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Distinctive Krebs cycle remodeling in iPSC-derived Neural and Mesenchymal Stem Cells

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Abstract

Mitochondria play a vital role in proliferation and differentiation and their remodeling in the course of differentiation is related to the variable energy and metabolic needs of the cell. In this work, we show a distinctive mitochondrial remodeling in human induced pluripotent stem cells differentiated into neural or mesenchymal progenitors. While leading to upregulation of the slow-running segment of the Krebs cycle and increased respiratory chain activities and respiration in the mesenchymal stem cells, the metabolic switch in the neural stem cells resulted in downregulation of α -ketoglutarate dehydrogenase and upregulation of isocitrate dehydrogenase 2 concomitant with the accumulation of α -ketoglutarate. The distinct, lineage-specific changes indicate an involvement of these Krebs cycle enzymes in cell differentiation.

Keywords

Mitochondria, Krebs cycle, differentiation, induced pluripotent stem cells

Introduction

The embryonic and fetal development requires active metabolism to undergo the multiple stages of proliferation and differentiation leading to a fully developed organism. The mechanisms underlying metabolic shift during specific cell lineage commitment have been the focus of many studies in recent years especially since the development of human pluripotent stem cells cultures including embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC). The ESC and iPSC are highly proliferative cells that use aerobic glycolysis as the primary source of ATP [1,2]. When exiting pluripotency, they are frequently reported to undergo obligatory metabolic switching to oxidative phosphorylation (Oxphos) for more efficient energy production [1,2,3,4,5]. However this metabolic prerequisite for pluripotency exit has recently been challenged, as it was shown that a high glycolytic flux was maintained in the early ectoderm [6] thus indicating the lineage specific requirements for ATP production during differentiation. In addition to the shift in energy supply, differentiation may necessitate and/or induce germ layer-specific changes in mitochondrial metabolites especially in Krebs cycle intermediates known to be at the crossroad of multiple signaling and metabolic pathways. Indeed, Krebs cycle fuels biosynthesis of lipids, proteins and carbohydrates, it affects the activities of proteins through allosteric regulation or postranslation modifications, regulates the availability of ions or acts as hormone-like molecules activating metabolitespecific receptors (reviewed in [7,8]). Interestingly, several metabolites of the Krebs cycle, including α -ketoglutarate (aKG), succinate and fumarate are respective cofactor and inhibitors of HIF prolyl hydroxylases (PHDs) and for DNA and histone demethylases thus participating directly in the epigenetic modulation of gene expression and providing a possible connection to the cell fate specification [9,10]. In this work, using iPSC and iPSC-derived neural and mesenchymal stem cells, we evaluated the activity of the Krebs cycle enzymes and report here their distinct, unexpected remodeling.

Material and Methods

Fibroblast Reprogramming and pluripotent Stem Cell Culture

Human fibroblasts were cultured in fibroblasts medium consisting of DMEM, high glucose, GlutaMAX Supplement, supplemented with 10% of fetal bovine serum (Sigma), 1% nonessential amino acids (Invitrogen) and 1mM Sodium pyruvate (Invitrogen). The Human iPSCs control line (WT, i90c16) was derived from IMR-90 lung fibroblast cell (ATCC CCL-186). Human iPSCs were obtained as previously described by Yu et al. [11] using Addgene plasmid 20925, 20926 and 20927. The iPSCs lines were amplified up to the 15th passage before differentiation. Molecular characterization of pluripotency and self-renewal capacities of these cells was performed as described previously [11]. Human iPSCs (passages 25–40) were cultured on matrigel coated dishes in StemMacs iPS-Brew XF, human (Miltenyi). Cultures were fed daily and passaged every 4–5 days with TrypLE Express (Life technologies). Cultures were verified for mycoplasma monthly using Mycoalert kit (Lonza LT07-318).

Pluripotent Stem Cell differentiation

Differentiation into Neural Stem Cells (NSCs) was performed in the following way: commitment of iPSCs to the neural lineage was performed as described in [12]. At day 8-10, neural rosettes were manually collected and plated in poly-ornithine/laminin-treated culture dishes in N2B27 medium consisting of DMEM/F12, neurobasal, N2 and B27 supplement, 50 μ M β -mercaptoethanol and Penicilline/Streptomycin (Life technologies) supplemented with fibroblast growth factor-2 (FGF2, 10ng/ml) and epidermal growth factor (EGF, 10ng/ml). At confluence, the cells were passed using trypsin as a single-cell suspension with a density of 50 000 cells cm2. Mass amplification of NSCs was performed in N2B27 medium until passages 6-8 and cells were frozen in a mixture containing 10% dimethyl sulfoxide and 90% fetal bovine serum (Invitrogen). The Rock inhibitor Y27632 was used at the time of thawing to enhance cell recovery.

Mesenchymal stem cells (MSCs) were obtained using an adaptation of a previously described protocol [13]. Briefly, differentiation was induced by plating 4 to 6 iPSCs colonies/cm2 on 0.1% gelatin coated dishes in MSCs medium consisting of DMEM/F12 supplemented with 20% fetal bovine serum (FBS) (Sigma), 1% nonessential aminoacids, 50 μ M β ⁻⁻ mercaptoethanol, 10 ng/mL recombinant human FGF2 (all from Life technologies) and 1 mM L⁻⁻ Ascorbic acid 2⁻⁻ phosphate (Aa2⁻⁻P) (Sigma⁻⁻ Aldrich). Medium was changed every other day and cells were passaged with TrypLE Express (Life technologies) to new gelatin coated dishes when they reached confluence. MSCs were frozen after 5 passages. Cultures were verified for mycoplasma monthly using Mycoalert kit (Lonza LT07-318).

mFISH karyotype analysis

Cells were conditioned with colchicine (Eurobio) for 90 min, warmed with a hypotonic solution (5mg/mL KCL) and fixed with a Carnoy fixative. mFISH 24Xcite probe (Metasystem) and ProLong Gold Antifade Mountant with DAPI (Life technologies) were used for mFISH staining. 30-70 metaphases were acquired with Metafer MetaSystems software coupled to an AxioImager Zeiss Z2 microscope equipped with a camera cool cube and 10X and 63X objectives. Images were analyzed with Isis software (MetaSystems).

Immunocytochemistry

Cells were seeded on glass coverslips and grown inside wells of a 12 well-plate until 70-90% confluency in standard growth media at 37 °C, 5% CO2. Cells were fixed in 4% paraformaldehyde (15 min, room temperature) before permeabilization and blocking in PBS supplemented with 0.1% Triton X-100 and 1% BSA (Sigma-Aldrich St. Quentin, Falavier, France). Primary antibodies were incubated overnight at 4°C in 1% BSA (Sigma-Aldrich). Antibodies used were: Nestin (Milipore MAB5326), Ki67 (Dako M7240), Oct4 (Santa Cruz), Tra-1-60 (Cell signaling 4746), Nanog (Cell signaling 4903), SSEA4 (Cell signaling 4755), Sox2 (Milipore AB5603). Secondary antibodies and DAPI or Hoechst counterstain were applied for 1 h at room temperature. Images acquisitions were performed on a fluorescence microscope equipped with epi-fluorescence illumination (ImagerZ1; Carl Zeiss, LePecq, France) using the Axiovision image capture equipment and software or with Leica TCS SP8 confocal laser microscope and LasX Leica software.

Enzymatic analyses and Cellular Respiration

Cells were collected by enzymatic digestion with TrypLE Express (Life technologies), washed twice with cold PBS and frozen as dry pellets of 1x106 cells. Cells were subsequently thawed using 100 µl of ice-cold solution consisting of 0.25 M sucrose, 20 mM Tris-HCl (pH 7.2), 2 mM EGTA, 40 mM KCl, 0.01% digitonin (w/v). After 5 min incubation on ice, cells were centrifuged (2500g, 5 min, 4 °C), and the pellets suspended in 100 \Box 1 of the same solution. Activities of respiratory chain complexes and Krebs cycle enzymes were spectrophotometrically measured in both fractions (soluble cytosolic fraction and mitochondria containing membrane fraction) with 50-100 µg of proteins using a Cary 60 UVvisible spectrophotometer (Varian Inc, Les Ulis, France) as previously described [14,15,16]. Cellular respiration was measured with intact cells collected immediately before the analysis using the Optode device fitted to a handmade cap in either 50 µl or 250 µl thermostated cell as previously described [22]. For the aKG and succinate levels analysis, cell pellets were collected as previously and organic acids were extracted with 0.05N Perchloric acid in PBS incubated in ice for 5 min then sonicated and cleared by centrifugation (16000g, 5 min, $4 \,^{\circ}$ C). The levels of aKG were measured spectrofluorometrically with alpha-ketoglutarate assai kit according to manufacturer instructions (Sigma-Aldrich MAK054). The levels of succinate was measured spectrophotometrically by a coupled reaction in which succinate was first converted to succinyl-CoA with Succinyl-CoA thiokinase (Succinate converter MAK184B, Sigma-Aldrich St. Quentin, Falavier, France) in the presence of 35 µM Coenzyme A, 2.5Mm MgCl2, 0.5mM ATP in 100mM KPO4 pH 7.8. Then ADP produced in this reaction was determined following NADH oxidation in PEP+PK/LDH assai as described previously [14, 15]. All chemicals were from Sigma–Aldrich (St. Quentin, Falavier, France). Protein was estimated using the Bradford assay (Sigma-Aldrich, St. Quentin, Falavier, France).

Gel electrophoresis and Immunohistochemistry

For the Western blot analysis, total protein extracts (50 μ g) were separated by SDS–PAGE on a 6, 10 or 12% polyacrylamide gel [18], transferred to a nitrocellulose membrane and probed overnight with primary antibodies against the protein of interest: IDH2 (Abcam ab129180), OGDH (Abcam ab137773) and CS (Abcam ab 96600), Aco2 (Abcam ab110321). Secondary antibodies (Thermofisher Scientific 32460 and 32430) were applied for 1 h at room temperature. The antibody complexes were visualized with the Western Lightning Ultra Chemiluminescent substrate kit (Perkin Elmer, France) and images acquisitions were performed with G-Box Chemi XT16 camera.

Results and Discussion

Human induced pluripotent stem cells differentiation

As a cellular model for studying the impact of mitochondrial metabolism on differentiation, we used human induced pluripotent stem cells (iPSC) differentiated into mesenchymal or neural progenitors. The iPSC were generated from primary fibroblasts by using the episomal vector based reprogramming strategy [11]. Homogenous populations of neural stem cells (NSC) and mesenchymal stem cells (MSC) have been obtained using previously developed and validated protocols [12,13]. All cell types were phenotypically verified (Fig S1).

Respiratory capacity of iPSC and iPSC-derived neural and mesenchymal stem cells

In order to assess mitochondrial involvement in differentiation we first analyzed the mitochondrial network, as changes in mitochondrial morphology may reflect the respiratory capacity of the cell. Using antibodies against mitochondrial citrate synthase, we immunostained the mitochondrial network in iPSC and iPSC-derived stem cells. We detected a large amount of mainly spherical mitochondria localized perinuclearly in iPSC cells (Fig 1). Upon neural and mesenchymal differentiation, we observed a reshaping of the mitochondrial network with the appearance of more elongated, reticular mitochondria in the NSC accompanying important changes in cell morphology of these cells and a highly

interconnected network in MSC. Apparently immature mitochondria are frequently associated with elevated glycolytic metabolism of the plutipotent stem cells [1,19]. In contrast, the well-developed network is generally observed in the lineage-commited progenitors and in the differentiated somatic cells and is thought to indicate the activation of Oxphos [1,20,23]. The better developed and connected mitochondria observed in NSC and MSC could thus point to a change in energy production.

We next measured cellular respiration and the activities of the respiratory chain (RC) in these cells. As revealed by a 2 fold increase in the rate of oxygen consumption and in the activities of mitochondrial Respiratory Complexes II (Succinate Quinone DCPIP Reductase, SQDR), III (QCCR, Quinone Cytochrome c Reductase) and IV (COX), MSC appeared to have undergone remodeling towards higher mitochondrial activity (Fig 2). However, we did not detect any changes in cellular respiration or RC activities in NSC (Fig 2) indicating the lack of Oxphos activation in these cells.

These results show that although Oxphos was activated during differentiation, this occurred in the lineage-specific manner. While observed in the mesenchymal progenitors, Oxphos switch was not required for neural induction and NSC proliferation arguing against the obligatory shift from glycolysis to Oxphos for pluripotency exit and differentiation [1,2,4,5]. Importantly, a sustained high aerobic glycolytic flux was recently reported in early ectoderm, and Oxphos switching was reported only after neural progenitor cells transition to midbrain floor plate precursors [6]. As the apparent Oxphos repression persists in the late cortical progenitors, used in our study, this suggests a differential Oxphos switching during cortical differentiation.

Krebs cycle profile in iPSC and iPSC-derived neural and mesenchymal stem cells

The Krebs cycle not only fuels the RC with reducing equivalents, used for ATP production, but is also a source of several key metabolites involved in cell proliferation and differentiation. To test whether its activity changes after lineage commitment, we analyzed Krebs cycle enzymes in iPSC and iPSC derived progenitors.

The analysis revealed a 2-fold increase in the activity of citrate synthase (CS) and a 4-fold induction of the mitochondrial aconitase 2 (ACO2), NAD-dependent isocitrate dehydrogenase 3 (IDH3) and a-ketoglutarate dehydrogenase (aKGDH) activities in MSC compared to the parental iPSC cells (Fig 3A). In contrast, the activities of mitochondrial fumarate hydratase (mt-FH) and mitochondrial malate dehydrogenase (MDH2) as well as mitochondrial NADP-dependent isocitrate dehydrogenase 2 (IDH2) were unchanged. Interestingly, the strongly

upregulated enzymes are part of the slow running segment of the Krebs cycle, the latter being shown functionally split into two complementary mini-cycles between oxaloacetate and α -ketoglutarate (Fig 4B) [21]. Induced activity of this segment in MSC could allow for more efficient use of pyruvate in the Krebs cycle. Interestingly, low expression of ACO2 and IDHs has been reported in human pluripotent stem cells and it has been associated with preferential glycolysis in these cells [22]. Increased activity of the CS – aKGDC segment, which we measured in MSC, might therefore allow Oxphos switching and induced respiration in mesenchymal progenitors.

An important, yet distinct Krebs cycle remodeling also occurred during neural differentiation. Indeed, in the NSC, we observed no change in the activity of the CS–IDH3 segment of the Krebs cycle, strongly induced in MSC. Instead, the activity of the aKGDH, which catalyzes the oxidative decarboxylation of alpha-ketoglutarate to succinyl-CoA, was significantly reduced. The 3-fold reduction of this rate-limiting step, regulating the entry of aKG into the suggested fast-running segment of the Krebs cycle, was accompanied by a 3-fold induction of aKG-forming IDH2 (Fig 3A).

These activity profiles were then confirmed by western blot analysis (Fig 3B). Accordingly, the MSC displayed a significant increase in the amount of CS, IDH2, ACO2, and OGDH (E1 component of aKGDH). In contrast, 3-fold upregulation of IDH2 and 3-fold downregulation of aKGDH could be detected in the NSC resulting in nearly 10-fold difference in the ratio of both enzymes as compared to the parental iPSC cells. To exclude the influence of the genetic background, this remodeling was further confirmed in NSC derived from another iPSC cell line, as shown by western blot analysis of IDH2 and OGDH (Fig 2S).

The opposite changes in the activities of IDH2 and aKGDH in the NSC could lead to increased accumulation of aKG in these cells. To verify it, we have measured its level in iPSC, NSC and MSC. We found the intracellular level of aKG significantly (3-fold) higher in the NSC compared to iPSC cells, while the level of succinate was unchanged (Fig 4A). In contrast, we did not detect any aKG increase or succinate accumulation in MSC, which is consistent with an overall upregulation of Krebs cycle and respiratory activities in these cells (Fig 4A). Since the increased metabolism of glutamine could be an additional cellular source of aKG, through the action of glutamate dehydrogenase (GlutDH), we also measured the activity of this enzyme that didn't significantly change in NSC (Fig 3A). However, we observed a slight but significant GlutDH induction in mesenchymal stem cells, consistent with induced mitochondrial activities in these progenitors (Fig 3A).

Our results showed a robust, 3-fold increase in the activity of aKG-forming IDH2 and a 3-fold decrease in the activity of aKG-consuming aKGDH, in NSC. We also detected significantly elevated level of this metabolite in neural progenitors. As a cofactor for the oxoglutarate dependent dioxygenases (OGDDs), aKG is known to modulate gene expression and affect the pluripotency exit and differentiation [9,10; reviewed in 24] which suggests that the remodeling of the Krebs cycle and the accumulation of aKG in the NSC, reported here, may be associated with neural commitment and cortical differentiation.

In conclusion, using iPSC-derived neural and mesenchymal stem cells, we showed an important mitochondrial remodeling during both lineages differentiation. In MSC, we detected a significant upregulation of the slow-running segment of Krebs cycle, simultaneously with increased respiratory chain activities and cellular respiration, indicating the involvement of Oxphos switching in the differentiation of these progenitors. Inverse changes in the mitochondrial activities occurred during neural differentiation. There was no activation of the CS – IDH3 segment of the Krebs cycle and no induction of the respiratory chain activities. Instead, we detected a significant reduction in the activity of aKGDH, the rate limiting Krebs cycle complex, simultaneously with a strong induction of the mitochondrial IDH2. This opposite change in aKG-producing IDH2 and aKG-consuming aKGDH, might be responsible for increasing the intracellular level of aKG, detected in NSC. Taking into account the key signaling and epigenetic role of this metabolite, this remodeling might be a neural specific checkpoint during differentiation and future work will help to describe its molecular basis.

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References

[1] S. Varum, A.S. Rodrigues, M.B. Moura, O. Momcilovic, C.A. Easley, et al. 2011. Energy Metabolism in Human Pluripotent Stem Cells and Their Differentiated Counterparts, PLoS ONE 6(6):e20914. https://doi.org/10.1371/journal.pone.0020914

[2] J. Zhang, I. Khvorostov, J.S. Hong, Y. Oktay, L. Vergnes, et al., UCP2 regulates energy metabolism and differentiation potential of human pluripotent stem cells, EMBO J. 30(2011)4860-73. https://doi.org/10.1038/emboj.2011.401

[3] A. Prigione, J. Adjaye, Modulation of mitochondrial biogenesis and bioenergetic metabolism upon in vitro and in vivo differentiation of human ES and iPS cells, Int J Dev Biol. 54(2010)1729-41. https://doi.org/10.1387/ijdb.103198ap

[4] W. Gu, X. Gaeta, A. Sahakyan, A.B. Chan, C.S. Hong, et al. Glycolytic Metabolism Plays a Functional Role in Regulating Human Pluripotent Stem Cell State, Cell Stem Cell. 19(2016)476-490. https://doi.org/10.1016/j.stem.2016.08.008

[5] A. Moussaieff, M. Rouleau, D. Kitsberg, M. Cohen, G. Levy, et al. Glycolysis-mediated changes in acetyl-CoA and histone acetylation control the early differentiation of embryonic stem cells, Cell Metab. 21(2015)392-402. https://doi.org/10.1016/j.cmet.2015.02.002

[6] T.S. Cliff, T. Wu, B.R. Boward, A. Yin, H. Yin, J.N. Glushka, J.H. Prestegaard, S. Dalton, MYC Controls Human Pluripotent Stem Cell Fate Decisions through Regulation of Metabolic Flux. Cell Stem Cell, 21(2017)502-516.e9. https://doi.org/10.1016/j.cmet.2015.02.002

[7] P. Bénit, E. Letouzé, M. Rak, L. Aubry, N. Burnichon, J. Favier, A.P. Gimenez-Roqueplo,
P. Rustin, Unsuspected task for an old team: succinate, fumarate and other Krebs cycle acids in metabolic remodeling, Biochim Biophys Acta. 1837(2014):1330-7. https://doi.org/10.1016/j.bbabio.2014.03.013

[8] C. Frezza, Mitochondrial metabolites: undercover signalling molecules, Interface Focus. 7(2017)20160100. https://doi.org/10.1098/rsfs.2016.0100 [9] B.W. Carey, L.W. Finley, J.R. Cross, C.D. Allis, C.B. Thompson, Intracellular α -ketoglutarate maintains the pluripotency of embryonic stem cells, Nature. 518(2015)413-6. https://doi.org/10.1038/nature13981

[10] T. TeSlaa, A.C. Chaikovsky, I. Lipchina, S.L. Escobar, K. Hochedlinger, J. Huang, T.G. Graeber, D. Braas, M.A. Teitell, α-Ketoglutarate Accelerates the Initial Differentiation of Primed Human Pluripotent Stem Cells, Cell Metab. 24(2016)485-493. https://doi.org/10.1016/j.cmet.2016.07.002

[11] J. Yu, K. Hu, K. Smuga-Otto, S. Tian, R. Stewart, I.I. Slukvin, J.A. Thomson, Human induced pluripotent stem cells free of vector and transgene sequences, Science. 324(2009) 797-801. https://doi.org/10.1126/science.1172482

[12] C. Boissart, A. Poulet, P. Georges, H. Darville, E. Julita, R. Delorme, T. Bourgeron, M. Peschanski, A. Benchoua, Differentiation from human pluripotent stem cells of cortical neurons of the superficial layers amenable to psychiatric disease modeling and high-throughput drug screening, Transl Psychiatry. 2013 20;3:e294. https://doi.org/10.1038/tp.2013.71

[13] K. Giraud-Triboult, C. Rochon-Beaucourt, X. Nissan, B. Champon, S. Aubert, G. Piétu, Combined mRNA and microRNA profiling reveals that miR-148a and miR-20b control human mesenchymal stem cell phenotype via EPAS1, Physiol Genomics. 43(2011)77-86. https://doi.org/10.1152/physiolgenomics.00077.2010

[14] P. Rustin, D. Chretien, T. Bourgeron, B. Gérard, A. Rötig, J.M. Saudubray, A. Munnich, Biochemical and molecular investigations in respiratory chain deficiencies, Clin Chim Acta. 228(1994)35-51.

[15] P. Bénit, S. Goncalves, E. Dassa, JJ Brière, G. Martin, P. Rustin, Three spectrophotometric assays for the measurement of the five respiratory chain complexes in minuscule biological samples, Clin Chim Acta. 374(2006)81-6. https://doi.org/10.1016/j.cca.2006.05.034

[16] S. Goncalves, V. Paupe, E.P. Dassa, J.J. Brière, J. Favier, A.P. Gimenez-Roqueplo, P. Bénit, P. Rustin, Rapid determination of tricarboxylic acid cycle enzyme activities in biological samples, BMC Biochem. 28(2010)11:5. https://doi.org/10.1186/1471-2091-11-5

[17] P. Bénit, D. Chrétien, M. Porceddu, C. Yanicostas, M. Rak, P. Rustin, An Effective, Versatile, and Inexpensive Device for Oxygen Uptake Measurement. J Clin Med. 6(2017)pii: E58. https://doi.org/10.3390/jcm6060058

[18] U. K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature. 227(1970)680-685

[19] J.C. St John, J. Ramalho-Santos, H.L. Gray, P. Petrosko, V.Y. Rawe, C.S. Navara, C.R. Simerly, G.P. Schatten, The expression of mitochondrial DNA transcription factors during early cardiomyocyte in vitro differentiation from human embryonic stem cells, Cloning Stem Cells. 7(2005)141-53. https://doi.org/10.1089/clo.2005.7.141

[20] C.D. Folmes, T.J. Nelson, A. Martinez-Fernandez, D.K. Arrell, J.Z. Lindor, P.P. Dzeja,
Y. Ikeda, C. Perez-Terzic, A. Terzic, Somatic oxidative bioenergetics transitions into pluripotency-dependent glycolysis to facilitate nuclear reprogramming, Cell Metab. 14(2011)264-71. https://doi.org/10.1016/j.cmet.2011.06.011

[21] M. Yudkoff , D. Nelson, Y. Daikhin, M. Erecińska, Tricarboxylic acid cycle in rat brain synaptosomes. Fluxes and interactions with aspartate aminotransferase and malate/aspartate shuttle, J Biol Chem. 269(1994)27414-20. https://doi.org/10.1016/j.cmet.2011.06.011

[22] S. Tohyama, J. Fujita, T. Hishiki , T. Matsuura, F. Hattori, R. Ohno, H. Kanazawa, T. Seki, K. Nakajima, Y. Kishino , M. Okada, A. Hirano, T. Kuroda, S. Yasuda, Y. Sato, S. Yuasa, M. Sano, M. Suematsu, K. Fukuda, Glutamine Oxidation Is Indispensable for Survival of Human Pluripotent Stem Cells, Cell Metab. 23(2016)663-74. https://doi.org/10.1016/j.cmet.2016.03.001

[23] S. Mandal, A.G. Lindgren, A.S. Srivastava, A.T. Clark, U. Banerjee U, (2011) Mitochondrial function controls proliferation and early differentiation potential of embryonic stem cells. Stem Cells, 29(2011)486-95. https://doi.org/10.1002/stem.590 [24] K.A. Tran, C.M. Dillingham, R. Sridharan, The role of α-ketoglutarate-dependent proteins in pluripotency acquisition and maintenance, J Biol Chem. 2018 Sep 4. pii: jbc.TM118.000831.
 doi: 10.1074/jbc.TM118.000831

Figure legends

Figure 1. Mitochondrial network in iPSC and iPSC-derived neural and mesenchymal stem cells. A) Punctuate perinuclear mitochondria in iPSC and reshaped mitochondrial network with appearance of elongated, reticular mitochondria in NSC and MSC as visualized by immunofluorescence staining of the mitochondrial Citrate Synthase (green; Abcam ab96600). The nuclei were labelled with Hoechst (blue, Invitrogen 33342). B) Morphological features of iPSC, NSC and MSC as observed with phase contrast microscopy.

Figure 2. Respiratory activities in iPSC and iPSC-derived neural and mesenchymal stem cells. A) Cellular respiration was measured with Optode device; n=5 and B) Activities of mitochondrial Respiratory Chain Complexes II (Succinate Quinone DCPIP Reductase, SQDR), III (QCCR, Quinone Cytochrome c Reductase) and IV (COX) were analyzed spectrophotometrically; n=5 (CIII, CIV), n=4 (CII); n is one independent culture; Values are means \pm SD. Data were analyzed with Graphpad Prism using two-tailed t-test.

Figure 3. Krebs cycle remodeling in neural and mesenchymal stem cells. A) Activities of Krebs cycles enzymes were analyzed spectrophotometrically; Citrate Syntase (CS), Isocitrate dehydrogenase 2 (IDH2), n=5; Aconitase 2 (ACO2), Isocitrate dehydrogenase 3 (IDH3), mitochondrial Fumarate Hydratase (mt-FH), a-ketodehydrogenase complex (aKGDH), n=4; Malate dehydrogenase 2 (MDH2), n=3; Aspartate Aminotransferase (ASAT), n=5; Values are means \pm SD; B) Accumulation of IDH2, OGDH (Oxoglutarate dehydrogenase component E1 of aKGDH; CS and ACO2 as visualized by Western blot (left panel - representative picture showing two independent cultures for each cell type), quantified with ImageJ and reported as percentage of iPS; n=4; n is one independent culture; Values are means \pm SD. Data were analyzed with Graphpad Prism using two-tailed t-test.

Figure 4. Intracellular levels of aKG and remodeled Krebs cycle in iPS and iPS-derived stem cells. A) Intracellular levels of aKG were measured spectrofluorometrically in NSC and MSC (n=3) and in iPS (n=4). Succinate levels were measured spectrophotometrically (n=3); n is one independent culture; Values are means \pm SD; Data were analyzed with Graphpad Prism using two-tailed t-test. B) Summary of Krebs cycle remodeling in the iPS-derived NSC and MSC. The functional splitting of the Krebs cycle reactions represented as two complementary mini-cycles: the fast running cycle from aKG to oxaloacetate (large arrow, left cycle) and the slow running segment (narrow arrow, right cycle) (see text for explanation).

Supp Figure 1: Molecular verification of i90c16 human-induced pluripotent stem cells and its derivatives. (A) mFISH chromosome analysis of i90c16-iPSCs showing a normal caryotype (46, XX). (B) Immunostaining showing the expression of the pluripotency markers OCT4, SSEA4, TRA1-60 and NANOG. Scale bars = 200 μ m. (C) Representative phase-contrast image of i90c16-iPSCs colonies on feeder cells. Scale bars = 200 μ m. (D) Sample bright-field image of i90c16-iPSCs derived neural stem cells (NSCs). Scale bars = 50 μ m. (E) Immunostaining showing the expression of the neural (NESTIN, SOX2) and proliferating (Ki67) markers in NSCs. (F) Phase-contrast image of i90-iPSCs derived MSCs. (G) Flow cytometry analysis of MSCs markers (CD29, CD44, CD73, CD105 and CD166) expression in i90c16-iPSCs derived MSCs. Scale bars = 200 μ m.

Supp Figure 2: The aKGDH/IDH2 remodeling in iPS1869-derived NSC.

Accumulation of IDH2, OGDH and CS as visualized by Western blot (left panel - representative picture showing three independent cultures for each cell type), quantified with ImageJ and reported as percentage of iPS. n=3; n is one independent culture; Line containing Molecular weight marker is covered. Values are means \pm SD. Data were analyzed with Graphpad Prism using two-tailed t-test. This control iPSC line was published previously as 1869 (Boissart et al., 2013) and was reprogrammed from GM01869 fibroblasts obtained from the Coriell's biorepository (Coriell Institute for Medical Research, NJ, USA). It was differentiated into NSC using the same procedure as describe for i90c16.

Figure 1.



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Figure 2.









Figure 3.



Figure 4.

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