LETTER TO THE EDITOR

Mitochondrial metabolism and glutamine are essential for mesoderm differentiation of human pluripotent stem cells

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Dear Editor,

Human pluripotent stem cells (hPSCs) generate energy mainly by aerobic glycolysis, with glutamine oxidation in the tricarboxylic acid (TCA) cycle providing additional ATP required for survival.1–3 During the exit from pluripotency and initial differentiation into multiple germ lineage precursors, energy production shifts from mainly aerobic glycolysis to mitochondrial oxidative phosphorylation (OXPHOS).1 Until recently, consensus in the field was that as hPSCs exit pluripotency, a metabolic switch from aerobic glycolysis to OXPHOS is required. However, a more detailed examination of nutrient-balanced and chemically de lineages using RNA-Seq was equivalent to a previous study using variance in nutrient composition (Supplementary information, that intrinsic metabolic preferences were not derived from a established pro

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revealed widespread cell death under Gln deprivation, suggesting that Gln is indispensable for ME formation (Supplementary information, Fig. S2f). In contrast, Gln withdrawal led to an increase in EC specifying biomarker proteins PAX6 and MAP2B during 21 days of EB differentiation (Fig. 1h, Supplementary information, Fig. S2e). Increased EC production may be linked to a compensation for Gln deprivation by recovery of glycolytic metabolites (Fig. 1g, h, Supplementary information, Fig. S2g).

Together, this EB differentiation profile suggests that Gln withdrawal skews potentiation of ME negatively and EC positively. This report provides a deeper understanding of distinct metabolic shifts in early germ lineages by showing that mitochondrial respiration and Gln oxidation is essential for ME differentiation. Further studies are needed to elucidate the functional roles of metabolic reprogramming in early germ lineages, with possibilities including transcriptional program
Fig. 1 Human pluripotent stem cell-derived mesoderm differentiation requires glutaminolysis and distinct mitochondrial metabolism. a Oxygen consumption rate (OCR) measured by mitochondrial stress test of H9 hPSC and H9-derived endoderm (EN), mesoderm (ME), and ectoderm (EC) lineages at 5 days of directed differentiation. Data are normalized by micromgram of protein content per well. Injections of 1 µM oligomycin, 0.33 µM FCCP, 0.5 µM FCCP, and 1 µM each Antimycin A/Rotenone were performed. Data represent $n = 6$ technical replicates of 1 biological experiment with additional $n ≥ 2$ biological experiments provided in Supplementary information, Fig. S1e. b Media footprint analysis (relative metabolite consumption and production into media) of nutrient-balanced EN, ME, and EC samples, quantifying levels of (i) glucose consumption, (ii) lactate production, (iii) glutamine consumption, and (iv) glutamate production at 5 days of directed differentiation. Positive values indicate increased production of cellular metabolites into spent media, whereas negative values indicate cellular consumption of media metabolites. Data are normalized to initial unspent fresh media levels as measured by UHPLC-MS, and represent $n = 3$ independent experiments. Media of differentiated H9 hESCs were changed 24 h prior to spent media metabolite extraction. c Normalized single colony biomass accumulation and growth rates (%/hour) of nutrient-balanced H9-derived EN/ME/EC samples measured over 24 h. Data represent $n = 29$ EN, $n = 45$ ME, and $n = 37$ EC colonies. d, e Fractional contribution of $^{13}$C$_5$-labeled metabolites from [U-$^{13}$C$_5$]glutamine (d) or $^{13}$C$_5$-labeled metabolites from [U-$^{13}$C$_5$]glutamine (e) after 18 h quantified by UHPLC-MS. f Mass isotope ratio distribution (MID) of TCA cycle-associated metabolites from [U-$^{13}$C$_5$]glutamine. The carbon labeling of TCA cycle-associated metabolites from [U-$^{13}$C$_5$]glutamine is schematically illustrated in black for the first (1st) and second (2nd) turn or $α$-ketoglutarate ($α$-KG) reductive carboxylation (RC) into citrate. Media of differentiated H9 hESCs were changed 18 h prior to intracellular metabolite extraction. g Relative levels of TCA cycle metabolites in H9 hPSCs under 36 h and 48 h spontaneous differentiation in the presence or the absence of Glu in the culture media co-treated or untreated with 1 mM DCA, quantified by UHPLC-MS. h Immunoblot of ME (SLUG) and EC (PAX6, SOX2 and MAP2) markers in H9-derived EB at 21 days of differentiation in the presence or the absence of Glu in the culture media co-treated or untreated with 1 mM DCA, or carbon source-specific dependencies.

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AUTHOR CONTRIBUTIONS

ADDITIONAL INFORMATION
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REFERENCES

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