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Human pluripotency
A difficult state to make smart choices

Shravanti Rampalli and Mickie Bhatia*

McMaster Stem Cell and Cancer Research Institute; McMaster University; Ontario, Canada

Pluripotent cells have unique properties of self-renewal while maintaining developmental potential to differentiate into all three germ layers. By definition, pluripotent cells have no predetermined lineage program, thereby forming the fundamental basis of enquiry and experimental investigations to understand cell fate decisions. Molecular and cellular insights into human pluripotent stem cell (hPSC) fate hold the potential to impact a broad range of human disease for cell replacement therapies and disease modeling and therefore are highly prioritized in current research efforts. The majority of concepts regarding ground state pluripotency have been derived from large-scale genomic,[1] epigenomic[2] and proteomic[3] experimentation using a variety of hPSC lines cultured and derived from very divergent culture and derivation methods. In turn, these global analyses are unique to the human and differ from sources used for the study of mouse ESCs or iPSCs, which are derived from inbreed strains and standardized culture conditions. Although this provides consistent results using mouse PSCs, the surrogacy that can be translated to hPSCs to improve differentiation toward applications is completely unclear. Nevertheless, commonality in pluripotent state emerges from a core set of transcription factors, including Oct4, Sox2 and Nanog.[4] Unique to hPSCs, these factors regulate pluripotency by associating with epigenetic (e.g., Polycomb and Trithorax) regulators to establish bivalent marks.[5] However, the complex interplay among transcription factors, cell signaling and bivalent epigenetic marks has not yet been fully described in the context of hPSC differentiation.

Human ESCs (hESCs) have been shown to possess unique chromatin structure to ensure ground state of pluripotency termed "bivalent domains". These domains have both active (H3K4Me3) and repressive (H3K27Me3) histone modifications thought to control key developmental regulators and maintain a silent, but poised, transcription state.[6] These observations depend on the assumption that all hPSCs harvested for these molecular analyses are homogenous, despite the fact that the field of somatic stem cell biology has shown the stem cell compartment is arranged purposefully as a hierarchy with unequivalent developmental potential.[6] Although a bivalent hypothesis for each individual hPSC is attractive to explain its pluripotent potential and cell fate decisions, the validity of this model is best questioned by increasing evidence of heterogeneity among hPSCs, and there is a lack of evidence to demonstrate this behavior at the single-cell level to date. Using transgenic mouse models, at least two independent laboratories have indicated clonal lines of mouse ESCs are not homogenous; rather they are comprised of dynamic and interdependent subpopulations.[7,8]

Similar to mouse, and perhaps even to a larger extent, hPSCs also exhibit phenotypic and molecular heterogeneity.[9] Using unbiased clonal tracking assays, subpopulations of hESCs were shown to participate in in vitro vs. in vivo differentiation.[10] Furthermore, at the molecular level, the complexity of hESC cultures using cell surface markers such as cKIT and A2B5 was diversely expressed in hPSCs that continue to equally express core pluripotent factors.[11] Direct isolation of these subfractions demonstrated their propensity toward hematopoietic and neural lineages with reduced self-renewal at a functional level of developmental potential. It is commonly believed that acquisition of lineage markers is associated with loss of pluripotency; however, our current understanding argues against this idea as a unifying theme of hPSC cell fate control. As such, we have observed robust self-renewal potential from hESCs harboring protein expression of lineage-specific Brachury/cKIT or A2B5. Investigation of histone marks in isolated hESC subfractions revealed resolution of bivalent domains into monovalent marks.[11] If cells were not fractioned, bivalent marks could readily be observed similar to previous reports, cautioning against the interpretation of bivalency as it relates to hPSC cell fate control. This is consistent with the idea that bivalent domains are not restricted to PSCs, as studies have observed them in adult stem cells.

If bivalent marks were found in all hPSCs and had equal opportunity to make lineage choice, the direct differentiation protocols of hESCs toward specific lineages would generate purer differentiation vs. the spectrum of lineages and be more efficient in nearly all differentiation protocols to date. Equally important is to determine if, in fact, heterogeneity in pluripotent cultures remains a requirement and not merely a byproduct of culture methods to ensure a balance of differentiation and self-renewal. Nevertheless, how and why PSC heterogeneity in mouse and human cultures is achieved and its biological requirements in vitro remain important questions worthy of further in-depth investigation. Since hPSCs represent a

*Correspondence to: Mickie Bhatia; Email: mbhatia@mcmaster.ca
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captured state of pluripotency in vitro, the choice to make truly appropriate lineage commitment decisions during differentiation can only be functionally determined using in vivo readouts, and feature rarely measured to date.

We believe that the bivalent model to describe single-cell behavior and cell fate decisions is overly simplistic and not reflective of the complexity of hPSC fate decisions. Alternatively, we suggest that the frequent fluctuations within the stem cell compartment give rise to a spectrum of inter-converting metastable states that allow lineage priming and self-renewing balance at the level of hPSC culture and niche. Accordingly, it will be important to understand how these seemingly stochastic changes are governed by epigenetic and transcriptional regulators that translate the overall pluripotency of human PSCs.

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