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Energy Metabolism in the Acquisition and Maintenance of Stemness

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Abstract

Energy metabolism is traditionally considered a reactive homeostatic system addressing stage-specific cellular energy needs. There is however growing appreciation of metabolic pathways in the active control of vital cell functions. Case in point, the stem cell lifecycle – from maintenance and acquisition of stemness to lineage commitment and specification – is increasingly recognized as a metabolism-dependent process. Indeed, metabolic reprogramming is an early contributor to the orchestrated departure from or reacquisition of stemness. Recent advances in metabolomics have helped decipher the identity and dynamics of metabolic fluxes implicated in fueling cell fate choices by regulating the epigenetic and transcriptional identity of a cell. Metabolic cues, internal and/or external to the stem cell niche, facilitate progenitor pool restitution, long-term tissue renewal or ensure adoption of cytoprotective behavior. Convergence of energy metabolism with stem cell fate regulation opens a new avenue in understanding primordial developmental biology principles with future applications in regenerative medicine practice.

Keywords

Glycolysis; oxidative metabolism; nuclear reprogramming; induced pluripotent stem cells; embryonic stem cells; hematopoietic stem cells; mitochondria; metabolic remodeling

1. Introduction

The resurgent interest in intermediary metabolism combined with advances in techniques for both the high throughput examination of the global metabolome and stable isotope tracing of specific metabolic pathways, has begun to resolve the metabolic fingerprint of the stem cell life cycle. While energy metabolism has long been considered a homeostatic system, responsive to match with high fidelity stage specific energetic demands, recent studies have revealed that metabolism can also feedback to regulate the epigenetic and transcriptional identity of a cell [1]. Therefore changes in energy metabolism and metabolite content may represent a physiological mechanism by which stem cells interact both locally within their niche and respond to changes in their systemic environment. Understanding these

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interactions will contribute to define how modulation of energy metabolism can be harnessed to regulate stem cell self-renewal and lineage-specific differentiation, critical concepts for cell biology with ramifications in regenerative medicine. This review highlights the major metabolic pathways associated with stem cell function and how they may fuel the transition between cellular fates.

2 Metabolism driving cell fate conversions

Inherent plasticity in the ability of cells to shift from one metabolic pathway to another, allows selection of the most appropriate pathway to match current energetic requirements. However an increasing body of evidence indicates that metabolic remodeling occurs early during transitions between cell states, prior to the establishment of the particular cell fate. The early transitions in energy metabolism may be required to energetically prime the cells to ensure they are competent to meet the metabolic demands of emerging cell fates, as well as to establish the epigenetic state required for a particular cell type. Several recent studies examining the temporal changes in the global gene and protein expression during nuclear reprogramming of somatic cells back to the pluripotent ground state have enabled the further characterization of this metabolism-dependent process. Indeed the first wave of gene induction during reprogramming is enriched for genes related to metabolism and proliferation in both mouse [2] and human systems, which precedes induction of embryonic genes and the core pluripotency network [3]. Temporal proteomic profiling has confirmed similar waves of protein expression changes during reprogramming, including stoichiometric changes of the mitochondrial electron transport chain within the initial wave [4]. This is characterized by a reduction in subunit expression of complex I and IV, and an increase expression of complex II, III, V of the mitochondrial electron transport chain [4, 5], which complements the early upregulation of glycolytic genes that precede induction of pluripotency genes [5–7].

Functionally, a metabolic burst of both oxidative and glycolytic metabolism occurs early in the reprogramming process in support of a temporary hyperenergetic state, following which oxidative metabolism declines, while glycolysis continues to increase [8]. The estrogen-related nuclear receptors, $ERR\alpha$ and $ERR\beta$, and their co-activator $PGC-1\alpha/\beta$ are transiently expressed early in the reprogramming process and support the burst in oxidative metabolism. A $Sca1^+CD34^-$ subpopulation with 10 and 7-fold higher expression of $ERR\gamma$ and $PGC-1\beta$, respectively, display a 50-fold greater efficiency at generating iPSCs than the remaining cells [8]. Transcriptional analysis demonstrated a global upregulation of metabolic genes with oxidative phosphorylation genes representing the most significantly altered pathway in $Sca1^+CD34^-$, further supporting the importance of the hyperenergetic state for induction of pluripotency. Recent evidence also demonstrates a role of the oocyte factors $Tcl1$ and $Tcl1b1$ in supporting metabolic remodeling and enhancing reprogramming efficiency independent of changes in cell proliferation [9]. Mechanistically, $Tcl1$ increases $Akt1$ activity and may support an increased glycolysis, while $Tcl1b1$ suppresses the mitochondrial polynucleotide phosphorylase, thus impairing mitochondrial biogenesis and contributing to the switch from oxidative metabolism to glycolysis during reprogramming [9]. Remodeling of energy metabolism also plays a role in the transition between naïve and primed pluripotent stem cells (PSCs). Human naïve PSCs display higher oxygen utilization compared to their primed

counterparts, displaying a similar bivalent use of both glycolysis and oxidative metabolism as mouse embryonic stem cells (ESCs) [10, 11]. Indeed the transition between these pluripotent states is associated with a remodeling of the metabolome, including changes in glycolysis, fatty acid and amino acid metabolism. This includes an elevated abundance of 1-methylnicotinamide, a product of nicotinamide N-methyltransferase, which is upregulated in the naïve state and consumes S-adenosylmethionine (SAM) making it unavailable for histone methylation reactions [12]. By competing for SAM availability, this suppresses methylation of repressive histone 3 lysine 27, thus linking cellular metabolism with the naïve state. Therefore, it appears that metabolic remodeling during acquisition of pluripotency is not simply a consequence of transition between cell identities, but may represent an initiating event.

3 Metabolic pathways fueling stem cell function and fate

3.1 Glycolysis

High metabolic flux through glycolysis is a common feature across a number of stem cell populations [13–21]. Indeed, stimulation of glycolysis itself or regulators of glycolysis facilitates the acquisition of the pluripotent state during nuclear reprogramming [5, 22–30] and supports the maintenance of stemness [16, 31–35]. Generation of induced pluripotent stem cells (iPSCs) stimulates a significant remodeling of the glycolytic pathway early during the reprogramming process [5], including an up regulation of genes for the initial steps of glucose uptake and phosphorylation, as well as the distal portions of the pathway [36]. Proteomic analysis has demonstrated a progressive increase in glycolysis during nuclear reprogramming [4], with greater expression of the majority of glycolytic enzymes observed in the resultant iPSCs compared to parental cells, and resemble their embryonic counterparts [5]. PSCs also display an isoform switch from hexokinase I to II [5], which supports elevated glycolytic flux due to the twin catalytic domains of this isoform as well as the preferential access to mitochondrial ATP production and reduction in product inhibition by glucose-6-phosphate due to its mitochondrial localization [20, 37].

The glycolytic phenotype is not limited to PSCs but is also characteristic of a number of tissue specific stem cell populations, such as long-term hematopoietic stem cells (LT-HSCs) [17, 35] and mesenchymal stem cells (MSCs) [18]. LT-HSCs are maintained in a quiescent slow cycling state to ensure long-term tissue renewal by protecting them from life-long accumulation of cellular damage and mutations [38]. Compared either to whole bone marrow or their committed progenitors, HSCs display a metabolic profile consistent with elevated glycolytic flux including high levels of fructose-1,6-bisphosphate and pyruvate, the products of the rate limiting steps of glycolysis, in line with high pyruvate kinase activity [17, 35]. MSCs also have a greater dependence on glycolysis due to elevated expression of glycolytic enzymes compared to their differentiated osteoblasts [18]. HSCs are dependent on expression of pyruvate dehydrogenase kinase (PDK) 2 and 4, which phosphorylate and inactivate pyruvate dehydrogenase thus preferentially shunting pyruvate to lactate production instead of entering the tricarboxylic acid cycle for further oxidation. Indeed deletion of PDK2 and 4 results in reduced dependence on glycolysis and leads to HSC functional exhaustion, including loss of quiescence and transplantation capacity [35].

Restoration of glycolysis in deficient HSCs through either PDK overexpression or pharmacologic inhibition of mitochondrial pyruvate entry was sufficient to restore glycolysis in defective HSCs and reinitiate cell cycle quiescence and reconstitution capacity [35], thus supporting the critical importance of glycolysis for maintenance of HSC function and fate.

3.1.1 Hypoxia and Hypoxia-inducible factors in regulating glycolysis in stem cells

—A number of transcriptional and signaling pathways converge to regulate glycolysis in stem cell populations, with hypoxia and hypoxia-inducible factor 1 α (HIF1 α) and 2 α (HIF2 α) pathway being the well characterized (*Ruohola-Baker this issue*). Under normoxic conditions, oxygen-regulated prolyl hydroxylases hydroxylate proline residues in HIFs, thereby priming these proteins to undergo ubiquitination and proteasomal degradation [39, 40]. Thus hypoxia stabilizes HIF1 α and 2 α through suppression of prolyl hydroxylation, allowing them to form nuclear heterodimers with HIF1 β and bind to hypoxia response elements in the promoter regions of genes, including many glycolytic genes such as GLUT1, LDHA and PDKs [38–40]. Indeed, many tissue specific stem cells reside within in vivo niches with low oxygen tensions, which maintain these cells in a glycolytic quiescent state to ensure tissue regenerative capacity by limiting oxidative metabolism generation of reactive oxygen species (ROS) and subsequent accumulation of ROS-induced cellular damage [32, 38, 41, 42]. In the hematopoietic system, loss of HIF1 α results in decreased glycolytic flux leading to impaired HSC quiescence and eventual HSC exhaustion [43], implicate the importance in HIF1 α for HSC maintenance. This phenotype can be rescued by overexpression of PDK2/4 or using 1-aminoethylphosphonic acid to promote glycolysis and reestablish stem cell quiescence [35]. HIF1 α can also be transcriptionally activated in HSCs by MEIS1 leading to elevated HIF activity and downstream functions [17]. Some of HIF1 α action on glycolysis may also be mediated via transcriptional activation of CRIPTO, which binds its receptor GRP78 to stimulate glycolytic proteins [44, 45]. MSCs and neuronal stem cells, which normally reside within an in vivo hypoxic niche, also lose hypoxia-dependent quiescence when cultured in normoxia resulting in accelerated proliferation associated with oxidative metabolism and loss of stem cell function due to increased cellular senescence [46–48].

Embryonic stem cells (ESCs) within the blastocyst inner cell mass also reside in a hypoxic environment of the lumen of the oviduct and uterus with oxygen tension ranging from 1.5 to 9% [49]. Although PSCs can be propagated in high oxygen in vitro, a hypoxic environment promotes glycolysis [50] and contributes to their stemness maintenance and self-renewal capacity [31, 32, 51, 52]. Indeed hypoxia alone is sufficient to de-differentiate early lineage committed progeny from ESCs and iPSCs back to a self-renewing pluripotent state and is associated with enrichment of HIF1 α /HIF2 α target genes and restoration of a glycolytic phenotype [53]. Consistent with this observation, stabilization of HIF1 α and HIF2 α during early reprogramming of somatic cells to iPSCs supports an early glycolytic shift through activation of their target genes PDK1–3 and pyruvate kinase isoform M2 [6, 7]. However, HIF2 α stabilization after day 12 of reprogramming impairs reprogramming efficiency partially due to upregulation of TNF-related apoptosis-inducing ligand [6]. HIF1 α -induced glycolytic genes are also critical for the transition between ESCs and epiblast stem cells (EpiSCs), the later of which display a characteristic HIF1 α gene expression profile

associated with a greater reliance on glycolysis for energy production [11]. Stabilization of HIF1 α chemically or through expression of a non-degradable isoform in mouse ESCs shifted metabolism to a more glycolytic phenotype and increased the percentage of EpiSC-like colonies [11]. Therefore hypoxia and signaling through the HIF pathway appears to be a critical rheostat in directing the stem cell fate and function through regulation of glycolysis.

3.1.2 Glycolysis fuels stemness—While the reliance on glycolysis in tissue specific stem cells fits well with the lower energetic demands of the quiescence state and helps to limit oxidative metabolism-dependent generation of ROS and mitigate accumulation of ROS-induce cellular damage to ensure life-long tissue renewal, the importance of utilizing glycolysis in highly proliferative PSCs is less obvious. Yet, a glycolytic phenotype appears to be a consistent feature of highly proliferative cells [54, 55]. While glycolysis is inherently less efficient in terms of energy generation, producing only a fraction of the 36 ATP that can be generated via complete oxidation of glucose, it enables a faster rate of ATP generation and in some cases can outpace ATP production from oxidative metabolism [56, 57]. Generation of ATP does not appear to be limiting in proliferating cells as glycolytic cells maintain high ATP/ADP ratios even when then are stimulated to divide at an accelerated rate [58, 59]. Consistent with this observation is that cancer cells can utilize an alternative pathway to metabolize phosphoenolpyruvate to pyruvate that supports anabolism yet uncouples glycolysis from PKM-dependent ATP production [55, 60]. Therefore in the presence of abundant resources, for instance in cell culture where stem cells are bathed in high levels of glucose, utilization of glycolysis may be advantageous as it maintains pools of carbon intermediates required for biosynthesis of cellular contents to enable the generation of new daughter cells [54, 55]. Glycolytic and pentose phosphate pathway intermediates serve as precursors for a number of essential biosynthetic pathways including purine and pyrimidine nucleotides, amino acids and triacylglycerols (Fig. 1). At least half of the carbon atoms required for de novo purine and pyrimidine synthesis are derived from the pentose phosphate pathway intermediate ribose-5-phosphate, which is subsequently activated to 5-phosphoribosyl- α -pyrophosphate. Meanwhile, carbon atoms for non-essential amino acid synthesis are directly derived from glycolytic intermediates, with cysteine, glycine and serine being synthesized from 3-phosphoglycerate and alanine synthesized from pyruvate. Dihydroxyacetone phosphate also serves as a precursor for glycerol-3-phosphate, which is critical for triacylglycerol and phospholipid biosynthesis. The detailed anabolic requirements of proliferating cells and the economics of macromolecule biosynthesis have been extensively reviewed elsewhere [55].

3.2 Hexosamine Biosynthesis Pathway

Alternatively a small proportion of glucose can be shunted into the hexosamine biosynthesis pathway to generate UDP-N-acetylglucosamine (UDP-GlcNAc), a substrate for post-translational modification of proteins with O-linked-N-acetylglucosamine (O-GlcNAc) [61]. This pathway acts as a site of convergence of carbohydrate, amino acid, fatty acid and nucleotide metabolism, making it a critical nutrient sensing pathway to link metabolic status with cell function and fate. Members of the core pluripotency network and nuclear reprogramming factors, including Oct4, Sox2 and cMyc, actively undergo O-GlcNAcylation [62, 63]. These modifications have direct impact on stem cell function as PSC self-renewal

and nuclear reprogramming is impaired by either reducing UDP-GlcNAc generation by limiting glucose availability or blocking O-GlcNAc transferase (OGT), while stem cell differentiation is blocked by increases in global O-GlcNAc levels [62]. OGT may also mediate its impact on ESCs by binding and GlcNAcylation of ten-eleven translocation (TET) proteins TET1 and TET2 [64, 65], which catalyze the conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) and leads to DNA demethylation, particularly in CpG rich promoter regions [64]. The OGT-TET complex may also help to recruit repressive complexes, such as Sin3A and NuRD, to suppress expression of developmental genes and maintain the pluripotent state [65]. Hematopoietic cells may also utilize glucose dependent UDP-GlcNAc for N-glycosylation to regulate growth factor surface expression to coordinate glucose and glutamine metabolism and stimulate cell growth and proliferation [66].

3.3 Mitochondrial function and metabolism

3.3.1 Mitochondria structure and function in stem cells—Associated with the greater dependence on glycolysis for stemness maintenance, stem cells display immature mitochondrial infrastructure and may repurpose their mitochondria away from canonical ATP generators. In this context, stem cells largely express low copy numbers of mitochondrial DNA (*St. John this issue*) and display a sparse mitochondria infrastructure that has been proposed to represent a marker of stemness, consisting of immature spherical structures with poorly developed cristae that are predominantly localized in the perinuclear space [13, 15, 18, 67–73]. As a consequence of this immature mitochondrial infrastructure, stem cells have lower levels of mitochondria respiration and reduced oxidative reserve capacity compared to their differentiated counterparts [5, 14–19]. Tissue specific stem cells may minimize their reliance on mitochondria function due to lower energetic demands of the quiescent state and to reduce the potentially toxic effect of ROS. LT-HSCs can be isolated from whole bone marrow by gating for low mitochondrial membrane potential, and display greater colony formation capacity and bone marrow reconstitution capacity compared to the low potential subpopulation [17]. Consistent with a lower requirement for oxidative metabolism in HSCs, depletion of the mitochondrial protein tyrosine phosphatase (PTPMT1) leads to reduced oxidative capacity and an expansion of the HSCs pool and an associated block in stem cell differentiation that leads to hematopoietic failure [74]. While simulation of mitochondrial biogenesis through deletion of the tuberous sclerosis complex results in impaired HSCs quiescence and HSC pool maintenance, as well as defective hematopoiesis, which can be restored with rapamycin treatment [75]. Indeed, accumulation of mitochondrial DNA mutations due to expression of a proofreading defective mitochondrial DNA polymerase (POLG), leads to impaired mitochondrial function associated with a premature aging hematopoietic phenotype [76]. While the mtDNA mutations result in distinct differentiation blocks, it had little functional effect on maintenance of the HSC pool, further supporting the divergent role of mitochondria in HSCs versus progenitors [76]. However, some level of mitochondrial function is required for maintenance of HSC function, as loss of FOXO3 function, resulting in impaired mitochondrial respiration and elevated glycolysis, ultimately leads to loss of HSC quiescence [77]. Mitochondrial features may be a consistent indication of the differentiation capacity of tissue specific stem cells as cardiac progenitor cells display efficient

cardiomyocyte differentiation when abundant mitochondria are available compared to poor differentiation capacity in cells with few mitochondria [78]. Manipulation of the mitochondrial infrastructure by stimulating mitochondria biogenesis was sufficient to overcome this differentiation block [78].

While mitochondrial structure and function appear to be critical regulators of stem cell function and fate, they may play divergent roles in pluripotent versus tissue specific stem cells. Despite the immature nature of stem cell mitochondrial structure/function and evidence that autophagic clearance of mitochondria is critical for induction of pluripotency [79], impaired mitochondrial homeostasis is associated with loss of stemness properties. For example, knockdown of POLG in ESCs leads to loss of pluripotency and induction of differentiation [80], while knockdown of growth factor erv1-like in ESCs induces expression of GTPase dynamin-related 1 (Drp1) leading to excessive mitochondrial fission and loss of pluripotency, impaired differentiation capacity and a reduction in cell viability [81]. In addition, stem-like cells asymmetrically segregate their mitochondria between daughter cells, with daughter cells receiving a greater proportion of young mitochondria maintaining their stem cell traits, while impaired segregation caused by disruption of mitochondria fission results in the loss of stem cell characteristics [82]. In addition, high mitochondrial membrane potential has been linked with stem cell properties [15, 71, 83, 84] and may be actively maintained through hydrolysis of glycolytically-derived ATP by ATP synthase as electron transport activity is reduced in PSCs [85]. The role of high mitochondrial membrane potential plays in maintenance of the pluripotent state remains unresolved, but it has been proposed to help maintain a more fragmented mitochondrial network [86, 87], maintain optimal redox potential for lipid and amino acid synthesis [88] or to prime stem cells to quickly respond to the energetic demands of cellular differentiation [21]. Indeed, mitochondrial potential may represent a potential tool to select reprogramming intermediates [5] and robust stem cell populations for cell therapy [17, 89]. ESCs sorted into low and high mitochondrial membrane potential subpopulations have similar expression of pluripotent markers yet display distinct differentiation capacity with the high subpopulation more efficiently forming teratomas (a defining trait of PSCs) than their low subpopulation counterparts [90]. In addition, cells undergoing nuclear reprogramming also obtain a high mitochondrial potential associated with the pluripotent state [5]. Therefore PSCs appear to actively regulate mitochondrial distribution and activity in support of mitochondrial functions beyond canonical oxidative metabolism that are critical for stemness maintenance.

3.3.2 Tricarboxylic acid cycle and catapleurosis—Typical of a proliferative phenotype, PSCs avidly need anabolic precursors to ensure the *de novo* synthesis of cell constituents, which competes with complete substrate oxidation within the mitochondrial tricarboxylic acid cycle (TCA) [55]. To support stemness, stem cells can commission catapleurosis, extracting partially oxidized mitochondria substrates for anabolic purposes [91]. In fact, ESCs incompletely oxidize pyruvate in the TCA to generate exportable intermediates [92]. In this way, citrate is exported from mitochondria, processed by ATP-citrate lyase to form cytosolic acetyl-CoA, which serves as substrate for protein/histone acetylation along with fatty acid/cholesterol biosynthesis [93]. Initial ESCs differentiation is linked to reduced acetyl-CoA production and loss of histone H3K9 and H3K27 acetylation,

suggesting that TCA-derived cytosolic acetyl-CoA facilitates histone acetylation and an open chromatin state [92]. Inhibition of ATP citrate lyase compromises acetyl-CoA content and histone acetylation enabling myogenic differentiation [94], while acetate excess blunts early differentiation and histone deacetylation [92]. Not exclusive to pluripotency, ATP-citrate lyase also contributes to adipogenic differentiation proficiency [93].

Glutamine is also a major energy substrate for stem cells and can also contribute its carbons to the TCA cycle in support of stem cell function and fate regulation. Case in point, in hematopoietic lineage specification, use of glutaminolysis and nucleotide biosynthesis versus glucose catabolism selects for erythroid versus myeloid fates [95]. Glutaminolysis contributes to early erythroid commitment but is not required for lineage maintenance, suggesting that specific metabolic states enable and initiate differentiation along specific lineages, not simply matching the energetic demands of the lineage destination [95, 96]. Both glucose and glutamine be utilized by ESCs to generate alpha-ketoglutarate – a distinct metabolite-linking metabolism with stemness regulation [97]. Alpha-ketoglutarate serves as a substrate for a number of enzymes, including HIF α prolyl hydroxylase and alpha-ketoglutarate dependent dioxygenases that include Jumonji C-domain-containing histones demethylases and the ten-eleven translocation family of DNA demethylases. A high alpha-ketoglutarate/succinate ratio thus favors demethylation of repressive chromatin marks including H3K9/K23. How these TCA cycle intermediates contribute to stemness acquisition during nuclear reprogramming, where changes in DNA methylation and histone marks are robust, remains unexplored.

3.3.3 Thr/single carbon metabolism—The metabolic state of PSCs is associated with a high requirement for the catabolism of specific amino acids. For example removal of threonine from cell culture media [98] or pharmacologic inhibition of threonine dehydrogenase [99] leads to loss of stemness, cell cycle arrest and cell death in mouse PSCs, while threonine dehydrogenase induction promotes pluripotent induction through nuclear reprogramming [100]. Indeed, threonine dehydrogenase and downstream enzymes in threonine catabolism, including glycine C-acetyltransferase (GCAT) and glycine decarboxylase (GLDC) are highly expressed in mouse PSCs and are quickly suppressed during stem cell differentiation [98, 101]. This pathway is critical for coupling the breakdown of threonine with supplying single carbon equivalents to the folate pool. The folate pool can then donate these carbon equivalents to a number of anabolic pathways including the biosynthesis of purine nucleotides, which is consistent with the observation that DNA synthesis is suppressed when threonine is removed during mouse ESC culture [98]. In addition, threonine catabolism also helps maintains a high SAM to S-adenosylhomocysteine ratio to promote histone 3 lysine 4 methylation, and ultimately support proliferation and self-renewal of mouse PSCs [101]. In an analogous fashion, human PSCs rely directly on methionine catabolism by methionine adenosyltransferase to support the SAM productio as threonine dehydrogenase is only expressed as a non-functional pseudogene in humans [102].

3.3.4 Fatty acid metabolism—Beyond glucose and glutamine, the metabolism of fatty acids can contribute to both catabolic energy generation and anabolic precursor generation.

Although fatty acid oxidation is critical for embryonic development and for specific cell lineages, its role in stem cell biology has been relatively unexplored. Initial work has demonstrated that peroxisome proliferator-activated receptor (PPAR) δ is highly expressed in HSCs and is associated with high rates of fatty acid oxidation, which is critical for maintaining the balance between HSC self-renewal and lineage specification [103]. Indeed genetic depletion of PPAR δ or its upstream regulator promyelocytic leukaemia protein (PML) or pharmacologic inhibition of fatty acid oxidation stimulated the exit of HSCs from quiescence and lead to their symmetric commitment into lineage specified daughter cells. This leads to a short-term expansion of HSCs followed by exhaustion of this compartment, which ultimately impairs the reconstitution capacity of these cells. In contrast, activation of this pathway using PPAR δ agonists promotes asymmetric cell division to support HSC maintenance and self-renewal and was sufficient to rescue the quiescence and maintenance defects in PML^{-/-} cells [103]. Therefore regulation of fatty acid oxidation may play a critical role partitioning cells between self-renewal and lineage specification.

Lipid biosynthesis also appears critical to maintain stem and progenitor cell proliferation in the adult brain [104]. Fatty acid synthase (FAS), the rate-limiting enzyme for fatty acid synthesis, is highly expressed in neural stem/progenitor cells (NSPCs) within active regions of neurogenesis, and endows these cells with a distinct metabolic phenotype in support of de novo lipogenesis and the generation of new lipid membranes required for cell division. Deletion or inhibition of FAS impairs NSPC proliferation resulting in fewer NSPCs in neurogenic zones and less newly formed neurons [104]. Rates of lipid synthesis are dependent both on FAS activity, as well as the generation of its substrate, malonyl-CoA, by acetyl-CoA carboxylase (ACC). Indeed, Mig12-interacting protein (Mig12), an activator of ACC, and Spot14/thyroid hormone responsive protein, which dimerizes with Mig12 to prevent ACC activation, are also critical for regulating neurogenic NSPCs, thus further supporting the importance of de novo lipogenesis. Overexpression of Spot14 impairs NSPCs proliferation, associated with reduced malonyl-CoA levels and impaired lipogenesis from glucose and acetate, however this phenotype can be rescued by overexpressing Mig12 [104]. Therefore Spot14 may ensure neural stem cell quiescence by reducing lipogenesis and placing a metabolism-dependent brake on neurogenesis [105]. Interestingly, malonyl-CoA also regulates long-chain fatty acid β -oxidation by serving as an endogenous inhibitor of carnitine palmitoyl transferase 1 (CPT1), the rate-limiting enzyme for long-chain fatty acid transport into the mitochondria. Therefore by regulating the equilibrium between fatty acid oxidation and lipogenesis, malonyl-CoA may serve as a rheostat of stem cell function and fate [105]. In this context, naïve PSCs oxidize fatty acids as a source of energy, while their primed counterparts do not oxidize fatty acids and shift to fatty acid synthesis [12].

4 Summary

Recent advances in metabolomics have unmasked a fundamental role for intermediary metabolism in securing the fitness of stemness acquisition and maintenance. This underappreciated dimension of metabolism leverages an inherent metabolic plasticity to pre-emptively 'condition' the epigenetic and transcriptional landscape for cell fate conversions and matches the anabolic and catabolic demands of the destination cell fate. In turn, modulation of energy metabolism emerges as a legitimate target to optimize stem cell self-

renewal and lineage-specific differentiation. The impact of metabolism on stem cell function and fate has potential implications across aging and disease, thus this initial dissection of underlying metabolic mechanisms paves the way to a more comprehensive appreciation of stem cell biology with potential applications for regenerative medicine.

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Fig. 1.

Metabolic remodeling of glycolysis and oxidative metabolism fuels acquisition, maintenance and departure from pluripotency. Nuclear reprogramming of somatic cells through induction of reprogramming factors induces dramatic metabolic remodeling, characterized by an initial metabolic burst of both glycolysis and oxidative metabolism, following which oxidative metabolism declines, while glycolysis continues to increase. Conversion between the primed and naïve pluripotent states in human cells is also associated with changes in the metabolic infrastructure in support of a transition from predominantly glycolytic to bivalent metabolism utilizing both glycolysis and oxidative metabolism. In contrast, upon a differentiation stimulus, pluripotent stem cells activate mitochondria biogenesis to generate a robust network of mitochondria in support of oxidative metabolism to match lineage-specific energetic demands.

Fig. 2.

Common intermediary metabolic pathways that proliferating stem cells utilize to match the anabolic demands of cell division. Rapid proliferation places unique demands on the cell, with the requirement for anabolic precursors, such as nucleotides, amino acids and lipids, outpacing that of ATP. Therefore, stem cells extract glycolytic intermediates and repurpose their mitochondria from efficient energy generators to cataplerotic engines, in order to supply hydrocarbons for the generation of cellular biomass. ACP acyl carrier protein, CPT1 carnitine palmitoyl transferas 1, dATP deoxyadenosine triphosphate, dCTP deoxycytidine triphosphate, dGTP deoxyguanosine triphosphate, dTTP deoxythymidine triphosphate, IMP

inosine monophosphate, PRPP 5-phosphoribosyl- α -pyrophosphate, UMP uridine monophosphate, THF tetrahydrofolate.

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