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MINIREVIEW ARTICLE

Intercellular protein expression variability as a feature of stem cell pluripotency

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Abstract The expression of pluripotent stem cell protein markers, self-renewal, the potential to differentiate in cell types of all three germlines and teratoma formation in nude mice form the spectrum of the stringent pluripotency criteria for human stem cells. Currently, intercellular variability is discussed as an additional putative defining property of pluripotent stem cells. In future, it will be of relevance to clarify the genesis of intercellular variability for each stem cell line/population before its application in basic science or therapy. Furthermore, for a better understanding of stemness it will be indispensable to separately investigate the issue of intercellular variability for each feature of pluripotency.

Keywords Stem cells · Pluripotency · Intercellular variability · Stemness · Stem cell protein markers

Abbreviations

AFSCs	Amniotic fluid stem cells
ESCs	Embryonic stem cells
iPSCs	Induced pluripotent stem cells
SCNT	Somatic cell nuclear transfer

Introduction

To allow the comparison of different stem cell types with regard to their applicability for disease modeling or human therapy, standards for the definition of "pluripotency" are essential (Ben-David and Benvenisty 2011; Mattis and Svendsen 2011; Iilic and Polak 2012). For the designation "pluripotency" in its loosest sense stem cells must express specific pluripotency markers, harbour the potential of self-renewal, and must be able to differentiate into cells of all three embryonic germ layers. Stem cells, whose progeny can reconstitute an entire organism, are considered to fulfil the pluripotency criteria in their strictest sense. In this context murine pluripotent stem cells are routinely tested for their transmission through the germline of chimeric animals to yield live pups. Ethical and practical limitations prompted the scientific community to agree that human stem cells must form teratomas derived from all three embryonic germ layers in immune-deficient mice to fulfil the stringent pluripotency criteria (Maherali and Hochedlinger 2008; Ellis et al. 2009; Slipp 2009; Müller et al. 2010). In accordance with these widely used definitions, human embryonic stem cells (ESCs), derived from normal "surplus" in vitro fertilization embryos (Thomson et al. 1998) or via somatic cell nuclear transfer (SCNT; cloning) (Tachibana et al. 2013), and induced human pluripotent stem cells (iPSCs) (Takahashi et al. 2007; Yu et al. 2007) fulfil the stringent pluripotency criteria, whereas stem cells derived from amniotic fluid (AFSCs) (Prusa et al. 2003; Tsai et al. 2006; De Coppi et al. 2007; Siegel et al. 2008) are pluripotent according to the loose criteria (Daley et al. 2009; Rosner and Hengstschläger 2012).

Intercellular variability as "new" criteria for pluripotency

Recently, a very important aspect has been added to this discussion. It is argued that all the above described criteria define "pluripotency" as a statistical property for the entire

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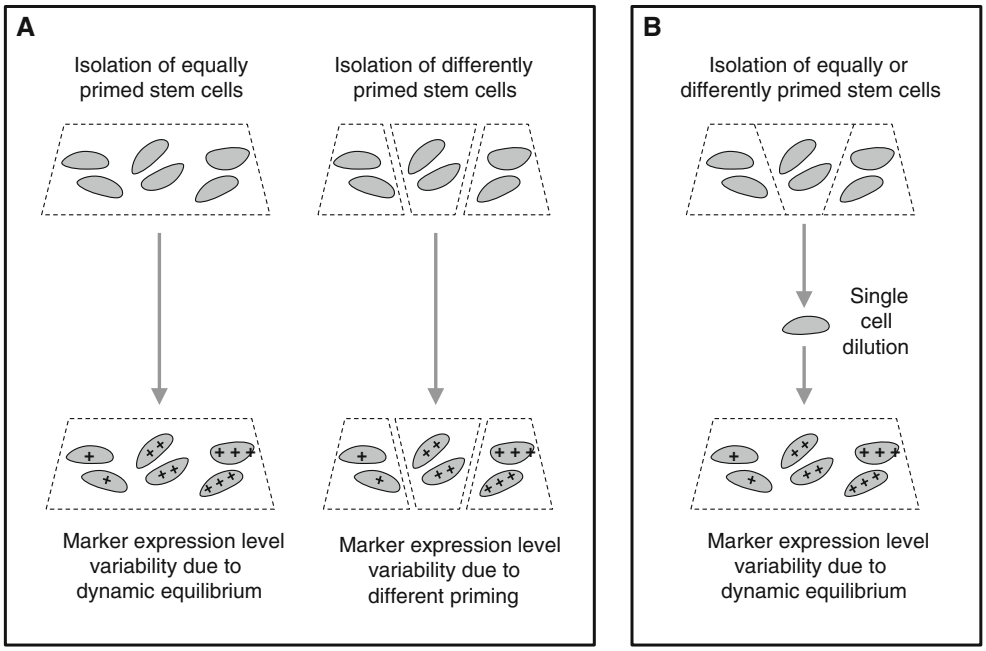

Fig. 1 The genesis of protein expression variability in pluripotent stem cell populations. **a** Pluripotent stem cell populations contain cells expressing low (+), middle (++) and high (+++) levels of the same stem cell marker. The starting stem cell population, from which the stem cell line has been generated, could have been equally primed and during propagation the individual growing stem cells interconvert between different metastable states of varying marker expression levels (dynamic equilibrium). Alternatively, the starting material could consist of a mixture of differently primed stem cells with varying marker expression levels and the proportions of these different cohorts of cells could fluctuate during cultivation. **b** The detection of intercellular expression variability in a monoclonal pluripotent stem cell line, which has been generated upon minimal dilution to the single cell level, can prove the dynamic equilibrium hypothesis for each protein of interest

stem cell population, similar to a macrostate in statistical physics. However, a bulk of literature shows that a pluripotent stem cell population consists of a wide variety of molecular microstates on the individual cell level. For example, many different stem cell protein markers have been shown to exhibit significantly different expression levels on the single cell niveau within one pluripotent stem cell population/line. In contrast to differentiated cells with defined fates and therefore more homogenous expression patterns, this expression variability within one stem cell population might even be a defining property of "pluripotency" allowing the appropriate response to environmental signals by avoiding premature lineage commitment (MacArthur and Lemischka 2013).

The genesis of intercellular variability in stem cell populations

It is a well reported phenomenon that ESC, iPSC and also AFSC lines in culture consist of different individual cells with varying expression levels of the same stem cell marker (Thomson et al. 1998; Takahashi et al. 2007; Yu et al. 2007; De Coppi et al. 2007; Hayashi et al. 2008; Rosner et al. 2010, 2012, 2013; Valli et al. 2010; Moschidou et al. 2012). Here we want to point out that in general two putative underlying processes can be discussed: First, it is possible that the pluripotent cell population has been generated from an equally primed cluster of stem cells and the variable expression levels are the consequence of the individual cell's potential to increase and decrease the intracellular marker expression during propagation. Alternatively, the stem cell pool of origin could consist of differently primed cell cohorts with different marker expression levels and the proportions of these different cohorts could fluctuate in culture. In the first model, which has earlier been named "dynamic equilibrium" (Hayashi et al. 2008), an individual pluripotent stem cell harbours the potential to transit between distinct metastable marker expression states (regulation on the microstate). In the second assumption the single cell would never alter its marker expression level and the observed fluctuations in culture are rather caused by variations in the numbers of cells with high or low levels of expression (regulation on the macrostate) (Fig. 1a).

Secondly, we want to highlight that monoclonal cell lines generated by minimal dilution of pluripotent stem cells to the single cell level are an optimal tool for future investigations of this issue (Fig. 1b). In contrast to ESCs and iPSCs, several pluripotent AFSC lines already exist, which are indeed monoclonal. Such clonal AFSC lines are relatively easy to

establish, grow with high proliferation rates and have already been used in many different molecular biology studies (see e.g., Valli et al. 2010; Siegel et al. 2010; Fuchs et al. 2012; Gundacker et al. 2012). These cell biological tools could be of help to verify existing intercellular variabilities as a consequence of dynamic equilibrium. In fact, since the first description of AFSCs (Prusa et al. 2003; Rosner and Hengstschläger 2013) monoclonal AFSC lines have already been found to express intercellular variability for stem cell marker expression (see e.g., De Coppi et al. 2007; Rosner et al. 2013).

Future perspectives

It is the hope of investigators and patients alike that human pluripotent stem cells can efficiently be used for disease modeling (Zhu et al. 2011) and for stem cell-based human therapies (Wu and Hochedlinger 2011). In summary, (1) the already existing experimental proof that one single AFSC exhibits the potential to interconvert between distinct metastable states during its propagation provides strong evidence that intercellular variability is of high relevance for the pluripotency of a stem cell population, (2) whether this variability as a feature of pluripotency is regulated on the microstate (single cell) level or on the macrostate (cell cohort) level is not necessarily a question of either/or and most probably depends on the molecular target of interest and on the investigated stem cell type, (3) we think it is indispensable that in future this relevant question must separately be investigated for each feature of pluripotency and we emphasize that clonal stem cell lines are an optimal tool.

Conflict of interest The authors declare that they have no conflict of interest.

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