Cell Biology: Cellular Organization, Division, and Processes

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Lecture 30

Stem cells Part I Introduction-SL

Hello everyone, I am Shikha Laloraya, Professor of Biochemistry, at IISc. Welcome to this introductory lecture on stem cells, which is a part of my ongoing course on cell biology.

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In multicellular organisms, early development occurs by rapid mitotic divisions of embryonic cells, which later differentiate into specialized cells that form tissues and organs. Most differentiated cells in adult animals are arrested in the G_0 stage of the cell cycle; for example neurons-they remain arrested in the G_0 stage of the cell cycle and do not come out of that. Loss of cells due to programmed cell death or due to injury has to be compensated for by formation of new cells during the life of an adult animal.

To achieve this balance, most of the tissues have got cells that can divide to replace the cells that have died or have been lost. The proliferation of differentiated cells can occur in some cases, for example in case of fibroblasts, which are already arrested in G_1, can re-enter the cell cycle if required when an injury occurs for example if there is an injury then PDGF is produced at the site and in response to PDGF (which is platelet-derived growth factor) the proliferation of fibroblasts occurs and they also secrete collagen, which helps in repair.

Endothelial cells are cells which line the blood vessels and they can also proliferate in response to VEGF (vascular endothelial growth factor) that is produced in response to lack of oxygen. So, because of this new capillaries canbe formed where they are required. Epithelial cells can also proliferate in some internal organs and also in case of liver regeneration, after surgical removal of a part of the liver it can be regenerated to a large extent.

Stem cells are present in many tissues; many fully differentiated cells cannot divide themselves but within those tissues there is a subpopulation of cells, which are referred to as stem cells that can proliferate and they can replace the differentiated cells.

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Now some of the tissues are not at all renewable. So, some of the tissues, they do not have any stem cells at all and they cannot be renewed even when the cells are lost. For example, in mammalian system this is true for the auditory epithelium and also for the retinal epithelium. So, if the sensory receptors in these organs, for example the hair cells in the ear, and the photoreceptors, are lost or damaged, then they cannot be replaced. Once they are lost, they are irreplaceable.

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So, what exactly are stem cells? Stem cells are self-renewing cells that are present in adult tissues and they help in their maintenance. These cells can proliferate and they can replace the differentiated cells throughout the life of an adult organism. And these cells, when they divide, they give rise to one daughter cell that remains a stem cell and another one that divides rapidly and then the resulting cells differentiate.

Stem cells are present within distinct micro-environments term niches that provide the signals that are required for maintenance of stem cells throughout life and balance the self-renewal versus differentiation. So, examples of such cells are actually, these are present in tissues where the cells have a short life span, for example blood cells or sperm or epithelial cells of the skin and digestive tract. Replacement of damaged tissues in skeletal muscle or the nervous system can also happen with the help of stem cells.

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So, this slide shows the formation of blood cells. In fact stem cells were first identified in this hematopoetic system. Many different types of blood cells can form from the hematopoietic stem cells, which are actually present in the bone marrow. And the cells in these in the blood, they have distinct structures and also various specialized functions as you can see here. For example oxygen and CO_2 transport by erythrocytes, then there are also immune cells such as lymphocytes and then granulocytes and macrophages and various other kinds of cells are part of this system.

So, during the production of these cells from the stem cells of course, the stem cell is renewed, there is self-renewal of the hematopoietic stem cell. Their precursors that are formed they may undergo many rounds of cell division prior to differentiation. And then the differentiated cells that are formed, they stop proliferating, and they also have a limited life span and. So, they have to be replaced by the hematopoietic stem cells.

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The property of stem cells to repair or replenish damaged tissue has application in medical treatments. An example of this is hematopoietic stem cell transplantation, or it is also referred to as bone marrow transplantation. This procedure is useful after chemotherapy of cancer patients that

kills rapidly dividing cancer cells as well as dividing normal cells, including the hematopoietic stem cells.

So, variation of this procedure is autologous hematopoietic stem cell transplantation, in which the stem cells they are actually obtained from the patient, before the chemotherapy, and they are stored. And then after the treatment is over they can be returned back to the patient to help in producing the new cells.

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The basic character of specialized cells can be experimentally altered or reprogrammed. For example in a very important classic experiment by Gordon, nuclei from a differentiated cell could be reprogrammed by transplantation into an enucleated oocyte or egg cell. So, in this experiment in 1962 using the *Xenopus* system, the nucleus of a differentiated cell from a tadpole's gut was introduced into an e-nucleated oocyte.

The hybrid cell could develop into a complete normal frog as you can see here but the success rate was low, it was not all the time but only a subset of these injected cells could in fact form an entire organism. And this experiment had two implications, one was that the cell nucleus of differentiated cells has a complete genome and it has a complete genetic information, which is capable of supporting development of all normal cell types. And also that cytoplasmic factors can reprogram a nucleus, that is the oocyte cytoplasm drives the gut nucleus into an early embryonic state that can undergo the changes in gene expression, which are required for normal development. Now this reprogramming involves many epigenetic changes, for example there is alteration in patterns of DNA methylation and also the histone H1 that is the linker histone is replaced by an oocyte specific H1 variant. And the histone H3 at many locations in the chromatin is also replaced by another H3 isoform.

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Embryonic stem cells were first cultured from mouse embryos by Dr Gail Martin in 1981 and also by Evans and Kaufman in the same year. In this study the cells from the inner cell mass of a normal mouse blastocyst were cultured and in fact cell lines could be established that could then be propagated indefinitely in culture. These were termed embryonic stem cells or ES cells. ES cells are pluripotent that is they can give rise to most tissue types excluding very few extra embryonic tissues such as the placenta, which of course is not derived from the inner cell mass.

The ES cells could also be induced to differentiate in culture into a variety of different cell types for example endodermal or cartilage or neuron-like cells. And when these cells were introduced into early mouse embryos, they could also give rise to cells in all the tissues. Mouse ES cells are grown in the presence of a growth factor, the leukemia inhibitory factor or LIF, that helps maintain these cells in an undifferentiated state. When this factor is removed then the cells aggregate to form embryoid bodies that can differentiate into various cell types such as blood cells, epithelial cells, nerve cells, vascular smooth muscle cells, and beating heart muscle cells. The ES cells can be directed to

differentiate along particular pathways also by addition of specific growth factors or small molecules or combinations of them.

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Now human ES cells were also isolated later on from human embryos in 1998, and this raised the possibility of their use in clinical transplantation therapies. These cells could also be differentiated into most types of specialized cells using the specific combinations of growth factors, which are not shown here but this had tremendous promise for medicine.

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Given the technical and ethical difficulties in deriving embryonic stem cells, a big advance in the field came when it was shown that adult somatic cells can also be directly converted into pluripotent stem cells in culture, and this was shown by Takahashi and Yamanaka in 2006. So, at that time it was known that the gene Oct4 is exclusively expressed in ES cells, this codes for a transcriptional regulator and it was also known that when Oct4 is lost from ES cells they lose their ES like character. So, co-expression of a set of four transcriptional regulators, Oct4, Sox2, Klf4 and Myc, referred to as OKSM, this combination, could reprogram the mouse fibroblasts converting them into cells which were similar to ES cells in their properties. These ES cell like cells, which were derived in this way were termed induced pluripotent stem cells or IPS cells. These cells could also divide indefinitely in culture and they can contribute to development of any tissue when introduced into a mouse blastocyst. And IPS cells can also be induced to generate specific adult cell types or an even whole organ, that is the promise, by suitable signalling proteins and growth factors similar to ES cells.

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So, here is an interesting example of differentiation of induced pluripotent stem cells into cardiomyocytes in culture. Cardiomyocytes are very interesting cells, they are beating heart cells, they beat synchronously. Cardiomyocytes can be generated from induced pluripotent stem cells by mimicking the requirements for embryonic cardiogenesis. And in this process the Wnt signalling pathway plays an important role in cardiac tissue differentiation. Wnt signalling during the first days of cell culture increases the half-life of beta-catinin. And it enables its action as a transcription factor, which helps in the formation of cardiac mesoderm. And then inhibiting this pathway at the mesoderm phase allows differentiation to continue until the spontaneous beating of these cells begins; following this the Wnt activation is resumed and you get mature beating cardiomyocytes in culture. A video of these beating cardiomyocytes derived from induced pluripotent stem cells can be seen in the second lecture on stem cells in this course.

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Induced pluripotent stem cells are useful in understanding defective phenotypes of specialized cells at the cellular level in genetic disorders or in in vitro disease modelling. An example of the use of such cells for pediatric neurogenetic disorders is shown here. First, somatic cells from patients and also healthy controls are reprogrammed into induced pluripotent stem cells using the methods described.

The defect in these cells could also be corrected by gene editing using the CRISPR/Cas9 system. The induced pluripotent stem cells from patients as well as these gene edited patient-derived IPS cells, as well as the ones from the normal controls, can then be differentiated into the relevant neuronal cell type. These neurons can then be used for in vitro assays to elucidate the molecular mechanism, which causes the disease by investigating the various defects in the cellular function, or in their organization using many different techniques and assays.

And finally, different drugs can be pre-clinically tested on these patient-derived cells to test for rescue of the disease phenotypes, in vitro, with the ultimate goal of course, of clinical translation.

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In conclusion, embryonic stem cells and induced pluripotent stem cells have tremendous potential in the field of regenerative medicine. They are useful for disease modeling in vitro, that is understanding the disease phenotypes at a cellular level by analysis of specialized cell type specific phenotypes in a genetic disease. And they are also useful in drug discovery using these in-vitro disease models, and of course they are very useful in cell biology research. Thank you.