BIOL446
Lab 10.
Cell Differentiation

Objective: To gain experience in inducing differentiation of one cell type into another,.

Background

Differentiation is a key feature of most multicellular organisms, including all higher eukaryotes. By becoming multicellular, organisms can allow certain cells to specialize in certain tasks, and thereby gain efficiency. This requires various cells to be constructed differently, depending on the task to which they are devoted. In higher organisms this process is invariably one way. Organisms begin life as embryos made up of a small number of pluripotent stem cells. These cells have the ability to become any type of cell in the adult (or other life cycle stages of the) organism. As they grow and divide, certain cells begin to exhibit different morphology and become specialized. Once this has begun, the cell is usually committed to proceeding down a lineage to become a specific cell type. The process may have a number of forks, so that for instance an embryonic cell may eventually become a pluripotent blood stem cell in the bone marrow. At this stage, it can still become any one of terminal blood cell types, but will never become a liver cell, for instance.

Thus it can bee seen that this process is closely allied with development, the process whereby all the various tissues and organs are formed during the growth of a new individual organism from a zygote. Obviously, this process required the zygote to divide many times, and in the process the daughter cells must change into many different types. However, the process of differentiation is an ongoing process in adult organisms as well, most notably in tissues that need rapid and continual replacement, such as skin, various epithelial cells, and blood. The process by which these features are produced in the final cell type is known as differentiation. This involves the coordinated transcription of many different genes, and translation and processing of various proteins.

In this experiment you will examine the neuronal differentiation by inducing SH-SY5Y bone marrow neuroblastoma cells, in vitro, into neuron cells. Retinoic acid (RA), the most commonly used compound for differentiation, will be used for the induction.

This laboratory involved tissue culture techniques. Absolute sterility is essential. The level of attention to detail required is much greater than for bacterial work. Never touch anything without gloves on. Always wash your hands with 70% ethanol before work, and wash them again once they are gloved to sterilize the gloves. Never allow any pipette or other object that will come into contact with the media to touch non-sterile surfaces. Open the cultures and media bottles as briefly as possible. Besides using sterile techniques for not contaminating the cells, the cells will use here and the cells we use throughout the rest of this lab course are transformed immortal cells that may also have transforming ability, that is, to course cancer. Thus all the sterile procedures are to be followed to protect the cell and, more importantly, to protect you. Never touch the cells

even if you are wearing gloves. Any spill in the hood will need to be treated with 70% ethanol all 100% methanol.

Methods

You will be given a plate of culture of the SH-SY5Y bone marrow neuroblastoma cell (ATCC_CRL-2266). The TAs will also prepare a 1000-time concentrated RA stock solution for you.

SH-SY5Y cells attaches to the culture plate, but are not adhered tightly. It can be detached from the plate by trypsinization, but over-trypsinization will reduce the viability of the cells, or even lyse the cells. Since this is the only lab that you are going to split the cells during class, it may take more time for you to carry out the procedure. To avoid lengthy trypsinization, you will detach the cells by pipetting as soon as you add the trypsin solution.

Protocols:

Lab day 1,

1. Follow the procedures described above and clean up the cell culture hood.
2. Get a 100 mm plate with SH-SY5Y cells from the CO_2 incubator which is operating at 37 °C and 5% CO_2.
3. Aspirate the medium in the plate with an aspirator. .
4. Slowly add 4 ml HBSS without Ca^{+2} and Mg^{+2} to the plate and aspirate the HBSS after a brief, gentle swirling.
5. Add 3ml Trypsin-EDTA solution to detach the cell. Do not trypsinize the cells for a long time, since this would lyse the cells. Gently tap the side of the plate to help the cells to detach. Check the cells occasionally and stop the trypsinization by adding 27 ml of the growth medium (1:1 of DMEM/F12) as soon as the cells detach.
6. Pipette 2-3 times to separate clumps of cells.
7. If the cell is close to confluent before trypsinization, plate the cells for a 1:3 split. This means that you will plate four 100 mm plates. That is why you added 27 ml of medium to the plate. Add 1.25 ml of the culture to each of a set of four 35 mm plates. (The surface area of a 100 mm plate is about 8 times that of a 35 mm plate).
8. Add 1.7, 3.5, and 7 µl of the 1000X RA to three of the 35 mm plates, respectively. Label your plates to indicate how much RA was added.
9. Incubate the plates in the CO_2 incubator.
10. Collect the waste in a waste bucket with a bio-safety bag.
11. Clean up the cell culture hood.

Lab day 2,

1. Exam all four of your plates under the inverted microscope and take snapshots of each of the plates.

Results: You will need to combine snapshots of every plate into one figure, with more than one panel.