**UNIT 5 TEACHER BACKGROUND INFORMATION**

**Note: Terms in bold are defined in teacher and student glossaries.**

**BACKGROUND INFORMATION**

Embryonic stem cells have the potential to transform medicine. Scientist and doctors believe they could potentially treat a myriad of human conditions and diseases, such as spinal cord injury, type I diabetes, neurodegenerative diseases and many others. However there are many issues and barriers to fulfilling this potential. Chief among these is that human embryonic stem cells are derived from human embryos. Many people find the destruction of a human embryo for the purpose of stem cell research to be unethical. Also there are currently a number of restrictions on embryonic research funding. Currently no federal funding can be used in research that destroys or harms a human embryo, so no new embryonic cell lines can be created with federal funds. The lines themselves can be studied with federal funds if the individual cell line is approved by the National Institutes of Health and meets several criteria. One of these criteria is that all lines must have been created from excess embryos following therapeutic *in vitro* fertilization. Despite the large number of frozen excess embryos, few are available for research purposes. (ref) Also, if embryonic stem cells are to be used in cell transplant therapies it is necessary that the donor cells match the recipient. There aren't currently enough stem cell lines to match every person in the population. In particular, there is little representation of minorities in existing stem cell lines, meaning finding an appropriate match for a minority patient would be even more difficult.

Recognizing issues with embryonic stem cell research Dr Shinya Yamanaka of Japan sought to create embryonic stem cell-like cells by reprogramming **somatic cells**. The function and identity of a cell is dictated by the specific genes expressed in that cell type. Yamanaka theorized that if somatic cells were induced to express genes that regulate pluripotency he could re-create a stem cell identity. He found that **transgenic** expression of four **transcription factors**, Oct3/4, Sox2, Klf4 and c-Myc, was sufficient to reprogram mouse fibroblasts into embryonic stem cell-like cells termed **induced pluripotent stem (iPS) cells**. Transcription factors bind to DNA and regulate the expression of other genes. These four genes are capable of activating a network of gene expression sufficient to reproduce pluripotency function. These genetically-engineered (or transgenic) cells can differentiate into all other types of cells in the body just like human embryonic stem cells. This procedure has since been performed on human fibroblasts, as well as other cells types such as stomach, liver and neural stem cells.

This technique is advantageous to human embryonic stem cells in a couple of key points. First and most critically for many in the field; *no human embryos had to be harmed or destroyed in the process*. This technology also gives researchers more flexibility in

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producing cells for research. As cells can be isolated from any individual to create iPS cells, researchers can specifically select individuals with a genetic disease. Differentiating the iPS cells into the affected cell types could potentially allow researchers to examine disease progression in culture. For example, a paper summarized in this lesson describes the creation of iPS cells from ALS patients. These iPS cells were then differentiated into motor neurons, the cell type that degenerates in ALS. IPS could eventually be particularly useful in cell transplant therapies. IPS cell technology could be used to create patient specific cell lines, in which iPS cells are generated from the patients own cells so they are a perfect genetic match and eliminate the risk of immune system rejection.

IPS is a very new technology and currently cannot be used in human cell transplant therapies. The biggest concern at the moment is that iPS cells are more likely to develop cancer than human embryonic stem cells. One of the four transgenes, c-Myc, is a known oncogene. If c-Myc continues to be expressed progeny of iPS cells are likely to become cancerous. In addition, retroviral vectors are the most common method for transfection of the four factors. A retrovirus is an RNA virus that in the course of its life cycle reverse-transcribes (makes DNA out of RNA) its genetic information and inserts it into the DNA of the host cell. These insertions could interrupt normal gene sequences and are therefore potentially mutagenic. This could also potentially cause cancer formation. Scientists are currently investigating alternate methods to create iPS cells. IPS cells can be made without the use of c-Myc, but the process is much less efficient. Other gene delivery methods utilizing adenoviral vectors, plasmids, and even substitution of the gene with small molecules have been shown to create iPS cells, but again much less efficiently than with retroviral vectors. In general iPS is extremely inefficient. Approximately 0.0001-0.1% of all cells become iPS cells depending on the method.

IPS is such a new technology the majority of information about it is in scientific publications. The majority of this lesson is based on summaries of scientific publications. In this unit, we will explore several aspects of iPS cell technology. First we will introduce iPS by Yamanaka protocol used to create iPS cells from skin cells, and explain the methods and assays used to confirm the cells' new identity. Understanding of this first paper will be critical to understanding the rest of the material in the lesson. We will explore several aspects of stem cell iPS in a jigsaw: various gene delivery methods such as retroviral, adenoviral, and plasmid as well as small molecules amplifying gene expression; the use of and potential advantages of using cell types other than fibroblasts; iPS without the use of c-Myc which produces cells less prone to cancer; producing disease specific iPS cells from patients with ALS; and finally treatment for a mouse model of sickle cell anemia with genetically modified patient specific iPS cells.

IPS is a very new technology and there are still many aspects of it that are not understood. Part of the value of this lesson is it demonstrates the process of scientific discovery. The original Yamanaka paper is critical to establishing a new and exciting technique with much promise. However much more research needs to be done to perfect this technique and to fully realize its potential.

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