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Nuclear reprogramming with c-Myc potentiates glycolytic capacity of derived induced pluripotent stem cells

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Abstract

Reprogramming strategies influence the differentiation capacity of derived induced pluripotent stem (iPS) cells. Removal of the reprogramming factor c-Myc reduces tumorigenic incidence and increases cardiogenic potential of iPS cells. c-Myc is a regulator of energy metabolism, yet the impact on metabolic reprogramming underlying pluripotent induction is unknown. Here, mitochondrial and metabolic interrogation of iPS cells derived with (4F) and without (3F) c-Myc demonstrated that nuclear reprogramming consistently reverted mitochondria to embryonic-like immature structures. Metabolomic profiling segregated derived iPS cells from the parental somatic source based on the attained pluripotency-associated glycolytic phenotype and discriminated between 3F versus 4F clones based upon glycolytic intermediates. Real-time flux analysis demonstrated a greater glycolytic capacity in 4F iPS cells, in the setting of equivalent oxidative capacity to 3F iPS cells. Thus, inclusion of c-Myc potentiates the pluripotent glycolytic behavior of derived iPS cells, supporting c-Myc-free reprogramming as a strategy to facilitate oxidative metabolism-dependent lineage engagement.

Keywords

Cardiogenesis; cardiac differentiation; glycolysis, iPS cells; metabolomics; mitochondria; oxidative phosphorylation; stem cell metabolism

Introduction

Nuclear reprogramming enables the redirection of somatic tissue back to pluripotency through expression of a set of reprogramming factors that are normally dormant in differentiated populations [1,2]. Induction with reprogramming cocktails, such as Oct4, Sox2, Klf4, and c-Myc, are sufficient to reset the somatic gene expression pattern and epigenetic landscape to an embryonic-like state enabling production of induced pluripotent stem (iPS) cells, which recapitulate features of embryonic stem cells [1,3-5]. The ability to produce a pluripotent cell population without the requirement for an embryo source offers a platform tailored to examine the underlying mechanisms of disease, stratify disease susceptibility and provide personalized biotherapeutics [6-9]. With the prospect of bioengineering functional tissues for regenerative applications from somatic sources, reprogramming strategies must be optimized to reduce tumorigenic potential, while

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potentiating the ability to differentiate into tissue-specific lineages [10-12]. A case in point, eliminating the proto-oncogene c-Myc from the reprogramming cocktail reduces the efficiency of pluripotent induction [13], yet produces iPS cells with decreased risk of transformation [14] and with a greater aptitude for cardiogenic lineage specification [15-17].

c-Myc is a master transcription factor that not only regulates pathways essential for pluripotency [18,19] and cardiogenesis [20-22] but also has a fundamental role in integrating cell proliferation with the regulation of energy metabolism [23]. Indeed, c-Myc binds and regulates metabolic gene targets, influencing glycolysis [24-26], mitochondrial biogenesis [27,28], glutamine [29] and proline catabolism [30]. The ability of c-Myc to regulate metabolic reprogramming is highlighted in cancer, where dysregulation of c-Myc contributes to the Warburg effect, the propensity of cancer cells to metabolize glucose via glycolysis despite sufficient oxygen availability to support oxidative metabolism [31,32].

Metabolic reprogramming has recently been identified as an enabling step in pluripotency induction [33-37]. Nuclear reprogramming induces a dramatic remodeling of the mitochondrial and metabolic infrastructure, which underlies a metabolic shift from predominantly oxidative metabolism in parental somatic cells to a glycolytic metabolite characterizing iPS cells [33,34,38-40]. However, the contribution of c-Myc to the metabolic behavior of derived iPS cells has not been established.

Herein, we reprogrammed fibroblasts to the pluripotent state in the presence (4F iPS cells) or absence (3F iPS cells) of c-Myc to examine the effect of exogenous c-Myc on the metabolic remodeling during induction of pluripotency. Nuclear reprogramming induced a glycolytic phenotype in both 3F and 4F iPS cells when compared to parental fibroblasts, yet inclusion of c-Myc in the reprogramming process potentiated the glycolytic capacity of derived iPS cells.

Methods

Nuclear reprogramming

Mouse embryonic fibroblasts were reprogrammed as described [17,33,41]. In brief, 10⁵ primary fibroblasts were transduced with integrating viral vectors encoding OCT4, SOX2, and KLF4 (viPS kit, Open Biosystems) in the presence (4F iPS) or absence (3F iPS) of c-MYC for 12 h. Individual iPS clones were selected based upon morphology within four weeks of viral induction and clonogenically expanded. iPS cell clones met pluripotent criteria including expression of stem cell markers, embryoid body differentiation, teratoma formation, diploid aggregation and contribution to organogenesis [15,41,42]. Cells were maintained in embryonic stem cell medium consisting of DMEM supplemented with 15% FBS, leukemia inhibitory factor (LIF), 25 mM glucose, 2 mM Glutamax (Invitrogen) and 1 mM sodium pyruvate. Experiments were performed between passage 20 and 25.

Pluripotent validation

Pluripotent and cardiac markers were determined by RT-PCR of cDNA prepared using a Superscript II First strand kit (Invitrogen) on total RNA extracted using an RNeasy kit (Qiagen). Analyzed genes included *Sox2* (Mm00488369_s1), *Pou5f1* (Mm00658129_gH), *Fgf4* (Mm00438917_m1), *Lhx1* (Mm00521776_m1), *Gsc* (Mm00650681_g1), *Sox17* (Mm00488363_m1), *Cxcr4* (Mm01292123_m1), *Kdr* (Mm00440099_m1), *Nkx2.5* (Mm00657783_m1), *Tbx5* (Mm00803521_m1), *Mef2c* (Mm01340839_m1) and *Myocardin* (Mm00455051_m1; Applied Biosystems). Mouse *Gapdh* (4352932E, Applied Biosystems) was used as a control. *In vivo* differentiation capacity of iPS cells was assessed by teratoma formation by injecting 500,000 3F and 4F iPS cells into opposite sides of athymic nude mice. Mice were observed weekly, with tumors being visually detected and animals

sacrificed when the tumor exceeded 10% of body weight. *In vitro* differentiation was performed using a hanging-drop method to produce embryoid bodies. Drops (25 μ l) from a 25,000 cell/ml suspension in differentiation medium supplemented with 20% FBS were suspended on the lid of a plate for 48 h. Embryoid bodies were flushed and kept in suspension for 2 days to enable spontaneous differentiation, following which they were transferred into cell culture plates coated with 0.1% gelatin, where beating activity was monitored daily [17].

Microarray Analysis

To examine the effect of exogenous Myc on glycolytic gene expression in iPS cells, we queried the Myc Cancer Gene Database (<http://www.myc-cancer-gene.org>) to identify Myc targets within glycolysis [43]. Gene expression was investigated using Mouse 430 2.0 GeneChip (Affymetrix). Total RNA was isolated using an RNeasy Mini Kit (Qiagen). Labeled complementary cRNA was obtained from isolated total RNA, and hybridized to the microarrays (Affymetrix). Arrays were scanned using an argon-ion laser, and data visualized using MAS 5.0 Affymetrix software to assess quality of hybridization. Gene expression data were analyzed using Genespring GX 12.0 (Agilent Technologies) [44,45]. In addition to *Myc* (1424942_a_at) and *Mycn* (1417155_at), specific probesets included *Eno1* (1419022_a_at), *Gapdh* (AFFX-GapdhMur/M32599_5_at), *Hk2* (1422612_at), *Ldha* (1419737_a_at), *Ldhb* (1434499_a_at), *Pdha1* (1418560_at), *Pfkfb* (1416780_at) and *Pgk1* (1417864_at).

Mitochondrial morphology and membrane potential

Mitochondrial density and morphology were examined in ultramicrotome sections of 1% glutaraldehyde and 4% formaldehyde fixed cells on a JEOL 1200 EXII electron microscope [46]. Mitochondrial membrane potential was assessed by incubating with 1 μ g/mL JC-1 (Invitrogen) for 30 min at 37°C in live cells. Images were acquired with a LSM 510 Axiovert laser confocal microscope (Zeiss).

Metabolomic footprinting

Extracellular metabolites ("metabolomic footprint") were quantified using proton nuclear magnetic resonance spectroscopy [33]. In brief, iPS cells were incubated in embryonic stem cell medium and media samples serially collected at 4, 8, 12 and 24 h. Media (540 μ L) was then added to 60 μ L of D₂O (Sigma) containing 5 mM sodium 3-(trimethylsilyl)propionate-2,2,3,3-d₄ (TSP) (Sigma) for chemical shift reference and 81.84 mM formate (Sigma) for peak quantification reference [47]. p-Toluenesulfonic acid (Sigma) was utilized as a reference standard to calibrate the formate concentration for quantitative analysis [48]. Samples were filtered through Costar Spin-X filters and added to 5 mm NMR tubes (Wilmad Labglass) for ¹H NMR analysis on a Bruker Ultrashield 700 MHz spectrometer using a *zgpr* water pre-saturation pulse with an 11160.7 Hz spectral width, 32,000 points, acquisition time of 1.4680 s, relaxation delay of 14 s and 64 scans. Spectra were processed with exponential line broadening to 0.3 Hz and zero filling to 65,000 points. Following Fourier transformation, spectra were autophased with metabonomic phase correction, baseline corrected using a Bernstein polynomial fit and referenced to the TSP peak (0.00 ppm) using MestReNova 5.3.2 (MestRelab Research). Metabolite identities were assigned by comparison to reference values for chemical shift and multiplicity, and confirmed by comparison to spectra of pure compounds in the Human Metabolome database [49,50]. Net fluxes of metabolites were calculated by subtracting normalized concentrations of metabolites in basal media from concentrations of metabolites in 24-h conditioned media. Rates were normalized to total cellular protein content determined by a Bradford protein assay (Bio-rad).

Live cell metabolic analysis

Oxygen consumption rates (OCR) and extracellular acidifications rates (ECAR) were measured using a XF24 Extracellular Flux Analyzer (Seahorse Biosciences) as described [51,52]. In brief, cells were plated into wells of a XF24 cell culture microplate based upon cell titration experiments and maintained overnight to ensure 80% confluence. Prior to assay, OCR plates were equilibrated in the absence of CO₂ for 1 h in unbuffered XF assay medium supplemented with 25 mM glucose, 2 mM glutamax, 1 mM sodium pyruvate, 1x nonessential amino acids and 1% FBS, while ECAR plates were equilibrated in unbuffered DMEM supplemented with 143 mM NaCl, 3 mg/L phenol red and 2 mM L-glutamine. Glycolytic processes were interrogated by serial addition of glucose (10 mM), oligomycin (0.5 µg/ml) and 2-deoxyglucose (100 mM) to calculate basal glycolytic rate, glycolytic capacity (in response to oligomycin) and glycolytic reserve (glycolytic capacity – basal rate). Mitochondrial processes were interrogated by serial addition of oligomycin (0.5 µg/ml), carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP, 1 µM) and rotenone (0.5 µM) to calculate basal respiration rates, oxidative capacity (respiration in response to FCCP) and oxidative reserve (oxidative capacity – basal respiration). Each plotted value is the mean of at least 8 replicate wells, and is normalized to total protein quantified using a Bradford protein assay (Bio-rad).

Statistical analysis

Data are presented as mean ± SEM. Student *t*-test was used to evaluate two group comparisons. Principal component analysis and partial least squares discriminant analysis was performed on Pareto scaled data [18] using MetaboAnalyst [53,54]. A value of *p* < 0.05 was considered significant.

Results

3F iPS display a greater propensity for cardiac differentiation than 4F iPS cells

iPS cells derived in the presence (4F) or absence (3F) of c-Myc expressed pluripotent genes (Sox2, Oct4, Fgf4, Lhx1 and Gsc; Fig 1a) and were indistinguishable based upon cellular morphology of small and condensed compact clusters, in line with previous characterization of these cell lines including the expression of pluripotent markers, capacity for multilineage differentiation in teratomas and contribution to chimeric embryos [15,17,41,42]. Transcriptome profiling of Myc (1424942_a_at) and Mycn (1417155_at and 1425922_a_at) was similar upon derived iPS cell clones (Fig 1b). These 4F and 3F cells lines maintained similar rates of *in vivo* differentiation as documented by teratoma formation in immunodeficient mice (Fig 1c). Upon *in vitro* differentiation (Fig 1d-g), 3F iPS cells rapidly downregulated pluripotent genes, while 4F iPS cells maintained pluripotent gene expression at day 5 of differentiation (Fig 1f) despite similar morphology of embryoid bodies at this stage (Fig 1d). Gene expression analysis further demonstrated an upregulation of cardiac markers following 12 days of differentiation, with 3F iPS cells displaying higher expression than 4F counterparts (Fig 1g), correlating with a greater degree of spontaneous beating activity in the absence of c-Myc (Fig 1d).

Nuclear reprogramming induces mitochondrial infrastructure remodeling in 3F and 4F iPS cells

Nuclear reprogramming, irrespective of the presence of exogenous c-Myc, induced a regression of the mature cristae rich and tubular mitochondrial of parental fibroblasts into immature cristae poor and spherical mitochondria of iPS cells (Fig. 2A). 3F and 4F iPS cells demonstrated similar mitochondrial abundance with localization predominantly to the perinuclear space. Mitochondrial function was assessed in live cells using the dual

fluorescence mitochondrial potential sensitive probe JC-1, which accumulates in hyperpolarized mitochondria to form aggregates that fluoresce red, while at lower potentials is maintained in the monomeric form that fluoresces green. The distribution of the red to green fluorescence signal gives a readout of the mitochondria functional state, indicating a similar mitochondrial function in the 3F and 4F iPS cells, which is elevated compared to parental fibroblasts (Fig. 2B). Nuclear reprogramming thus remodels mitochondrial prevalence and anatomy to a similar extent in 3F and 4F derived iPS cells.

c-Myc-free nuclear reprogramming induces an intermediate glycolytic metabotype

Nuclear magnetic resonance spectroscopy (NMR) enables high-resolution quantification of extracellular metabolites in a reproducible, non-targeted manner with minimal sample preparation and allows for compound identification from inherent structural information [55]. ^1H NMR quantification of 18 extracellular metabolites (Fig. 3A) demonstrated that the metabolomic footprint of iPS cells segregated from that of parental fibroblasts, confirming a metabolic transition during nuclear reprogramming (Fig. 3B). Principal component analysis also separated the metabolite profiles of 3F and 4F iPS cells, indicating a c-Myc dependent effect on energy metabolism. Acetate, lactate and glucose were identified as distinguishing metabolites based on variable importance measures in a partial least square discriminant analysis (Fig. 3C). 3F iPS cells displayed a metabotype distinct to parent fibroblasts and approaching 4F iPS counterparts based upon the calculation of the rates of production of glycolytic endproducts, acetate (0.41 ± 0.03 versus 0.02 ± 0.02 and 0.69 ± 0.02 nmol/h/ μg protein, respectively, $n = 6$, $p < 0.05$, Fig. 4A) and lactate (4.1 ± 0.1 versus 3.0 ± 0.1 and 4.7 ± 0.1 nmol/h/ μg protein, respectively, $n = 6$, $p < 0.05$, Fig. 4B). 4F iPS cells also demonstrated elevated utilization of glucose compared to parental fibroblasts and 3F iPS cells (2.3 ± 0.1 versus 2.0 ± 0.1 and 2.0 ± 0.1 nmol/h/ μg protein, respectively, $n = 6$, $p < 0.05$, Fig. 4C). Exclusion of c-Myc thus influences the nuclear reprogramming-induced glycolytic metabotype.

Live cell metabolic behavior reflects an elevated glycolytic capacity of iPS cells reprogrammed in the presence of c-Myc

Agglomerative clustering of glycolytic enzyme expression demonstrated that 3F iPS cells have an intermediate phenotype between parental fibroblasts and 4F iPS counterparts [33]. Myc regulated glycolytic genes, including Hk2, Pfkfb3, Gapdh, Pfkfb1, Eno1, Pdhf1, Ldha and Ldhd, were largely upregulated in the pluripotent lines compared to parental fibroblasts, yet did not differ between 3F and 4F iPS clones (Fig. 5). Assessment of glycolytic and oxidative metabolism through measurement of extracellular acidification rates (ECAR) and oxygen consumption rates (OCR), respectively, enables the real-time monitoring of cellular metabolic status. Sequential supplementation of glucose, an ATP synthase inhibitor (oligomycin) and glycolysis inhibitor (2-deoxyglucose) facilitated interrogation of key parameters of glycolytic function (Fig. 6A), demonstrating that 4F iPS cells have an elevated basal glycolytic rate (3.3 ± 0.2 versus 2.2 ± 0.2 mpH/ μg protein/min, respectively, $n = 8$, $p < 0.05$, Fig. 6B) and glycolytic capacity (6.4 ± 0.5 versus 4.6 ± 0.4 mpH/ μg protein/min, respectively, $n = 8$, $p < 0.05$, Fig. 6C), with no significant difference in glycolytic reserve (1.4 ± 0.2 versus 1.8 ± 0.2 mpH/ μg protein/min, respectively, $n = 8$, Fig. 6D) compared to 3F iPS cells. In contrast, key parameters of mitochondrial physiology dissected through stepwise supplementation of oligomycin, electron chain uncoupler (FCCP) and complex 1 inhibitor (rotenone) did not differ between 4F and 3F iPS cells (Fig. 6E), including basal oxygen consumption (30.1 ± 2.7 versus 26.2 ± 1.2 pmol/ μg protein/min, respectively, $n = 10$, Fig. 6F), oxidative capacity (33.7 ± 2.7 versus 28.3 ± 1.6 pmol/ μg protein/min, respectively, $n = 10$, Fig. 6G) and oxidative reserve (3.2 ± 1.0 versus 2.2 ± 0.7 pmol/ μg protein/min, respectively, $n = 10$, Fig. 6H). Real-time assessment of energy metabolism thus

pinpoints a c-Myc induced glycolytic flux, without concomitant effect on oxidative metabolism.

Discussion

Emerging evidence indicates that metabolic reprogramming is required for induction of pluripotency [33-36]. This study addresses the metabolic consequences of reprogramming to pluripotency in the presence versus absence of c-Myc. Nuclear reprogramming induced a metabolic switch from oxidative metabolism of parental somatic cells to glycolysis of derived pluripotent stem cells, in both the 3F and 4F iPS cells. Inclusion of c-Myc in the reprogramming cocktail potentiated the resultant 4F iPS cell glycolytic capacity, independent of changes in the mitochondrial infrastructure or oxidative capacity.

Nuclear reprogramming-induced remodeling of the metabolic and mitochondrial infrastructure is a critical contributor to pluripotent induction [33-36,56]. This includes the transcriptional and epigenetic reconfiguration of the pathways of glucose and oxidative metabolism, resulting in an upregulation of glycolytic enzymes and selective down regulation of electron transport chain subunits at the protein level [33,34,38,57]. A concomitant reduction in mitochondrial DNA content leads to a regression in mitochondrial anatomy and prevalence through remodeling of the network of mature mitochondria in parental somatic cells to a predominantly perinuclear localization of immature mitochondria in iPS cells [33,36,38-40,58,59]. These infrastructural changes manifest functionally in iPS cells as an acceleration of glycolysis at the expense of oxidative metabolism [33,34,38-40]. Much of this initial work has been performed in iPS cells reprogrammed with c-Myc, and a direct comparison of the metabolic properties of 3F and 4F iPS cells have not been performed despite the well-established role of c-Myc in regulating energy metabolism.

Herein, we document that iPS cells reprogrammed in the absence of c-Myc demonstrate a preferential utilization of glycolysis over oxidative metabolism associated with a remodeling of mitochondrial structure and function in line with previous observations [33]. Consistent with the established role of c-Myc in regulating glycolysis [23-26], head-to-head comparison of 3F and 4F iPS cells using metabolomic footprinting analysis and real-time extracellular flux analysis indicated that the inclusion of c-Myc in the reprogramming process potentiates the glycolytic metabotype of resultant iPS cells without affecting mitochondrial function and oxidative metabolism. As c-Myc target genes broadly control diverse aspects of cellular metabolism, ranging from glycolysis to mitochondrial biogenesis to amino acid metabolism, directly and through interaction with metabolic transcription factors [24-30], a single downstream target of c-Myc is unlikely. c-Myc glycolytic target genes were upregulated in 3F and 4F iPS cells compared to their parental fibroblasts, but did not differ between pluripotent lines. This suggests that the different glycolytic capacity between 3F and 4F iPS cells may not simply be due to changes at the level of expression, but may impact post-translational modification or protein turnover. Indeed, label-free proteomic analysis demonstrated that 3F iPS cells have an intermediate phenotype between parental fibroblasts and 4F iPS cells based upon agglomerative clustering of glycolytic enzyme expression [33], corroborating the pattern observed in metabolomic footprinting and extracellular flux analysis provided herein.

The c-Myc stimulation of glycolysis may have important implications in the reprogramming process itself as glycolytic gene expression precedes the induction of pluripotent genes, indicating that stimulation of glycolysis may be an early event during reprogramming [33]. Indeed somatic cells with lower oxidative capacity and greater glycolytic capacity produce iPS cells with a higher efficiency [34]. In addition, rates of glycolysis are tightly correlated with reprogramming efficiency, with strategies that promote glycolysis through direct

supplementation with glycolytic intermediates [33,35], pharmacologic activators [35], use of hypoxia [60] or inhibition of the p53 pathway [61-66] augment nuclear reprogramming efficiency. Modest changes in metabolism can significantly impact downstream biological processes, with the greater efficiency and rate of reprogramming documented in the presence of c-Myc [13,14] attributable to stimulation of glycolysis.

Metabolic remodeling is also essential for differentiation of pluripotent stem cells to align energy production with the evolving bioenergetic requirements of increasingly specialized tissues [67]. Cell lineages with high energetic demands, including the cardiomyocyte, drive the requirement for more efficient oxidative ATP generation [68-71]. Indeed, stem cell differentiation stimulates mitochondrial DNA replication and biogenesis, supporting the maturation of individual mitochondria into elongated and cristae rich structures, that are highly interconnected to form extensive mitochondrial networks through the cytosol [39,59,69,72-75]. Concomitant upregulation of enzymes involved in the tricarboxylic acid cycle and electron transport chain enables the acceleration of oxidative metabolism to increase ATP production [68,69,73,74]. Inhibition of mitochondrial maturation and oxidative metabolism impairs differentiation and promotes maintenance of the pluripotent state, while inhibition of key glycolytic enzymes promotes differentiation [69,76-80]. In fact, stem cells that contain mitochondria with low mitochondrial potential, or greater number of mitochondria with a more dispersed distribution have a greater propensity for spontaneous differentiation [67,81-83]. Therefore the baseline metabolic state of stem cells significantly impacts their capacity for differentiation. In this context, 3F iPS cells show greater cardiogenic potential than their 4F-induced counterparts [15-17], suggesting that c-Myc promotion of glycolysis may hold 4F iPS cells in the pluripotent state hindering lineage specification.

A limitation of the present study was the limited number of 3F and 4F iPS clones examined. Although clonal variability of iPS cell lines has been recognized, it should be noted that we consistently observed a glycolytic switch during nuclear reprogramming, validated in previous studies [33,34,38]. The murine model system was chosen to initially inspect the metabolic properties of iPS cells, as there is little known with respect to the metabolic requirements for lineage specific differentiation and the impact of reprogramming factor selection on the metabolic phenotype of bioengineered pluripotent cells. These insights will enable subsequent studies to examine energy metabolism in a human system in a more directed manner. Indeed, the metabolic switch from oxidative metabolism to glycolysis observed in murine iPS cells has also been reported in human iPS cells [34,38], indicating that nuclear reprogramming induced metabolic remodeling is conserved across species. The metabolic impact of different reprogramming strategies observed in the murine system may thus be translatable to the human system, and would represent a critical criterion for selecting the most appropriate cell line to elicit repair response. In addition, as reactivation or residual activity of viral transgenes have been observed to interfere with differentiation potential of iPS cells [1,84,85], we cannot exclude the possibility that residual exogenous c-Myc may contribute to the metabolic differences observed.

In summary, nuclear reprogramming in the presence or absence of c-Myc results in a shift in energy metabolism from oxidative phosphorylation characteristic of somatic cells to glycolysis that defines pluripotent cells. Head-to-head comparison of 3F and 4F derived iPS cells, demonstrates that a c-Myc inclusive reprogramming strategy potentiates the glycolytic capacity of the resultant iPS cells. Thus beyond impacting the efficiency of nuclear reprogramming [13,14], the present study reveals that the choice of reprogramming factors significantly impacts the metabolic state of derived iPS cells, which in turn may significantly influence their propensity for metabolism-dependent lineage engagement.

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Fig.1.

3F iPS cells harbor a greater cardiogenic potential despite displaying similar pluripotent traits to 4F iPS cells. (a, b) Nuclear reprogramming induces expression of pluripotent genes in 3F and 4F derived iPS cells and demonstrated similar levels of Myc expression. (c, d) 3F and 4F iPS cells display similar rates of *in vivo* teratoma formation and have similar embryoid body morphology at day 5 of differentiation. (e) 3F iPS cells display a greater degree of spontaneous beating activity during embryoid body differentiation. (f) 3F iPS cells rapidly downregulated pluripotent genes upon *in vitro* differentiation, while 4F iPS cells maintained pluripotent gene expression at day 5 of differentiation. (g) Gene expression analysis further demonstrated an upregulation of cardiac markers following 12 days of differentiation, with 3F iPS cells displaying higher expression. Values are mean $\pm$ SEM, $n = 3$, except for teratoma formation where $n = 7$. In (a) * $p < 0.05$ versus fibroblast, in remaining figures * $p < 0.05$ versus d0.

Fig. 2.

iPS cells display equivalent mitochondrial infrastructure following nuclear reprogramming with and without c-Myc. (a) Nuclear reprogramming induced a regression of mitochondrial morphology to spherical and cristae poor structure in both 3F and 4F iPS cells. (b) Live cell assessment of mitochondrial membrane potential with JC-1 demonstrated an equivalent distribution of red aggregates (high potential) and green monomers (low potential) in 3F and 4F iPS cells.

Fig. 3.

3F and 4F iPS cells have similar but distinct metabolomic footprints. **(a)** ^1H NMR spectra of extracellular metabolites from parental fibroblasts (MEF), 3F and 4F iPS cells. **(b)** Principal component analysis segregated the 3F and 4F iPS cell metabolite landscapes from each other and their parental fibroblasts. **(c)** Partial least squares discriminant analysis variable importance measures ranked acetate, lactate and glucose as the most distinguishing metabolites for segregating cell groups.

Fig. 4.

Nuclear reprogramming in the presence of c-Myc potentiates the resultant iPS glycolytic metabotype. 4F iPS cells display a greater rate of production of the glycolytic endproducts, acetate (**a**) and lactate (**b**), and consume greater amounts of glucose (**c**) than 3F iPS cells and parental fibroblasts. 3F iPS cells also demonstrate greater production of glycolytic endproducts, indicating that nuclear reprogramming induces glycolysis, but induction with c-Myc enhances the glycolytic response. Values are mean $\pm$ SEM, $n = 6$. * $p < 0.05$ versus MEF, # $p < 0.05$ versus 3F iPS cells.

Fig. 5.

Upregulation of c-Myc glycolytic target genes in iPS cells. Transcriptional profiling of glycolysis demonstrated the upregulation of c-Myc target genes in 3F and 4F iPS cells compared to parental MEFs. However gene expression did not differ between pluripotent cell lines. Values are mean $\pm$ SEM, n = 3 for MEF and n = 4 for 3F and 4F iPS cells. * p < 0.05 versus MEF.

Fig. 6. c-Myc potentiates the basal glycolytic rate and glycolytic capacity in iPS cells. Real time analysis of extracellular acidification rate (a), a surrogate marker of glycolysis, demonstrates a greater basal glycolytic rate (b) and glycolytic capacity (c) in 4F iPS compared to 3F iPS cells, with no significant difference in glycolytic reserve (d). Analysis of oxygen consumption demonstrates that 3F and 4F iPS cells display a similar oxidative profile (e) consisting of basal oxygen consumption rate (OCR, f), oxidative capacity (g) and oxidative reserve (h). Values are mean $\pm$ SEM, $n = 8$ biological replicated for extracellular acidification rates and $n = 10$ biological replicates for oxygen consumption rates. * $p < 0.05$ versus 3F iPS cells.