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Metabolic Remodeling in early development and cardiomyocyte maturation

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Abstract

Aberrations in metabolism contribute to a large number of diseases, such as diabetes, obesity, cancer, and cardiovascular diseases, that have a substantial impact on the mortality rates and quality of life worldwide. However, the mechanisms leading to these changes in metabolic state – and whether they are conserved between diseases – is not well understood. Changes in metabolism similar to those seen in pathological conditions are observed during normal development in a number of different cell types. This provides hope that understanding the mechanism of these metabolic switches in normal development may provide useful insight in correcting them in pathological cases. Here, we focus on the metabolic remodeling observed both in early stage embryonic stem cells and during the maturation of cardiomyocytes.

Keywords

metabolism; developmental transitions; hESC; iPSC; naive to prime transition; cardiomyocyte; fetal to adult maturation

Metabolism and cell fate

Metabolic signatures are highly characteristic for a cell and may contribute to the fate of the cell [1–8]. The recent demonstrations that mitochondria, redox state and metabolic intermediates can profoundly affect transcriptional programs and, thereby, cell fates show

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the regulatory function of cellular metabolism. Furthermore, some metabolites are shown to act as ligands in cellular signaling pathways [9]. The nematode *C. elegans* exhibits specialized intermediary metabolism in long-lived mutants and Dauer states, involving glycolytic, glyoxyate, branched chain amino acid, and fumarate metabolism [10–12]. In mammalian systems, redox state and hypoxia regulate self-renewal and mesoderm specification [13–16], while T lymphocyte differentiation requires the bioenergetic sensor pathway involving LKB1 and AMPK [17,18]. The signals arising from metabolic programs include redox and reactive oxygen species, as well as metabolic intermediates [19–22], allowing for a wide variety signaling mechanisms involved in the regulation and the effect of metabolic signatures in cell fate and maturation. While the alterations in metabolism underlie key developmental transitions in a number of different cell types, in this review, we will discuss the metabolic changes observed in the naïve to primed transition in embryonic stem cells and the maturation of cardiomyocytes from fetal to adult stage [7,22,57].

Naïve-to-primed ESC transition

Pluripotent stem cells – mouse and human embryonic stem cells (mESCs and hESCs, respectively) or induced pluripotent stem cells (iPSCs) – demonstrate the remarkable capacity to remain unspecialized in culture. Depending on chemical cues received from their environment and their internal genetic programs, they can also differentiate into a number of different cellular lineages. The recently stabilized naïve and primed hESCs are biologically equivalent *in vitro* representations of pre- or post-implantation embryos, respectively, and, hence, form excellent models for studying early normal and pathological developmental processes. The naïve hESCs have more developmental potential than primed ESCs [24,25] and are marked by significantly lower H3K27me3 levels compared to primed hESCs. While, the molecular mechanisms that affect the transition between these two states are incompletely understood, recent studies have revealed that metabolic signatures of these stages play an important role in their fate.

Pluripotent stem cells are thought to acquire specific metabolic signatures important for stemness through common means. Since pluripotency does not represent a single defined state, subtle stages of pluripotency provide an experimental system for studying potential regulators of the developmental capacity of human and mouse ESCs [24–32]. Both naïve and primed stem cells have been derived in mouse as well as in humans, albeit with some heterogeneity among the populations. We and others have shown that the earlier developmental stage, naïve ESC has highly active mitochondria, but the naïve to primed ESC transition accompanies a dramatic metabolic switch from bivalent to highly glycolytic state both in mouse and in human [22,28,31]. Importantly, we have shown that a gene expression signature indicative of the metabolic switch is observed in mouse *in vivo* inner cell mass (ICM) to post-implantation embryonic cells [31]. The unique metabolic signature of each pluripotent stage led us to postulate that the downregulation of the electron transport chain (ETC) in the epiblast stage must have a tremendous beneficial value for the pluripotent cell population. While reduction of complex IV (cytochrome c oxidase) activity was previously shown to associate with pathological cases, the developing pluripotent stem cell can harness this reduction to its benefit, possibly to protect its pluripotent state against oxidative stress [5].

Similar reduction of mitochondrial activity is observed in the context of cancer in the Warburg effect. The Warburg effect, increased glycolysis in cancer cells, leads to increased metabolic flux of glucose carbons into biosynthetic precursors. This is thought to be beneficial for fueling anabolic processes and control of redox potential and reactive oxygen species (ROS) that are required for rapid tumor cell growth and division. The developmental suppression of oxidative phosphorylation in post-implantation Epiblast stem cells (EpiSCs)/hESCs may serve a similar function in preparation for embryonic growth and the formation of germ cell layers. However, in normal development, this state of low mitochondrial activity is exceedingly transient, since the primed state of inert mitochondria rapidly changes to highly respiring mitochondria when cells begin to differentiate. It is not yet understood how and why the primed, post-implantation stage pluripotent cells enter the highly glycolytic, metabolically cancer-like stage and how a differentiating cell leaves this stage.

Recent data show that metabolites may play a more significant role in regulating embryonic stem cell fate than previously appreciated. In mouse embryonic stem cells, threonine and S-adenosyl methionine (SAM) metabolism are coupled resulting in regulation of histone methylation marks [33]. Methionine and SAM are also required for the self-renewal of hESCs, since depletion of SAM leads to reduced H3K4me3 marks and defects in maintenance of the hESC state [34]. SAM, therefore, is a key regulator for maintaining the ESC undifferentiated state and regulating their differentiation. We have shown that SAM levels, controlled by Nicotinamide N-Methyltransferase (NNMT), are also critical during the naïve-to-primed hESC transition, where the epigenetic landscape changes through increased H3K27me3 repressive marks [22]. NNMT consumes SAM in naïve cells, making it unavailable for histone methylation. Histone methylation (H3K27me3) further regulates the key signaling pathways important for the metabolic changes that are necessary for early human development. However, while NNMT regulates the substrate levels for Polycomb repressive complex, the regulators for positional methylation have not yet been identified. Differential metabolites between pluripotent stages may control epigenetic dynamics and signaling.

Hypoxia and HIF in stem cell acquisition

The emerging role of hypoxia and the hypoxia-inducible factors (HIFs) in the acquisition of stemness is an example of metabolic context in cell fate and its impact on pathological conditions [13, 14, 31,33, 34]. We have shown that hypoxia can induce the reversal of the early steps in human ESC differentiation [13]. In tumors, aggressive cancer cells display gene expression signatures characteristic of ESCs and are commonly exposed to hypoxic environments. These two processes may be mechanistically linked via HIFs. Hypoxia, through HIFs, can induce a human embryonic stem cell-like transcriptional program in cancer cells [36]. Furthermore, HIF is required for acquisition of pluripotency, early during reprogramming of somatic cells to induced pluripotent stem cells [14,35]. HIFs reprogram cellular metabolism, affecting substrate and energy utilization, redox state and mitochondrial TCA cycle flux. As metabolic states can be propagated once the initial conditions for their establishment change, the metabolic stability is associated with maintenance of stemness under normoxic conditions [31].

We have shown that stem cells acquire a common characteristic metabolic signature through exposure to stabilized HIF activity [14,31,36,37]. Further, this metabolic signature may be determinative for stemness. The dependency of stem cells on glycolysis to produce ATP could be an adaptation to low oxygen tensions *in vivo* since hypoxia is a key feature of the stem cell niche [38]. Further, low oxygen levels are beneficial for hESC, adult stem cells [39–43] and cancer cells [36,38]. HIF1 α and HIF2 α are stabilized in low oxygen, dimerize with HIF1 β and control the transcription of multiple target genes, including genes involved in glucose metabolism [44,45]. Human iPSC are usually reprogrammed from somatic cells to a primed pluripotent stage and hence are very similar metabolically to hESC [2,46,47]. Therefore, a metabolic switch from oxidative to highly glycolytic needs to take place during iPSC formation. Supporting this idea, inhibition of glycolysis reduces the reprogramming efficiency while stimulation of glycolytic activity enhances iPSC generation [1,2,48]. This metabolic switch occurs very early in the reprogramming process and requires HIF1 and HIF2 proteins, each independently and stage-specifically [14,35].

Metabolism during cardiomyocyte differentiation and maturation

Cultured pluripotent stem cells also provide a powerful model for studying the role of metabolism in the maturation of differentiated cells, such as cardiomyocytes. Well established protocols allow for the reliable differentiation of both hESCs and iPSCs into cardiomyocytes. These cells closely resemble embryonic cardiomyocytes, in that they beat spontaneously, display sarcomeric proteins, and have calcium transients that closely resemble cardiac action potentials [23]. Human pluripotent stem cell derived cardiomyocytes (hPSC-CMs) have high therapeutic potential. However, the long maturation period of hPSC-CMs poses a substantial roadblock for the development of any prospective therapies. Thus, knowledge of and the ability to manipulate the mechanisms that regulate cardiomyocyte development to accelerate cardiomyocyte maturation are valuable research targets. Recent work demonstrates that changes in cardiomyocyte metabolism are a vital component in the regulation of both cardiomyocyte differentiation and maturation.

Mature cardiomyocytes consume an exorbitant amount of ATP to meet the high metabolic demands placed on these cells by continuous beating [49]. However, they have an extremely limited ability to store high energy phosphates, requiring efficient ATP production machinery, in order to continually fuel the cell [50]. They, therefore, undergo several changes in metabolic processes to increase their metabolic capacity as they age. One of the most significant metabolic changes in cardiomyocytes is the transition from anaerobic to oxidative metabolism [51]. The fetal heart is adapted to a low oxygen environment where levels of circulating fatty acids are low, fetal cardiomyocytes are highly dependent on glycolysis to produce ATP [50]. Because lactate is also plentiful in the fetal heart, fetal tissue will also produce a large part of its ATP via lactate oxidation. In the neonatal heart, though, the metabolic profile of cardiomyocytes undergoes a substantial shift. After birth, there is an increased workload placed on the heart, as well as a period of massive cellular growth. As such, the metabolic demands placed on the cardiomyocytes increase greatly, outstripping the cells' ability to keep pace via glucose and lactate oxidation alone [52]. There is also a concomitant increase in circulating levels of free fatty acids. This, combined with transcriptional regulation of proteins involved in fatty acid oxidation, mediates a switch in

the metabolic profile of cardiomyocytes from being glycolysis-dependent as immature cardiomyocytes to predominately relying on oxidative metabolism as mature cardiomyocytes [53]. While immediately after birth, over half of the ATP in cardiomyocytes is produced via glycolysis, by seven days after birth, glycolysis decreases, accounting for less than 10% of ATP production [50]. As lactate levels fall during development, cardiac tissue also relies less on lactate oxidation, resulting in a tissue that utilizes fatty acids as its primary fuel source [53].

Changes in metabolic substrates are coupled with mitochondrial reorganization in order to more efficiently power beating cardiomyocytes. As cardiomyocytes mature, the number of mitochondria in each cell increases [54,55]. Furthermore, mature cardiomyocytes have mitochondria that are more uniform in size and that have cristae that are more densely packed than young cardiomyocytes.

This switch in metabolic substrate and reorganization is a key component of cardiomyocyte development. When respiratory chain function is disrupted during cardiac development, it compromises the ability of cardiomyocytes to reorganize their mitochondria and switch their metabolic profiles, resulting in impaired contractile machinery [56]. This suggests that the switch in metabolic profile is not only an accompanying phenomenon during cardiomyocyte maturation, but also a potential regulator of cardiomyocyte maturation. Thus, understanding maturational mechanisms that also contribute to changes in metabolic profile may yield important insight into entry points in the control of cardiomyocyte maturation.

Recent work has identified a number of factors that regulate cardiomyocyte metabolic maturation, acting both inter- and intracellularly. We have shown that the let-7 family of microRNAs work endogenously in cardiomyocytes to accelerate maturation, largely through a significant impact on cardiomyocyte metabolism [7]. When comparing the microRNA profile of immature and mature cardiomyocytes, the let-7 family of microRNAs is the most highly upregulated. Furthermore, inhibition of let-7 family members by antimirs or the let-7 family via the overexpression of Lin28 led to decreased maturational markers, while overexpression of let-7 increases maturational markers. This included an increase in respiratory capacity and an increase in the efficiency of using palmitate as an energy source. The let-7 miRNA family targets genes are heavily involved in the PI3/AKT/insulin pathway. This suggests that the let-7 family of microRNAs may act as regulators of cardiomyocyte maturation by directly controlling the developmental switch in metabolic profile.

A number of exogenous signals have also been shown to regulate cardiomyocyte development [55,56, 57]. One such example is thyroid hormone Tri-iodo-L-thyronine (T3). Circulating thyroid hormones are known to be essential for a number of heart processes, including the regulation of other developmental switches that are required for cardiomyocyte maturation from fetal to adult [55, 57]. Treatment of cardiomyocytes with T3 led to an increase in a number of maturational markers, including cell size, calcium handling, and contractile kinetics [59]. Importantly, T3 treatment also led to an increase in both basal and maximum respiratory rate, demonstrating that treated cells adapt better to increased energy demands. Again, this suggests that regulation of cardiomyocyte metabolic profile is a key component of being able to manipulate cardiomyocyte maturation.

Cardiac metabolism and disease

Cardiac hypertrophy is the increase of cardiomyocyte size in response to an increase in biomechanical stress that results in an overall increase in the size of the heart [60,61]. This increased biomechanical stress can be either extrinsic, caused by elevated blood pressure or valvular heart disease, or intrinsic, caused by inherited heart defects, cardiomyopathies. The resultant hypertrophy works to normalize the augmented wall tension in the myocardium due to the increased biomechanical stress being applied.

While hypertrophy is considered an adaptive response to increased cardiac stress, prolonged hypertrophy is associated with increased risk for sudden death or progression to heart failure [61]. During the early stages of heart failure, several adaptive responses manifest, including the activation of the renin-angiotensin system, increased sympathetic nervous system activity, and cardiomyocyte hypertrophy. Initially, these compensatory mechanisms ensure the heart is able to perform its necessary function. However, these same mechanisms eventually result in the pathological remodeling of the left ventricle, as evidenced by increased left ventricle chamber volume, wall thinning, and myocardial fibrosis [62].

One of the hallmarks of pathological hypertrophy and heart failure is a re-expression of the fetal gene program. Common fetal genes that are upregulated include: atrial natriuretic factor (ANF), brain natriuretic peptide (BNP), and α -myosin heavy chain (α MHC). From a metabolic standpoint, there is also a reversion to a fetal-like metabolic state. However, it is not a complete reversion. As previously discussed, the fetal and neonatal heart is reliant on glycolysis as a source of energy due to the increased activity of glycolytic pathway associated enzymes [63,64] and altered pathway regulation in fetal hearts [65,66]. Following birth, there is a 10-fold increase in fatty acid oxidation along with a decrease in glycolytic rates [67]. In some forms of heart failure, this pattern reverses itself and there is a reversion of metabolic state from one that relies on fatty acids back to one that has increased glucose uptake and glycolysis [68,69] with either no change or a decrease in glucose oxidation [53,70].

Another interesting metabolic switch during heart failure is the activation of fructose metabolism. During heart failure there is an increased demand for oxygen due to changes in coronary resistance, cardiac vascularization, and perfusion. The hypoxic environment of the myocardium activates HIF1 α , which, in turn, activates transcriptional programs to alleviate the low oxygen environment, such as myocardial angiogenesis, cardiomyocyte growth, and reprogramming of metabolism [71]. However, in the case of continued pathological stress, this compensatory mechanism of HIF1 α 's ability to rescue the hypoxic environment becomes detrimental. Specifically, it was shown that HIF1 α activation of splice factor 3b subunit 1 (SF3B1) mediated splicing of the central fructose-metabolising enzyme ketohexokinase (KHK) pre-mRNA, resulting in the expression of KHK-C (instead of KHK-A) which exacerbates the pathological cardiac response [72].

There are a number of metabolic based cardiomyopathies that result in disrupted metabolism [73]. With the advent of iPSC technology, we can now study human patient specific diseases to better understand the disease using a human model and elucidate novel therapies. This

iPSC technology has been recently harnessed to provide closer examination of two cardiac metabolic diseases: Barth syndrome and diabetic cardiomyopathy.

Barth syndrome, an x-linked cardiac and skeletal myopathy, is the result of a mutation in the gene tafazzin [74]. Tafazzin is the gene responsible for producing the enzyme acyltransferase. When acyltransferase has reduced function, the normal acylation of cardiolipin, the major phospholipid of the mitochondrial inner membrane [75], is decreased and leads to mature cardiolipin depletion [76]. Using a combination of patient derived cardiomyocytes and Cas9-mediated genome editing, it was shown that a mutation in tafazzin was sufficient to recapitulate the patient disease phenotype in wild type cells. Furthermore, it was shown that the contractile deficit found in the Barth syndrome hiPSC-CMs was not a result of global cellular energy depletion, but rather tafazzin deficiency resulted in impaired sarcomere assembly and contractile stress generation. However, tafazzin deficiency did lead to increased ROS production, while the suppression of ROS ameliorates the metabolic, sarcomerogenesis and contractile phenotypes in Barth syndrome hiPSC-CMs [76], demonstrating the underlying role of metabolic regulation in this disease.

Diabetic cardiomyopathy (DCM), a complication of type 2 diabetes mellitus (T2DM), results in dilated cardiomyopathy and heart failure [77]. T2DM induces a pathological metabolic state in cardiomyocytes that is different from non-diabetic complicated heart failure. As previously discussed, heart failure results in a reversion of cardiomyocytes from fatty acid β -oxidation to a fetal like profile that has increased reliance on glucose oxidation. However, since there is insulin resistance in T2DM myocardium, T2DM instead promotes fatty acid β -oxidation in cardiomyocytes with pathological consequences. The three main perturbations are: 1) decreased ATP per O₂ produced during fatty acid β -oxidation, 2) accumulation of toxic lipid metabolites, and 3) mitochondrial dysfunction and ROS production that elevate proteases which results in cleaved myofilament proteins. Drawnel *et al.* generated two hiPSC lines from patients of different clinical histories to best test the likelihood that genetic/epigenetic predisposition would affect phenotype. A fast progression (FP) patient that showed cardiovascular disease within 5 years of initial diabetes diagnosis and a slow progression (SP) with no cardiovascular disease despite 15 years of T2DM were generated [78].

The authors showed that their *in vitro* diagnosis model of DCM was able to distinguish differences in disease severity between the FP and SP hiPSC-CMs. They were also able to use this platform to investigate novel therapeutics that may rescue the DCM phenotype. Many traditional pathways common to treating diabetic cardiac dysfunction, such as molecules that prevent calcium entry, reduce calcium in the sarcoplasmic reticulum, or inhibit calcium-regulated proteins, were identified by a drug screen. Additionally, a novel pathway of DCM rescue was identified: small molecules related to protein synthesis inhibition. It is known that DCM cardiomyocytes experience lipotoxicity and lipid peroxidation, which suggests the activation of proteolytic enzymes, promotion of ER stress, and leakage of calcium from the sarcoplasmic reticulum [79]. Since the promotion of ER stress leads to an increase in the unfolded protein response to increase cellular resilience, the authors postulated that using inhibitors of protein synthesis prevents accumulation of

nascent unfolded proteins and reduces load on the ER. As a result, protein synthesis inhibition would protect the cell from further loss of sarcomeric integrity [78].

Metabolic similarities between naïve-to-primed hESC transition and cardiomyocyte maturation

Inspired by the fact that metabolic changes are critical components of both the naïve to primed transition for ESCs and the fetal to adult transition in cardiomyocytes, we asked whether we could identify any common mechanisms between the two cell types.

In a recent study [22], we performed principal component analysis (PCA) on RNA-seq samples of different naïve and primed hESC lines from several published studies using all genes. We found that naïve and primed hESC lines form two distinct clusters (Figure 1A and supplemental table 1). The genes that contribute to the naïve versus primed separation are plotted in Figure 1B. Since metabolic transformation is a well-known component of the naïve to primed transition, we performed PCA on the same samples using only metabolic genes (metabolic enzymes and transporters [80], supplemental table 2), and found that naïve and primed samples cluster the same way as in Figure 1A. This suggests that in early development, during the metabolic reorganization, metabolic genes are critical contributors in naïve versus primed hESC differences. The top metabolic genes that contribute to the naïve versus primed separation along the first principal component (x-axis) are plotted in Figure 1C (see also Table 1 and supplemental table 2 for more genes).

We performed the same PCA analysis on RNA-seq samples of human adult and fetal heart samples, as well as 20-day and 1-year old cardiomyocyte derived from H7 hESC using all genes (Figure 2A) and found that these samples are clearly separated by their degree of maturity, as seen along the first principal component (x-axis), from right (least mature) to left (most mature). Interestingly, the top genes contributing to the separation of fetal versus adult samples include many metabolic genes in lipid and fatty acid metabolism and oxidative phosphorylation (Figure 2B and supplemental table 3, e.g., LPL, PLA2G2A, FABP4, PDK4, COX6A2, COX7A1). Motivated by this observation, we used only metabolic genes for a PCA of the same samples and found that these cardiac-related samples were still well separated based on their degree of maturity, similar to Figure 2A. The top metabolic genes that contribute to fetal vs adult cardiomyocyte separation along the first principle component are plotted in Figure 2C. This clearly demonstrates that the expression of metabolic genes (Figure 2C, Table 1 and supplemental table 4), and thus cellular metabolic states, is a biomarker of cardiomyocyte maturity.

Table 1 lists the top metabolic genes that contribute to the naïve versus primed hESC and adult versus fetal cardiomyocyte separation in PCA plots (25 genes in each direction, i.e., expressed higher in one condition vs. the other). Among the top 50 contributors in each comparison, many genes share similar functions between the two very different transitions (highlighted in Table 1). For example, glucose transporters (HEPH and SLC7A/SLC2A3) are upregulated in both primed vs naïve hESC and fetal vs adult cardiomyocytes (Table1), as expected since both primed hECS and fetal cardiomyocytes have a higher demand for glycolysis compared to their counterparts. Similarly, N-nicotinamid methyl transferase

(NNMT) is developmentally regulated in both hESC and cardiomyocyte development. NNMT is expressed significantly higher in naïve hESC lines compared to primed hESCs, and also higher in more mature cardiomyocytes compared to fetal-like samples. While we showed that NNMT represses Wnt and activates the HIF pathway in primed hESCs by controlling substrate availability for H3K27me3 histone methylation [22], its role in cardiomyocyte maturation remains to be identified.

We further performed Gene Set Enrichment Analysis (GSEA) on all genes used in the PCA plots (Figure 1A and 2A) that are expressed higher in naïve hESC (samples towards the right in Figure 1A) compared to primed hESC (samples towards the left in Figure 1A), as well as genes that are expressed higher in mature human heart samples (samples toward the left in Figure 2A) compared to fetal-like heart samples (samples toward the right in Figure 2A) to identify naïve-enriched and mature cardiomyocyte-enriched Gene Ontology terms. Table 2 lists metabolic-related GO terms enriched in naïve hESC and mature cardiomyocytes (FDR<0.1). It is clear that many metabolic processes undergo changes in cardiomyocyte maturation. Interestingly, two GO terms (highlighted in red) related to oxidative phosphorylation are enriched in both naïve hESC and mature cardiomyocytes, suggesting a similar up-regulation of aerobic respiration in both naïve hESC lines compared to primed hESC, as well as mature cardiomyocyte compared to fetal-like cardiomyocytes. Up-regulated genes in mature cardiomyocytes also showed significant enrichment in lipid and fatty acid metabolism, which agrees with the observation that fatty acids are the main energy substrate for mature cardiomyocytes.

The similarities between metabolic transitions of naïve to primed hESCs and normal to cancer tissues, as well as primed to naïve hESCs and cardiomyocyte maturation suggest that shifts in metabolic profiles is a recurring theme in normal physiological conditions and pathological conditions. The intriguing question is whether same metabolic shifts (e.g., increased reliance on glycolysis) are a result of response to the same environmental context (e.g., hypoxia), intracellular demand (e.g., fulfill requirements of cell proliferation), or a combination of both.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Differential expression of genes between primed and naïve human embryonic stem cell lines

(a) Principal component analysis (PCA) of RNA-seq data from published primed and naïve hESC lines, using all genes [22]. Each dot represents a sample. Different primed and naïve lines are color- and shape-coded. Primed hESC lines form a cluster on the left, naïve hESC lines form a cluster on the right. (b) Genes contributing to principal components separating primed versus naïve hESCs. Each dot represents a gene. The size of the dots is proportional to the contribution of the gene to the primed versus naïve separation along PC1 (x-axis in a, [22]). Top contributing genes are darker. (c) Metabolic genes contributing to principal components separating primed versus naïve hESCs when only metabolic genes are used in the PCA analysis. The size of the dots is proportional to the contribution of the gene to the primed versus naïve separation along PC1 (x-axis). Top contributing genes are darker. Genes in b and c are listed in supplemental tables 1 and 2.

Table 1
Top genes contributing to the separation of maturing cell types

Listed here are the top 25 genes that are enriched in each cell type during the maturation of both naïve embryonic stem cells compared to primed embryonic stem cells (left) and fetal (fetal, 20 day, 1 year old) cardiomyocytes to adult cardiomyocytes (right). Genes that are differentially developmentally regulated during the maturation of both cell types are highlighted in red, irrespective of the direction of enrichment.

Naïve vs. Primed			Fetal vs. Adult	
Naïve-enriched	Primed-enriched		Fetal-enriched	Adult-enriched
ALPPL2	SLC7A3		SULT1E1	PLA2G2A
CHST2	HEPH	Genes in Common	MOXD1	LPL
CDA	AASS	Between the Two Transitions	DPYSL3	PTGDS
ASRGL1	PTGIS		HEPH	GGT5
A4GALT	PLA2G3	DPYSL2/DPYSL3	ATP1B2	COX7A1
SOAT1	CBR1	NNMT	TYMS	GPX3
MAN1C1	DPYSL3	CA2/CA4/CA14	DGKI	NNMT
GAD1	PCYT1B	GPX2/GPX3	GUCY1A3	COX6A2
OLAH	PIPOX	MAN1C1	SLC2A3	SLCO4A1
SLC5A9	B3GAT1	NDUFAB1/NDUFA4L2	HMGCS1	CYP4B1
B3GNT7	DPYSL2	Solute Carrier Family	ALAS2	CA4
SLC7A7	DNMT3B	DNMT3B/DNMT3A	SQLE	PDE2A
GPX2	CA14	HEPH	OGDHL	TYMP
CA2	SEPHS1	PDE4B/PDE2A	RRM2	NDUFA4L2
ABCA1	SLCO4A1	PLCH1/PLCB4	GYG2	COMT
PYGB	SLC38A5	PTGIS/PTGDS	TK1	MAOA
ABCD1	B3GALT5	PLA2G3/PLA2G2A	SLC27A6	GPT
UAP1L1	SMS		PLCB4	CKB
UPP1	PDE4B		LOXL2	MGLL
NDUFAB1	GALNT3		HMGCR	COX4I2
NNT	PLCH1		MAN1C1	CKMT2
SHMT1	ALDOC		MSMO1	SLC1A3
GALNT6	CLC		TUSC3	CAT
AGL	XYLT1		DNMT3A	NDUFB7
NNMT	SLC38A4		IREB2	SLC6A6

Table 2
Gene ontology terms of genes enriched in ESC and cardiomyocyte maturation

GO terms highlighted in red represent GO terms enriched in both naïve hESCs compared to primed hESCs and mature cardiomyocytes compared to fetal, 1 year, and 20 day cardiomyocytes.

Naïve-enriched pathways		Adult cardiomyocyte-enriched pathways	
GO term	FDR	GO term	FDR
CELLULAR_RESPIRATION	0.094	MITOCHONDRION_ORGANIZATION_AND_BIOGENESIS	0.003
AEROBIC_RESPIRATION	0.092	LIPID_METABOLIC_PROCESS	0.008
		CELLULAR_LIPID_METABOLIC_PROCESS	0.013
		ORGANIC_ACID_METABOLIC_PROCESS	0.017
		CELLULAR_RESPIRATION	0.024
		CARBOXYLIC_ACID_METABOLIC_PROCESS	0.027
		AEROBIC_RESPIRATION	0.032
		CELLULAR_BIOSYNTHETIC_PROCESS	0.035
		GENERATION_OF_PRECURSOR_METABOLITES_AND_ENERGY	0.034
		AMINO_ACID_METABOLIC_PROCESS	0.033
		ELECTRON_TRANSPORT_GO_0006118	0.032
		OXYGEN_AND_REACTIVE_OXYGEN_SPECIES_METABOLIC_PROCESS	0.043
		PHOSPHOLIPID_METABOLIC_PROCESS	0.044
		MONOCARBOXYLIC_ACID_METABOLIC_PROCESS	0.052
		COFACTOR_METABOLIC_PROCESS	0.053
		FATTY_ACID_METABOLIC_PROCESS	0.054
		MEMBRANE_LIPID_METABOLIC_PROCESS	0.061
		COENZYME_METABOLIC_PROCESS	0.060
		BIOSYNTHETIC_PROCESS	0.088
		NITROGEN_COMPOUND_METABOLIC_PROCESS	0.091
		STEROID_METABOLIC_PROCESS	0.089