

Project title

Generation and characterization of induced pluripotent cells (iPSCs) and studying checkpoint mechanism in human somatic cells and iPSCs”

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Research Academy theme/s

Biotechnology and stem cell research

The problem“

Cell Therapy has the potential to provide cures for a number of currently incurable diseases. Stem cells from both adult and embryonic sources offer a promising resource to generate a wide range of tissue types for the treatment of a variety of degenerative and auto-immune diseases, such as Parkinson's Disease, Multiple Sclerosis, spinal cord injuries, diabetes and myocardial infarction. A key issue in the clinical use and commercialization of SC-based therapies is the potential for these cells and their derivatives to elicit immune rejection by receiving patient. Generally, compatibility is less than absolute and even tissue matched organ transplant patients need to take immuno-suppressant drugs for the rest of their lives to prevent rejection of the transplanted organ. This is both expensive and has multiple undesirable side effects for the recipient.

While a number of routes have been proposed to overcome or circumvent immune rejection of transplanted cells, the most attractive option for cell therapy is to transplant tissue derived from SC lines genetically identical (autologous) to individual patients, thereby overcoming immune rejection issues. Animal studies have demonstrated that ESCs, autologous to the somatic cell donor, can be derived from blastocysts produced by nuclear transfer of adult cells into enucleated oocytes, a process known as somatic cell nuclear (SCNT). In humans this approach raises numerous practical, logistical and biological questions that need to be overcome; hence SCNT-ESC lines have not yet been established.

Recently numerous research papers have reported the generation of human ES-like cells (called iPS cells) by over-expressing 4 key genes in somatic cells.⁶ We believe that while this research is incredibly exciting and promising, several issues need to be addressed for iPS technology to be clinically relevant- 1) The approach requires genetic modification of the adult cells. 2) One of the genes used for generation of the human iPS cells is a cancer gene and it was originally reported in mouse studies to result in tumours in mice generated from these cells, and 3) .The only method of gene transfer that has worked in both mice and human uses retroviral vectors, that require high levels of infection.

The process by which a cell gives rise to a new daughter cell is called cell division. During the cell cycle several parameters are important for successful division. There are several composite proteins and protein kinases, such as cyclin dependent kinases which efficiently co-ordinate this event. The cell has to pass through several checkpoints, to ensure that any damage prior to the event has been rectified. So the checkpoint mechanism is to assess DNA damage, repair the damage and if the damage is irreparable then stall the cell cycle. Several studies have investigated the aspect of checkpoints in cell cycle of yeast cells, but the checkpoint mechanism in induced or reprogrammed pluripotent stem cells has not been studied so far. It will be

interesting to study this mechanism and its associated proteins in somatic cells and induced pluripotent stem cells (iPSCs) from humans.

Induced pluripotent stem cells, commonly abbreviated as iPS cells or iPSCs, are a type of [pluripotent stem cell](#) artificially derived from a non-[pluripotent](#) cell, typically an adult [somatic cell](#), by inducing a "forced" expression of certain [genes](#). The generation of iPS cells is crucial on the genes used for the induction. iPS cells are typically derived by [transfection](#) of certain stem cell-associated genes into non-pluripotent cells, such as adult [fibroblasts](#). Transfection is typically achieved through viral vectors, such as [retroviruses](#). Transfected genes include the master transcriptional regulators [Oct-3/4](#) (Pou5f1) and [Sox2](#), although it is suggested that other genes enhance the efficiency of induction. After 3-4 weeks, small numbers of transfected cells begin to become morphologically and biochemically similar to pluripotent stem cells, and are typically isolated through morphological selection, doubling time, or through a [reporter gene](#) and antibiotic selection. [Oct-3/4](#) and certain members of the [Sox gene family](#) (Sox1, Sox2, Sox3, and Sox15) have been identified as crucial transcriptional regulators involved in the induction process whose absence makes induction impossible.

The project will also give an insight into the genes p53, ATM, MAD genes that play an important role in the control of checkpoint mechanism during each phase of the cell cycle. p53 protein can sense DNA damage and halt the progression of the cell cycle in G1 by blocking the activity of Cdk2. P53 protein is also a key player in apoptosis. ATM is ataxia telangiectasia mutated, getting its name from the human disease, also detects DNA damage and therefore interrupts the cell cycle. MAD, i.e. mitotic arrest deficient, gene encodes two proteins that bind to kinetochore. If there is any failure to attach it then MAD blocks entry into anaphase. The study of these genes in both somatic and iPS cells will help us in understanding whether the checkpoint mechanism is maintained in each phase of the cell cycle. If this aspect is studied then we could fine-tune the rate of cellular division to control tumor development, aging etc.

Project aims

1. Generation and characterization of iPSCs
2. Comparison of iPSC lines with Embryonic Stem Cells (ESCs)
3. Investigate approaches to generating clinically relevant iPS cells, without stable genetic modifications using non-integrating viruses and/or nanoparticle delivery.
4. To determine the activity of cyclin dependent kinase (Cdk) involved in each phase of the checkpoints in cell cycle, monitor each phase of the cell cycle in both somatic and iPS cells and study the genes MAD, ATM, p53 etc in checkpoint mechanism in cell cycle. The project also aims to check each cell cycle phase of both the somatic and iPS cell cycle.

Expected outcomes

Understanding the process of reprogramming of iPSCs at the cellular level by comparison with ESCs, which is essential for translation to future clinical outcomes. The ability to generate non-genetically modified reprogrammed somatic cell with therapeutic potential will significantly impact on the application of cell therapy. The ability to understand the checkpoint mechanism in somatic and iP cells will be beneficial in understanding stem cells and give a clear understanding of how these cells maintain its integrity during cell cycle. In iPSCs we can understand how these pluripotent stem cells efficiently maintains its cell cycle and avoid DNA damage, which is imperative for eventual clinical use of the cells.

Project plan

The student will need training with stem cell culture and viral vector generation and transfection for maintenance and generation of iPSCs before generating the initial data. This would be provided at Monash

University under the supervision of Drs Verma and Sumer. The training will also include characterization of stem cells by *in vitro* and *in vivo* methods including-

- Vector design and generation
- Viral generation and transduction of target cells
- Isolation and maintenance of pluripotent cells
- In vitro differentiation as embryoid bodies (EBs)
- Characterization by protein localization, gene expression
- In vivo differentiation by teratoma formation in Severe Combined Immune Deficient (SCID) mice

Following this period the student will return to IITB and analyse the cell lines generated. She will also use the acquired skills to transfer stem cell expertise to the laboratory of Prof. Panda and generate additional iPSC lines for analysis. The investigations at IITB will include- To analyse the somatic cells and iPSCs for checkpoints mechanism in cell cycle regulation. To determine the activity of cyclin dependent kinase (Cdk) involved in each phase of the checkpoints in cell cycle, monitor each phase of the cell cycle in both somatic and iPS cells and study the genes MAD, ATM, p53 etc in checkpoint mechanism in cell cycle.

Once proficient with generation, culture and analysis of pluripotent cells, the project will examine alternative approaches to generating iPSCs without the use of integrating vectors.

Budget: \$40,000 for 1st year and **\$30,000** for subsequent years.

Instruments required at IITB: Biosafety Cabinet, 5% - CO₂ Incubator etc.

REFERENCES

1. Pralong D, Mroziak K, Occhiodoro F and Verma PJ. (2006) Reprogramming differentiated nuclei with pluripotent cytoplasm In: Cell Reprogramming and Transgenesis by Nuclear Transfer. Eds: Verma, P.J. and Trounson, A.O. Humana Press, NY, USA.
2. Pralong D, Lim ML, Vassiliev I, Mroziak K, Wijesundara N, Rathjen P and Verma PJ (2005). Tetraploid embryonic stem cells contribute to the inner cell mass of mouse blastocysts *Cloning and Stem Cells* 7(4):272-8
3. Taylor CJ, Bolton EM, Pocock S, Sharples LD, Pedersen RA, Bradley JA. Banking on human embryonic stem cells: estimating the number of donor cell lines needed for HLA matching. *The Lancet* 2005; 366: 2019-2025.
4. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. *Cell* 2007; 131, 861-872.

5. Karp, Gerald (2005). *Cell and Molecular Biology: Concepts and Experiments*, 4th Edition. Hoboken, NJ: John Wiley and Sons. pp. 598–599. ISBN 0-471-16231-0.
6. Hartwell, LH; Weiner, TA. Checkpoints: controls that ensure the order of cell cycle events. *Science*. 1989 Nov 3; 246(4930): 629–634.
7. Weinert, TA; Hartwell, LH. Characterization of RAD9 of *Saccharomyces cerevisiae* and evidence that its function acts posttranslationally in cell cycle arrest after DNA damage. *Mol Cell Biol*. 1990 Dec; 10(12): 6554–6564.
8. Tolmach, LJ; Jones, RW; Busse, PM. The action of caffeine on X-irradiated HeLa cells. I. Delayed inhibition of DNA synthesis. *Radiat Res*. 1977 Sep; 71(3): 653–665.