Introduction to Radiotherapy and DNA Damage

Radioactivity is a naturally occurring part of our world, and has been since Earth was created. Naturally occurring radioactive materials are present in the walls and household appliances (microwave, TV, wifi) of our homes, and in the food and water we ingest. There are also radioactive gasses in the air we breathe.

All forms of radiation are damaging to our cells if they are exposed to high doses of it. Even UV-A radiation from the sun, which is indispensable for production of Vitamin D, results in sunburns and cataract if you stay for too long in the sun. However, we can use the harmful effects of radiation to treat cancer, where the aim is to damage and ultimately kill the cancer cells.

Most cells of our bodies divide and grow to produce new cells as replacements for the old and used cells. As an example, we all lose strands of hair, but do not go bald (at least not until we reach a certain age!). We are also capable of donating blood regularly and the outer layer of our skin is constantly shed and subsequently renewed. Cancer cells also divide and grow, but do so in an uncontrolled manner, compared to healthy cells (figure 1).

The difference in growth rate between cancer cells and healthy cells is exploited when cancer patients receive chemotherapy because chemotherapy kills dividing cells – without considering if it is a cancer cell or a healthy cell. In contrast to chemotherapy, where the entire body is exposed to treatment, radiotherapy is a local treatment. Radiotherapy is specifically directed at the body part that contains the tumour and the aim is to destroy the cancer cells, while leaving as many of the healthy cells unharmed as possible.

Radiotherapy works by inducing small breaks in the cells' DNA that can lead to large errors when cells divide and distribute their DNA during cell division. Radiotherapy targets all cells in the radiated area including both cancer and healthy cells. DNA damage can be repaired to a certain extent by the cells' own DNA repair mechanisms, which handle all DNA damage occurring during the cells' lifetime. If the DNA repair mechanism does not work optimally, the radiation dosage required to induce permanent damage to the DNA will be lower than if the DNA repair was operating flawlessly. Some cancer cells have faults in their DNA repair mechanism, that renders them more susceptible to radiotherapy, but it is not all of them. It is a common misconception

that all tumours are more susceptible to radiotherapy than healthy cells, and that is why radiotherapy must be targeted to the precise area.

The aim of this experiment is to understand what occurs to a cell exposed to radiation, and to understand how distinct types of cells react differently to the same dose of radiation. In this experiment, we will directly detect how cells are affected using a microscopy technique known as immunofluorescence (IF). IF is a commonly applied laboratory technique used in all areas of cell biology, both for research and clinical diagnostic purposes. Using this method, we will be able to detect if the cells express specific proteins/protein modifications. To visualise proteins by IF, we use antibodies that specifically recognise the protein of interest (antigen). These antibodies are conjugated to a fluorescent marker, which enables the entire antibody-protein complex to be detected by fluorescence microscopy.

In our case, we are interested in observing DNA damage, that we expect will occur, when we expose the cells to radiation. We will detect the protein γ-H2AX, which is associated with a specific type of DNA damage known as double strand break (DSB), where both strands of the DNA helix are broken. We will expose the cells to different Grey (Gy) and observe what happens over time.