**The Evolution of Cancer**

*TO DO BEFORE CLASS: (Introduction and questions to be posted online by their teacher)*

Cancer is a term used for diseases in which abnormal cells divide without control and are able to invade other tissues. All cancers begin in cells, the body's basic unit of life. To understand cancer, it's helpful to know what happens when normal cells become cancer cells[[1]](#footnote-1).

The body is made up of many types of cells. These cells grow and divide in a controlled way to produce more cells, as they are needed to keep the body healthy. When cells become old or damaged, they die and are replaced with new cells1. However, sometimes this orderly process goes wrong1.

LEARNING GOALS:

* To understand that evolution also occurs at the cell level (Somatic Evolution).
* To understand how some mutations are related to the evolution (development) of cancer.
* To be able to differentiate between a cancer cell and a normal cell.

BRIDGE: Identifying the traits (mutations) that help in the progress of cancer is important for the development of treatment and a cure.

Brainstorm before class (Bring your answers on a piece of paper to obtain feedback).

1. What makes cancer cells different from normal cells? What kind of special traits do cancer cells have?

Ex: Cancer cells display uncontrolled growth (They do not senesce).

(Hint: Think about challenges cancer cells have to face in order to survive).

2. How would you test if a cell is a cancer cell or a normal cell? (Your own experimental design, keep it short: one paragraph, just the general idea).

3. Familiarize yourself with the Western Blot technique.

INTRODUCTION (*Slides, 15 min, IN class*)

Cells in our body grow and divide in a controlled way to produce more cells. However this process is not perfect and each time they divide mutations occur in their genome. Some mutations are harmless while others may divert cells into acquiring abnormal phenotypes.

Normal cells and tumour cells evolve by [natural selection](http://en.wikipedia.org/wiki/Natural_selection). This accounts for how cancer develops from normal tissue. There are three necessary and sufficient conditions for natural selection, all of which are met in a tumour[[2]](#footnote-2)[[3]](#footnote-3):

1. Variability (generated through random mutations)
2. Variability must be inheritable (from parental cell to daughter cell).
3. Variability affects survival or reproduction (fitness)[[4]](#footnote-4).

Cells in tumours compete for resources, such as oxygen and glucose, as well as space. Thus, a cell that acquires a mutation that increases its fitness (i.e. ability to invade other spaces) will generate more daughter cells than competitor cells that lack that mutation. In this way, a population of mutant cells can expand in the tumour.

Cancer cells need to overcome many barriers like:

* Cell death.
* Immune system.
* Acidic environment (due to high production of lactate).
* Normal cells are not very motile (cancer cells need to metastasize).

REMEMBER: The information to produce any type of protein is encoded in the genome of every cell. In normal cells there’s a tight control of what is expressed and what isn’t (a protein important in the kidney might need to be silenced in the liver, or a protein important during embryogenesis might need to remain silenced in adulthood). Cancer cells take advantage of this and express proteins that will help them proliferate without control, overcome the barriers mentioned above and metastasize.

Scientists have discovered that some proteins are over-expressed or under-expressed in cancer cells and they’ve called them “Markers”. The advantage of the presence or absence of each is different depending on the function of the protein (Think what would it be as you read the table).

|  |  |
| --- | --- |
| **Marker** | **Function in NORMAL cells** |
| Ras | Controls appropriate cell growth, differentiation and survival.  |
| CAIX | Maintains an adequate intracellular and extracellular pH. |
| MT1-MMP | Degrades the extracellular matrix (the space outside the cell) for example during embryonic development when there’s a need for tissue remodelling.  |
| MyosinX | Localizes in cell protrusions involved in the movement (migration) of cells.  |

*NOTE: Some of these markers will be used for the Western-blot.*

*PRE-ASSESMENT:* Will discuss some of their ideas for how they would differentiate a cancer cell from a normal cell?

Will ask them to make teams of 3-4, will assign one marker per team (CAIX, MT1-MMP and myosinX) and will ask them to use the previously explained information to come up with a hypothesis per team (and to not to share it). It should be something like:

“A cancer cell expresses more Ras protein than a normal cell and this allows for indefinite growth”

PROTOCOL

*Differentiate Cancer Cells from Normal Cells using Western Blot*

Each team will be given one membrane that contains different types of cells in each lane (normal and cancer cells). These membranes have been incubated with antibodies against proteins that are expressed in different amounts by cancer and normal cells (markers discussed above). The name of the antibody used and the molecular weight of the protein will be written at the bottom of the membrane.

You will be in charge of exposing the membrane and developing the film. This will allow you to see a pattern of bands on the film (see image below, each number represents one lane):

4

3

1

2

Ras, 21 kDa

MATERIALS (per team – 5 teams only)

* Protocol
* 1 PVDF membranes pre-incubated with single antibody
* Saran wrap
* 1 Kodak cassette
* 2 pieces of film (pre-trimmed)
* SuperSignal/ECL (aliquots, pre-mixed by guest teacher)
* Marker

MATERIALS (common use)

* Molecular marker image
* Eppendorf tubes
* P200 pipette and tips
* Developer in tray
* dH20 in tray
* Fixer in tray
* dH20 in tray
* Tongs
* Timer

PROCEDURE

*Wear lab coat and gloves:*

1. Place the membrane on top of a piece of saran wrap (use tongs).
2. Place the membrane and saran wrap inside the Kodak cassette, close to one of the corners.
3. Wait for signal from teacher to do this step: add 100 ul of SuperSignal/ECL across the membrane at the right level (see MW image, solution pre-mixed by guest teacher).
4. Close the cassette and go to dark room.

*Inside the dark room (have in there films, timer, tongs).*

1. Open the box containing the films.
2. Open the Kodak cassette and place one film on top of the area containing the membrane.
3. Close the cassette. Expose for 1 min (*This needs to be pre-determined by teacher*).
4. Remove the film with tongs and place it in the developer solution until the bands start to appear.
5. Rinse in dH20 for a few seconds.
6. Place the film in the fixer solution until film becomes clear.
7. Rinse the film in dH20 for a few seconds.
8. Hang the film for 5 min.
9. Repeat with a different exposure time if needed (3-5 min).

*Back on your bench:*

1. Place the developed film on top of the membrane again and use the marker to label the molecular weight marker on the film.
2. Write down numbers below each lane or where a lane is supposed to be (1,2,3,4). Remember some lanes may appear empty.
3. Write down the date, exposure time, and antibody used for blotting.

POST-ANALYSIS: *Please* *answer these questions for next class.*

1. Determine if the cells present in the different lanes are cancer or normal cells.
2. How does the presence/absence of that protein confer an advantage to the cancer cell?
3. How could you determine if same amount of sample was loaded in all the lanes and therefore your results are not false positives?
4. What is the purpose of the molecular weight marker?
5. What challenges did you face during this experiment?

SUMMARY

Individual cells in our body are also capable of evolution. Evolution occurs when cells obtain random mutations that improve cell survival or cell reproduction (fitness). Sometimes these mutations can turn a normal cell into a cancer cell, for example, by granting them immortal and these cells end up outgrowing normal cells and forming big and irregular masses (tumours).

Some mutations cause proteins inside the cells to be overproduced while other mutations have the opposite effect: they silent the production of some protein. Depending on the function of the protein this may have different implications for the fate of that particular cell.

IMPORTANT NOTE: It’s always good to test the membranes in the lab beforehand to make sure they will work on the day of the class. Another good thing to do is to bring films that were successfully developed beforehand and give them to the students if the films developed during class turn out not so good.

1. National Cancer Institute. www.cancer.gov [↑](#footnote-ref-1)
2. Nowell, P. C. (1976). "The clonal evolution of tumor cell populations".*[Science](http://en.wikipedia.org/wiki/Science_%28journal%29%22%20%5Co%20%22Science%20%28journal%29)* **194** (4260): 23–28 [↑](#footnote-ref-2)
3. Merlo, L. M.; Pepper, J. W.; Reid, B. J.; Maley, C. C. (2006). "Cancer as an evolutionary and ecological process". *Nature Reviews Cancer* **6** (12): 924–935 [↑](#footnote-ref-3)
4. Hanahan, D.; Weinberg, R. (2000). "The hallmarks of cancer". [*Cell*](http://en.wikipedia.org/wiki/Cell_%28journal%29) **100**(1): 57–70 [↑](#footnote-ref-4)