Commentary

Stem Cell INVESTIGATION

Metabolic plasticity complements the unique nature and demands of distinct pluripotency states

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Pluripotency, the ability to differentiate into all somatic cell types, is a pertinent feature of embryonic stem cells (ESCs). However, during early ontogenesis, *in vivo*, this capacity only exists transiently (1,2). Within this window of development, extracellular signals governing embryogenesis grant pluripotency a dynamic nature, while molecular changes in cellular identity occur in preparation for further lineage specification (2). By precisely coordinating the cellular microenvironment and embryonic derivation stage, ESCs can be stabilized in distinct states of pluripotency, *in vitro*. Conventional derivation of human ESCs (hESCs) from the inner cell mass (ICM) of the pre-implantation blastocyst gives rise to stem cells, which are markedly different from mouse ESCs (mESCs). *In vitro*, hESCs originate from a post-ICM intermediate, a transient epiblast-like structure (3,4). As such, hESCs adopt the distinct primed state of pluripotency, sharing more similarities with mouse epiblast stem cells (mEpiSCs) derived from post-implantation stage embryos (5). Conversely, mESCs, adopt the naive state, which constitutes the functional *in vitro* equivalent of the pre-implantation epiblast (5).

Primed ESCs display distinct pluripotency associated gene patterns, DNA hyper-methylation, X-chromosome inactivation and are inefficient in forming chimeras. In contrast, naive ESCs display lower variability in pluripotency linked gene expression, global DNA hypo-methylation, two active X-chromosomes in female cells and readily form chimeras (1,2,6). Their restricted gene profile, predisposition to certain lineages, and resistance to clonal expansion often make primed hESCs less favorable for future regenerative applications (1,7). The more homogenous naive state of pluripotency, with seemingly unbiased differentiation potential, has driven research towards the identification of *in vitro* conditions that preferentially stabilize a similar ground state, in human. To date, various conversion protocols have been established [reviewed in (6)], while direct derivation of naive hESCs from the ICM has also been achieved, although mostly at low efficiency (7-9) and commonly resulting in an abnormal karyotype (9,10).

Primed and naive hESCs are defined by unique transcriptional and epigenetic landscapes, which inherently translate to distinctive shifts in metabolic pathway utilization (7,9,11). Therefore, alterations in metabolism further distinguish these states and support their specific energy and biosynthesis demands (12-14). While primed hESCs depend on glycolytic metabolism, those in the naive state have the capacity to switch their energy dependency between glycolysis and oxidative phosphorylation (13-15). Previous studies have suggested that conversion of hESCs towards the naive state is accompanied by dramatic changes in mitochondrial activation and metabolic realignment (7,16).

By further exploring the notion that metabolic plasticity complements variations in cellular identity and global transcriptomic landscape, Gu *et al*. establish valuable links between cellular metabolic mechanisms, self-renewal and

cell-fate specification in both primed and naive hESCs. The authors demonstrate that shifts in both glycolytic flux and mitochondrial respiration, accompany manipulations of the pluripotency network, with primed and naive hESCs displaying different glycolytic rates. Furthermore, Gu *et al*. are the first to reveal intriguing differences in response to metabolic changes, relating to the presence of feeder cells in primed hESC conditions. Finally, they examine the potential value of altering cellular metabolism for directing preferential lineage specification.

By investigating metabolic profiles of naive hESCs converted in 5i/LAF conditions (9), as well as naive/reset cells (17), Gu *et al*. reveal that naive hESCs display an increased rate of glycolysis, coupled with higher *MYC* transcriptional activity and increased nuclear *N-MYC* and *C-MYC* levels, compared to their primed counterparts. In the mouse the opposite is true. Mouse EpiSCs have been shown to have a higher glycolytic rate, when compared to mESCs (15). Gu *et al*. suggest that this may be due to species specific differences relating to *C-MYC* expression, which serve to promote glycolysis. Nuclear *C-MYC*, is higher in mEpiSCs and naive hESCs, while lower levels of *C-MYC* were found in primed hESCs and naive mESCs. Despite broad similarities, mEpiSCs and primed hESCs exhibit crucial differences in gene expression (18). Differences in metabolic regulation, further confirm that these cell types are not developmentally equivalent.

The authors also observed higher glycolytic rates in hESCs derived directly from human blastocysts in naive 5i/LAF conditions. Gene enrichment analysis, revealed elevated *MYC* targets in both naive conditions, as well as a similarly significant enrichment in pre-implantation blastocysts. Hence, Gu *et al*. not only establish a link between increased glucose consumption and the naive state *in vitro*, but propose that such glycolytic pathway gene expression may characterize naive pluripotency, *in vivo*. However due to the dynamic nature of metabolism, defining unique metabolic signatures for specific developmental contexts may prove challenging (19).

Gu *et al*. stress that the aforementioned changes were only observed following complete transition to the naive state, emphasizing that increased glycolytic flux requires the acquisition of naive cell identity. As the stabilization of the human naive state requires precise coordination of the pluripotency network by targeting signaling pathways, investigating the role of specific signaling molecules in regulating naive hESC metabolism will also be of interest. Notable differences have been described in the growing number of reported hESC naive conditions, suggesting that they may represent a broader spectrum of developmental states (2,6,9,20). Interestingly, the findings by Gu *et al*. suggest a conserved metabolic profile across various naive conditions. These data are in agreement with the meta-analysis performed by Huang *et al*., describing common mitochondrial, RNA processing and ribosomal biogenesis genes in naive hESCs that were generated by different protocols (1,20). More extensive comparative transcriptomic and epigenetic profiling combined with metabolic analysis will further elucidate this hypothesis.

Gu *et al*. highlight the importance of glycolysis for self-renewal of primed and naive hESCs and are the first to investigate, in detail, the impact of feeders in response to glycolysis inhibition in primed hESCs. Inhibiting glycolysis, resulted in impaired proliferation in naive and feeder free (FF) primed hESCs, but not in feeder supported (FS) primed hESCs. The authors show that MEF-secreted factor regulation decreased the reliance of primed hESC on glycolysis for proliferation, which in turn decreased *MYC* and *N-MYC* levels. Conversely, FF primed hESCs utilized glucose more for biosynthesis than FS primed hESCs, leading to an increased reliance on glucose, with higher *MYC* and *N-MYC* transcription. Gu *et al*. employ Matrigel for their FF cultures. It remains to be elucidated how metabolism is affected by other FF systems, such as laminin isoforms, which constitute the extracellular matrix of the ICM. These have been found to be more optimal for long-term survival of both primed and naive hESCs (21). With the aim of further improving naive culture conditions, establishing the effects of FF conditions on naive hESC metabolism also seems relevant.

Changes in cellular demands during hESC differentiation, promote variations in metabolic regulation. Gu *et al*. show that manipulating glycolysis can, in fact, direct cell fate towards specific lineages. Transcriptome sequencing revealed that inhibition of glycolysis in FF primed hESC, induced neuronal lineage specification. Moreover, glycolysis inhibition increased the efficiency of directed neuronal differentiation. Therefore, understanding the processes regulating hESC metabolism will undoubtedly serve to further optimize hESC specification protocols.

Gu *et al*. skillfully highlight the dynamic nature of metabolism and its role in facilitating changes in cellular identity and function. Such insights are of immense value not only for understanding the biology of ESCs, but also their developmental contexts. Elucidating the underlying mechanisms regulating pluripotency, development, self-

renewal and cellular function is essential for future clinical applications of hESCs.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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