulators of glycolysis. hESCs cultured under hypoxic conditions were shown to consume less oxygen, utilize more glucose and less pyruvate, and produce more lactate, suggesting elevated levels of glycolysis [33]. Expression of glucose transporters GLUT1 and GLUT3 in hESCs are both upregulated under hypoxic conditions [5, 21, 33], suggesting increased glucose uptake under decreased oxygen tension. Multiple metabolic enzymes have been shown to be regulated by HIF, including pyruvate dehydrogenase kinase [51], lactate dehydrogenase, hexokinase, and several other glycolytic enzymes [50, 81, 85, 86]. The increased glycolysis flux maintains ATP generation, while PDK and LDH activation decreases substrate availability for the TCA cycle and attenuates mitochondrial ROS production to protect cells against oxidative stress under hypoxia [51].

As hESCs possess a unique metabolic program with high glycolytic flux [31, 100], metabolic changes under hypoxia are beneficial for hESC maintenance and generation.

Hypoxic culture conditions were reported to enhance the expression of pluripotency markers *SOX2*, *NANOG* and *POU5F1* in hESCs, mediated by HIF-2α [34]. Spontaneous differentiation of hESCs were reduced when cultured under low oxygen tension, indicating beneficial effects of hypoxia on hESC maintenance [29]. Reprogramming of mouse fibroblasts to induce pluripotent stem cells was also enhanced by hypoxia [111].

In addition to carbohydrate metabolism, hypoxia and HIFs are also extensively involved in lipid metabolism [70], although the functional implications for hESCs are not well understood. In recent years, HIFs have also been reported to participate in epigenetics, exosome release as well as biogenesis of non-coding RNAs [20], and novel mechanisms may be revealed in the near future which underlies the impact of oxygen tension on hESCs.

## Mitogenic regulation of hPSC energy metabolism

Given the pivotal role of metabolic homeostasis in hPSCs, the control of substrate and environmental factors are still not sufficient to control the balance of glycolysis and oxidative phosphorylation. Mitogenic regulation is an obvious candidate to coordinate metabolism and stem cell function. The maintenance of hPSCs requires continuous mitogenic stimulation, including FGF2, TGFβ and insulin [15]. All these mitogens have been implied in energy metabolism in somatic cells, but their roles in hPSCs are often difficult to study. The suppression of any of these pathways could lead to cell differentiation, which often complicates the interpretation of metabolic phenotypes. The immediate measurement of metabolic profile upon signal manipulation is essential for people to understand a mitogen's impact on energy metabolism without the interference from potential cell type changes.

Among all essential mitogens, the insulin/IGF family is the most important factor for hPSC survival and function, and is utilized in various lineage-specific induction protocols, such as neural cell fate induction [14], skin keratinocyte [119] and retinal pigmented epithelium differentiation [9]. IGF signaling also shifts cell fate during mesoderm induction [109]. During hESC maintenance, insulin/IGF is the only factor important for immediate metabolic regulation [80]. Continuous insulin stimulation is essential to sustain oxidative phosphorylation. If insulin stimulation is removed, oxidative phosphorylation level decreases immediately. Insulin promotes oxidative metabolism associated with pyruvate, glutamine and lipid. Oxidative phosphorylation is enhanced by insulin through the PI3K/AKT/GSK3 pathway. Insulin activates PI3K and AKT, which subsequently inhibits GSK3. Inhibition of GSK3 is able to partially rescue oxidative phosphorylation in the absence of insulin. The insulin-dependent oxidative phosphorylation is stem cell specific, and the phenomenon disappears during differentiation. FGF2 and TGFβ removal for two days is sufficient for the loss of insulin-dependent oxidative phosphorylation. It suggests that FGF2 and TGFβ are in play with metabolic regulation but in an indirect manner. Interestingly, insulin does not promote glycolysis, probably due the fact that hPSCs express the insulin-independent glucose transporter GLUT1 [27, 80].

Besides the insulin pathway, other pathways could also play a role in metabolic control in hPSCs. OCIAD1, a mitochondrial protein, was reported to actively regulate oxidative phosphorylation in hPSCs. Reduction of OCIAD1 expression levels led to increased activity of mitochondrial complex I and promoted oxidative phosphorylation, enhancing hPSC differentiation upon induction [90]. OCIAD1 was previously reported to regulate the JAK/STAT signaling pathway and promote STAT3 activation in mESCs [93], and also shown to modulate NOTCH signaling in *Drosophila* [52]. It is plausible that these pathways may be involved in the regulation of hPSC energy metabolism.

## Regulation of metabolism by nuclear factors

In addition to HIFs that were discussed earlier, other nuclear factors may also contribute to the regulation of hESC metabolism. High mobility group A (HMGA) proteins, including HMGA1 and HMGA2, are chromatin factors involved in global chromatin remodeling. Both HMGA1 and HMGA2 are highly expressed in hESCs, and can regulate pluripotency and differentiation by controlling gene expression [18, 75]. The expression level of HMGA1 is high in undifferentiated hESCs and declines during differentiation. Its ectopic expression maintains pluripotency markers, prevents hESC differentiation and enhances reprogramming [88]. At the same time, HMGA1 is also an important regulator of glucose production and metabolism. HMGA1 is essential for the transcription of both *INS* and *INSR* gene, and is also a downstream target of insulin action through the PI-3K/AKT pathway [19]. Consistently, *Hmga1* knockout mice display glucose intolerance due to down-regulation of insulin receptors [35].

Another group of nuclear factors known to regulate metabolism are the Forkhead box protein O (FOXO) transcription factors, which regulate a diverse array of cellular functions, including DNA repair, cell proliferation, cell death, and metabolism [1, 39]. FOXO1 is a key regulator of glucose production. FOXO1 is downstream of insulin signaling and the PI-3K/AKT pathway, and the phosphorylation by AKT inhibits FOXO1's nuclear localization and function [72]. Under fasting conditions, FOXO1 is upregulated by the cAMP-PKA

pathway to maintain glucose levels [107]. In hESCs, FOXO1 is abundantly expressed, and the expression level decreases upon differentiation, coinciding with the metabolic transition [59]. FOXO1 is essential for maintaining the expression of pluripotency genes, and remains active in hESCs despite its phosphorylation by AKT [117]. The mechanism through which FOXO1 regulates hESC metabolism remains to be explored. HMGA1 was reported to be a positive modulator of FoxO1 [4], potentially enabling the crosstalk between metabolism and pluripotency.

Besides nuclear gene expression, changes in mitochondria DNA can also have significant impacts on metabolism. Pathogenic mtDNA variations have been reported to alter the process of oxidative phosphorylation and lead to disorders involving multiple organ systems. iPSCs offer an invaluable model system to study mitochondrial disorders and test potential therapeutics [45, 49].

## Summary

Although we discuss all the energy metabolism regulators separately, they actually cross talk with each other. Oxygen and pH fluctuations lead to changes in HIF and PI3K/AKT pathway [3, 16], which are directly related to substrate utilization and final energy production. Meanwhile, the amount of substrate available can also regulate signaling pathways, such as the mTOR and AMPK pathway that could affect overall metabolism beyond just energy production. Many current studies imply the association between metabolism and cell fate determination through epigenetics [25, 82]. Given the intermingled relationships between metabolism, signal transduction, nuclear signals and epigenetic modifications, it is possible that more complicated regulatory mechanisms are involved at multiple levels. Further analysis of the regulators of hPSC metabolism and their roles in stem cell function is necessary and may provide new insights into the applications of stem cell technology.

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## Declarations

**Conflict of interest** The authors declare no conflict of interest.

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