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The openness of pluripotent epigenome - Defining the genomic integrity of stemness for regenerative medicine

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Editorial

Human pluripotent stem cells have the theoretic ability to differentiate into all the somatic cell types in the human body, whereas human somatic stem/progenitor/precursor cells (hSSCs) are multipotent, indicating that their potential is restricted to a particular germ layer, organ, or tissue of origin.[1-3] Pluripotent human embryonic stem cells (hESCs), derived from the inner cell mass or epiblast of the human blastocyst, have both the unconstrained capacity for long-term stable undifferentiated growth in culture and the intrinsic potential for differentiation into all the somatic cell types in the human body, holding tremendous potential for restoring human tissue and organ function.[1-3] These properties offer pluripotent hESCs as an unlimited source to generate the diversity of human somatic cell types for regenerative medicine and as a model system for studying mechanisms underlying human embryonic development.[1-3] Although human somatic stem/progenitor/precursor cells have traditionally been isolated directly from such tissue sources of origin in vivo (primary hSSCs), the field is becoming increasingly adept at deriving multipotent human somatic stem/progenitor/precursor cells in vitro from pluripotent cells (secondary hSSCs).[2-5] However, not all pluripotent cells are stem cells. The scientific definition and proof for human pluripotent stem cells are that they have the intrinsic ability of both unlimited or long-term self-renewal and unrestricted differentiation into all the somatic cell types in the human body. To circumvent the ethical issues surrounding the derivation of hESCs, in the last few years, pluripotency-inducing factors, most of which are known oncogenes or toxic cancerogenic chemicals, have been used to artificially reprogram somatic cells to induced pluripotent stem (iPS) cells.[1-3] Embryo-originated pluripotent hESCs, which display high levels of expression of Oct-4, SSEA-4, Tra-1-60, and Tra-1-81, can maintain prolonged normal stable growth or self-renewal in non-hostile growth environments containing the essential developmental components that sustain hESC pluripotency and self-renewal.[1-3, 6] However, so far, there is no evidence that pluripotent cells derived from other sources harboring adult nuclei by transcription-factor- or small-molecule-based reprogramming or somatic cell nuclear transfer, such as iPS cells or pluripotent cells derived from cloned embryos, can maintain prolonged normal stable growth or self-renewal.[1-3] In addition, some techniques that those reports used for their analysis of pluripotent sticky cells, such as FACS designed for sorting non-sticky adult cells or western blot analysis designed for detecting weakly expressed molecules that cannot be detected by immunocytochemical analysis of strongly expressed markers, would give false positive to a heterogeneous population or a colony of cells where the majority of cells might be negative.[2, 3] The artificially reprogrammed adult cells, such as iPS cells or pluripotent cells derived from cloned embryos, are characterized by the expression of embryonic markers that are initially identified in embryonic tumor/cancer cells and forming teratomas in vivo, which shows these reprogrammed adult cells might be either pluripotent stem cells or pluripotent cancer cells. These reports have not provided the essential proofs for human pluripotent stem cells, such as evidences for maintaining long-term genomic integrity or stability and for differentiation into all normal somatic cell types in the human body.[1-3] Although pluripotent, the artificially reprogrammed adult cells are made from adult cells, therefore, they carry many negative repressive chromatin remodeling factors and unerasable genetic imprints of adult cells that pluripotent hESCs do not have.[1-3] In fact, the iPS cells differ dependent on cell type of origin and display abnormal gene silencing of somatic cells, and iPS cell-derived cells show accelerated senescence.[1-3] Unlike the tightly-regulated in vivo biological reprogramming in the human reproduction process, insertion or transient expression of foreign oncogenes in adult somatic cells at a

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a non-physiological level tends to induce cancer phenotypes and malignant transformation of iPS cells, resulting in low survival rates and genetic-defects of iPS cell-derived fetus.[1-3] It is known that somatic cell nuclear transfer and factor- or small-molecule-based reprogramming are incapable of restoring a correct epigenetic pattern of pluripotent hESCs, which accounts for abnormal gene expression, accelerated senescence, and immune-rejection following transplantation of reprogrammed cells.[1-3] In view of the growing interest in the use of human pluripotent stem cells, these major drawbacks have raised serious concerns about the genomic integrity of artificially reprogrammed adult cells and, thus, have diminished the utility of reprogramming somatic cells as viable therapeutic approaches. So far, the pluripotent hESCs remain as the only genetically-stable human pluripotent stem cell source with full-developmental potential in deriving somatic elements for tissue and function restoration.

The pluripotent state of hESCs is associated with the expression of a unique group of genes, including Oct-4, alkaline phosphatase, SSEA-4, Tra-1-60, Tra-1-80, though none of these markers, in isolation, is exclusively expressed by undifferentiated hESCs.[1-3] Rather, their presence as a group is associated with the undifferentiated state of pluripotent hESCs. Gene expression analysis has indicated that stem cells do not seem to have a common core transcription profile that dictates the undifferentiated self-renewing state of all stem cell derivatives, known as stemness.[3, 4, 7] There exist overlaps in gene expression between cells of varying lineages yet a lack of overlap in the gene expression profiles of various types or derivations of stem cells, in spite of their apparent phenotypic similarity.[3, 4, 7] Recent studies reveal that discerning the intrinsic plasticity and regenerative potential of human stem cell populations reside in chromatin modifications that shape the respective epigenomes of their derivation routes.[3-5, 7]

The pluripotency of hESCs that display normal stable expansion is associated with a globally active acetylated chromatin, as evident by high levels of expression and nuclear localization of active chromatin remodeling factors; weak expression or cytoplasmic localization of repressive chromatin remodeling factors that are implicated in transcriptional silencing; and residual H3 K9 methylation.[3-5, 7] This normal pluripotency of hESCs is characterized by an epigenome comprised of a globally open conformation of chromatin remodeled by Oct-4 primed for unrestricted lineage choices.[3-5, 7] The wide distribution pattern of Oct-4 is coincident with sites of active chromatin modification genome-wide, suggesting that Oct-4 might play an essential role in the interface of chromatin and transcription regulation to maintain a pluripotent epigenome enabled by a globally active open chromatin.[3-5, 7] The progressive narrowing of potency is associated with the gradual restriction in chromatin openness, hence, lineage choices as a result of global increases in chromatin-silencing.[3-5, 7] The intrinsic plasticity and regenerative potential of human stem cell derivatives can be differentiated by their epigenomic landscape features.[3-5] Human stem cell derivatives retain more open epigenomic landscape, therefore, more developmental potential for scale-up regeneration, when derived from the pluripotent hESCs in vitro than from the tissue in vivo.[3-5]

The openness of pluripotent epigenome differentiates the active pluripotency of normal hESCs from the repressive pluripotency of abnormal cells, such as the iPS cells reprogrammed from adult cells, pluripotent cells derived from cloned embryos, and pluripotent embryonic carcinoma cells.[3-5, 7] The hESCs are not only pluripotent, but also incredibly stable and positive, as evident by that only the positive active chromatin remodeling factors, but not the negative repressive chromatin remodeling factors, can be found in the open epigenome of pluripotent hESCs.[3-5, 7] The association of pluripotent epigenome of hESCs with a globally open chromatin state conforms to highly dynamic active epigenomic remodeling, which provides the molecular foundation for the normal stable pluripotency of hESCs and for the genomic integrity of pluripotent stem cells.[3-5, 7] Although undifferentiated hESCs display the bivalent histone marks that include the H3K4me3 activation and the H3K27me3 repressive modifications, only residual nucleosomal H3 K9 methylation, a chromatin modification implicated in transcriptional repression during development, was observed in the open pluripotent epigenome of hESCs.[3-5, 7] Residual repressive chromatin remodeling implicated in chromatin silencing and transcriptional repression might be essential for stabilizing the pluripotent state of hESCs with a globally active open epigenome at a normal developmental stage.[3-5, 7] In fact, aberrant H3 K9 methylation at embryonic stage has been associated with abnormal DNA hypermethylation and cell malignant transformation in the pluripotent embryonic carcinoma cells.[3]

Recent developments in hESC research have overcome some major obstacles in moving stem cell research from current studies in animals towards humans for clinical trials, including resolving minimal essential human requirements for de novo derivation and long-term maintenance of clinically-suitable stable hESC lines and direct conversion of such pluripotent hESCs into a large supply of clinical-grade functional human neuronal or cardiomyocyte cell therapy products to be translated to patients for CNS or heart repair.[1-15] Without an understanding of the essential developmental components for sustaining pluripotency and self-renewal of hESCs, hESC lines are at risk for becoming unhealthy and unstable after prolonged culturing under animal feeders, feeder-conditioned media, or artificially-formulated chemically-defined conditions.[1-3, 6] Resolving minimal essential human requirements for sustaining embryonic pluripotency allows all poorly-characterized and unspecified biological components and substrates in the culture system, including those derived from animals, to be removed, substituted, and

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optimized with defined human alternatives for de novo derivation and long-term maintenance of good manufacturing practice (GMP)-quality xeno-free stable hESC lines and their human stem cell therapy derivatives.[1-3, 6] Formulation of minimal essential defined conditions renders pluripotent hESCs be directly and uniformly converted into a specific neural or cardiac lineage by small signal molecule induction.[1-15] Retinoic acid (RA) was identified as sufficient to induce the specification of neuroectoderm direct from the pluripotent state of hESCs maintained under the defined culture, without going through a multi-lineage embryoid body (EB) stage, and trigger a cascade of neuronal lineage-specific progression to human neuronal progenitors (Xcel-hNuP) and neurons (Xcel-hNu) of the developing CNS in high efficiency, purity, and neuronal lineage specificity by promoting nuclear translocation of the neuronal specific transcription factor Nurr-1.[1-5, 9-11] Neuroectoderm specification transforms pluripotent hESCs uniformly into a more neuronal lineage-specific nuclear Nurr1-positive embryonic neuronal progenitor than the prototypical neuroepithelial-like nestin-positive human neural stem cells (hNSCs) derived either from CNS or hESCs.[1-5, 9-11] Genome-scale profiling of microRNA (miRNA) differential expression showed that the expression of pluripotency-associated hsa-miR-302 family was silenced and the expression of Hox miRNA hsa-miR-10 family that regulates gene expression predominantly in neuroectoderm was induced to high levels in those hESC-derived neuronal progenitors.[2, 3, 10, 11] Following transplantation, those hESC neuronal derivatives engrafted widely and yielded well-dispersed and well-integrated human neurons at a high prevalence within neurogenic regions of the brain.[3, 5, 11] This technology breakthrough enables well-controlled generation of a large supply of neuronal lineage-specific progenies across the spectrum of developmental stages direct from the pluripotent state of hESCs with small molecule induction, providing an adequate neurogenic human cell source for developing safe and effective stem cell therapy for CNS repair in a wide range of neurological disorders.[1-5, 9-11]

Similarly, nicotinamide (NAM) was identified sufficient to induce the specification of cardiomesoderm direct from the pluripotent state of hESCs maintained under the defined culture, without going through a multi-lineage embryoid body stage, by promoting the expression of the earliest cardiac-specific transcription factor Csx/Nkx2.5 and triggering progression to cardiac precursors (Xcel-hCardP) and beating cardiomyocytes (Xcel-hCM) with high efficiency.[1-3, 6, 8, 12-15] Cells within the beating cardiospheres expressed markers characteristic of cardiomyocytes and electrical profiles of the cardiomyocytes confirmed their contractions to be strong rhythmic impulses reminiscent of the p-QRS-T-complexes seen from body surface electrodes in clinical electrocardiograms.[1-3, 6, 8] This novel approach of hESC cardiac lineage-specific differentiation direct from the pluripotent stage using small molecule induction is a major milestone towards human trials of hESC cardiac cell therapy derivatives, offering the benefits in efficiency, purity, stability, safety, and scale-up production of clinical-grade hESC cardiac cell therapy products in cGMP facility over all other existing conventional approaches.[1-3, 6, 8, 12-15] Currently, these hESC cardiomyocyte cell therapy derivatives are the only available human cell sources in large commercial scales with adequate cellular pharmacologic utility and capacity to regenerate the contractile heart muscle, vital for heart repair in the clinical setting.[1-3] Nuclear translocation of NAD-dependent histone deacetylase SIRT1 and global chromatin silencing lead to hESC cardiac fate determination, while silencing of pluripotency-associated hsa-miR-302 family and drastic up-regulation of neuroectodermal Hox miRNA hsa-miR-10 family lead to hESC neural fate determination.[2, 3, 10, 11] Embedding lineage-specific genetic and epigenetic programs into the open epigenomic landscape of pluripotent hESCs offers a new dimension for direct control and modulation of the pluripotent fate of hESCs when deriving a large supply of clinical-grade genetically-stable somatic cell lineages for regenerative therapies.

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