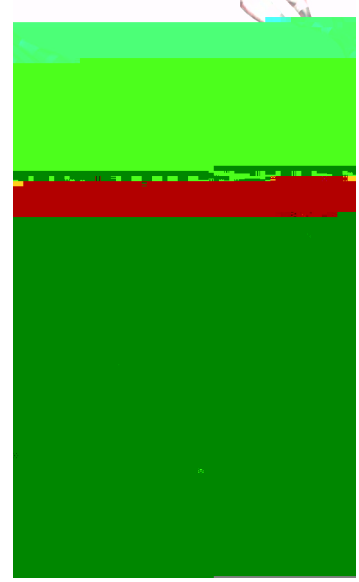
Commentary



Naive pluripotent stem cells as a model for studying human developmental epigenomics: opportunities and limitations

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“A a a d g cha e ge e a e he he a e hESC a e a d c a d ab e a e, he he he a e a e e he g g a g c d ha ed a a a e hESC e.”

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Next year will mark the 20th anniversary since the discovery of human embryonic stem cells (hESCs) and the demonstration that these remarkable cells can differentiate into all lineages of the body [1]. Impressive progress since then has delivered hESC-derived therapies to the clinic, established hESCs as a cellular platform for drug screening, toxicology assays and disease modeling, and advanced our understanding of human developmental biology.

Although derived from epiblast cells of the preimplantation human embryo, the biological properties of hESCs more closely reflect the early postimplantation embryo that is formed several days later in development [2–5]. This distinction in timing is important because it helps to evaluate the characteristics of hESCs by comparing with their equivalent cells *in vivo*. Classifying the current hESC lines as similar to postimplantation cells also opens up an obvious need to capture hESCs in an alternative state that recapitulates the preimplantation embryo.

Several reports of preimplantation-like human stem cells, broadly termed ‘naive’ hESCs, have raised the hope that deriving such a cell type is achievable [6–11]. These recent additions to the stem cell hierarchy are particularly exciting for developmental epigeneticists. Access to an *in vitro* model that reproduces the epigenetic state of preimplantation epiblast cells would enable new opportunities to study the underlying mechanisms of many key epigenetic processes, such as the regulation of X-chromosome inactivation in humans.

As with all novel cell types, there are on-going discussions about how to evaluate this new cell state. The task is

DNA methylation: characteristic feature of naive pluripotency or consequence of culture?

An important, outstanding challenge is to evaluate whether naive hESCs are a distinct and stable state, or whether they are a response to the strong signaling conditions that are used to maintain naive hESCs in culture. For example, the addition of a MEK1/2 inhibitor (a universal component of naive embryonic stem cell [ESC] media) to conventional mouse and human ESCs leads to a rapid, global loss of DNA methylation through the downregulation of UHRF1 and DNA methyltransferases [16]. This occurs independently of an initial cell-state change. These observations lead to the question of whether the hypomethylated epigenome reflects the biology of the preimplantation human embryo and is an informative readout of the naive state, or alternatively, is it a direct effect of MEK1/2 inhibition that simply mimics our expectations? Comparing the methylomes between naive hESCs and human embryos reveals similar levels of low, global DNA methylation [11,13,17]. However, a closer examination of methylation levels at CpG sites between naive hESCs and human embryos showed that the methylomes are actually quite different; naive hESCs have fairly uniform and low levels across the genome, whereas the embryo retains regions with higher levels of DNA methylation [17]. Although an important first step, one caveat here is that this comparison used data from whole embryos, of which the pluripotent epiblast cells are a relative minority compared with the extraembryonic cells. Additional embryo methylation datasets, preferably from single cells of defined lineage, are required for a more detailed comparison.

The naive hESC methylation profiles fit with a model in which the DNA methylation machinery is continually suppressed. It is important to remember that epiblast cells in the embryo are only transient, and if epiblast cells were maintained with inhibited DNA methylation activity then they too would probably display uniformly low levels of methylation across the genome. There are important implications of this however, for example, the methylation marks at imprinted gene control regions are erased in naive hESCs [13,17]. This contrasts with the human preimplantation embryo where methylated imprints are intact, despite the strong demethylating environment [18]. Indeed, initial experiments suggest that imprints are relatively resistant to demethylation compared with other regions in the genome when hESCs are challenged with MEK1/2 inhibition, and prolonged inhibitor treatment is

naive hESCs recapitulate closely the pre-X-inactivation state of the human embryo [22] and, therefore, provide a tractable cell culture model to study the process of X-chromosome inactivation in humans.

The human embryo ensures X-chromosome dosage compensation using mechanisms distinct from the mouse, potentially through the competition of opposing long noncoding RNAs, *XIST* and *XACT* [14,22]. The presence

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