



## Excessive Cellular Proliferation Negatively Impacts Reprogramming Efficiency of Human Fibroblasts

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**Key Words.** Reprogramming • Insulin signaling • Cell proliferation • Human pluripotency

### ABSTRACT

The impact of somatic cell proliferation rate on induction of pluripotent stem cells remains controversial. Herein, we report that rapid proliferation of human somatic fibroblasts is detrimental to reprogramming efficiency when reprogrammed using a lentiviral vector expressing *OCT4*, *SOX2*, *KLF4*, and *cMYC* in insulin-rich defined medium. Human fibroblasts grown in this medium showed higher proliferation, enhanced expression of insulin signaling and cell cycle genes, and a switch from glycolytic to oxidative phosphorylation metabolism, but they displayed poor reprogramming efficiency compared with cells grown in normal medium. Thus, in contrast to previous studies, our work reveals an inverse correlation between the proliferation rate of somatic cells and reprogramming efficiency, and also suggests that upregulation of proteins in the growth factor signaling pathway limits the ability to induce pluripotency in human somatic fibroblasts. *STEM CELLS TRANSLATIONAL MEDICINE* 2015;4:1–8

### SIGNIFICANCE

The efficiency with which human cells can be reprogrammed is of interest to stem cell biology. In this study, human fibroblasts cultured in media containing different concentrations of growth factors such as insulin and insulin-like growth factor-1 exhibited variable abilities to proliferate, with consequences on pluripotency. This occurred in part because of changes in the expression of proteins involved in the growth factor signaling pathway, glycolysis, and oxidative phosphorylation. These findings have implications for efficient reprogramming of human cells.

### INTRODUCTION

Breakthrough discoveries from the Yamanaka laboratory [1, 2] and the establishment of human induced pluripotent stem cells (hiPSCs) have opened new avenues for generating patient-specific stem cell derivatives that can be used for in vitro modeling of human disease, drug development, and cell replacement therapy. However, current methodologies of induced pluripotent stem cell (iPSC) generation continue to face technical challenges, in part because of relatively poor reprogramming efficiencies. As efforts to make iPSCs more useful in human transplantation studies continue, many groups have contributed to significant progress in this field, including the use of reduced numbers of reprogramming factors, and adopting nonintegrating methods of their delivery, cell permeable proteins, and stand-alone small molecules or direct reprogramming [3–7]. Despite these efforts, reprogramming efficiency and its relationship with cell proliferation continues to remain poorly understood in

the iPSC field. For example, vitamin C has been suggested to promote reprogramming by limiting cell senescence and indirectly promoting proliferation [8], and mitochondrial regression has been reported to be associated with a pluripotent state [9]. Other studies suggest that a high proliferation rate of human somatic fibroblasts is essential for efficient reprogramming by decreasing apoptosis rates and limiting reprogramming barriers, including senescence [10, 11]. In contrast, Xu et al. [12] reported that the slow proliferation of mouse somatic cells is beneficial for reprogramming. Consistently, several small molecule inhibitors of cell proliferation have also been reported to enhance somatic cell reprogramming [13–15].

To directly address the significance of proliferation for reprogramming, we cultured primary human fibroblasts in either defined insulin-rich AmnioMAX (Ax) medium or in normal, conventional Dulbecco's modified Eagle's medium (N) (both from Thermo Fisher Scientific Inc., Waltham, MA, <http://www.thermofisher.com>). AmnioMAX,

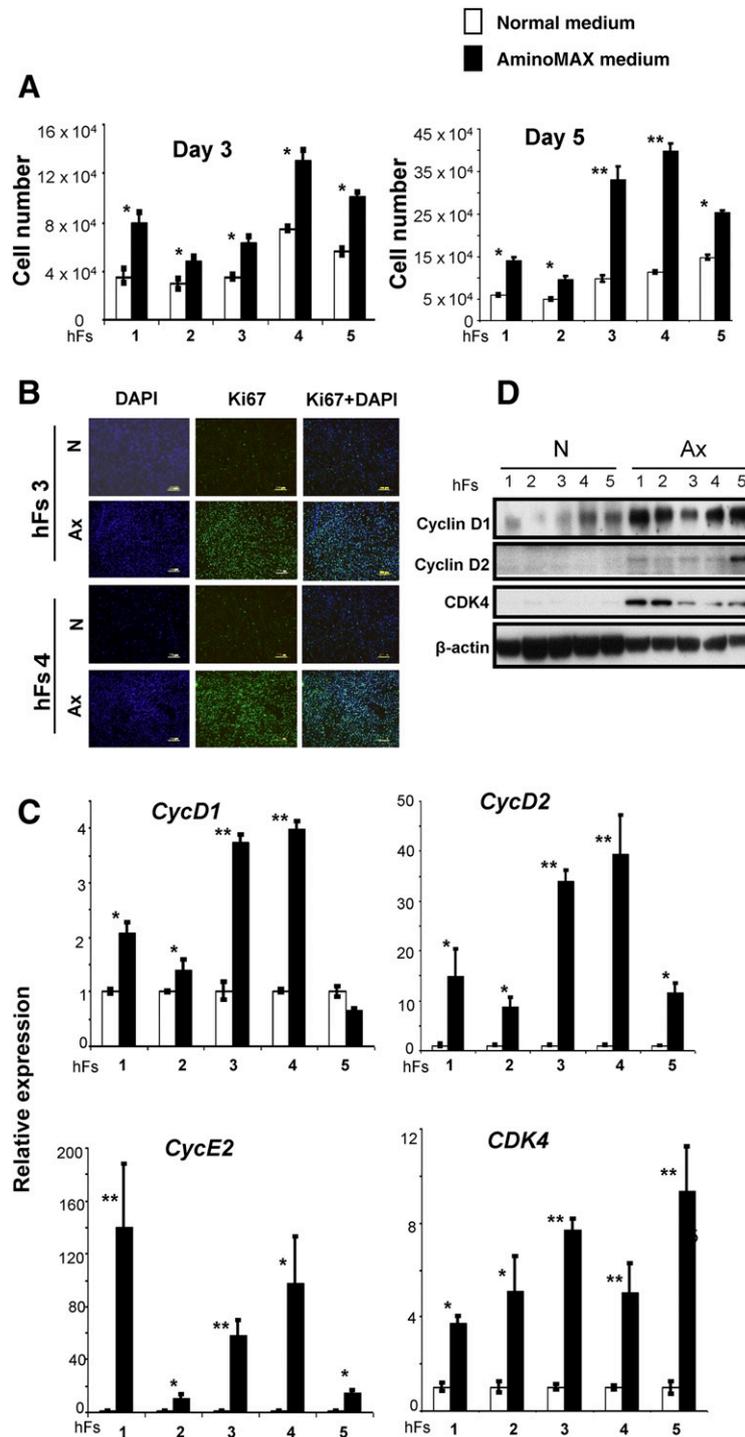
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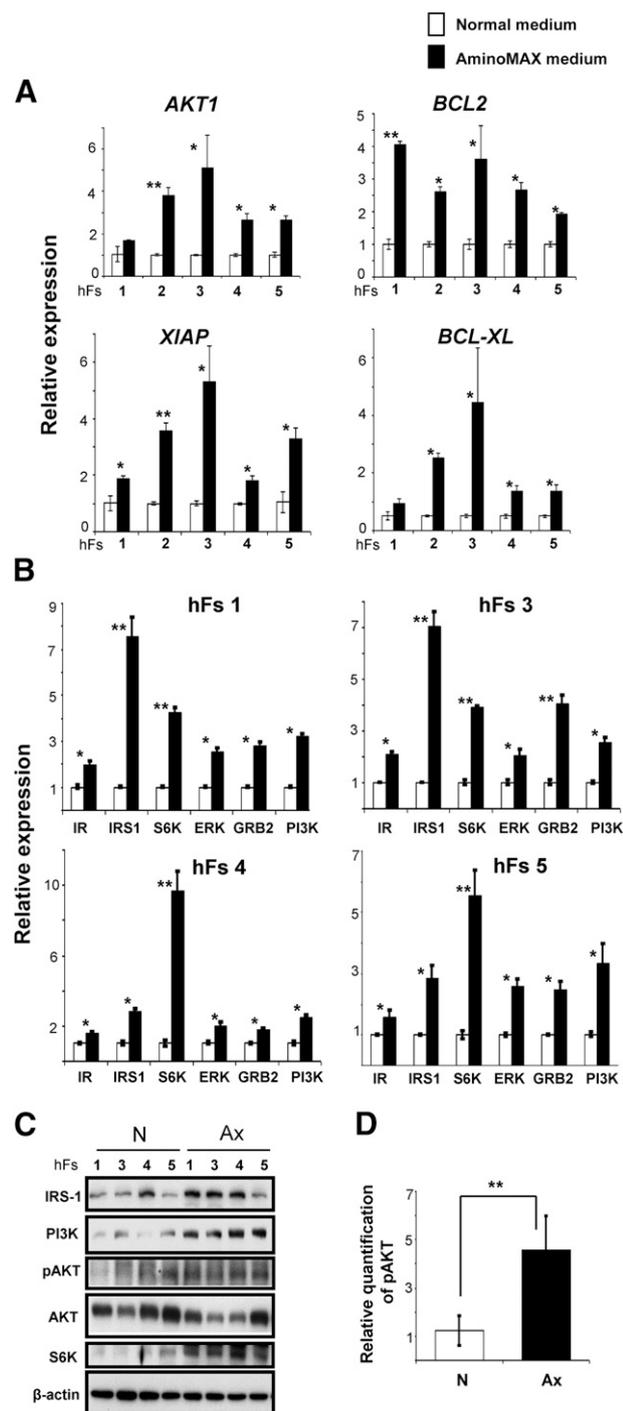
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**Figure 1.** The growth medium determines growth kinetics of human somatic fibroblasts. **(A):** The cell numbers of 5 primary hFs—AG16104 (hFs 1), AG16086 (hFs 2), 120111 (hFs 3), 120116 (hFs 4), and AG16102 (hFs 5)—were measured by a hemocytometer on day 3 and day 5 after excluding dead cells by trypan blue staining. (White boxes represent cell numbers in N and black boxes indicate cell numbers in defined Ax.) The cells were seeded at equal densities (30,000 cells per well) on day 0 and counted on day 3 and day 5 in triplicate wells. The x-axis denotes the hFs. **(B):** hFs 3 and hFs 4 were seeded at equal densities (30,000 cells per well) and cultured in N or Ax medium. Cells were harvested on day 3 and subjected to Ki67 immunostaining to identify cycling cells. DAPI was used to stain nuclei. Scale bars = 200  $\mu$ m. **(C):** The relative expression of cell cycle genes *CycD1*, *CycD2*, *CDK4*, and *CycE2* in hFs grown in N (white bars) or Ax medium (black bars). Expression was normalized to the  $\beta$ -actin gene and is shown relative to the average N medium level. The x-axis denotes the number of hFs. **(D):** Expression of *CycD1*, *CycD2*, and *CDK4* proteins was demonstrated by Western immunoblot analysis.  $\beta$ -actin was used as an internal control. All experiments were performed three times. Data are shown as mean  $\pm$  SD. Statistical significance was determined by Student's *t* test. \*,  $p < .05$ ; \*\*,  $p < .01$  for Ax versus N. Abbreviations: Ax, AminoMAX medium; DAPI, 4,6-diamidino-2-phenylindole; hF, human fibroblast; N, Dulbecco's Modified Eagle's medium.



**Figure 2.** Fibroblasts cultured in Ax growth medium exhibit altered expression of genes in cell survival and growth factor (insulin/insulin-like growth factor-1 [IGF-1]) signaling pathways. **(A):** The relative expression of *AKT1*, *BCL2*, *XIAP*, and *BCL-XL* genes by real-time polymerase chain reaction in human fibroblasts (hFs 1, 2, 3, 4, and 5) cultured in N or Ax medium. In both **(A)** and **(B)**, expression was normalized to the  $\beta$ -actin gene and is shown relative to the average N medium level. The x-axis denotes the number of hFs. **(C):** Western blot analysis of IRS1, PI3K, S6K, pAKT, and AKT proteins in hFs 1, hFs 3, hFs 4, and hFs 5 cultured in N or Ax medium.  $\beta$ -Actin was used

a well-defined medium, has been used for culturing human amniotic fluid cells and fibroblasts [16, 17]. Our study demonstrates a direct inverse correlation of high cell proliferation and reprogramming efficiency for human somatic cells in Ax medium. These results have important implications for utility of these cells for translational studies in humans.

## RESULTS AND DISCUSSION

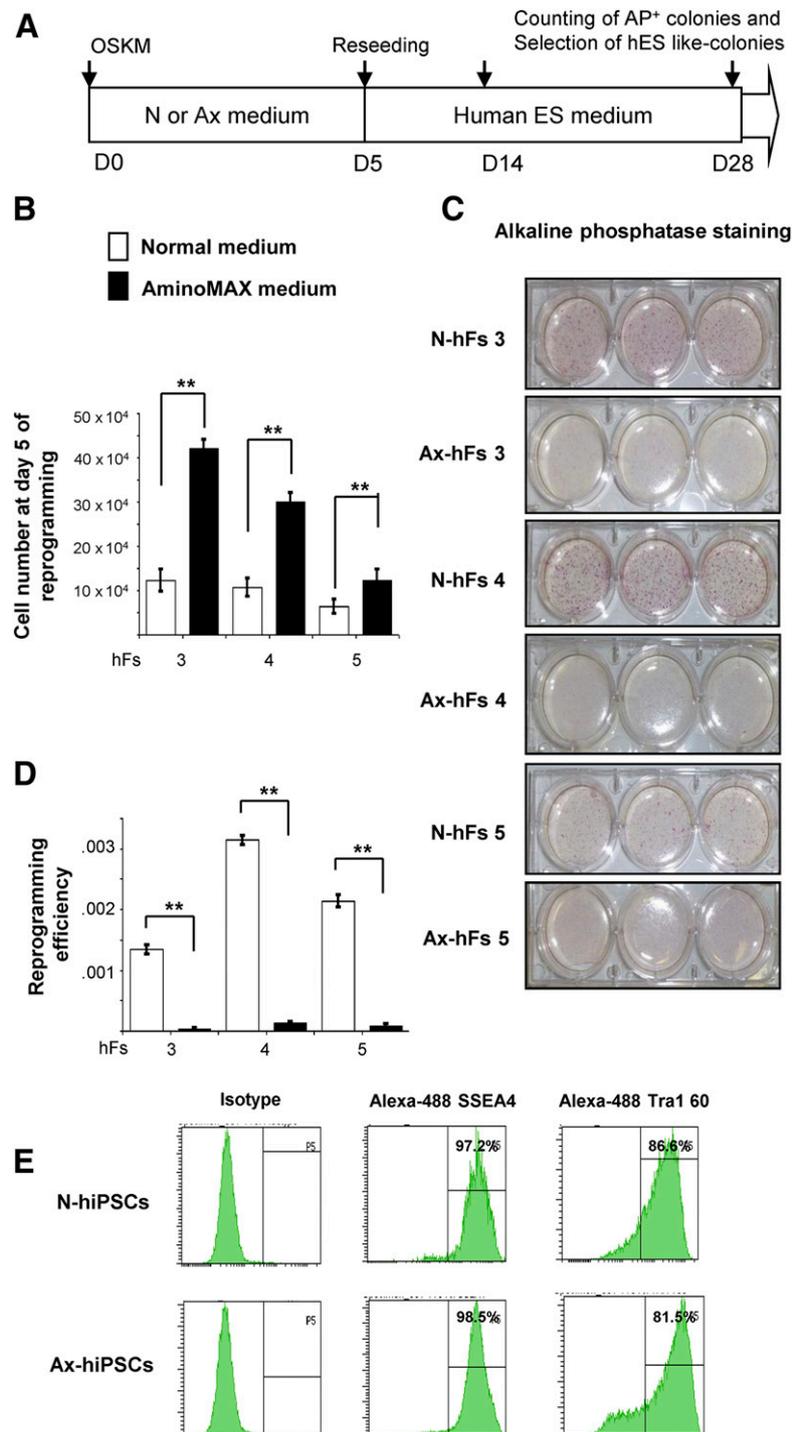
### AmnioMAX Medium Accelerates the Growth Kinetics of Somatic Fibroblasts

Human fibroblasts (hFs) obtained from healthy individuals—AG16104 (hFs 1), AG16086 (hFs 2), 120111 (hFs 3), 120116 (hFs 4), and AG16102 (hFs 5)—were either grown in conventional normal growth medium (N) or Ax medium. Strikingly, fibroblasts grew faster when grown in Ax medium compared with cells grown in N medium (supplemental online Fig. 1A). To quantify proliferation, we seeded an equal number of fibroblasts ( $2 \times 10^4$ ) on day 0 and counted the fibroblasts at days 3 and 5. We observed a 1.5- to 2-fold increase at day 3 and a 2- to 3-fold increase in number of cells at day 5, when cultured in Ax medium compared with N medium (Fig. 1A). An increased rate of proliferation in Ax medium was confirmed by Ki67/4',6-diamidino-2-phenylindole immunostaining in hFs 3 and hFs 4 (Fig. 1B). Further quantification by flow cytometry analysis of hFs 3, hFs 4, and hFs 5 at day 5 revealed an average 2- to 3-fold increase in Ki67-positive cells grown in Ax medium (supplemental online Fig. 1B, 1C). We also observed an increase in expression of cell cycle genes *CycD1* (2- to 5-fold), *CycD2* (2- to 10-fold), *CycE2* (20- to 40-fold), and *CDK4* (2- to 6-fold) in human fibroblasts grown in Ax medium compared with fibroblasts cultured in N medium (Fig. 1C). Detection of increased *CycD1*, *CycD2*, and *CDK4* proteins by Western immunoblotting confirmed enhanced cell cycle progression in fibroblasts cultured in Ax medium (Fig. 1D). Attempts to culture human fibroblasts in mTeSR human medium were not successful and the cells failed to grow in contrast to robust growth when cultured in N or Ax medium (supplemental online Fig. 1D). Together, these data suggest that growth of human fibroblasts in Ax medium leads to a greater rate of proliferation and the enhanced expression of cell cycle proteins.

### Enhanced Growth Factor (Insulin) Signaling Contributes to Higher Proliferation of Fibroblasts Cultured in Ax Medium

The more rapid proliferation of somatic fibroblasts in defined Ax medium led us to investigate the expression of genes associated with cell survival and growth factor (insulin/insulin-like growth factor-1 [IGF-1]) signaling pathways. We observed a

as an internal control. **(D):** Graph representing the relative quantity of phosphorylation of AKT normalized to total AKT band density by ImageJ software (US National Institutes of Health, Bethesda, MD, <http://imagej.nih.gov/ij>). All experiments were performed three times, represented as mean  $\pm$  SD. Statistical significance was determined by Student's *t* test. \*,  $p < .05$ ; \*\*,  $p < .01$  for Ax versus N. Abbreviations: Ax, AminoMAX medium; D, day; ERK, extracellular signal-regulated kinase; GRB2, growth factor receptor-bound protein; IR, insulin receptor; IRS1, insulin receptor substrate-1; N, Dulbecco's modified Eagle's medium; PI3K, phosphatidylinositol 3-kinase; S6K, S6 kinase.



**Figure 3.** Cellular state of fibroblast growth affects cellular reprogramming. **(A):** Scheme of reprogramming of human somatic fibroblasts into hiPSCs using a cocktail of a Cre-excisable STEMCCA lentivirus vector expressing OSKM. **(B):** Human fibroblasts 120111 (hFs 3), 120116 (hFs 4), and AG16102 (hFs 5) were subjected to reprogramming by STEMCCA lentiviral vector. On day 5, at the time of further splitting during reprogramming process, cells were counted growing in N or Ax medium. **(C):** On day 5 of reprogramming, hFs 3, hFs 4, and hFs 5, grown in N or Ax medium, were seeded at a density of  $3 \times 10^4$  fibroblasts in triplicate in 6-well plates. AP staining was performed using a Stemgent kit (Stemgent Inc., Cambridge, MA, <https://www.stemgent.com/products/227>) between days 21 and 28 after iPSC colonies were visualized in reprogramming culture. **(D):** Reprogramming efficiencies from hFs 3, hFs 4, and hFs 5 grown in N or Ax medium were determined by dividing the number of AP-positive colonies by the number of fibroblasts that were initially seeded and transduced ( $5 \times 10^4$  cells). **(E):** hiPSCs from both the groups were subjected to flow cytometry analysis to evaluate the surface pluripotency markers SSEA4 and Tra-1 60, using Alexa Fluor 488 antibodies. All experiments were performed three times, represented as mean  $\pm$  SD. Statistical significance by Student's *t* test. \*,  $p < .05$ ; \*\*,  $p < .01$  for Ax versus N. Abbreviations: AP, alkaline phosphatase; Ax, AminoMAX medium; ES, embryonic stem cell; hF, human fibroblast; hiPSC, human induced pluripotent stem cell; N, Dulbecco's modified Eagle's medium; OSKM, OCT4, SOX2, KLF4, and cMYC.

**Table 1.** Small molecules that suppress cellular proliferation increase reprogramming efficiency

Small molecules	Effect on reprogramming	Molecular targets	Effects on cell proliferation
Valproic acid [25]	Promotes reprogramming	HDAC inhibitor	Suppresses cell proliferation in various cell types
Tricostatin A [23]	Promotes reprogramming	HDAC inhibitor	Inhibits cell proliferation in various cancer cells
5-Azacytidine [26]	Increases reprogramming	DNA methyltransferase inhibitor	Inhibits cell proliferation
Kenpauillone [24]	Promotes reprogramming	Inhibitor of GSK-3 $\beta$ and cell cyclins	Suppresses cell cycle progression
BIX-01294 [26]	Improves reprogramming	G9a histone methyltransferase inhibitor	Decreases cancer cell proliferation
CHIR99021 [24]	Promotes reprogramming	GSK3 inhibitor	Suppresses proliferation of various cancers
PD0325901 [24]	NA	MEK inhibitor	Antiproliferative against various cancer lines
Butyrate [23]	Improves reprogramming	Small-chain fatty acids	Suppresses proliferation in various cancers

Abbreviations: GSK, glycogen synthase kinase; HDAC, histone deacetylase; MEK, mitogen-activated protein kinase; NA, not available.

2- to 6-fold upregulation of genes involved in blocking apoptosis, including *AKT1*, *BCL2*, *XIAP*, and *BCL-XL* in human fibroblasts grown in Ax medium (Fig. 2A). Furthermore, growth factor (insulin/IGF-1) signaling pathway-related genes, including *IR*, *IRS1*, *S6K*, *ERK*, *GRB2*, and *PI3K*, were also significantly upregulated (2- to 3-fold) in fibroblasts grown in Ax medium (Fig. 2B). Western immunoblot analysis confirmed an increased phosphorylation of AKT (approximately 2.5-fold) and higher protein expression of phosphatidylinositol 3-kinase (PI3K), IRS-1, and S6K (approximately 5-fold) in human fibroblasts grown in defined Ax medium (Fig. 2C, 2D; supplemental online 2A). However, we did not observe any significant change in the protein levels of GRB2 or ERK (supplemental online Fig. 2B), suggesting Ax medium has a more prominent effect on the PI-3 kinase pathway than the MAP kinase pathway.

Our findings are consistent with previous studies [18] reporting increased expression of cell survival genes (*AKT1*, *BCL2*, *XIAP*, and *BCL-XL*) during increased proliferation. Consistent with the observation that insulin is a potent inducer of cell proliferation during development [19], we detected an upregulation of phosphorylated AKT and of proteins in the insulin/IGF-1 signaling pathway, including PI3K, IRS1, and S6K, in fibroblasts grown in defined Ax medium.

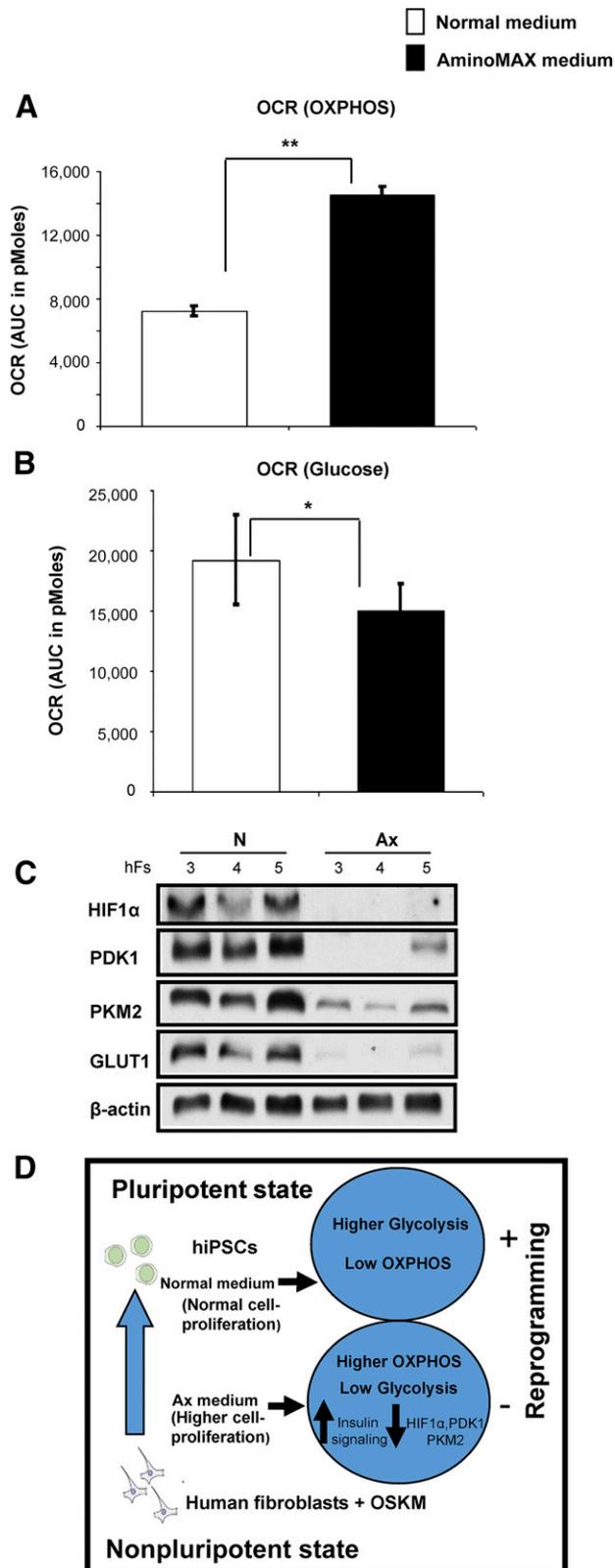
Our focus on examining the insulin signaling pathway and cell proliferation in the context of reprogramming of human somatic fibroblasts gains significance given several opposing reports in this field. Ruiz et al. [11] reported that high proliferation of somatic cells is beneficial to reprogramming by providing evidence that expression of CycD1, CycD2, and CycE2, but not CDK1, CDK2, and CDK4, increased the reprogramming efficiency of human keratinocytes by more than 2-fold. On the contrary, Xu et al. [12] reported that low proliferation of somatic cells is helpful to induce pluripotency. They demonstrated that removing cMyc from among the four reprogramming factors led to a 10-fold increase in reprogramming efficiency of mouse fibroblasts, whereas forced expression of cMyc led to hyperproliferation and correlated negatively with overall reprogramming efficiency. In contrast to these two studies, our observations implicate increased cell cycle markers and an upregulation of proteins in the insulin/IGF-1 signaling pathway in the reprogramming process.

### High Proliferation Rate and Upregulation of Insulin Signaling in Somatic Fibroblasts Correlates With Lower Reprogramming Efficiency

Next, to examine whether cell cycle progression and increased growth determine somatic cell reprogramming,

we subjected fibroblasts individually cultured in N or Ax medium to reprogramming into iPSCs, using a protocol reported previously [20] (Fig. 3A). As expected, even after human lentiviral transduction, we observed a 2- to 3-fold increase in viable cell numbers of both transduced and nontransduced fibroblasts in Ax medium (Fig. 3B). However, surprisingly, this increase in cell numbers was associated with a significant reduction in reprogramming efficiency of cells cultured in Ax medium compared with those cultured in N medium, as demonstrated by alkaline phosphatase staining. A similar outcome in three independent samples (hFs 3, hFs 4, and hFs 5) confirmed a uniform effect (Fig. 3C, 3D; supplemental online Fig. 2C). Real-time polymerase chain reaction analysis showed no difference in the expression levels of *OCT4* or *NANOG* between N-hiPSCs and Ax-hiPSCs, and there was no detection of *OCT4* and *NANOG* in their respective parental fibroblasts (supplemental online Fig. 2D). Furthermore, we observed a similar level of expression of SSEA4 (>90%) and TRA1 60 (>80%) pluripotent surface markers by flow cytometry (Fig. 3E) and *OCT4* expression by immunohistochemistry (supplemental online Fig. 2E) in hiPSCs derived from the fibroblasts cultured in either medium. The hiPSCs from both groups were able to form embryoid bodies as well as develop teratomas that included cells from the three lineages, as shown by immunostaining (supplemental online Fig. 2F, 2G). These results indicate that a higher proliferation and an upregulation in expression of proteins in the growth factor (insulin/IGF-1) signaling pathway does not impact pluripotency of the derived hiPSCs that are successfully reprogrammed, but does influence the frequency of cells that undergo reprogramming. Consistent with our results, Xu et al. [12] reported that low proliferation of mouse fibroblasts is beneficial for reprogramming. Although these authors did not explain the precise mechanism, their data reveal that different small molecules that are antiproliferative agents (e.g., amphidicolin, cisplatin, aloisine A, CDK9 inhibitor II) enhanced the reprogramming efficiency of mouse somatic fibroblasts. One possible explanation for the altered reprogramming is that higher proliferation rates affect some epigenetic markers and/or influence the heterochromatin stage of the cells to eventually limit cellular reprogramming.

Vitamin C, a small molecule, was reported to improve somatic cell reprogramming by enhancing cell proliferation. In contrast, valproic acid has been shown to increase reprogramming efficiency and to induce pluripotency in human amniotic-fluid cells alone, without ectopic expression of reprogramming factors [15, 21, 22]. Indeed, several small molecules, such as kenpauillone,



**Figure 4.** Metabolic shift and the expression of genes regulating reprogramming in fibroblasts grown in N or Ax medium. **(A):** OCR during basal respiration, displayed as AUC, of human hFs 3 and hFs 4 grown in N or Ax medium **(B):** OCR in the presence of glucose as sole energy substrate, displayed as AUC, of human somatic fibroblasts hFs 3 and hFs 4 grown in N or Ax medium. **(C):** Western blot

trichostatin A, 5-azacytidine, and CHIR99021, have all been identified as antiproliferative agents in various cell types [23–26] that also promote reprogramming of fibroblasts [13, 15] (Table 1; supplemental online data).

To further validate the role of IGF-1 and insulin signaling, we reprogrammed hFs 3, 4, and 5 cultured in N medium with or without supplementation with IGF-1 (100 nM) or insulin (43 ng/ml). We used the same concentration of insulin as that present in Ax medium. Interestingly, we observed a significant decrease in reprogramming efficiency in the presence of either IGF-1 or insulin (supplemental online Fig. 3A–3C). This supplementation experiment further supported a potential role for insulin or IGF-1 signaling in reprogramming of human fibroblasts.

#### Activation of Metabolic Switch From Glycolysis to Oxidative Phosphorylation Leads to Significant Decrease in Reprogramming Efficiency of Somatic Fibroblasts

Previous reports indicating that cell-fate conversion is associated with a transition between oxidative phosphorylation and glycolytic metabolism [9], coupled with the observation that insulin/IGF-1 is known to regulate mitochondrial function [27, 28], prompted us to explore whether a similar switch appears in the phenotype of human fibroblasts that show altered insulin/IGF-1 signaling. To this end, we undertook metabolic profiling by investigating cellular metabolism in the context of reprogramming, using the Seahorse Bioflux Analyzer (Seahorse Bioscience, Billerica, MA, <http://www.seahorsebio.com>). This analysis revealed that human fibroblasts cultured in defined Ax medium exhibit increased basal respiration, as shown by a 2-fold higher oxygen consumption rate (OCR) compared with fibroblasts grown in conventional N medium (Fig. 4A; supplemental online Fig. 4A). Interestingly, fibroblasts cultured in N medium displayed increased glycolytic capacity compared with fibroblasts grown in Ax medium. Thus, in response to glucose stimulation, fibroblasts grown in N medium showed a higher OCR and extracellular acidification rate than fibroblasts grown in Ax medium (Fig. 4B; supplemental online Fig. 4B). Consistent with a role for altered glycolysis and hypoxia in the regulation of reprogramming [29], we observed a significantly reduced protein expression of HIF1α (93%), PDK1 (77.1%), PKM2 (91.6%), and GLUT1 (95.9%) in total cell extracts

analysis of HIF1α, PDK1, PKM2, and GLUT1 in hFs 3, hFs 4, and hFs 5 cultured in N or Ax medium. β-Actin was used as an internal control. **(D):** A proposed model for the effects of high cell proliferation and insulin signaling on reprogramming of human fibroblasts. In fibroblasts with normal proliferation, cells maintain higher glycolysis and low OXPHOS. Cells cultured in Ax medium, with higher cell proliferation, exhibit increased growth factor (insulin/IGF-1) signaling and a higher OXPHOS by downregulating the expression of HIF1α, PDK1, PKM2, and GLUT1 proteins, leading to a significant decrease in the efficiency of induction of pluripotency (hiPSCs). All experiments were performed three times, represented as mean ± SD. Statistical significance was determined by Student's *t* test. \*,  $p < .05$ ; \*\*,  $p < .01$  for Ax versus N. Abbreviations: AUC, area under the curve; GLUT1, glucose transporter-1; Ax, AminoMAX medium; HIF1α, hypoxia inducible factor-1α; hiPSC, human induced pluripotent stem cell; N, Dulbecco's modified Eagle's medium; OCR, oxygen consumption rate; OSKM, OCT4, SOX2, KLF4, and cMYC; OXPHOS, oxidative phosphorylation; PDK1, phosphoinositide dependent kinase-1; PKM2, pyruvate kinase M2 isoform.

of fibroblasts grown in Ax medium (Fig. 4C; supplemental online Fig. 4C).

HIF1 $\alpha$  signaling enhances reprogramming efficiency via metabolic switch toward glycolysis by upregulating expression of PDK1. Therefore, activation of HIF1 $\alpha$  regulates Oct4 expression and augments the induction of human stemness signature in various tumor cell lines [29, 30]. Consistent with this notion, our findings demonstrate that reduction in HIF1 $\alpha$  protein in highly proliferating somatic fibroblasts grown in Ax medium promotes refractoriness to reprogramming. Previous reports implicated an upregulation of PDK1 by small molecules in an increase in reprogramming [3]. Similarly, PKM2 may be involved in positive regulation of OCT4 and GLUT1 in glycolysis [31]. In our study, we noted that key regulators of glycolysis (e.g., PDK1, PKM2, and GLUT1) are all decreased in human fibroblasts that are rapidly proliferating when cultured in Ax medium and, consequently, exhibit a significant loss of reprogramming efficiency (Fig. 4D).

To further validate the role of PDK1 in reprogramming, a central regulator of glycolysis, we knocked down PDK1 in hFs 3, hFs 4, and hFs 5 using scrambled or PDK1-specific siRNAs (supplemental online Fig. 5A). Knocked-down PDK1 human fibroblasts showed significantly reduced reprogramming efficiency as compared with fibroblasts cultured in scrambled control small interfering RNA (supplemental online Fig. 5B, 5C). This loss-of-function study further validated our findings in regard to a potential role of PDK1 and glycolysis in reprogramming of human fibroblasts.

## CONCLUSION

We report that stimulation of cell proliferation limits human somatic cell reprogramming via upregulation of proteins in the insulin/IGF-1 signaling pathway and by promoting a metabolic switch from glycolysis to oxidative phosphorylation. These data provide a previously unidentified perspective on the roles of cell proliferation and growth factor signaling in induction of pluripotency and have implications for studies aimed at

reprogramming of cells derived from humans with pathological states associated with impaired metabolism and/or cell proliferation, such as diabetes or cancer.

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## AUTHOR CONTRIBUTIONS

M.K.G.: conception and design, collection and assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; A.K.K.T. and T.N.R.: collection and assembly of data, data analysis and interpretation, final approval of manuscript; S.B, J.S., T.T., J.H., D.F.D.J., and R.W.: collection and assembly of data, final approval of manuscript; A.K.: collection and assembly of data of metabolic study, data analysis and interpretation, final approval of manuscript; A.J.W.: provision of suggestions, manuscript editing, final approval of manuscript; R.N.K.: conception and design, manuscript writing, financial support, final approval of manuscript.

## DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

A.J.W. is on the scientific advisory board of FATE Therapeutics. R.N.K. has compensated research funding from AstraZeneca. The other authors indicated no potential conflicts of interest.

## REFERENCES

- 1 Takahashi K, Tanabe K, Ohnuki M et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007;131:861–872.
- 2 Daley GQ. Stem cells: Roadmap to the clinic. *J Clin Invest* 2010;120:8–10.
- 3 Zhu S, Li W, Zhou H et al. Reprogramming of human primary somatic cells by OCT4 and chemical compounds. *Cell Stem Cell* 2010;7:651–655.
- 4 Hou P, Li Y, Zhang X et al. Pluripotent stem cells induced from mouse somatic cells by small-molecule compounds. *Science* 2013;341:651–654.
- 5 Kim JB, Greber B, Arauzo-Bravo MJ et al. Direct reprogramming of human neural stem cells by OCT4. *Nature* 2009;461:649–643.
- 6 Park IH, Zhao R, West JA et al. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature* 2008;451:141–146.
- 7 Hanna J, Markoulaki S, Schorderet P et al. Direct reprogramming of terminally differentiated mature B lymphocytes to pluripotency. *Cell* 2008;133:250–264.
- 8 Esteban MA, Wang T, Qin B et al. Vitamin C enhances the generation of mouse and human induced pluripotent stem cells. *Cell Stem Cell* 2010;6:71–79.
- 9 Folmes CD, Nelson TJ, Martinez-Fernandez A et al. Somatic oxidative bioenergetics transitions into pluripotency-dependent glycolysis to facilitate nuclear reprogramming. *Cell Metab* 2011;14:264–271.
- 10 Son MJ, Son MY, Seol B et al. Nicotinamide overcomes pluripotency deficits and reprogramming barriers. *STEM CELLS* 2013;31:1121–1135.
- 11 Ruiz S, Panopoulos AD, Herreras A et al. A high proliferation rate is required for cell reprogramming and maintenance of human embryonic stem cell identity. *Curr Biol* 2011;21:45–52.
- 12 Xu Y, Wei X, Wang M et al. Proliferation rate of somatic cells affects reprogramming efficiency. *J Biol Chem* 2013;288:9767–9778.
- 13 Li W, Zhou H, Abujarour R et al. Generation of human-induced pluripotent stem cells in the absence of exogenous Sox2. *STEM CELLS* 2009;27:2992–3000.
- 14 Lyssiotis CA, Foreman RK, Staerk J et al. Reprogramming of murine fibroblasts to induced pluripotent stem cells with chemical complementation of Klf4. *Proc Natl Acad Sci USA* 2009;106:8912–8917.
- 15 Huangfu D, Maehr R, Guo W et al. Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. *Nat Biotechnol* 2008;26:795–797.
- 16 Ye L, Chang JC, Lin C et al. Induced pluripotent stem cells offer new approach to therapy in thalassemia and sickle cell anemia and option in prenatal diagnosis in genetic diseases. *Proc Natl Acad Sci USA* 2009;106:9826–9830.
- 17 Staunstrup NH, Madsen J, Primo MN et al. Development of transgenic cloned pig models of skin inflammation by DNA transposon-directed ectopic expression of human beta1 and alpha2 integrin. *PLoS One* 2012;7:e36658.
- 18 Yu X, Zhang X, Dhakal IB et al. Induction of cell proliferation and survival genes by estradiol-repressed microRNAs in breast cancer cells. *BMC Cancer* 2012;12:29.
- 19 Strassburger K, Tiebe M, Pinna F et al. Insulin/IGF signaling drives cell proliferation in part via Yorkie/YAP. *Dev Biol* 2012;367:187–196.
- 20 Teo AK, Windmueller R, Johansson BB et al. Derivation of human induced pluripotent stem cells from patients with maturity onset

diabetes of the young. *J Biol Chem* 2013;288:5353–5356.

**21** Moschidou D, Mukherjee S, Blundell MP et al. Valproic acid confers functional pluripotency to human amniotic fluid stem cells in a transgene-free approach. *Mol Ther* 2012;20:1953–1967.

**22** Moschidou D, Mukherjee S, Blundell MP et al. Human mid-trimester amniotic fluid stem cells cultured under embryonic stem cell conditions with valproic acid acquire pluripotent characteristics. *Stem Cells Dev* 2013;22:444–458.

**23** Medina V, Edmonds B, Young GP et al. Induction of caspase-3 protease activity and apoptosis by butyrate and trichostatin A (inhibitors of histone deacetylase): Dependence on protein synthesis and synergy

with a mitochondrial/cytochrome c-dependent pathway. *Cancer Res* 1997;57:3697–3707.

**24** Tighe A, Ray-Sinha A, Staples OD et al. GSK-3 inhibitors induce chromosome instability. *BMC Cell Biol* 2007;8:34.

**25** Xia Q, Sung J, Chowdhury W et al. Chronic administration of valproic acid inhibits prostate cancer cell growth in vitro and in vivo. *Cancer Res* 2006;66:7237–7244.

**26** Weller EM, Poot M, Hoehn H. Induction of replicative senescence by 5-azacytidine: fundamental cell kinetic differences between human diploid fibroblasts and NIH-3T3 cells. *Cell Prolif* 1993;26:45–54.

**27** Liu S, Okada T, Assmann A et al. Insulin signaling regulates mitochondrial function in pancreatic beta-cells. *PLoS One* 2009;4:e7983.

**28** Zarse K, Schmeisser S, Groth M et al. Impaired insulin/IGF1 signaling extends life span by promoting mitochondrial L-proline catabolism to induce a transient ROS signal. *Cell Metab* 2012;15:451–465.

**29** Yoshida Y, Takahashi K, Okita K et al. Hypoxia enhances the generation of induced pluripotent stem cells. *Cell Stem Cell* 2009;5:237–241.

**30** Prigione A, Rohwer N, Hoffman S et al. HIF1 $\alpha$  modulates reprogramming through early glycolytic shift and upregulation of PDK1-3 and PKM2. *STEM CELLS* 2014;32:364–376.

**31** Lee J, Kim HK, Han YM et al. Pyruvate kinase isozyme type M2 (PKM2) interacts and cooperates with Oct-4 in regulating transcription. *Int J Biochem Cell Biol* 2008;40:1043–1054.



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