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Pluripotent Stem Cells

Few areas of biology currently garner more attention than the study of human pluripotent stem cells (hPSCs). This interest has arisen because of their potential to form the basis of cellular therapies for diseases affecting organ systems with limited regenerative capacity, to provide enhanced systems for drug screening and toxicity testing as well as to gain insight into early human development. There are currently two major methods for generating cells with pluripotent properties. The first involves isolating the inner cell mass from an early human blastocyst and culturing the resulting cells in appropriate culture conditions (see below) to generate human embryonic stem cells (hESCs).[1] The second involves artificially expressing key developmental transcription factors in somatic cell types, which, with the appropriate culture conditions, causes the cells to be reprogrammed into induced pluripotent stem cells (iPSCs).[2–4]

Generation of Induced Pluripotent Stem Cells

Much effort has been dedicated to understanding the transcriptional state of undifferentiated pluripotent stem cells. For example, OCT-3/4,[5] KLF-4, SOX2,[6] and NANOG[7,8] have been shown to be central to the specification of pluripotent stem cell identity due to their unique expression patterns and their essential roles in early development. These efforts, along with others, enabled the discovery of defined factors for the reprogramming of somatic cells. The specific molecular processes by which somatic cells are reprogrammed into pluripotent stem cells are not understood, although recent findings suggest it is a stepwise process,[9,10] and that the stochastic nature of reprogramming can be explained in part by the multiple molecular and genetic events required for full reprogramming to occur.[11] Current research focuses on improving reprogramming efficiency by better understanding several variables in the process of reprogramming: (1) the choice of factors used; (2) the delivery methods; (3) the target cell type; (4) the timing and levels of factor expression; and (5) the culture conditions. In addition to this, methods to identify and characterize truly reprogrammed pluripotent cells are also important.[12]

The original cocktail of factors described by Yamanaka[13], OCT4, SOX2, c-MYC and KLF-4, continue to be the major factors that are used for reprogramming. Originally, delivery of the factors was achieved through the use of viruses that integrated into the genome. However, concerns over the clinical use of these cells and the potential for insertional mutagenesis have led to the exploration of non-integrating methods of factor delivery including transient transfection,[14] non-integrating viral approaches[15] and protein transduction.[16] Other recent methods such as the use of polycystronic minicircles,[17] synthetic mRNA,[18] self-replicating RNA,[19] RNA based viruses such as Sendai,[20] and synthetic microRNA[21] have also been shown to be successful. Particularly exciting are recent research efforts to identify small molecules that replace some of these factors by either modifying genome methylation patterns or inhibiting key signaling pathways.[22,23] The ultimate goal of this research is to define stepwise protocols by which cells can be fully reprogrammed solely by chemical means.

The choice of cell type to use for reprogramming is based on accessibility of tissue samples, genetic make-up of the target cells, and reprogramming efficiency. Skin-derived dermal fibroblast cells and peripheral blood cells are the most commonly used cell types due to the limited invasiveness of sample collection and availability of banked tissue samples representing a variety of diseases. Peripheral blood cell types have varying and often reciprocal efficiencies of reprogramming versus frequency in blood. For example, $CD34^+$ hematopoietic stem and progenitors have relatively high reprogramming efficiencies[24] but are rare in circulating blood (0.01-0.1%).[25] In contrast, T- and B-cells are more frequent and have acceptable reprogramming efficiency,[26] but are less ideal as target cells for reprogramming due to TCR and IgG gene rearrangements that may affect the downstream function of hiPSCs generated from them.[27] Thus, peripheral blood represents a promising and readily available source of cells for reprogramming.

Interestingly, not all cell types require all four factors to be delivered in order to successfully reprogram cells. For example,

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as neural stem cells endogenously express SOX2, KLF-4, and c-MYC, they were able to be reprogrammed solely through transduction of OCT-4.[28] Cell types also appear to have different reprogramming efficiencies. In mice, stomach and liver cells appear to be reprogrammed more efficiently and completely than fibroblasts.[29] Similarly, the reprogramming of human adipocytes is ~20-fold more efficient than fibroblasts and have the added advantage of being a readily available source of cells.[30,31] As a final consideration when choosing a starting cell type, several reports have noted retained gene expression from the parental cell types[32] and have also shown that the epigenetic state is predictive of the original somatic cell type.[33] Such epigenetic memory may increase the propensity of iPS cell lines to differentiate to the original cell lineage/type.[33-35]

A common phenomenon observed during reprogramming is the emergence of partially reprogrammed colonies which are usually associated with continued expression of the reprogramming factors. These cells exhibit a range of phenotypes but often fail tests of fully pluripotent cells.[37] Chan et al.[38] showed that while overall reprogramming efficiency was lower in feeder-free conditions, the only types of cells that emerged were fully reprogrammed cells. This indicates the importance of culture conditions in the process of reprogramming. We developed feeder-free, defined and xeno-free media for reprogramming fibroblasts (TeSR™-E7™) or blood cells (ReproTeSR™), which provide recognizable hiPSC colonies with less differentiated or partially reprogrammed background cell growth.

Culture Conditions for hPSCs

Initial methods to culture hESCs were modeled on techniques originally developed to culture mouse ESCs (mESCs).[39,40] These techniques involved culture on a layer of mitotically inactivated mouse embryonic fibroblasts (MEFs, or feeder cells) in medium supplemented with 20% fetal bovine serum (FBS). In these conditions, hESC lines could be propagated indefinitely with retention of their pluripotent properties.[1] From the initial development of these culture conditions, it was realized that the continued use of feeders and animal-derived components in hESC cultures would hinder the development of clinical applications due to: a) the presence of immunogenic material; b) the risk of transmitting animal virus or prion material; and c) difficulty with quality control of these undefined components.

Subsequently, improvements to these procedures have largely focused on removing the undefined and non-human components. Several groups have developed culture conditions for hESCs that are, to various degrees, serum- and MEF-free. It was discovered that, in MEF-dependent conditions, serum could be replaced with Knock-Out Serum Replacement,[41] a commercially available serum substitute. Xu et al. reported a culture system that utilized Matrigel® as a culture matrix and MEF-conditioned medium (consisting of serum replacement and basic fibroblast growth factor, bFGF) that allowed hESCs to be cultured without direct contact with feeders.[42] Another approach to MEF removal from the culture system was to replace them with human feeders.[43] As the feeders are of human origin, the possibility of the transmission of foreign pathogens is limited, but unfortunately the secreted factors are still undefined and subject to large variation between batches.

True feeder-free culture has been achieved using an extracellular matrix surface coating on the cultureware, and a combination of transforming growth factor-β (TGF-β) and bFGF or high levels of bFGF alone[44,45] together with a serum replacement in the medium. A number of publications have described defined xeno- or feeder-free media formulations for the maintenance of hESCs.[46-49]

The TeSR™ family of defined and serum-free media for feeder-free culture of hPSCs includes mTeSR™1, TeSR™2, and TeSR™-E8™. mTeSR™1 was developed by Dr. Tenneille Ludwig and colleagues at the WiCell™ Research Institute (Madison, WI) and supports long-term, feeder-free culture of hESCs and hiPSCs.[49] The formulation of mTeSR™1 includes key factors that support pluripotency including bFGF, TGF-β, γ-aminobutyric acid (GABA), pipecolic acid and lithium chloride, as well as bovine serum albumin (BSA). mTeSR™1 is now the most widely-published feeder-free medium, used in over 800 peer-reviewed publications. TeSR™2 is a more defined medium, based on the xeno-free formulation from the same group, containing recombinant HSA.[47] The WiCell™ Research Institute also developed a low protein, highly defined culture medium for hPSCs. This medium, TeSR™-E8™, contains only the most essential components required for maintenance providing a simpler medium for the culture of pluripotent stem cells.

hPSCs differ at the molecular and functional level from mESCs and are considered to more closely resemble post-implantation mouse epiblast stem cells (EpiSCs). mESCs and conventional hPSCs exhibit distinct gene expression patterns and different requirements in culture.[50,51] Specifically, mESCs are maintained by inhibiting MEK/ERK signaling, activating WNT signaling (by GSK3 inhibition), and stimulating with the leukemia inhibitory factor (LIF) cytokine, while hPSCs or mouse EpiSCs are cultured in FGF and Activin and are not responsive to LIF.[50] Several recent studies have identified conditions capable of maintaining hPSCs in a “ground state” resembling mESCs as opposed to the “primed state” that hPSCs are traditionally maintained in.[51-53]

A lot of effort has focused on finding surface matrices that are more

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defined than Matrigel®. Two of the more promising approaches are synthetic peptides chemically linked to the cultureware,[54,55] and recombinant proteins that interact with specific integrins and cell adhesion molecules.[56,57] A new defined surface, Vitronectin XF™, was developed and manufactured by Primorigen Biosciences and has been commercially released by STEMCELL Technologies. Vitronectin XF™ can be used with mTeSR™1 or, TeSR™2 or TeSR™-E8™ for a xeno-free culture system.

Clinical Applications of hPSCs

Because of their differentiation potential, it is hoped that hPSCs may form the basis of cellular therapies where tissue damage or malfunction is severe and irreversible. Cardiovascular diseases, type-1 diabetes, spinal cord injury, and Parkinson's disease are examples of diseases where it is hoped that hPSC-based therapies will provide a cure. Techniques have been developed to differentiate hPSCs into a variety of adult cell types including hematopoietic,[58-60] cardiac,[61,62] neural,[63-65] pancreatic,[66-71] retinal pigmented epithelia[72,73] and osteogenic lineages.[74] However, a number of obstacles currently impede the clinical application of hPSC-based therapies. At present, only limited testing of hPSC-derived cells has been performed to ensure full maturation and functionality of differentiated cells. Furthermore, protocols for the differentiation of hPSCs to functionally relevant progeny are generally inefficient, resulting in low differentiated cell yields and contamination by other cell types as a result of aberrant differentiation. Of greater concern is the possibility of the persistence of undifferentiated hPSCs in transplanted populations which may result in teratomas.[75,76]

Further concerns surround the possibility of immune rejection of transplanted cells either due to the expression of different major histocompatibility complex antigens on donor cells[77] or from the expression of foreign antigens as a result of culturing in animal products.[78] Using patient-specific iPSCs for cellular therapies would circumvent the need for histocompatibility matching. And while the potential of rejection due to foreign animal antigens remains controversial,[78,79] much effort is being devoted to developing xeno-free culture media and matrices for hPSC expansion and differentiation. STEMCELL Technologies offers the STEMdiff™ suite of defined and feeder-free products for efficient differentiation of hPSCs to cells of all three lineages.

Clinical application of hPSC-based therapies are moving closer towards becoming a reality. For example, the group led by Dr. Masayo Takahashi at RIKEN, Japan, recently started treating the first patients with age-related macular degeneration using autologous hiPSC-derived retinal pigmented epithelial cells. Similarly, early stage clinical trials are ongoing in the U.S. and the E.U. by Advanced Cell Technologies to utilize hESC-derived retinal pigmented epithelial cells to treat Stargart's macular dystrophy, a degenerative eye disease that causes blindness in children. Finally, ViaCyte has recently received FDA acceptance of IND for the candidate hESC-derived beta cell replacement therapy for type 1 diabetes and will begin phase 1 clinical trials soon.

Use of hPSCs in Drug Screening and Toxicological Testing

The most immediate impacts are likely to be gained from the use of hPSCs in the fields of drug development or toxicity testing. It has been estimated that the cost of bringing a new drug to market through development, clinical trials and FDA approval can be upwards of 800 million to 1.3 billion USD.[80] Furthermore the number of drugs that are ultimately successful is very low, and many drugs fail at the phase II or III clinical trial stages due to unexpected toxicities, after large investments have been put into their development. Given these costs and the high risk assumed by pharmaceutical companies, there are great advantages to having access to large numbers of biologically relevant human cells for early testing and screening. hPSCs in their undifferentiated state may be useful to identify teratogenic or toxic effects of potential compounds. Incorporating compounds into defined differentiation protocols may identify candidates that potentiate or skew differentiation towards a beneficial outcome. The potential to generate large numbers of end stage cells such as neurons and cardiomyocytes will ultimately provide directly relevant cell types for drugs being developed for cardiovascular or neurodegenerative disorders. In addition, the generation of cardiomyocytes and hepatocytes may be directly relevant to toxicity measurements. Finally, disease-specific iPSCs made by reprogramming relevant cell types from patients has the potential of revealing not only fundamental biological defects but also providing potentially unlimited cells with which to investigate potential therapeutic approaches.

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