Human-induced pluripotent stem cell

Induced pluripotent stem cells (also known as iPS cells or iPSCs) are a type of pluripotent stem cell that can be generated directly from adult cells. The iPSC technology was pioneered by Shinya Yamanaka's lab in Kyoto, Japan, who showed in 2006 that the introduction of four specific genes encoding transcription factors could convert adult cells into pluripotent stem cells.

He was awarded the 2012 Nobel Prize along with Sir John Gurdon "for the discovery that mature cells can be reprogrammed to become pluripotent."

The generation of human-induced pluripotent stem cells (iPSCs) from somatic cells using gene transfer opens new areas for precision medicine with personalized cell therapy and encourages the discovery of essential platforms for targeted drug development. iPSCs retain the genome of the donor, may regenerate indefinitely, and undergo differentiation into virtually any cell type of interest using a range of published protocols. There has been enormous interest among researchers regarding the application of iPSC technology to regenerative medicine and human disease modeling, in particular, modeling of neurologic diseases using patient-specific iPSCs. For instance, Parkinson's disease, Alzheimer's disease, and spinal cord injuries may be treated with iPSC therapy or replacement tissues obtained from iPSCs [1].

Limitations in genetic stability and recapitulating accurate physiological disease properties challenge the utility of patient-derived (PD) cancer models for reproducible and translational research.

Uhlmann et al. from the University Hospital of Düsseldorf, genetically engineered a portfolio of isogenic human-induced pluripotent stem cells (hiPSCs) with different pan-cancer relevant oncoprotein signatures followed by differentiation into lineage-committed progenitor cells. Characterization on molecular and biological level validated successful stable genetic alterations in pluripotency state as well as upon differentiation to prove the functionality of the approach Meanwhile proposing core molecular networks possibly involved in early dysregulation of stem cell homeostasis, the application of the cell systems in comparative substance testing indicates the potential for cancer research such as identification of augmented therapy resistance of stem cells in response to activation of distinct oncogenic signatures [2].

Numerous interventions have been explored in animal models using cells differentiated from human-induced pluripotent stem cells (iPSCs) in the context of neural injury with some success. The work of Lavoie sought to transplant cells that are generated from hiPSCs into regionally specific spinal neural progenitor cells (sNPCs) utilizing a novel accelerated differentiation protocol designed for clinical translation. They chose a xenotransplantation model because the laboratory is focused on the behavior of human cells in order to bring this potential therapy to translation. Cells were transplanted into adult immunodeficient rats after moderate contusion spinal cord injury (SCI). Twelve weeks later, cells derived from the transplanted sNPCs survived and differentiated into neurons and glia that filled the lesion cavity and produced a thoracic spinal cord transcriptional program in vivo. Furthermore, neurogenesis and ion channel expression were promoted within the adjacent host spinal cord tissue.

Transplanted cells displayed robust integration properties including synapse formation and myelination by host oligodendrocytes. Axons from transplanted hiPSC sNPC-derived cells extended both rostrally and caudally from the SCI transplant site, rostrally approximately 6 cm into supraspinal structures. Thus, iPSC-derived sNPCs may provide a patient-specific cell source for patients with SCI that could provide a relay system across the site of injury [3].

The human induced pluripotent stem cell (iPSC) line ZJUCHi002-A was established from renal epithelial cells present in urine (urinary cells) collected from an 8-year-old Charcot-Marie-Tooth disease type 2A (CMT2A) patient carrying point mutation in MFN2 (c.752C > T). Urinary cells were reprogrammed by retrovirus vectors containing reprogramming factors: OCT4, SOX2, KLF4 and c-MYC. The pluripotency, capacity of differentiation into 3 germ layers, silence of reprogramming factors and normal karyotype were all confirmed in this study [4].

Liu et al., developed a safe and cost-effective pipeline to generate clinically relevant NSCs. They first isolated cells from patients' urine and reprogrammed them into iPSCs by non-integrating Sendai virus vectors, and carried out experiments on neural differentiation. NSCs were purified by A2B5, an antibody specifically recognizing a glycoganglioside on the cell surface of neural lineage cells, via fluorescence activated cell sorting. Upon further in vitro induction, NSCs were able to give rise to neurons, oligodendrocytes and astrocytes. To test the functionality of the A2B5+ NSCs, they grafted them into the contused mouse thoracic spinal cord. Eight weeks after transplantation, the grafted cells survived, integrated into the injured spinal cord, and differentiated into neurons and glia.

The specific focus on cell source, reprogramming, differentiation and purification method purposely addresses timing and safety issues of transplantation to SCI models. It is Liu et al., belief that this work takes one step closer on using human iPSC derivatives to SCI clinical settings [5].

The field of stem cell biology has been catapulted forward by the startling development of reprogramming technology. The ability to restore pluripotency to somatic cells through the ectopic co-expression of reprogramming factors has created powerful new opportunities for modelling human diseases and offers hope for personalized regenerative cell therapies. While the field is racing ahead, some researchers are pausing to evaluate whether induced pluripotent stem cells are indeed the true equivalents of embryonic stem cells and whether subtle differences between these types of cell might affect their research applications and therapeutic potential [6].

Induced pluripotent stem cells are adult cells that have been genetically reprogrammed to an embryonic stem cell–like state by being forced to express genes and factors important for maintaining the defining properties of embryonic stem cells. Although these cells meet the defining criteria for pluripotent stem cells, it is not known if iPSCs and embryonic stem cells differ in clinically significant ways. Mouse iPSCs were first reported in 2006, and human iPSCs were first reported in late 2007. Mouse iPSCs demonstrate important characteristics of pluripotent stem cells, including expressing stem cell markers, forming tumors containing cells from all three germ layers, and being able to contribute to many different tissues when injected into mouse embryos at a very early stage in development. Human iPSCs also express stem cell markers and are capable of generating cells characteristic of all three germ layers.

Although additional research is needed, iPSCs are already useful tools for drug development and modeling of diseases, and scientists hope to use them in transplantation medicine. Viruses are currently used to introduce the reprogramming factors into adult cells, and this process must be carefully controlled and tested before the technique can lead to useful treatment for humans. In animal studies, the virus used to introduce the stem cell factors sometimes causes cancers. Researchers are currently investigating non-viral delivery strategies. In any case, this breakthrough discovery has created a powerful new way to “de-differentiate” cells whose developmental fates had been previously assumed to be determined. In addition, tissues derived from iPSCs will be a nearly identical match to the cell donor and thus probably avoid rejection by the immune system. The iPSC strategy creates pluripotent stem cells that, together with studies of other types of pluripotent stem cells, will help researchers learn how to reprogram cells to repair damaged tissues in the human body.

Peripheral nerve-derived adult pluripotent stem cells (NEDAPS) are readily induced to osteoblastic and endothelial cells, suggesting therapeutic potential for bone repair and other regenerative therapies [7)].

Genetic modification is an indispensable tool to study gene function in normal development and disease. The recent breakthrough of creating human induced pluripotent stem cells (iPSCs) by defined factors (Takahashi et al., Cell 131:861-872, 2007) provides a renewable source of patient autologous cells that not only retain identical genetic information but also give rise to many cell types of the body including neurons and glia. Meanwhile, the rapid advancement of genome modification tools such as gene targeting by homologous recombination (Capecchi, Nat Rev Genet 6:507-512, 2005) and genome editing tools such as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas (CRISPR-associated) system, TALENs (Transcription activator-like effector nucleases), and ZFNs (Zinc finger nucleases) (Wang et al., Cell 153:910-918, 2013; Mali et al., Science 339:823-826, 2013; Hwang et al., Nat Biotechnol 31:227-229, 2013; Friedland et al., Nat Methods 10(8):741-743, 2013; DiCarlo et al., Nucleic Acids Res 41:4336-4343, 2013; Cong et al., Science 339:819-823, 2013) has greatly accelerated the development of human genome manipulation at the molecular level. This chapter describes the protocols for making neural lineage reporter lines using homologous recombination and the CRISPR/Cas system-mediated genome editing, including construction of targeting vectors, guide RNAs, transfection into hPSCs, and selection and verification of successfully targeted clones. This method can be applied to various needs of hPSC genetic engineering at high efficiency and high reliability [8)].

As a potentially unlimited autologous cell source, patient induced pluripotent stem cells (iPSCs) provide great capability for tissue regeneration, particularly in spinal cord injury (SCI). However, despite significant progress made in translation of iPSC-derived neural stem cells to clinical settings, a few hurdles remain. Among them, non-invasive approach to obtain source cells in a timely manner, safer integration-free delivery of reprogramming factors, and purification of NSCs before transplantation are top priorities to overcome.

Reprogramming of somatic cells to induced pluripotent stem cells (iPSC) and subsequent differentiation opened up the opportunity of deriving cell types in vitro which (like neurons) had a

very restricted accessibility in the past. However, cell culture protocols for iPSC reprogramming, neural induction and differentiation tend to be labor and time intensive, costly and commonly depend on viral vector delivery. Single-step reprogramming to induced neural stem cells (iNSC) avoids many of the necessary intermediate steps of the aforementioned method but yields a cell type that proliferates over longer time spans and readily differentiates to mature neurons when required.

Azmitia et al. from the Department of Neurosurgery, University of Kiel, Germany, describe a plasmid based reprogramming protocol employing defined, commercially available components for induction and proliferation of iNSC, followed by a defined, small molecule based differentiation step toward mature neurons. The described method might be of particular interest for groups with limited resources and/or restricted access to higher biosafety level facilities required for viral transduction, but also for groups requiring a high throughput for dealing with large numbers of cell lines [9].

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