

Alpha-ketoglutarate: a “magic” metabolite in early germ cell development

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A causal relationship between cell metabolism and the fate of pluripotent stem cells through epigenome regulation is emerging. A recent study shows that the tricarboxylic acid cycle intermediate alpha-ketoglutarate (α KG) can both sustain naïve mouse embryonic stem cell pluripotency and promote primordial germ cell differentiation. This observation together with other studies provides intriguing possibilities for stabilizing ephemeral embryonic cell states and enhancing desired fate transitions through specific metabolite manipulations.

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See also: J Tischler *et al*

Primordial germ cells (PGCs) are precursors of gametes that connect one generation to the next. Modeling early mouse germ cell development *in vitro* begins with the transition of naïve mouse embryonic stem cells (mESCs) into transient, primed mouse epiblast-like cells (EpiLCs) (Fig 1). This is followed by conversion of EpiLCs into PGC-like cells (PGCLCs), which can produce sperm and oocytes. mESCs and PGCs are similar in expressing pluripotency regulators Oct4, Nanog, and Sox2, but are unique from epigenome and transcriptome changes that yield cell states with distinct developmental potentials (Hayashi *et al*, 2011). A key question under study in many lineage-specific developmental systems is what is the role for metabolism in cell fate transitions and outcomes?

Now, Tischler *et al* (2018) identify roles for alpha-ketoglutarate (α KG) in maintaining mESC pluripotency and enhancing PGCLC differentiation. Using single-cell RNA-Seq over a developmental time course referred to as a “pseudotime analysis”, they show a transition from mainly oxidative (OXPHOS) to glycolytic metabolism with progression from mESCs to EpiLCs. On conversion of EpiLCs to PGCLCs, OXPHOS increases and glycolysis decreases, consistent with a prior study (Hayashi *et al*, 2017). Repressing glycolysis or elevating α KG levels favors retention of mESC self-renewal over exit from naïve pluripotency. Adding α KG boosts PGCLC production by ~50%, and added α KG can replace 2i/Lif medium to maintain mESC self-renewal or can substitute for BMP4/8 in PGCLC fate induction. Remarkably, α KG addition also extends EpiLC competency to form PGCLCs without affecting cell quality, with H3K9me2 and H3K27me3 histone marks stabilized.

Alpha-ketoglutarate is a cofactor for Jumonji C (JmjC)-domain-containing histone demethylases (JHDMs) and ten-eleven translocation (TET) DNA demethylases. Cell-permeable dimethyl- α KG (dm- α KG) used to elevate α KG also has non-epigenome activities and can stabilize hypoxia-inducible factor 1 α (HIF1 α) to induce a pseudo-hypoxic state with altered gene expression (Hou *et al*, 2014). α KG can also inhibit the ATP synthase and mTOR (Chin *et al*, 2014), although added α KG did not affect the ATP synthase and could substitute for dm- α KG to promote human ESC differentiation in a prior study (TeSlaa *et al*, 2016). Consistent with an

epigenome effect, dm- α KG altered H3K9me3 and H3K27me3 levels to promote self-renewal of naïve mESCs in another study (Carey *et al*, 2015). Added α KG also enhanced spontaneous mESC differentiation (Hwang *et al*, 2016) and directed human neuroectoderm differentiation (TeSlaa *et al*, 2016) with altered histone and DNA methylation levels.

Using pseudotime modeling, the current RNA-Seq analysis revealed cell heterogeneity in differentiation capacity on EpiLC induction, with added α KG increasing PGC competency. Also, α KG preserved histone methylation patterns and prolonged a transient state for PGC competency. Whether succinate, a product and inhibitor of dioxygenase epigenome modifying enzyme reactions, would impair PGC competency was not examined. Also, the epigenetic state resulting from increased α KG may be physiologic or aberrant and requires further comparison studies. These results further suggest that α KG or other metabolites could prolong (or shorten) other transitory intermediate cell states. For example, cancer cells use glutaminolysis to generate α KG as an anapleurotic fuel for the TCA cycle and drug resistance may occur by induction of a transient transcriptional state via epigenome remodeling (Shaffer *et al*, 2017). Manipulating α KG levels by glutamine reduction could deplete TCA cycle intermediates and block transient drug-resistant cancer cell states.

In vivo studies of α KG and other metabolites on cell fate are largely unexplored, yielding questions about how diet and environment influence germ cell metabolite levels and outcomes. Most epigenetic marks

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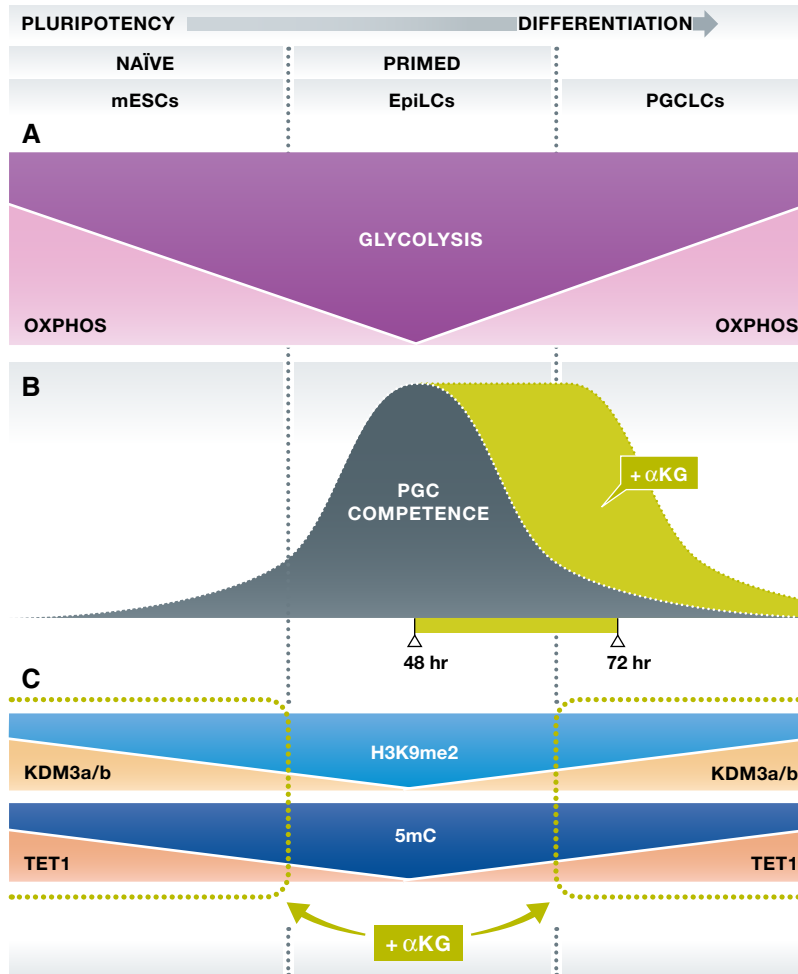
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shaped by short-term environmental conditions are not inherited even with known imprinting (Heard & Martienssen, 2014). Caloric restriction, high-fat diet, and malnourishment in parents can impact the epigenome and small noncoding RNAs transmitted by gametes to offspring (Sharma & Rando, 2017). Emerging genome editing technologies may abet *in vivo* manipulations of metabolite levels at specific times within a developing embryo to start addressing the role of metabolites *in vivo*. Indeed, large knowledge gaps exist for (i) how to assess the contribution of diet to key metabolite levels in both somatic and germ cells, (ii) the responsiveness of epigenetic regulators and cofactors to specific metabolite levels, and (iii) whether metabolite-influenced

epigenome modifications persist and manifest into adulthood and across generations. An impediment to early embryo studies is the paucity of starting materials, with advances in single-cell methods holding promise to bridge the gap between metabolism, epigenetics, and stem cell fates (Zhang *et al*, 2018). Advancing this emerging opportunity *in vitro* at first, Tischler *et al* (2018) deploy single-cell technologies to reveal how targeted metabolic manipulations augment (or potentially retard) dynamic cell state conversions during PGC development.

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