Naive embryonic stem cells: the future of stem cell research?

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There are now three reports concerning *de novo* establishment of naive human embryonic stem cells (hESCs) [1–3]. The future value of naive hESCs hinges on what we now know about mouse embryonic stem cells (mESCs). Two stages of mouse pluripotency have been stabilized to date through *in vitro* culture. ‘Naive’ represents the earliest stage, which can be enriched for a purer population, termed ‘ground state’ [4]. Naive most closely approximates embryonic epiblast in the preimplantation blastocyst. The primed state most closely approximates *in vivo* immediate postimplantation epiblast [5,6]. There are clear differences between these stages in culture, expression, epigenetics (including appropriate X-inactivation) and developmental competence. The fact that naive mESCs can generate whole animals through tetraploid complementation [7] while primed mESCs cannot [4,5], is a particularly compelling reason for heightened expectations from naive hESCs.

hESC lines are conventionally considered to be in a ‘primed’ state. As with mice, this state most closely resembles the *in vivo* early postimplantation epiblast prior to gastrulation [5,6]. This human epiblast population may be heterogeneous with a level of developmental intricacy that is not defined. Whether cells within the epiblast directly equate with hESCs and whether hESCs are a unique *in vitro* artifact is unknown. However, it is known from mouse literature that the epiblast-containing preimplantation blastocyst inner cell mass is a heterogeneous population [8] and that mESCs are derived from the

preimplantation epiblast [9]. mESCs achieve stability *in vitro* in a preimplantation ‘naive’ state [10,11]. Derivation of hESCs rely on preimplantation embryo culture, but the cells do not stabilize until a postimplantation stage.

An understanding of the deficits inherent in the currently available hESC lines has generated interest in the potential value of naive hESCs. These include culture methods, genetic drift in culture and differences among lines for differentiation ability. hESCs are usually passaged in clusters to maintain culture stability. However, single cell passage is superior for ease and for multiple applications where single cells are preferable such as cloning, genetic manipulation, flow cytometry and cryopreservation. Genetic drift induced by culture requires close attention to passage number and frequent karyotyping. Minimizing drift induced by culture greatly improves overall culture efficiency. Variable differentiation ability is seen among the current hESC lines. This is partially due to individual genetics, but is also influenced by the exact point in pluripotency where the culture was stabilized. The field needs hESCs that routinely passage using trypsin, have improved chromatin stability in culture and predictably, robustly and completely differentiate. Because mESCs have the traits desired in hESCs, it has been a goal for several years to establish hESCs at a preimplantation equivalent stage similar to mESCs.

Naive human pluripotent cells were first described as pluripotent stem cells induced from somatic cells (iPSCs) by transgenic manipulation [12]. This was followed by

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reverse toggling of transgene-free primed hESCs to naive without the use of transgenes [1–3,13] and by *de novo* generation of naive hESCs directly from human embryos [1–3]. The compiled data from these reports are remarkably consistent with regard to the character of naive hESCs and reflect what we know about mESCs. Species differences arise in the media used to achieve a stable naive state. A primary difference is the human requirement for FGF to stabilize and protect the culture from background differentiation [1–3]. For this reason, and given what we know about the influence of the media components on establishing a pluripotent state, media differences to generate naive hESCs may well predict subtle differences between naive lines. In our hands, *de novo*-derived naive hESCs appear to reach a stable, uniform early phenotype and are as easy as naive mESCs to maintain. Cell cultures of naive hESCs derived from embryos appear uniform both as naive and when cultured as primed, while reverse toggled hESCs can be quite heterogeneous. Expression data indicate that reverse toggled cells, from primed to naive, do not reach quite as early a state as *de novo*-derived naive cells, likely due to culture heterogeneity, and that *de novo*-derived naive cells toggle forward to a primed state that falls short of *de novo*-derived primed hESCs [2]. Thus, it is *de novo* derivation of naive hESCs that will provide the field with its sought after naive pluripotent standard.

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What value is there in having and understanding the naive state? Because of the heightened developmental capacity seen both through teratoma formation [2] and through mouse/human fetal chimeras [1], naive hESCs will allow characterization to develop a yardstick to measure the naive state, will allow robust generation of somatic tissues of value for regenerative medicine, will unveil a greater understanding of human development and will provide a resource to generate new tools. Specifically, we will now be able to

understand normal early developmental changes that can correlate to cancer via understanding the array of gene expression, metabolic and protein changes that define these early stages of stemness. Cells that better tolerate genetic manipulation will prove useful for a broad array of possible functional questions and pragmatically, for creating a universal donor cell line. The possibilities and value will slowly unveil over time, as they have with mESCs. Thankfully, mESCs have given us a major leg-up with regard to understanding the value of naive hESCs.

There is much yet to be done before defining the normal naive hESCs state. We need to understand optimal culture conditions, develop means to achieve efficient *de novo* generation and obtain a biological understanding of the human ground state. New protocols for *in vitro* differentiation need to be developed before the extent of clinical utility is realized. Cumulative evidence on karyotype stability using perfected culture conditions with naive lines will determine if the naive state is more stable *in vitro*. However, the answer is now clear as to the value naive hESC will bring to the field of pluripotent stem cell research and regenerative medicine. Naive and primed stages are profoundly different. Having two, rather than one stage will allow a far richer understanding of pluripotency. Thus, quite simply, two is better than one in providing the clues we need to solve the puzzle that drives stem cell research.

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