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Elaine Fuchs' Lecture Part 1:
Stem Cell Biology

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Contents

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1. Keywords and Terms

Embryonic stem cells, adult stem cells, self-renewal, differentiation, transit-amplifying cells, blastocyst, totipotent, multipotent, unipotent, ethics, somatic cell nuclear transfer, induced pluripotent stem cells, regenerative medicine

2. Lecture Notes

What are stem cells?

There are two basic types of stem cells: embryonic stem (ES) cells, and adult stem cells. They share the ability to endlessly self-renew – they can divide continuously to generate daughter stem cells of the same type. However, they can also divide to produce transit amplifying (TA) cells which proliferate rapidly to quickly increase in number before differentiating into specialized cell types. Normally, a stem cell divides to

produce daughter cells with different fates – one daughter remains as a stem cell, while the other becomes a TA cell.

Embryonic stem cells are totipotent and can be cultured in vitro

ES cells are totipotent because they give rise to all of the tissues in an animal. ES cells make up the 'Inner Cell Mass' of early embryos. At the embryonic stage called the blastocyst, the inner cell mass is surrounded by a layer of cells called the trophectoderm. The trophoblast cells in the trophectoderm provide support to the ES cells in the inner cell mass as they develop into the fetus.

Scientists can remove ES cells from blastocysts and culture them *in vitro*. Once these ES cells have been isolated, they can be maintained in culture indefinitely, because, as stem cells, they can divide and self-renew endlessly. However, the growth conditions can be manipulated to induce the ES cells to reform blastocysts. A single ES cell can be induced to form a blastocyst (including the trophectoderm layer) which can then be implanted back into a 'pseudopregnant' mouse, where it will develop normally and be born three weeks later. Thus, a single ES cell has the potential to give rise to an entire animal.

Cultured ES cells can also be induced to differentiate *in vitro* into many different tissues by treating them with different cocktails of growth factors. For example, treating ES cells with Nerve Growth Factor cause them to develop into neurons. Many other cell types can be formed in this way too – the only limitation is knowing the precise culture conditions to use. This is why ES cells hold such great promise for regenerative medicine (see below).

Adult stem cells are either multipotent or unipotent.

During development, stem cells become increasingly restricted in what they can make. Adult stem cells can't give rise to all the tissues of the body. Instead, they are restricted to particular tissues. They allow these tissues to undergo homeostasis (natural turnover throughout the tissue's lifetime) and to be repaired following wounding or other types of injury. Adult stem cells may be multipotent (can develop into several different cell types and lineages) or unipotent (can only differentiate into one specific cell type).

Examples of multipotent adult stem cells include:

Hematopoietic stem cells (give rise to all the cells of the blood, including both red blood cells and immune cells)

Hair follicle stem cells (give rise to hair follicles, sebaceous glands and the epidermis)

Examples of unipotent adult stem cells include:

Epidermal stem cells (only give rise to the epidermis, which is constantly turning over and so needs to be continuously replenished with new cells)

Liver stem cells (only give rise to hepatocytes of the liver).

Adult tissues may contain large or small numbers of stem cells, depending on the tissue's requirement for new cells in maintaining homeostasis (for example, the pancreas and the central nervous system have few adult stem cells).

Stem cells and regenerative medicine

Because stem cells (particularly ES cells) can be manipulated *in vitro* to form a variety of different cell types, they hold great potential for regenerative medicine. ES/totipotent cells are of most interest as they have the most developmental potential, including the ability to differentiate into tissues in which adult stem cells are only present with very low abundance.

Stem cells could be differentiated into cells able to replace defective or damaged cells in many different diseases and conditions. Examples include:

- Nerve cells to treat patients with Parkinson's disease, Alzheimer's disease or spinal cord injuries.
- Pancreatic islet cells for diabetics.
- Skeletal muscle for patients suffering from muscular dystrophy.
- Heart muscle to replace damaged or diseased heart tissue.
- Immune cells for patients with immunodeficiencies.

- Skin and hair cells for patients with skin disease, baldness or with burned or otherwise damaged skin.

A greater understanding of stem cells may also help us better understand cancer and provide new treatments. The ‘cancer stem cell’ hypothesis holds that stem cells within tumors produce, by self-renewal and differentiation, the cancerous tissue. These stem cells may persist after normal anti-cancer treatments, thereby causing relapses and metastases.

Ethical issues surrounding human ES cell research

The major ethical dilemma associated with human embryonic stem cell research is the concern that the derivation of human ES cells from fertilized embryos often entails the destruction of that embryo and thus, in some people’s view, the destruction of a human life.

In vitro fertilization (IVF) technologies allow fertilized embryos and blastocysts to be created in the laboratory. The techniques often results in excess embryos that either go unused or are discarded. Some believe that using these embryos poses fewer ethical problems, since the embryos were not created in the mother’s womb, and were not destined to develop into a fetus anyway. However, this does not satisfy everyone: some believe that IVF embryos still constitute human life, and that they should only be used for implantation into mothers and not for research.

It is possible to isolate a single ES cell from an 8 cell stage IVF embryo and for the remaining 7 cell embryo to remain viable (although not without risk to that embryo). The single isolated ES cell can be cultured and expanded to provide material for stem cell research. This technique is, in fact, already in use for genetic screening of embryos derived from couples at high risk of passing on a serious genetic disease to their unborn child. Nevertheless, some people believe that the danger posed to the embryo in extracting one of its cells is not justified by the potential it offers for stem cell research.

Alternative methods for producing totipotent stem cells

Ultimately, generating stem cells free of any ethical issues may require using alternatives to the isolation of ES cells from human embryos. Note that finding a way to

produce totipotent stem cells holds the most promise for regenerative medicine as they have the capacity to differentiate into every possible tissue of the body. The lecture discusses one alternative method – nuclear transfer. Since this lecture was recorded, a second potential alternative has received much attention, namely induced pluripotent stem (iPS) cells. Both are discussed below.

Nuclear transfer

Nuclear transfer was the technique used to clone Dolly the sheep in 1996. The same approach was originally used by John Gurdon to clone frogs in 1962, and many animals, including mice, have been cloned in similar fashion since Dolly.

The technique involves removing the nucleus from an unfertilized oocyte and replacing it with the nucleus of an adult somatic cell. This produces a hybrid cell in which the oocyte cytoplasm somehow 'resets' the developmental history of the somatic nucleus, restoring its totipotent potential. Single hybrid cells can be cultured, induced to form blastocysts, and then implanted into mothers where they develop normally. The nuclei of mouse skin cells, for example, can be injected into enucleated oocytes and used to generate ES cell-like totipotent hybrid cells. This is actually a relatively efficient process, although subsequent attempts to generate cloned mice by implanting cloned blastocysts are much less efficient.

iPS cells

Induced pluripotent stem (iPS) cells are a relatively new approach to generating ES-like cells without running into so many ethical concerns. The technique was first demonstrated in mouse cells in 2006 in Shinya Yamanaka's laboratory, and with human cells in 2007 by Yamanaka and by James Thomson's group.

The technique involves reintroducing activated versions of a select few genes to adult somatic cells which then revert to an ES cell-like state. In Yamanaka's original work, just 4 transcription factors – Oct-3/4, SOX2, c-Myc, and Klf4 – could induce this reversion when transduced into adult mouse fibroblasts using retroviruses. However, mice derived from these iPS cells have a high incidence of tumor formation. Many researchers around the world are working to improve the efficiency and safety of iPS cell technology, using slightly different combinations of genes and different ways of introducing them into various types of adult cells. Importantly, this technology has been adapted to human

skin keratinocytes, raising the possibility of generating ES-like cells tailor made to human patients simply from a small skin biopsy.

Potential uses of nuclear transfer and iPS cell technologies

It should be stressed that scientists are not interested in using either of these technologies to clone humans. Instead, totipotent cells produced by these alternatives to ES cells have the potential to be differentiated *in vitro* to generate cells for research and regenerative therapy.

In terms of therapies, both of these techniques offer the intriguing advantage that stem cells could be derived from a patient's own tissue. This would avoid issues of immune rejection, as implanted cells would be a genetic match to the patient, preventing their immune system from recognizing the cells as foreign and eliminating them. On the other hand, if a patient's own cells are to be used as the source of totipotent cells, any genetic defect in the patient's cells will need to be corrected.

In terms of research, differentiated cells produced *in vitro* from either iPS cells or nuclear transfer hybrid cells will be useful tools for understanding disease processes and for drug development. For example, the skin cells of an Alzheimer's disease patient can be converted into iPS cells, which can then be differentiated into neurons. These genuine "Alzheimer's neurons" are invaluable, as we cannot take a brain biopsy from a living Alzheimer's patient, and we can presently only study the endpoint of the disease rather than study its onset.

Potential dangers of stem cell therapies

However they are attained – whether isolated from embryos, produced by nuclear transfer or by iPS techniques – totipotent cells have a danger, as well as benefits, in their developmental potential. These cells have the potential to form teratomas – tumors which derive from germ cells and contain multiple different tissue types. These tumors can form if ES/ES-like cells are implanted into animals due to uncontrolled differentiation. Future regenerative therapies must ensure that any ES cells derived by these technologies have been fully and irreversibly differentiated into the desired cell type prior to implantation into a patient. How long it will take before this research

becomes clinically applicable is currently uncertain. However, the research holds promise for the future.

3. Recommended Reading

1. Molecular Biology of the Cell (5th Edition), Alberts et al. Chapter 23
2. Blanpain, C and Fuchs, E. Epidermal homeostasis: a balancing act of stem cells in the skin. Nat Rev Mol Cell Biol. 2009. 10: 207-17.
3. Fuchs, E. The tortoise and the hair: slow-cycling cells in the stem cell race. Cell. 2009. 137: 811-9.
4. Fuchs, E. Finding one's niche in the skin. Cell Stem Cell. 2009. 4: 499-502.
5. Yamanaka, S. Strategies and new developments in the generation of patient-specific pluripotent stem cells. Cell Stem Cell. 2007. 1: 39-49.
6. Jaenisch, R and Young, R. Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. Cell. 2008. 132: 567-82.

4. Review Questions

1. Name 3 properties that all stem cells (both embryonic and adult) have in common.
2. What are transit-amplifying cells?
3. What are the 2 cell layers that exist in the embryonic blastocyst? Which of these layers contains ES cells?
4. Which of the following can describe an ES cell, and what does it mean? Totipotent, multipotent, unipotent.
5. Which of the following can describe an adult stem cell, and what does it mean? Totipotent, multipotent, unipotent.
6. Give an example of a multipotent adult stem cell and a unipotent stem cell.

7. Name two functions of adult stem cells.
8. What is the main concern with human embryonic stem cell research and their potential use in regenerative medicine?
9. Name a method for producing ES-like cells from an adult somatic cell.
10. What are the potential advantages of these alternative methods over ES cells obtained from human embryos?

5. Answers to Review Questions

1.
    a. They can divide endlessly
    b. They can self-renew
    c. They can produce transit-amplifying daughter cells that undergo differentiation.
2. TA cells are the progeny of stem cells that proliferate rapidly to increase in number before differentiating into specialized cell types.
3. The trophoectoderm and the inner cell mass. The latter of these contains ES cells.
4. Totipotent. An ES cell has the potential to differentiate into every type of animal tissue.
5. Adult stem cells can be multipotent (able to differentiate into a subset of cell lineages) or unipotent (able to differentiate into one particular cell type).
6. Many examples. Those mentioned in the lecture are hematopoietic stem cells and hair follicle stem cells (both multipotent) and epidermal stem cells and liver stem cells (both unipotent).
7. Homeostasis (natural turnover/ wear and tear) and wound repair.
8. The isolation of human ES cells requires either the destruction, or involves a risk of destruction, of human embryos, which some people believe constitutes a human life.

9. Nuclear transfer (discussed in the lecture) or induced pluripotent stem (iPS) cells.

10. No/fewer ethical issues around the destruction of human life.

    Can be derived from a patient and therefore are genetically matched. This avoids potential problems with immune rejection.

6. Discussion Questions

1. Initial attempts at producing mice from iPS cells resulted in mice with a high incidence of tumor formation. Why was this, and what might scientists do to overcome these problems?
2. Patient samples can be reverted to an ES-like state by either nuclear transfer or iPS technology, before being re-differentiated *in vitro*. For example, skin cells from an Alzheimer's disease patient might be used to create Alzheimer's diseased neurons *in vitro*, which can be used for research. How might this be an improvement over current mouse models of Alzheimer's disease?

7. Answers to Discussion Questions

1. There are two main reasons for the tumor formation in mice generated from iPS cells. Firstly, one of the genes used to revert adult somatic cells back to undifferentiated, totipotent iPS cells was the potent oncogene, c-myc. Successful, albeit less efficient, efforts at generating iPS cells without c-myc have now been made. Secondly, all 4 reverting genes were introduced into adult cells using retroviruses. The 4 genes were therefore permanently integrated into the cell's genome. This poses the risk that the genes may continue to function after they have reverted the cells to an ES-like state and the genes may integrate in an appropriate place, disrupting host cell gene function. Scientists are remedying this by temporarily transfecting in the genes on plasmids where they don't integrate into the genome and are lost after iPS conversion.
2. Current mouse models usually rely on a single genetic mutation and only replicate a subset of Alzheimer's disease symptoms. Sporadic Alzheimer's disease is, however, a complex disease involving multiple genetic defects. Obtaining (even indirectly) neurons from real Alzheimer's patients should much more accurately reflect the real

disease situation and its etiology. In the past, this has been impossible to study, as one can only obtain brain samples from deceased Alzheimer's patients.

8. Explain or Teach These Concepts to a Friend

1. What are the similarities and differences between embryonic and adult stem cells?
2. What do totipotent, multipotent and unipotent mean?
3. What are the ethical issues surrounding human ES cell research and their potential resolutions?
4. How can totipotent cells be obtained from adult tissues?

9. Research the Literature on Your Own

1. What is the current state of play in the iPS cell field? How does it compare to nuclear transfer as a potential source of totipotent cells for regenerative medicine?
2. What are 'cancer stem cells'? How may cancer therapies change as a result of their reported discovery?