

Human pluripotent stem cells decouple respiration from energy production

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Human pluripotent stem cells (hPSCs) rely heavily on glycolysis for energy metabolism, and because their mitochondria appear poorly developed, hPSCs have been assumed to be incapable of using oxidative phosphorylation (OxPhos). In this issue, Zhang *et al* (2011) demonstrate that hPSCs actually possess functional OxPhos machinery, but that the mitochondrial protein UCP2 decouples OxPhos from glycolysis. The study further suggests that regulation of glucose metabolism by UCP2 facilitates hPSC pluripotency and controls hPSC differentiation.

Pluripotent embryonic stem cells require an exceptionally high flux of glucose uptake and lactate production, even when these cells grow in aerobic conditions outside the hypoxic blastocyst (aerobic glycolysis) (Prigione *et al*, 2010). In contrast, differentiated cells often require lower rates of aerobic glycolysis and shunt most of the cytosolic pyruvate into mitochondria where it is oxidized via the Krebs cycle and the electron transport chain (ETC) to synthesize ATP, a process collectively known as OxPhos. Consistent with these observations, hPSC mitochondria possess poorly developed cristae that only enlarge to form a densely tubular structure upon differentiation, which led some to conclude that hPSCs lack functional mitochondria (Facucho-Oliveira *et al*, 2007). However, how this switch occurs and whether a specific mitochondrial physiology is required for maintenance of the pluripotent state remained unclear.

Zhang *et al* (2011) now show that hPSCs actually possess functional OxPhos machinery. In fact, hPSC mitochondria consume oxygen at rates similar to differentiated cell mitochondria. Unlike that of differentiated cells, glucose uptake is less coupled to OxPhos in hPSCs, and instead hPSCs predominantly use glycolysis to generate ATP. Furthermore, the authors inferred that ATP synthesis is also less coupled to the ETC in hPSCs and that ATP synthase may even be hydrolyzing ATP. Although more work is needed to establish this claim, it raises the intriguing possibility that ATP consumption in hPSCs is supporting an optimal membrane potential that promotes biosynthetic growth, just like in cancer cells (Racker 1976; Vander Heiden *et al*, 2010). Yet how is OxPhos decoupled from glycolysis in hPSCs? Zhang *et al* (2011) found that ectopic expression of UCP2 suppressed OxPhos during hPSC differentiation, while UCP2 knockdown decreased lactate production (Figure 1). Importantly, ectopic UCP2 also

impeded hPSC differentiation, suggesting that relieving UCP2-mediated suppression of OxPhos is required for differentiation.

UCP2 belongs to the uncoupling protein (UCP) family. UCP1 transports protons to dissipate the membrane potential and uncouples ATP synthesis from the ETC. In contrast, UCP2 is still a subject of controversy. UCP2 transports protons *in vitro*, but apparently not *in vivo* (Couplan *et al*, 2002). Instead, studies have shown that UCP2 decreases pyruvate oxidation, suggesting that UCP2 decouples glycolysis from OxPhos by shunting pyruvate out of the mitochondria (Emre and Nubel, 2010). Zhang *et al* (2011) provide evidence to support this view. By using ¹³C-isotope tracing, the authors

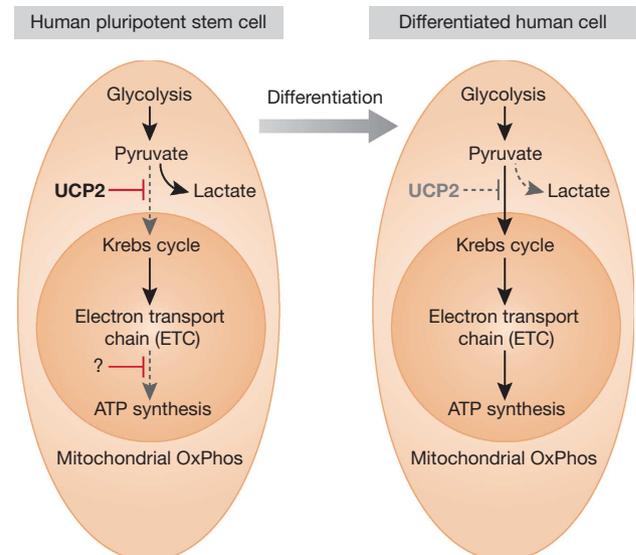


Figure 1 Decoupling of respiration from ATP synthesis in hPSCs. The high glycolytic flux in hPSCs generates cytosolic pyruvate and ATP. High levels of UCP2 (bold) in hPSCs suppress the channelling of glycolytic flux into the Krebs cycle (grey). Pyruvate is converted to lactate instead. Upon differentiation of hPSCs, UCP2 levels decline (grey), thus increasing the interaction between glycolysis and the Krebs cycle (bold). Lactate production (grey) drops and OxPhos increases. The fully active ETC is also less coupled to ATP synthesis in hPSCs, but coupling of this step increases with hPSC differentiation.

show that ectopic expression of UCP2 decreases glycolytic flux to the Krebs cycle in differentiating hPSCs. However, whether this reflects a *bona fide* pyruvate transport activity in UCP2 remains to be tested.

Glycolysis has recently been studied during the reprogramming of human fibroblasts into induced hPSCs (Zhu *et al*, 2010; Folmes *et al*, 2011). Although c-Myc is a well-known pluripotency factor that also promotes aerobic glycolysis, these studies have shown that aerobic glycolysis in hPSCs can be enhanced by factors other than c-Myc. It would be interesting to examine which pluripotency factors regulate UCP2 expression to control hPSC bioenergetics. Zhang *et al* (2011) also showed that ectopic expression of UCP2 prevented hPSC differentiation, but UCP2 knockdown failed to impair self-renewal or induce differentiation, suggesting that other mechanisms exist to suppress OxPhos or coordinate OxPhos with differentiation in hPSCs. In addition, it is still unclear

why or how the ETC is operating at maximal capacity, when ATP synthesis appears to be less coupled to the ETC in hPSCs. One possibility is that it is operating to recycle NAD and keep the Krebs cycle turning to generate biosynthetic intermediates. Another complementary possibility is that the high glycolytic ATP is forcing some ETC reactions to run in reverse, a phenomenon first observed in the 1960s (Forman and Wilson, 1982). This then functions to engage the ETC in an ATP-consuming futile cycle to maintain an optimal membrane potential. The study by Zhang *et al* (2011) raises many interesting questions with implications for understanding the bioenergetics in PSCs and perhaps also in other settings such as those found in cancer metabolism.

Conflict of interest

The authors declare that they have no conflict of interest.

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