

Stem cells and their applications; Environmental effects on development

Reading: 8 e: Chp 15: 489-493 (blood stem cells), Chp 21: 684-689 (stem cell applications), 666-675 (environmental effects on development)

9e: Blood stem cells: Chp 12: 466-471 (blood stem cells); Chp 17 649-653 (stem cell applications); 628-641 (environmental effects on development)

Learning Goals

Be able to:

- Define what a stem cell is and describe its general properties, using blood stem cells as an example.
- Describe to a lay person the potential and the current limitations of human stem cell research.
- Explain the difference between therapeutic and reproductive cloning.
- Compare the possible advantages and disadvantages for human tissue replacement therapy of adult, embryonic, and induced pluripotent stem cells.
- Explain the potential effects of environmental conditions on embryonic development

Stem Cells

Stem cells are frequently in the news. Some aspects of stem cell research are controversial as you know, but this research is some of the most exciting and practically promising now going on in developmental biology. In this class we will discuss the general role of stem cells in development and one example in some detail: how stem cells work in the blood forming system, as well as considering the potentials and problems associated with production and medical use of embryonic stem cells.

Terminally differentiated cell types are often no longer capable of division; red blood cells (erythrocytes) no longer even have functional nuclei. Some terminally differentiated cells, like muscle cells, have a lifespan as long as the animal. But many others, such as skin and red blood cells, have much shorter lifespans and must be continually replaced from a stem cell population. For example, a normal person loses and replaces more than 10^{11} red blood cells per day (!), loses and replaces all the cells that line the villi of the small intestine every 3-5 days, and sheds and replaces the entire surface area of the skin in a cycle that takes 2 to 4 weeks.

Stem cell divisions can be symmetric, giving rise to two daughter stem cells; or asymmetric, giving rise to a new stem cell and a cell that differentiates or produces only differentiated progeny. This choice of fates of the daughter cells must be carefully controlled. If too many daughter cells differentiate, the stem cell population will be depleted. If too many daughter cells remain stem cells, the stem cells can accumulate and proliferate too rapidly, sometimes causing a tumor.

All stem cells in later embryonic development are partially determined (i.e. no longer totipotent), and they vary in their lifetimes. Some, like myoblasts, are unipotent, that is, they produce progeny that differentiate into only one cell type: muscle cells. Myoblasts divide only a limited number of times before differentiating, because muscle cells do not generally have to be replaced in the adult animal. When muscle cells need to be regenerated following an injury, so-called satellite cells, a small quiescent stem cell population in muscle tissue, serve as a source of new myoblasts. Epidermal cells in the skin are also unipotent, but since skin cells must continually be replaced, these stem cells divide without limit throughout the lifetime of the animal. The stem cells of the blood forming system also divide without limit, but they are pluripotent, producing many different types of blood cells.

This system is discussed in more detail below as an example of how a pluripotent stem cell population functions in development.

Development of the blood forming system. There are two phases in vertebrate blood cell development. In the transient embryonic phase, mammalian hematopoietic stem cells (HSCs) arise from extra-embryonic ventral mesoderm in the blood islands of the yolk sac, induced by BMPs. In the second, definitive phase, HSCs that

will proliferate throughout the life of the animal arise from mesodermal cells that surround the aorta.

From the region around the aorta, HSCs migrate through a series of sites during fetal development, receiving different signals in each of these microenvironments. They first colonize the liver, a site of early fetal hematopoiesis, and then later the bone marrow and spleen. In humans, later fetal hematopoiesis occurs in both tissues, but by the time of birth it has shifted almost entirely to the bone marrow, where it continues throughout life.

The associations with different tissue microenvironments are accompanied by changes in the globin chains that are expressed during formation of red blood cells. Later in development, lymphoid cells are similarly induced to produce different classes of antibodies by the different microenvironments in which they reside (e.g. IgM and IgG in the lymph nodes, IgE in the intestinal Peyer's patches). These differences, as well as the controls on production of the various hematopoietic stem cell types, are mediated by growth factors bound to ECM components in the different tissue microenvironments in which hematopoiesis occurs.

Blood cell maintenance from pluripotent hematopoietic stem cells

In blood cell production, a large population of differentiating cells is maintained by a relatively small population of slowly dividing stem cells. To make this possible, primary hematopoietic stem cells (HSCs) first give rise progressively to more restricted stem cells and then to committed progenitor cells. These can no longer divide indefinitely, but still go through several amplifying rounds of cell division before their progeny differentiate. The number and rate of these amplifying divisions is probably an important control point for the regulation of the number of differentiated blood cells that can be produced.

Myeloid cells. Bone marrow cells injected into a lethally irradiated mouse will rescue the animal by providing hematopoietic stem cells, which colonize host tissues. Stem cell colonies can be seen in the spleen. Each nodule on the spleen derives from a single pluripotent stem cell of the so-called myeloid type, which can produce red blood cells (erythrocytes), monocytes (macrophages), megakaryocytes (source of platelets), and various granulocytes. The myeloid stem cells also produce more of themselves, as a stem cell should.

Lymphoid cells. Myeloid stem cells do not produce lymphocytes. B and T lymphocytes are derived from separate lines of lymphoid stem cells. However, both myeloid and lymphoid stem cells were eventually shown to arise from a common general hematopoietic stem cell, the HSC. The long-sought HSCs were first isolated in the mid-1990's: they are rare, found at a frequency of about 1 in 10,000 bone marrow cells. The entire hematopoietic system of a lethally irradiated mouse can be reconstituted from as few as 30 of these cells.

Amplification. The numbers of the various determined precursor cells are amplified by divisions that follow their commitment, so that a small number of pluripotent stem cells can give rise to a very large number of differentiated blood cells. These divisions are controlled by growth factors called colony stimulating factors (CSFs). For each different committed cell type, the amplifying divisions are controlled independently by specific CSFs, so that, for example, you can respond to high altitude (anoxia) selectively, by amplifying just the erythroid (red blood cell) precursor cells and not all other blood cell types.

Differentiation. The differentiation of the different kinds of hematopoietic stem cells depends on the microenvironment of the stem cells. Various cytokines and interleukins are secreted by mesenchymal cells (so called stromal cells) in the presence of the stem cells. These cells are primarily a kind of osteoblast that line the bone marrow. These cells can bind the HSCs through N-cadherin, and supply ligands that activate the Wnt pathway as well as the Notch and RTK pathways. Since each "niche" surrounding the stem cells is slightly different, the transcription factors that are activated in the different cells determine their ultimate fate.

Summary. Totipotent HSCs give rise to all the hematopoietic cell types by progressive determination, i.e. a series of stem-cell divisions at several levels from self-renewal to production of progressively committed proliferating stem cells, which then undergo several stages of differentiation. These stages of proliferation, determination, and differentiation are controlled by growth factors, generally termed CSFs, which are often restricted to certain microenvironments (e.g. spleen, bone marrow). Therefore, the pathway of determination

and differentiation that a precursor cell takes will depend largely on the microenvironment to which it is directed or in which it lands.

Stem cells for tissue replacement therapy

Embryonic stem (ES) cells can be obtained from the inner cell mass of a mammalian blastocyst and cultured indefinitely *in vitro* on appropriate media, as discussed previously. If reintroduced into another blastocyst, they are totipotent, capable of giving rise to any cell type in the animal. ES cells can also be caused to undergo controlled determination and differentiation in culture by exposing them sequentially to specific growth factors. In recent years, recipes have been worked out for producing several specific cell types in culture.

Stem cells have tremendous potential for replacing damaged, defective, or diseased tissues in human patients. Stem cell cultures can be obtained from some adult tissues (e.g. HSCs from bone marrow, epidermal stem cells from epidermis). These cells can be cultured, genetically manipulated if necessary, and can also be made to differentiate in culture before re-introducing into a patient. However, they have only limited developmental potential. Therefore, they are useful for some specific treatments but are not suited for general tissue replacement at present.

The problems with stem cells are first, obtaining cells with the same transplantation antigens (MHC haplotype) as the recipient, to avoid immune rejection of injected cells, and second, obtaining stem cells with the necessary developmental potential and administering them by methods that are safe and ethically acceptable to society.

Until last year, there were two practical sources of stem cells for tissue replacement therapy: adult stem cells and ES cells from therapeutic cloning. There is now a third potential source, induced pluripotent stem cells (iPS cells), which may bypass problems with the first two sources if future research shows they can be used without danger of tumor formation.

ES cells

Advantages: Highly pluripotent. Can make any tissue.

Disadvantages: Must have the same MHC haplotype as the recipient to avoid rejection. This can be achieved only by nuclear transplantation from a recipient's somatic cells into a human enucleated 1-cell embryo and development to the blastocyst stage, which will contain ES cells of the recipient's haplotypes – so-called therapeutic cloning (as opposed to reproductive cloning: attempting development of an individual from an engineered embryo).

Gene therapy can be applied effectively in combination with therapeutic cloning. ES cells made from an individual with a gene defect can be engineered in culture by knock-in of a normal gene at the affected locus, followed by tissue replacement with differentiated derivatives of the engineered ES cells. Some of the ethical objections to these techniques result from confusion between therapeutic and reproductive cloning.

Adult-derived stem cells

Advantages: If cultured from the recipient, stem cells will have the recipient's major histocompatibility (MHC) haplotype, so that transplanted stem cells or differentiated derivatives will not be rejected.

Disadvantages: The therapeutic possibilities with these cells are fewer, because their developmental potential is more restricted, though the extent of their potential is still unclear. Incorporation of cells derived from HSCs, for example, into other tissues (intestine, muscle) has been reported, but there are caveats to these experiments.

Induced pluripotent stem cells (iPS cells)

The finding that differentiated cell nuclei can be reprogrammed by oocyte cytoplasm, shown by the reproductive cloning of Dolly and other mammals, led researchers to attempt identifying the factors responsible,

in the hope of being able to reprogram such nuclei directly without cloning. By using micro-array technology to compare the expression profiles (the population of mRNAs they produce) of ES cells to those of differentiated cells derived from them, they found that expression of a small number of transcription factors distinguishes ES cells from later embryonic cells. In late 2007, the laboratory of James Thomson at UW Madison reported that a combination of just four transcription factors (Oct4, Sox2, Klf4, and c-Myc), when introduced into fibroblasts using lentiviral expression vectors, was sufficient to transform the fibroblasts into pluripotent cells with all the properties of ES cells, including the ability to differentiate into tissues of all three primary germ layers!

The present method of constructing these cells depends on insertion of the vector into the genomes of these cells. This leads to position effects causing variable expression of the transgenes, and the viral insertion events may disable genes that would be important in later developmental steps. Consequently, individual cell lines have somewhat different properties. In addition, lentivirus is a retrovirus and at least one of the transcription factor transgenes (c-Myc) is an oncogene, raising the possible danger of tumor formation in iPS derived tissues. Additional work on this system has used a different system for introducing these factors: episomal viral vectors, which are derived from viruses, but do not insert DNA into the host. When the vectors are present in a cell, the transcription factors are generated, but the DNA of the cells is not altered. Interestingly, some stem cells could be returned to pluripotency without c-Myc (blood stem cells), and neural stem cells require activation only of Oct4. This is a very promising technology, potentially promising treatments for a variety of human genetic diseases (and avoiding the technical complications and ethical objections associated with therapeutic cloning).

Environmental effects on development

In this course, we have primarily considered the embryo as a closed system, discussing the genetic control of different stages of development, the combinatorial effects of different transcription factors on commitment, differentiation, migration, etc. Of course, embryos are NOT a closed system: all embryos differentiate in an environment, and changes in the environment can affect development. As time permits today, I will discuss a few known disruptors of development, including alcohol and hormones (the synthetic ones we are exposed to). These topics are well covered in your book, so please consult those pages if you need more information.

Review questions

- 1) What are the characteristics of stem cell division?
- 2) Name two effects of different tissue microenvironments on the fates of hematopoietic cells.
- 3) What are the tissues in which HSCs originate and through which they migrate during embryonic and fetal development?
- 4) What experiment showed that the myeloid and lymphoid lines of stem cells are separate?
- 5) How were researchers able to isolate particular stem cell types from the many kinds of similar cells in bone marrow?
- 6) What kinds of factors control the commitment and differentiation of hematopoietic cells?
- 7) What are three possible sources of mammalian pluripotent stem cells?
- 8) What are the relative advantages and disadvantages of ES cells and adult-derived stem cells for tissue replacement therapy?
- 9) How is it possible to make ES cells from an adult recipient for tissue replacement therapy?
- 10) How could gene therapy be used in combination with therapeutic cloning to replace a genetically defective cell or tissue type in an individual?
- 11) What would be the potential advantages of induced pluripotent stem cells over either adult stem cells or ES cells obtained from human blastocysts made by therapeutic cloning?