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Ontological aspects of pluripotency and stemness gene expression pattern in the rhesus monkey

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ABSTRACT

Two essential aspects of mammalian development are the progressive specialization of cells toward different lineages, and the maintenance of progenitor cells that will give rise to the differentiated components of each tissue and also contribute new cells as older cells die or become injured. The transition from totipotentiality to pluripotentiality, to multipotentiality, to monopotentiality, and then to differentiation is a continuous process during development. The ontological relationship between these different stages is not well understood. We report for the first time an ontological survey of expression of 45 putative "stemness" and "pluripotency" genes in rhesus monkey oocytes and preimplantation stage embryos, and comparison to the expression in the inner cell mass, trophoblast stem cells, and a rhesus monkey (ORMES6) embryonic stem cell line. Our results reveal that some of these genes are not highly expressed in all totipotent or pluripotent cell types. Some are predominantly maternal mRNAs present in oocytes and embryos before transcriptional activation, and diminishing before the blastocyst stage. Others are well expressed in morulae or early blastocysts, but are poorly expressed in later blastocysts or ICMs. Also, some of the genes employed to induce pluripotent stem cells from somatic cells (iPS genes) appear unlikely to play major roles as stemness or pluripotency genes in normal embryos.

The progressive specialization of cells toward diverse cell lineages is an essential aspect of metazoan development. Equally essential is the maintenance of progenitor cells that will give rise to the differentiated components of each tissue and also contribute new cells as older cells die or become injured. There is a continuum in the transition from totipotentiality to pluripotentiality, to multipotentiality, to monopotentiality, and then to differentiation. The precise ontological relationship between these different stages is only partly understood, but it has become clear that this order of events is not strictly correlated with the chronology of the individual. Gametes are united to form a totipotent zygote. The zygote then passes through cleavage divisions that yield either totipotent blastomeres or lineage-restricted blastomeres, depending on the role of localized determinants. In mammals, blastomeres give rise to the blastocyst stage embryo containing an inner cell mass, which is an early progenitor of both embryonic and extraembryonic cells, and the trophectoderm, the earliest form of trophoblast that contributes to placenta formation. Inner cell mass cells have been successfully transformed to pluripotent embryonic stem cell lines in vitro in several species, including mouse, human, and monkey (Brook and Gardner, 1997; Evans and Kaufman, 1981; Ilic et al., 2009; Magin et al., 1992; Pau and Wolf, 2004; Thomson et al., 1998; Trounson, 2002). Trophoblast stem cells have been derived from blastocysts for mouse and monkey (Oda et al., 2009; Rielland et al., 2008; Tanaka et al., 1998; Vandevoort et al., 2007b). Additionally, pluripotent cells have been derived from early germ lineage cells isolated from fetal mice and humans (Durcova-Hills et al., 2001; Matsui et al., 1992), and germ lineage cells can also give rise to teratomas and embryonal carcinoma stem cells, which display pluripotency. Within the adult organism, many tissues harbor mono-potent, multi-potent, or pluripotent stem cells, which can be isolated and induced to give rise to a range of different cell types in vitro, as embryoid bodies, or as teratomas (Eguizabal et al., 2009; Wu et al., 2009). Thus, early ontogeny may witness a rapid transition to highly restricted fates in certain (e.g., extraembryonic) lineages, whereas adult tissues retain a variety of stem cells, some with very broad potentiality. This diversity of ontogenetic pathways leading to the establishment and maintenance of stem cells of different potencies precludes a simplistic explanation of the molecular and cellular mechanisms that establish stemness and pluripotentiality, and indeed suggest that molecular mechanisms that establish and maintain stem cells may operate within and be dependent upon the unique historical context for each stage, lineage and tissue.

There is great interest in developing technologies to enhance the ability to isolate and propagate stem cells. Mono-potent stem

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cells could be employed for gene therapy, and multipotent or pluripotent cells could in theory be differentiated to a myriad of derivatives for effecting repair of damaged or degenerating tissues, all of which could lead to new treatments for a wide range of diseases and injuries. Efforts to enhance the success of stem cell technologies have stressed the identification of genes that may be able to convert cells to a pluripotent or stem cell state. Molecular comparisons across stem cell types or between stem cells and oocytes have been employed to derive lists of putative "stemness" genes (Assou et al., 2009). More recently, small groups of genes were co-transfected into fibroblasts and other differentiated cell types to induce pluripotency at a low efficiency (Huangfu et al., 2008; Kim et al., 2008). The exact array of genes that can accomplish this "induced pluripotency" state appears to be expanding, and indeed it may be that many different combinations of genes may be able to convert somatic cells to pluripotency (Nakagawa et al., 2008; Takahashi and Yamanaka, 2006; Yu et al., 2007). Such exciting results lend a strong applied angle to complement the basic interest in understanding the molecular mechanisms that establish and maintain stem cells, and that limit their potentialities.

Understanding of early ontogenetic events that generate stem cell lineages facilitates both basic and applied aspects of stem cell biology. Studies that compare gene expression patterns solely between established stem cell lines or between stem cell lines and oocytes may fail to discriminate between stemness genes and genes that define other aspects of stem cells, such as cell cycle drivers, or may disregard unique molecular signatures that are responsible for the unique properties of oocytes or early embryos, such as meiotic arrest or reductional cleavage divisions. With regard to induced pluripotency, it is still unclear what role "pluripotency genes" play during normal development, or to what degree their normal functions are operating out of context to yield fortuitous effects on chromatin structure to enable stem cell characteristics to emerge. Ontogenetic data would be helpful in resolving these areas of uncertainty by providing a more clear understanding of developmental transitions that accompany the establishment of pluripotent stem cells in the embryo.

Comparisons that encompass oocytes, preimplantation stage embryos, early inner cell mass cells, trophoblast stem cells, and established embryonic stem cells should thus enhance our understanding of early stemness and pluripotency and reveal the precise temporal and ontological relationships between developmental stages and the activation/repression of individual genes. Due to a range of legal and ethical constraints, a non-human primate model offers the best choice for understanding early stem cell lineages in the human. In this study, we have undertaken an ontological survey of expression of a large number of putative "stemness" and "pluripotency" genes in rhesus monkey oocytes and preimplantation stage embryos, and compared that expression to expression in the inner cell mass, trophoblast stem cells, and rhesus monkey (ORMES6) embryonic stem cell line. Our results reveal the relationship between stemness and pluripotency genes and the developmental transitions from oocyte to embryo, and embryo to pluripotent stem cells and trophoblast stem cells in the rhesus monkey.

1. Results

The overall objective of this study was to understand how the expression of a range of stemness genes relates to specific stages in the ontogenetic establishment of pluripotent lineages and their conversion to established cell lines (Fig. 1). We focused our attention on following the progression (Fig. 2, Table S2) of genes during preimplantation development, during the conversion from inner cell mass (ICM) first to early outgrowths (EOs), and then to established embryonic stem cells (ESCs); from embryo to trophoblast stem (TSC) cell lines; during the conversion from non-differentiated (ND) to early differentiated (ED) ORMES6 ESCs in vitro and the differences between TSC and established ESCs. These comparisons were chosen in order to evaluate the relationship of the different genes to specific cell states in the ontogenetic series.

We examined the patterns of expression of 45 genes, including genes involved in inducing pluripotent cells (iPSC) (Fig. 3), maintaining pluripotency, self-renewal, growth regulation (Fig. 4),

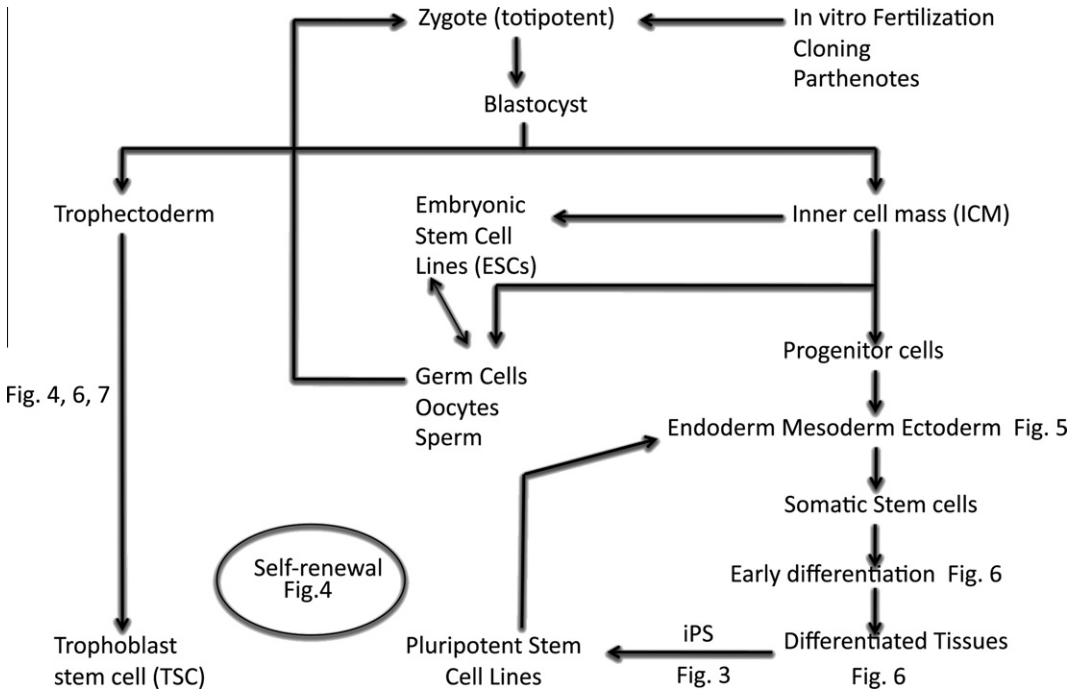

Fig. 1. Schematic summary of different groups of genes analyzed.