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Concise Review

Metabolic Determinants of Cardiomyocyte Proliferation

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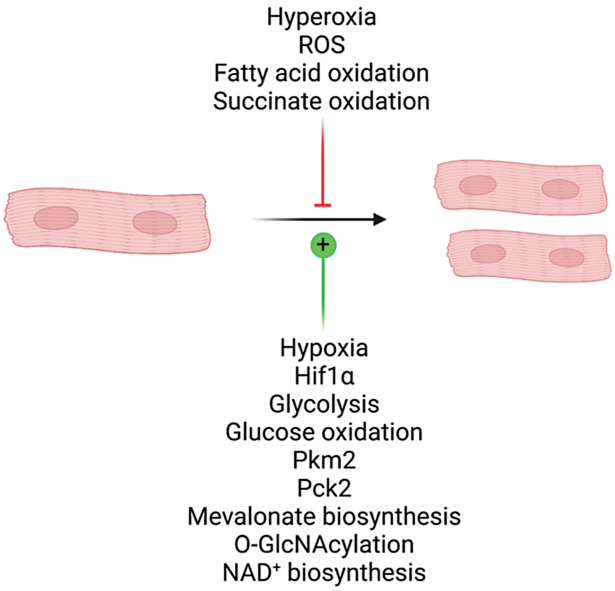
Abstract

The adult mammalian heart is recalcitrant to regeneration after injury, in part due to the postmitotic nature of cardiomyocytes. Accumulating evidence suggests that cardiomyocyte proliferation in fetal or neonatal mammals and in regenerative non-mammalian models depends on a conducive metabolic state. Results from numerous studies in adult hearts indicate that conditions of relatively low fatty acid oxidation, low reactive oxygen species generation, and high glycolysis are required for induction of cardiomyocyte proliferation. Glycolysis appears particularly important because it provides branchpoint metabolites for several biosynthetic pathways that are essential for synthesis of nucleotides and nucleotide sugars, amino acids, and glycerophospholipids, all of which are required for daughter cell formation. In addition, the proliferative cardiomyocyte phenotype is supported in part by relatively low oxygen tensions and through the actions of critical transcription factors, coactivators, and signaling pathways that promote a more glycolytic and proliferative cardiomyocyte phenotype, such as hypoxia inducible factor 1α (Hif1α), Yes-associated protein (Yap), and ErbB2. Interventions that inhibit glycolysis or its integrated biosynthetic pathways almost universally impair cardiomyocyte proliferative capacity. Furthermore, metabolic enzymes that augment biosynthetic capacity such as phosphoenolpyruvate carboxykinase 2 and pyruvate kinase M2 appear to be amplifiers of cardiomyocyte proliferation. Collectively, these studies suggest that acquisition of a glycolytic and biosynthetic metabolic phenotype is a *sine qua non* of cardiomyocyte proliferation. Further knowledge of the regulatory mechanisms that control substrate partitioning to coordinate biosynthesis with energy provision could be leveraged to prompt or augment cardiomyocyte division and to promote cardiac repair.

Graphical Abstract



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Significance Statement

The adult mammalian heart is incapable of complete repair after injury due in part to the postmitotic nature of cardiomyocytes. This review article covers the features of metabolism that are known to influence cardiomyocyte proliferation and cardiac regeneration. The vast majority of studies indicate that a high glycolytic and biosynthetic state is essential for cardiomyocyte proliferation. Expanding our understanding of the role of metabolism in cardiomyocyte proliferation could provide new avenues for maximizing the regenerative potential of the heart.

Introduction

Cardiomyocyte death is the principal cause of heart failure, with myocardial infarction (MI) comprising the primary contributor to loss of functional myocytes. Fortunately, advances in risk factor management have decreased rates of MI and acute MI-related fatalities[1]; however, non-lethal cardiac events and significant growth of the elderly population continue to escalate the burden of heart failure.[2,3] Projections in several countries indicate precipitation of a heart failure epidemic, which comes at the cost of upsurges in morbidity and higher healthcare expenditures.[4-8]

Notwithstanding prevention, cardiac repair presents a solution to this problem. Cardiac repair involves coordinated phases of extracellular matrix remodeling, neovascularization, and cardiomyocyte repopulation. Successful integration of these processes can lead to a completely regenerated heart. In mammals, cardiac regeneration occurs only in the fetal and early neonatal periods of mammals, when a significant loss of cardiomyocytes is countered by veritable heart regeneration; however, the capacity for cardiomyocyte cytokinesis appears to be lost by day 4 after birth. After this time, the cardiac repair response is limited to replacement fibrosis[9,10] as well as modest vascular regeneration and remodeling,[11,12] which are regulated in part by immune cell-mediated mechanisms.[13-15]

Metabolism influences each process involved in myocardial repair and regeneration. Metabolic pathways provide useable energy as well as the building blocks to build, repair, or remodel cells and tissues. Thus, catabolic and anabolic metabolic pathways must be appropriately synchronized to fulfill specific cellular tasks. For cell proliferation, nucleotide biosynthesis meets the demands for mRNA synthesis and DNA synthesis and repair; amino acid biosynthesis supports protein synthesis; and glycerophospholipid biosynthesis provides the structural components for making new membranes. Because metabolites have strong roles in cell signaling, metabolism also supports the informational architecture of cells and tissues. Although such universal roles of metabolism may be inferred to contribute to cardiac repair, it remains unclear how metabolic changes influence myocyte proliferation. The goal of this minireview is to integrate current information on how intermediary metabolism in the cardiomyocyte contributes to proliferation and to identify hurdles to gainful knowledge in the field of cardiac regeneration. For review of the role of metabolism in other cell types found in the heart (eg, fibroblasts, endothelial cells) and in broader aspects related to cardiac metabolism and remodeling, the reader is directed to the following reviews.[16-19]

Metabolic Requirements for Cell Proliferation

Cell division requires biosynthesis of cellular components to form daughter cells. Metabolism and its regulatory networks support this function and also provide useable energy and reducing power to maintain homeostasis and combat stress. Although catabolic processes are important for energy provision to sustain life and fuel work,[20,21] how anabolic pathways influence cellular processes such as cell growth and proliferation is less well understood. Studies in cancer cells suggest that, although ATP is required for homeostasis, additional ATP does not appear to be required to fuel proliferation. Rather, proliferating cells increase biosynthetic pathway activity.[22] The synthesis of nucleotides, nucleotide sugars, amino acids, and phospholipids integrate tightly with the catabolic pathways that provide ATP to fuel cellular work. In particular, the steps of glycolysis are optimally positioned to balance energy yield with enzyme cost, while concomitantly ensuring operability of ancillary branched pathways for biosynthesis.[23] Indeed, the canonical glycolytic pathway appears to be the shortest pathway that ensures both production of precursors for cellular biomass[24] and a high ATP yield capacity[25] (Fig. 1). Remaining unclear are the regulatory mechanisms that control substrate partitioning to coordinate biosynthesis with energy provision to animate cell proliferation.

The networked pathways of metabolism provide conduits that direct nutrients toward multiple fates. In the cell, these biochemical networks obey the second law of thermodynamics and rise to the challenge of partitioning free energy across numerous metabolic steps to meet requirements for biosynthesis and useable energy. Particularly important are reactions that control levels of metabolites that lie at branchpoint sites. Examples of these include glucose 6-phosphate (G6P), fructose 6-phosphate (F6P), dihydroxyacetone phosphate, and 3-phosphoglycerate, which provide precursors for pyruvate or enter ancillary pathways such as the pentose phosphate pathway (PPP), the hexosamine biosynthetic pathway (HBP), the glycerolipid synthesis pathway, and serine synthesis pathway[26]; some of these pathways branch further to direct carbon flow to additional fates. It is worth noting that these ancillary pathways appear to carry significant flux in the heart, given that at least 50% of glucose entering the myocardium and cardiomyocytes is partitioned for glycogen storage or biosynthetic pathways.[27-32] Nevertheless, it remains unclear how cells partition glucose-derived carbon to balance energy demand with biosynthetic need. Although non-equilibrium enzymes in glycolysis such as phosphofructokinase and pyruvate kinase are known to influence carbon fate,[33,34] the formation of metabolic enzyme complexes (eg, metabolons) that foster metabolic channeling also contribute to glucose carbon partitioning.[35-37] Understanding the interplay of these mechanisms and how they contribute to cell growth and proliferation is an exciting goal for the future.

In addition to partitioning of glucose-derived carbon to biosynthetic pathways, proliferating cells appear to use alternative means of driving carbon toward biosynthesis. Beyond glucose, glutamine contributes to core metabolic functions that support proliferation, as do branched chain amino acids, lactate, serine, and glycine, all of which can provide material

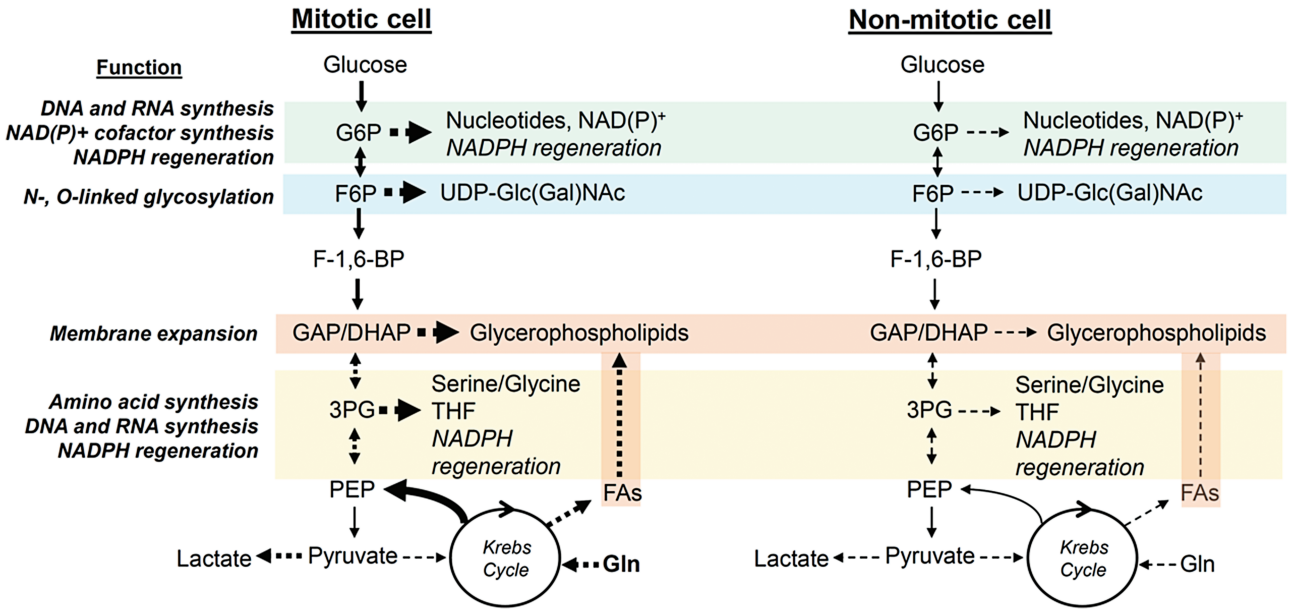

Figure 1. Fundamental metabolic differences in mitotic and non-mitotic cells. To support the demands of proliferation, dividing cells take up more glucose and amino acids such as glutamine, which provide the carbon and nitrogen sources for catabolic processes and biosynthetic reactions. The pentose phosphate pathway (light green) regenerates NADPH, which provides reducing power to biosynthesis and to combat oxidative stress, and branches into nucleotide biosynthetic and NAD(P)+ synthesis pathways. The hexosamine biosynthetic pathway (light blue) produces UDP-Glc(Gal)NAc, which is important for glycosylation reactions that regulate protein folding and for O-GlcNAcylation. Although the glycerol backbone of phospholipids is synthesized via the glycolytic precursor dihydroxacetone phosphate (DHAP) via the glycerolipid synthesis pathway (brown), the fatty acyl chains can be synthesized via carbon engendered in the Krebs cycle. The serine biosynthesis pathway (yellow) is also important for serine and glycine synthesis and the formation of methyl donors such as methylene tetrahydrofolate and for NADPH regeneration. Figure adapted from ref.[130]

resources required for replication.[38,39] Also important to biosynthesis and cell division are key enzymes that link metabolism in the mitochondrial and cytosolic compartments or that influence glucose catabolic rate. For example, the gluconeogenic enzyme, phosphoenolpyruvate carboxykinase 2 (Pck2), is commonly expressed in proliferating cells and drives carbon in the Krebs cycle into the glycolytic pathway, where it can be allocated for biosynthetic reactions.[40,41] Moreover, expression of pyruvate kinase (Pkm) splice variants have been shown to influence cell proliferation. Like Pck2, Pkm2 can help maintain the glycolytic intermediate pool, although this does not occur via the shuttling of carbon from the Krebs cycle; rather, Pkm2 is enzymatically slower than the Pkm1 variant and promotes accumulation of phosphoenolpyruvate and enriches or maintains levels of glycolytic intermediates.[22,42] These intermediates can then be allocated to ancillary biosynthetic pathways, where they contribute to building block synthesis and NADPH regeneration, both of which are required for cell growth and proliferation.[19,22,43]

Metabolism also controls the overall redox state of the cell. Numerous metabolic pathways influence the nicotinamide adenine dinucleotide NAD^+/NADH and nicotinamide adenine dinucleotide phosphate $NADP^+$/NADPH redox couples. Although catabolism couples substrate oxidation to the generation of NADH used to support oxidative phosphorylation, anabolism requires NADPH for synthesis of fatty acid acyl chains, nucleic acids, and cholesterol,[44-46] important for proliferating cells. NADPH also powers redox defenses and maintains protein thiol redox status.[47] NAD^+ is synthesized through the de novo synthesis pathway, the Preiss-Handler pathway and the salvage pathway, utilizing 4 precursor molecules: tryptophan, nicotinic acid, nicotinamide, and nicotinamide riboside. $NADP^+$ is biosynthesized via NAD kinase.[48] In proliferating cells, the oxidative pentose phosphate pathway appears to be the predominant source of cytosolic NADPH, where glucose 6-phosphate dehydrogenase catalyzes conversion of glucose 6-phosphate to 6-phosphogluconate; 6-phosphogluconate dehydrogenase further metabolizes 6-phosphogluconate to ribose 5-phosphate.[49] Also contributing to NADPH levels is serine-driven one-carbon metabolism, where methylene tetrahydrofolate oxidation to 10-formyl tetrahydrofolate is coupled to $NADP^+$ reduction.[50] Other enzymes, such as malic enzyme, glutamate dehydrogenase, nicotinamide nucleotide transhydrogenase and several shuttle systems (eg malate-aspartate, glycerol 3-phosphate, and isocitrate-α-ketoglutarate shuttles), also contribute to NAD(P)/NAD(P)H balance in cells.[48] Nevertheless, it remains unclear how many of these enzymes and their associated pathways influence cell proliferation, especially in cardiomyocytes.

Cardiomyocyte Metabolism in the Fetal Mammalian Heart

Unlike the adult mammalian heart, cardiomyocytes in the fetal heart are capable of avid growth and division,[9,10,51-53] which is associated with a unique metabolic state (Fig. 2). The fetal heart appears to depend mostly on glucose and lactate for energy,[54-56] with lactate both released and taken up by the myocardium.[56] In the growing fetus, the high reliance of the heart on glycolysis is a product of several factors, including but not limited to: a hypoxemic environment[57-60]; lower mitochondrial abundance and disorganized mitochondrial structure compared with adult heart[61,62]; lower fetal circulation of fatty acids and lower fat oxidation capacity of fetal cardiac mitochondria[63,64]; and a relatively low circulatory workload.[52]

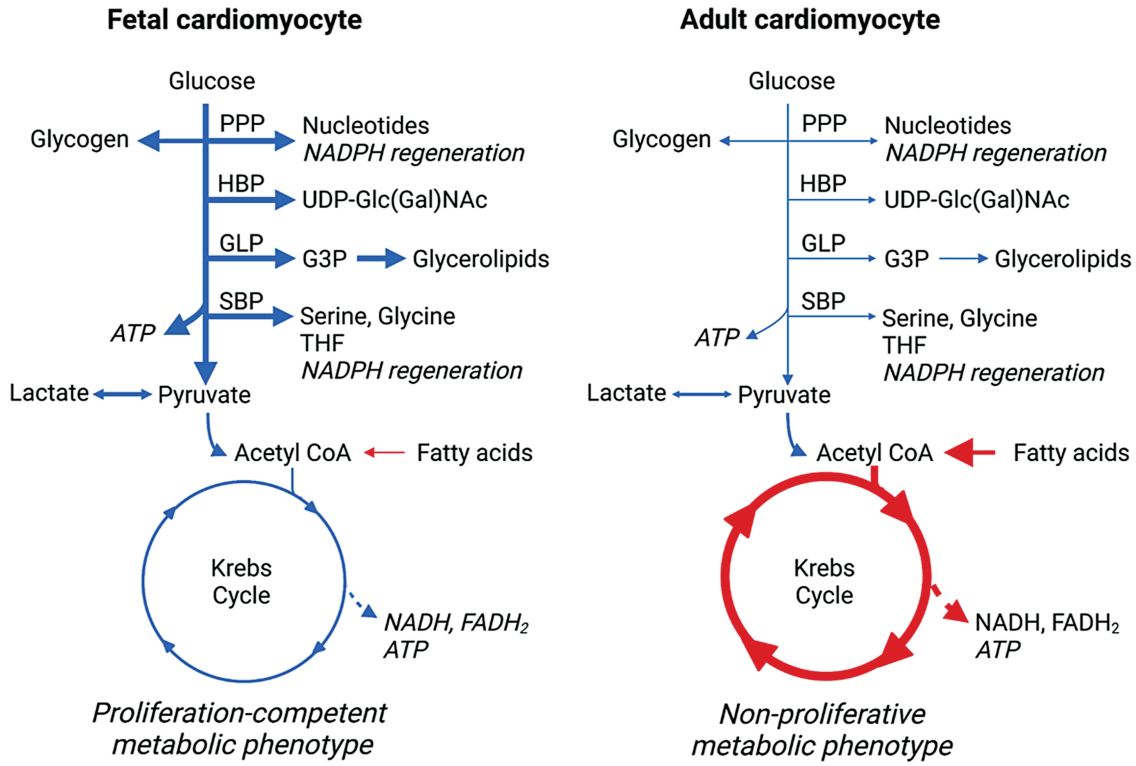

Figure 2. Metabolic phenotypes of fetal and adult cardiomyocytes. Fetal mammalian cardiomyocytes appear more reliant on glycolysis for energy compared with adult myocytes, which supply the majority of their ATP demand by oxidizing fatty acids. In addition, the fetal heart has been suggested to accumulate glycogen to higher levels and have higher pentose phosphate pathway (PPP) activity. Because end products of biosynthetic pathways are required for daughter cell formation, it is also expected that fetal hearts synthesize relatively more nucleotides and have higher hexosamine biosynthetic pathway (HBP), glycerolipid synthesis pathway (GLP), and serine biosynthesis pathway activity (SBP) than the adult heart.

Table 1. Evidence for a role of metabolism in cardiomyocyte proliferation.

Metabolic pathway	Intervention	Highlights	Reference
Glycolysis Glucose oxidation Fatty acid oxidation	Pdk4	Deletion of Pdk4 promotes cardiomyocyte proliferation, decreases DNA damage, improves cardiac repair after MI	77
	Fatty acid availability	Fatty acid deficient milk extends post-birth cardiomyocyte proliferation window	77
	Sdh	Provision of succinate inhibits cardiomyocyte proliferation and cardiac regeneration; Inhibition of Sdh promotes cardiomyocyte cell cycle entry and augments cardiac regeneration after MI	80
	Hypoxia Hif1α	Hypoxia diminishes ROS and promotes cardiomyocyte proliferation and regeneration after MI; Deletion of *Hif1a* downregulates glycolytic genes, decreases cardiomyocyte proliferation, and impairs cardiac development	[62],81-85
	Pkm2	Pkm2 overexpression promotes cardiomyocyte proliferation, improves cardiac function after MI, and activates the pentose phosphate pathway	90
	ErbB2	Activates glycolysis and promotes cardiomyocyte proliferation and cardiac repair	118,126
Anabolic pathways	Simvastatin	Inhibits the mevalonate pathway, which is important for isoprenoid synthesis, and impairs cardiomyocyte proliferation	95
	Oga	Overexpression of OGA, which is associated with the HBP, prevents cardiomyocyte cell cycle entry and progression	100
	Nampt	Knockdown of NAMPT diminishes cardiomyocyte cell cycle entry and progression	100
	Pck2	Overexpression of Pck2 augments 4F-triggered cardiomyocyte proliferation	100

4F, 4 factors, ie, cyclin D1, cyclin B1, Cdk1, and Cdk4; HBP, hexosamine biosynthetic pathway; Hif1α, hypoxia-inducible factor 1α; Nampt, nicotinamide phosphoribosyl transferase; Oga, protein O-GlcNAcase; Pck2, phosphoenolpyruvate carboxykinase 2; Pdk4, pyruvate dehydrogenase kinase 4; Pkm2, pyruvate kinase M2; Sdh, succinate dehydrogenase.

The fetal heart also accumulates glycogen to relatively high levels,[63] and the early neonatal heart has higher levels of glutamine,[65] which influences mTOR activation to help drive cardiac regeneration.

Because tissue accretion requires carbons derived from glucose for making nucleotides, amino acids, and glycerophospholipids, it is expected that the developing heart has high requirements for biosynthetic processes. Interestingly, this high anabolic demand occurs in the setting of progressively higher cardiac work load. In mice, the heart commences contraction at day 8 of gestation (40% of the gestation period), and in humans, contraction begins around day 22 of gestation[66] or even earlier[67] (equating to 8% of the overall gestation period). The heart grows in size during gestation in proportion with rising plasma volume and body weight.[68] Thus, it appears that the fetal mammalian heart has an extraordinary ability to coordinate anabolic processes for growth with energy-providing processes for physical work.

That the fetal and early neonatal heart maintains the ability to regenerate cardiomyocytes[10,51-53] also suggests a high level of metabolic robustness, which refers to the capacity to maintain metabolic homeostasis and tissue function in the face of external or internal perturbations.[69-72] This is likely a consequence of the high reliance of the fetal heart on glycolysis,[73] the steps of which ensure energy provision, while also maintaining sufficient levels of glycolytic precursors for biosynthesis.[23] Beyond the fact that the fetal heart has high PPP activity[74] and high levels of oxidative PPP enzymes,[75] little is known about biosynthetic pathway activity in the fetal heart, the extent to which it differs from the adult heart, and which specific aspects of the fetal heart metabolic configuration contribute to cell cycle entry and exit.

Influence of Metabolic Interventions on Mammalian Cardiomyocyte Proliferation

Although many studies investigating the metabolic requirements of proliferating cells have focused on cancer cell biology, accumulating evidence supports the relevance of metabolic changes to regenerative capacity of the heart (Table 1). Loss of the proliferative capacity of mammalian cardiomyocytes in adulthood is associated with a post-birth switch from predominantly glycolysis to mitochondrial oxidative phosphorylation and the use of fatty acids for energy.[73] Interestingly, feeding fatty acid-deficient milk to neonatal mice was found to extend the post-birth cardiomyocyte cell cycle activity window for up to 14 days after birth, which suggests that free fatty acid availability and fatty acid oxidation could influence the maturation of cardiomyocytes. In support of this, cardiac-specific pyruvate dehydrogenase kinase 4 (Pdk4) deletion (which increases glucose oxidation and could supplant the use of fats for energy[76]) increased cardiomyocyte proliferation.[77] It is possible that increasing glucose oxidation and inhibiting fatty acid oxidation promotes a phenotype amenable to proliferation by decreasing reactive oxygen species (ROS) production.[78,79]

A recent study highlighted the role of succinate in regulating cardiomyocyte cell cycle entry. Injection of succinate in neonatal mice for 7 days after MI was shown to inhibit cardiomyocyte proliferation and cardiac regeneration. Furthermore, injection of malonate, an inhibitor of succinate dehydrogenase, was shown to extend the proliferation window of neonatal cardiomyocytes. Daily injections of malonate in adult mice promoted adult cardiomyocyte cell cycle re-entry, revascularization, and heart regeneration after MI.[80] These changes were associated with metabolic reprogramming, leading to higher levels of some glycolytic and pentose phosphate pathway intermediates. These changes are interesting in light of the fact that glycolytic intermediates are used for several biosynthetic pathway products, which are required for daughter cell formation.

Hypoxia and its associated changes in metabolism also influence cardiomyocyte division and have been shown to contribute directly to the cell cycle. The relatively low blood oxygenation level of the fetal environment is conducive to cardiomyocyte proliferation, as the transition to the oxygen-rich postnatal environment has been suggested to promote reactive oxygen species (ROS) generation and cardiomyocyte cell cycle arrest, leading to loss of regenerative potential.[62] Moreover, exposure of adult mice to hypoxia diminishes ROS and allows cardiomyocyte cell cycle reentry and cardiac regeneration after MI.[81] Concordant with these findings are studies showing that hypoxia promotes proliferation of cardiomyocytes in culture[82,83] and in human cardiomyocytes in vitro and in vivo.[83] Thus, the processes of metabolism influenced by hypoxia are likely important in cardiac regeneration.

Hypoxia-inducible factor 1α (Hif1α) is stabilized by low oxygen environments and promotes a metabolic phenotype amenable to cardiomyocyte proliferation. *Hif1a* is expressed early in embryonic stages, and *Hif1a* deletion in mice promotes death at E10.5, which manifests as a defect in cardiac development.[84,85] Moreover, deletion of *Hif1a* downregulates glycolytic metabolism genes and decreases fetal cardiomyocyte proliferation.[86] Interestingly, a rare population of cardiomyocytes in the adult heart has been shown to have stable *Hif1a* expression and to contribute to basal cardiomyocyte turnover.[87] Intersecting with Hif1α is the transcription factor Meis1, which increases in the heart after birth and augments the expression of several cyclin-dependent kinase inhibitors (eg, p15, p16, and p21), leading to cardiomyocyte cell cycle arrest.[88] Because Meis1 influences expression of Hif1α-regulated genes,[89] it is likely that it also plays a key role in regulating levels of metabolic enzymes conducive to cardiomyocyte proliferation.

Similar to its effect in cancer cells,[22,42] the glycolytic enzyme Pkm2 has also been shown to influence cardiomyocyte proliferation. Interestingly, Pkm2 is expressed in cardiomyocytes during development and immediately after birth, but is lost in the transition to adulthood,[90] thereby paralleling loss of cardiomyocyte proliferative capacity. Cardiomyocyte-specific Pkm2 deletion during development was found to reduce cardiomyocyte cell number and diminish markers of the cell cycle. Gain-of-function studies suggest that cardiomyocyte-specific Pkm2 overexpression in models of MI promote cardiomyocyte cell division and significantly improve cardiac function and long-term survival. Interestingly, Pkm2 upregulation in cardiomyocytes was shown to activate the PPP and lower oxidative stress.[90] Conversely, a separate study suggested an antiproliferative function for Pkm2 in cardiomyocytes after MI.[91] The reasons for discrepancies between these studies remain unclear; however, it is interesting that both studies found an interaction between Pkm2 and β-catenin in regulating cardiomyocyte cell cycle entry,[90,91] especially in light of the importance of β-catenin to energy metabolism in cardiomyocytes.[92] The interaction between

Pkm2 and β-catenin is also required for recruitment of both proteins to the promoter of the cyclin D1 gene,[93] suggesting non-metabolic roles for Pkm2 in activation of the cell cycle as well. Of interest, Pkm2 also functions as a transcriptional co-activator by interacting directly with Hif1α,[94] highlighting feedback loops between metabolism and the transcriptional machinery.

High-throughput screening has been useful for understanding other ways that metabolism contributes to mammalian cardiomyocyte proliferation. In an effort to find a core proliferation program, screening in a human cardiac organoid platform revealed small molecules with pro-proliferative actions that activated the mevalonate pathway and the cell cycle network.[95] The mevalonate pathway is an anabolic pathway that converts acetyl-CoA into isopentenyl pyrophosphate, the essential building block of all isoprenoids, important for cholesterol synthesis and as precursors for protein prenylation.[95] The pathway is known to be important for proliferation and to affect the G1 and S phases of the cell cycle.[96,97] Indeed, the pro-proliferative effect of the small molecules was inhibited by simvastatin, which inhibits 3-hydroxy-3-methylglutaryl coenzyme A reductase, an essential enzyme in the mevalonate pathway.[95]

Similarly, another screening strategy identified a cocktail of cell cycle genes that, once expressed, activate the cardiomyocyte cell cycle in 15%-20% of adult rodent cardiomyocytes as well as in human iPSC cardiomyocytes and to promote cardiac regeneration in mice after MI.[98] Cell cycle induction using 4F also promoted cytokinesis in vivo, as indicated using fate-mapping MADM mice.[98] In a trial for preclinical application of 4F, Abouleisa et al cloned a polycistronic non-integrating lentivirus encoding 4F in which each factor is driven by the TNNT2 promotor (TNNT2-4F-NIL), leading to transient and specific activation of the cardiomyocyte cell cycle. Intramyocardial injection of TNNT2-4F-NIL improved systolic function and reduced scar size after ischemia-reperfusion in rats and pigs compared with respective controls.[99] Interestingly, transduction of the heart or human iPSC cardiomyocytes with 4F remarkably decreases expression of several genes important in fatty acid oxidation and increases expression of genes required for nucleotide, phospholipid, and amino acid biosynthesis, suggesting a shift from catabolic to anabolic pathway activity.[98,100] Indeed, respiration studies and stable isotope metabolomics revealed that 4F decreases mitochondrial oxidative phosphorylation and promotes partitioning of glucose-derived carbon to ancillary biosynthetic pathways of glucose metabolism, including NAD^+, hexosamine, phospholipid, and serine biosynthetic pathways. Furthermore, pharmacological or genetic interventions that diminish NAD^+ synthesis, serine synthesis, or protein O-GlcNAcylation decreased 4F-mediated cell cycle entry. Interestingly, overexpression of Pck2, which is known to support biosynthetic pathway activity in other cell types,[40,41] further augmented 4F-initiated cardiomyocyte proliferation.[100] Because Pck2 and Pkm2 have both been shown to augment biosynthesis and to promote cardiomyocyte proliferation, it is possible that they work in tandem through the phosphoenolpyruvate cycle[101-103] to coordinate metabolism to meet the demands of proliferating cells. Collectively, these findings suggest that induction of the cell cycle is associated with acquisition of a biosynthetic metabolic phenotype, sourced largely by glycolytic intermediates.

Evidence for a Role of Metabolism in Non-Mammalian Regenerative Models

Numerous non-mammalian species have been used as models of cardiac regeneration. Lower vertebrates, such as zebrafish and newts, regenerate their hearts through a coordinated process of cardiomyocyte dedifferentiation, proliferation, migration, and redifferentiation.[119-121] In studies that have examined metabolism, glycolysis has been identified as a metabolic pathway critical to the proliferative cardiomyocyte response to injury. Single-cell RNA sequencing approaches showed that proliferating cardiomyocytes in the border zone of the cryoinjured zebrafish heart have a distinct transcriptome characterized by downregulated mitochondrial genes and upregulated glycolytic genes.[118] This metabolic reprogramming to a glycolytic phenotype was found to be required for regeneration and is triggered by neuregulin 1/ErbB2 signaling,[118] which is known to be important in mammalian cardiomyocyte regeneration.[105,117,122-124] Interestingly, ErbB2 was found in zebrafish to activate glycolysis and promote trabeculation, and loss of Pkm2 impaired trabeculation.[125] Similarly, another study showed that transcripts for enzymes in glucose metabolism, eg, pyruvate kinase and pyruvate dehydrogenase kinase, are increased in zebrafish heart tissue bordering the damaged area and that inhibition of glycolysis decreases cardiomyocyte proliferation following injury.[126] Less metabolic information is available from other non-mammalian models; however, in newts, a hedgehog-triggered network of genes regulate heart regeneration, characterized in part by upregulation of the *Mycn* gene,[127] which is known to promote glycolysis.[128] Collectively, these data suggest that a

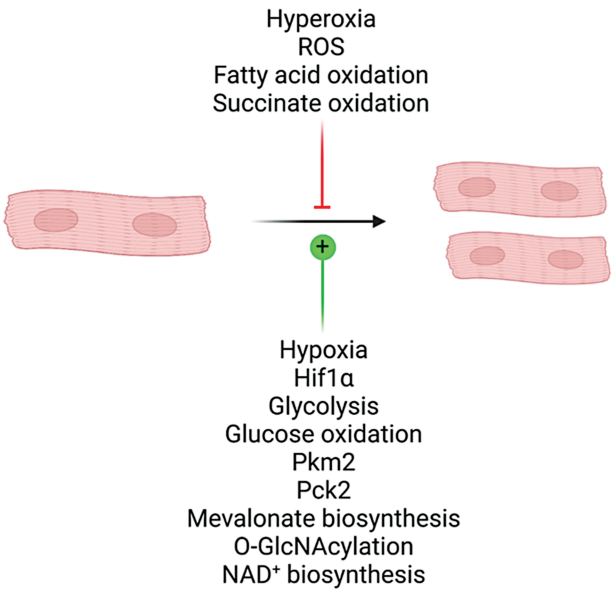

Figure 3. Working model displaying current knowledge of the influences of metabolism in cardiomyocyte proliferation. Conditions of high oxygen tension, high reactive oxygen species (ROS) levels, and high rates of fatty acid and succinate oxidation impede cardiomyocyte proliferation. Conversely, hypoxia and associated upregulation of Hif1α support high rates of glycolysis, which appears to be required for myocyte division. Enzymes that can support biosynthetic pathways such as pyruvate kinase M2 (Pkm2) and phosphoenolpyruvate carboxykinase 2 (Pck2) have been shown to augment cardiomyocyte proliferative capacity. Other processes related to biosynthesis, such as the mevalonate pathway, protein O-GlcNAcylation, and NAD^+ biosynthesis also appear to support a proliferative cardiomyocyte phenotype.

switch to a more glycolytic phenotype is a fundamental feature of cardiomyocyte proliferation. That even more fundamental models of tissue regeneration (eg, planarians) show the same glycolytic signature during tissue regeneration[129] suggests that changes in glycolysis, and likely the biosynthetic pathways it sources, are generalizable features of regenerating tissue.

Summary and Future Directions

The collective results from studies in models of cardiac regeneration indicate that conditions of relatively low fat oxidation, low ROS production, and high glycolysis are conducive for cardiomyocyte division. Although this metabolic phenotype appears to be evident in fetal and neonatal mammals, it is lost soon after birth. The proliferative cardiomyocyte phenotype is upheld physiologically in part by relatively low oxygen tensions and through the actions of critical transcription factors such as Hif1α and the Hippo-Yap and Nrg1-ErbB2 pathways, all of which remodel metabolism to a more glycolytic state. Because cellular building blocks (eg, nucleotides, amino acids, phospholipids, cholesterol) are required for the production of daughter cells, it is not surprising that biosynthetic pathway activation is also required for cardiomyocyte proliferation (Fig. 3).

Although the chemical logic of glycolysis provides branchpoint metabolites for several biosynthetic pathways, it remains unclear how proliferating cells partition substrate-derived carbon for material growth versus energy provision. Addressing this knowledge gap requires understanding how distinct metabolic enzymes coordinate the biosynthetic and transcriptional programs required for cardiac regeneration. Also required is fundamental knowledge of how biosynthetic pathways change during mitotic stages of heart development and whether poorly understood processes such as metabolic channeling influence cardiomyocyte proliferation. Harnessing such knowledge could provide the means to augment regenerative responses in the injured heart.

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Conflict of Interest

T.M.A.M. declared patent holder and stock ownership with Tenaya Therapuetics. The other authors indicated no financial relationships.

Author Contributions

R.A.: conception and design, manuscript writing, final approval of manuscript; T.M.A.M., B.G.H.: conception and design, financial support, manuscript writing, final approval of manuscript.

Data Availability

No new data were generated or analyzed in support of this research.

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