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Review

Cell surface markers for the identification and study of human naive pluripotent stem cells

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ABSTRACT

Characterisation of mouse pluripotent stem cells has revealed two distinct pluripotent states, naive and primed, that maintain characteristics of the pre and post implanted epiblast respectively. Recent studies have developed several culture systems that seek to recapitulate the naive phenomenon in human pluripotent stem cells. Therefore, robust methods to isolate these cells will be fundamental to assess their potential in modelling human development and disease. Here we review current methods for human naive pluripotent culture and collate a list of cell surface antigens that have been identified as markers to differentiate naive from primed human pluripotent stem cells. While these culture systems do display marker variability, and not all antigens mentioned were assessed in all methods, this review provides a resource for researchers of the human naive pluripotent stem cell state. SSEA-4, SSEA-3, CD24, CD75, CD7, CD77, CD130/GP130, CD57, CD90 and NLGN4X were all found to have a +/− expression profile in at least 2 methods, while +/− expression of Tra-1-81, CDH3, CD172a, CD107b, CD229 was reported in one method. Often it was reported that naive and primed cells could be defined using a low/medium/high expression of the following antigens TRA-1-60, PCDH1, GPR64, MHC Class I, however these markers were more likely to display expression pattern differences between methods. Studies using mouse naive cells indicate that they may have benefits over primed cells in modelling development and disease, and while it is yet to be determined if the same can be said about a human naive state, tools to identify this population should greatly further the field.

Contents

1. Introduction

Pluripotency refers to the potential of a cell to differentiate into all three germ layers of embryo development. Over time pluripotent stem cells (PSCs) differentiate to defined cell types and produce the increasing complexity of the developing organism (Chenoweth et al., 2010; Hackett and Surani, 2014; Nichols et al., 1998). These differentiated cells ultimately contribute to all somatic and germline cell types within the adult body (Hackett and Surani, 2014). PSCs are also capable of indefinite self-renewal in culture, thereby maintaining a pool of cells useful to study the development of potentially all cell types (Hackett and Surani, 2014; Shenghui et al., 2009). During early mouse development PSCs have distinct characteristics based on temporal and spatial factors (Hillman et al., 1972; Nichols and Smith, 2009; Nichols et al.,

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1998). Two distinct pluripotent states are described in the mouse that mark differences between the pre- and post-implantation epiblast (cells that form the embryo proper), the naive (or ground state) and primed states of pluripotency respectively (Davidson et al., 2015; Hackett and Surani, 2014; Nichols and Smith, 2009; Weinberger et al., 2016). It should be noted that the term ground state more accurately describes the transient state of naive pluripotency in vivo, and the term naive is used more commonly to describe pluripotent cells that do not have biased lineage specification (Hackett and Surani, 2014), and this is how these terms will be used in this review. Naive and primed states can be differentiated via differential gene expression, epigenetics and metabolic function (Davidson et al., 2015; Hackett and Surani, 2014; Nichols and Smith, 2009; Weinberger et al., 2016). Mouse embryonic stem cells (mESCs) are derived from the inner cell mass (ICM) of the developing blastocyst or the early preimplantation epiblast, and are described as existing in a naive state in culture. While post implantation mouse epiblast derived stem cells (mEpiSCs) are said to exist in a primed state (Bernemann et al., 2011; Nichols and Smith, 2012). These findings set the foundations for describing and comparing different states of pluripotency in mammals, of which there may be more transient states during development that are as yet uncharacterised (Manor et al., 2015; Smith, 2017; Weinberger et al., 2016). It has more recently been proposed that naive and primed states also exist in the human, however results so far have not been as definitive as in the mouse. Human embryonic stem cells (hESCs) derived in a similar fashion to mESCs, as well as human induced pluripotent stem cells (hiPSCs) (Takahashi et al., 2007; Yu et al., 2007) exhibit similar characteristics to mEpiSCs, and are also said to be in a primed state (Davidson et al., 2015; Tesar et al., 2007; Thomson et al., 1998). This has led to attempts to identify conditions that produce hESCs and hiPSCs with naive properties representative of an earlier stage of pluripotency in human development (Chan et al., 2013; Duggal et al., 2015; Gafni et al., 2013; Guo et al., 2017; Hanna et al., 2010; Qin et al., 2016; Takashima et al., 2014; Theunissen et al., 2014; Wang et al., 2014; Ware et al., 2014; Yang et al., 2017; Zimmerlin et al., 2016). Whether the naive state in the human has been successfully generated, and accurately represents a distinct stage of human pluripotency in vivo is currently being debated (Bates and Silva, 2017; Davidson et al., 2015; Manor et al., 2015; Zimmerlin et al., 2017).

The generation of "naive state" human PSCs has been proposed to confer advantages (e.g. single cell cloning, differentiation capacity) for the scientific experimentation and therapeutic application of resultant cell types, however these purported advantages are not yet proven (Bates and Silva, 2017; Hackett and Surani, 2014; Manor et al., 2015). Hence, the development of standardised culture conditions and characterisation criteria for the production of bona fide human naive PSCs will allow researchers to formally test the hypothesised advantages of human naive PSCs over primed human PSCs. Several methods, reviewed below, have recently been employed to distinguish between primed hESCs and the putative human naive PSCs generated by different groups (Collier et al., 2017; Liu et al., 2017; O'Brien et al., 2017; Pastor et al., 2016; Shakiba et al., 2015; Theunissen et al., 2016). These studies correlate molecular and phenotypic differences between human naive-like and primed PSCs with surface markers, to identify new criteria that better define these cell populations (Collier et al., 2017; Liu et al., 2017; O'Brien et al., 2017; Pastor et al., 2016; Shakiba et al., 2015). The use of surface markers to define pluripotent cell populations allows for the straightforward identification and selection of viable cells via fluorescence activated cell sorting (FACS) or magnetic sorting. These strategies can be easily and routinely combined with other markers to allow the isolation of viable cells in specific states of pluripotency for downstream research or medical applications (Choi et al., 2008; Collier et al., 2017; O'Brien et al., 2017). This review focuses on current methods of naive cell culture and the differentiation between the naive and primed states through the use of cell surface antigens.

2. Comparison of naive and primed pluripotent states in mouse and human

Naive mouse ESCs and "primed" mouse EpiSCs share many similar features (Tesar et al., 2007) while also displaying differences in genetic and epigenetic profiles (Nichols and Smith, 2009). In comparison to the primed state, naive mouse PSCs possess the capacity of chimaera-forming and a high single-cell cloning efficiency (Guo et al., 2009; Tesar et al., 2007; Ying et al., 2008). Other key features of naive stem cells include dome-like colonies, X chromosome reactivation in female cells, and dependence on leukaemia inhibitory factor (LIF)/Stat3 signalling. Also, DNA hypomethylation is a key epigenetic hallmark of the naive state (von Meyenn et al., 2016). In contrast, primed PSCs demonstrate a flattened morphology, random X chromosome inactivation in female cells and genome-wide methylation (Nichols and Smith, 2011). Although the expression of core pluripotency factors, Sox2, Oct4 and Nanog, are found in both pluripotent states, the expression levels of multiple transcription factors differ between naive and primed pluripotent cell populations (Guo et al., 2009). For example, KLFs, Oct4 and Nanog are upregulated in mouse naive PSCs as compared with primed PSCs (Guo et al., 2009).

To date, the definition of naive pluripotency is mainly based on the understanding of mESCs. Human ESCs, derived from preimplantation epiblasts, display characteristics reminiscent of the mouse primed state rather than the naive state (Hanna et al., 2010). Therefore, the generation of human naive pluripotency in vitro requires specific culture conditions and signalling pathways that differ from naive mESCs (Hanna et al., 2010). Moreover, naive PSCs from humans and mice have been demonstrated to show distinct epigenetic and genetic patterns (Huang et al., 2014). X-chromosome inactivation (XCI) is a hallmark of the successful acquisition of a naive state in mice, however timing and regulation of XCI initiation is different between mouse and human which means that XCI is not a hallmark of the human naive state (Okamoto et al., 2011). The gene expression profile of naive PSCs in humans and mice is also significantly different. For example, ESRRß is expressed in the mouse epiblast but not in the human epiblast reflecting species-specific embryological differences (Guo et al., 2016).

The potential of chimaera formation following blastocyst injection is a robust standard to define the naive pluripotent state in mice (Theunissen et al., 2014). While chimaeric studies using humans have far reaching ethical concerns, this defined pluirpotent state has also been demonstrated in other primates using ES cells derived from the Cynomolgus monkey maintained under naive conditions (Chen et al., 2015). Interspecies chimaeras have been successfully generated in a rodent system and show the potential gains of generating interspecies chimaeras using human cells. Interspecies chimaeras using rat naïve pluripotent cells injected into mouse pre implantation blastocysts, have generated developmentally normal chimaeras that can live for more than 2 years, and can correct for genetic defects (Wu et al., 2017). PDX1 knockout in mice leads to lack of pancreas formation and is lethal post birth. A chimaera using rat naive cells into the PDX1 null mouse background rescues this phenotype and gave rise to chimaeric mice that have a fully formed pancreas that reached adulthood (Wu et al., 2017). Human naive pluripotent cells, but not primed cells have been shown to integrate into the ICM of cows, which is an indication that chimaerism may occur, and a further study using these naive cells implanted into pigs showed integration but significant developmental retardation of embryos (Wu et al., 2017). Interspecies chimaera formation has also been attempted with primed hiPSC using a developmental staged matched approach (Mascetti and Pedersen, 2016). Primed hiPSC cells were injected into the late stage gastrula resulting in greater than 70% chimaera formation, a similar rate to mEpiSC at this stage (Mascetti and Pedersen, 2016). However, it is yet to be determined if a chimaera using human pluripotent cells could produce viable offspring.

In summary, there are multiple differences between the naive state in humans and mice, making it challenging to accurately describe the