Normal Cell Biology

There are two major compartments in the cell:

The **cytoplasm** contains the structures of the cell outside of the nucleus. The **cell membrane** forms the boundary of the cytoplasm and the cell.

The **nucleus** is the central, darker staining part of the cell which contains the chromosomes.

The Cytoplasm

Contains numerous organelles which perform cellular functions:

Mitochondria are energy producing organelles with their own mitochondrial DNA.

Endoplasmic reticulum is involved in the assembly of proteins. Rough endoplasmic reticulum contains ribosomes which translate messenger RNA (Ribonucleic Acid) into protein.

The golgi apparatus is involved in the packaging of protein into membrane bound organelles, either for storage or for delivery to the cell membrane

Centrioles are small assemblies of microtubles arranged in a cylinder. They are important localisation of the chromosomes during cell division.

The **cytoskeleton** extends through the cytoplasm, attached to cell membrane proteins. The cytoskeleton helps to cell to keep its shape and is also mobile in some cells (such as neutrophils), altering the shape of the cell and allowing movement. **Tumour cells** frequently co-opt the cytoskeleton to allow them to move through tissues.

The Nucleus

Contains the **DNA.**

DNA is stored in 44 somatic and two sex chromosomes.

may also contain a nucleolus, a site of ribosome manufacturing. The double-membrane of the nucleus contains numerous pores which allow proteins and RNA to communicate with the cytoplasm.

Eukaryotic Genes

A **gene** is a length of DNA, associated with some function, that may be inherited.

In **eukaryotes** (nucleated cells), a gene usually consists of:

A **transcribed segment**, which is translated into RNA for some effect:

The transcribed segment contains **introns** and **exons**; exons are **ex**pressed whereas introns are in between exons and are **in**cised by splicing mechanics after translation has occurred.

Each end of the transcribed segment contains a 5' and 3' untranslated region. This may allow the cell to recognise sequences of genetic code after they have been transcribed into RNA.

Non-transcribed parts, which include:

Regulatory segment(s), which are lengths of DNA allowing the cell to control which genes are expressed.

Start and stop segments which flank the transcribed segment, allowing transcription enzymes to bind.

The classical gene codes for a protein; the genetic code is transcribed into a strand of RNA by **RNA polymerase**.

This RNA, known as **pre-messenger RNA**, undergoes splicing where the introns are removed. Once completed, the messenger RNA is transported to the ribosomes in the cytoplasm for translation into protein.

Other genes code for RNA products that do not require translation into protein. This includes the RNA that forms the ribosome (**ribosomal RNA**), or **micro-RNAs** which are important in the post-transcription control of gene expression.

Gene regulatory segments need not be located immediately adjacent to the transcribed segment. Regulatory segments can exert an influence over thousands of base pairs, and different regulatory segments can also interact to enhance (or further suppress) the transcription of a gene.

Cell Culture Technique

A cell survival curve describes the relationship between the radiation dose and the proportion of cells that survive.

In the cell culture technique, a specimen of normal tissue is chopped into small pieces and treated with the enzyme trypsin.

This loosens the cells and separates them into a single cell suspension. A known number of the single cells are plated in culture medium in a petri dish and here they attach themselves to the bottom of the dish, which is incubated at 37°C.

After about 10 days, small isolated colonies of cells are seen in the dish. These are the result of individual cells having undergone a series of cell divisions.

If the single cells are irradiated soon after they are plated, some of them will be killed and so will not produce colonies.

The ability to undergo five or more cell divisions following irradiation is an indication of cell survival, since these cells are capable of almost indefinite cell multiplication.

Conversely, cell death is indicated by a cell's inability to proliferate and give rise to a visible colony of some 32 - 64 cells (five or six successive doublings).

This is called **reproductive death (or mitotic death)** to distinguish it from death of cells that do not proliferate (for example, nerve, muscle, secretory cells) where cell death may be defined as loss of specific function.

The process is repeated for a range of doses and from this data, we can plot a cell-survival curve. For higher radiation doses, more cells need to be plated in order to produce a statistically meaningful surviving fraction.

Plating efficiency (PE)

$$\%\ \text{plating efficiency (PE)} = \frac{\text{average number of colonies per dish}}{\text{number of cells plated per dish}} \times 100$$

The surviving fraction after dose D is then

$$\text{SF} = \frac{\text{average number of colonies after dose D per dish}}{\text{average number of cells plated per dish}} \times \frac{100}{\text{PE}}$$

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Cell Survival Curves

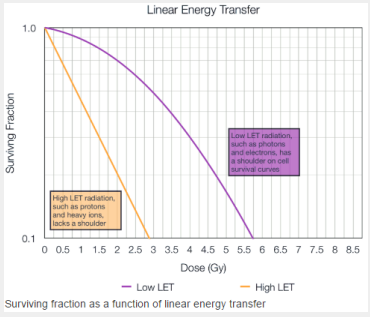
Survival curves are usually presented with dose plotted on a linear scale (x-axis) and surviving fraction on a logarithmic scale (y-axis).

When cells are irradiated by single exposures of varying doses of high-LET radiation (for example, α-particles), an exponential survival curve is obtained.

When cells are irradiated by single exposures of varying doses of ow-LET radiation (x-rays or γ-rays), the slope is not constant. Initially, the curve is relatively flat, but with a negative slope. This is followed by an inflection (called the shoulder) after which the curve also becomes exponential.

It is difficult to explain the shape of the cell-survival curve in terms of the biophysical events that have occurred and many theories have been proposed. Two models will be described here: **Multi-target Model** and **Linear-Quadratic Model**

High and Low LET survival curves



Surviving fraction as a function of linear energy transfer

Multi-target Model

In this model, the survival curve is described in terms of:

1. D1, the dose required to reduce the fraction of surviving cells to 0.37 on the initial portion of the curve **(single-hit killing),**

2. D0, the dose required to reduce survival from 0.1 to 0.037 or from 0.01 to 0.0037 on the final straight portion of the curve **(multi-hit killing),**

3. n, the extrapolation number, or Dq, the quasi-threshold, which are a measure of the width of the shoulder.

Multi-target Model (cont)

If n is large (for example, 10 or 12), the survival curve has a broad shoulder. If n is small (for example, 1.5 to 2), the shoulder is narrow. A threshold dose is the dose below which radiation produces no effect, so there can be no true threshold; Dq, the quasi- threshold dose, is the closest thing.

For high-LET radiation D1 = D0 and Dq = 0, however for low-LET D1 > D0.

The value of D0 usually falls in the range of 1 to 2Gy.

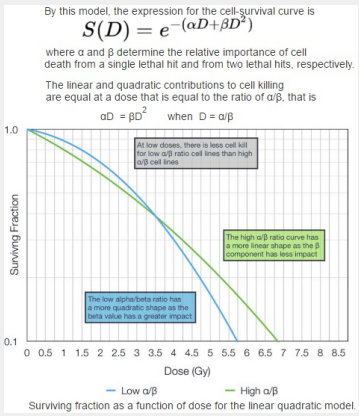
Linear-Quadratic Model

For some cell lines, the survival curve appears to bend continuously so that the linear-quadratic relation is a better fit. In this case, n has no meaning.

This model assumes that there are two components to cell killing by radiation, one of which is proportional to dose and the other proportional to the square of the dose.

The idea is consistent with results from chromosome work in which many chromosome aberrations (for example, dicentrics) are clearly the result of two separate breaks.

Linear-Quadratic Model



Surviving fraction as a function of dose for the linear quadratic model

Linear-Quadratic Model cont.

A characteristic of the linear-quadratic formulation is that the cell-survival curve is continuously bending.

The extent of the curviness is a function of the relative values of α and β.

Linear-Quadratic Model cont. (cont)

This does not coincide with what is observed experimentally when survival curves are determined down to 7 or more decades (powers of 10) of cell killing.

Here, the curve closely approximates to an exponential function of dose. However, in the first one or two decades of cell killing and up to any doses used as daily fractions in clinical radiotherapy, the linear-quadratic model is an adequate representation of the data.

Responses of tissues to radiotherapy can be predicted from the ratio α/β

The Cell Cycle

Every biological species has its own sensitivity to ionising radiation, that is, its own radiosensitivity.

his is not the same in all phases of a cell's life; cell death requires a greater or lesser dose, depending on when in the cycle radiation is given.

The basic division of the cell cycle is into that of **mitosis** (M) and **interphase** (G1, S, G2).

Cells may also be in a special state known as G0 or **'resting phase'**, where the cell is not making any effort to divide.

We can divide the cell cycle into four recognisable stages:

G1 is the stage between reproduction episodes.

S is the stage when new DNA is synthesised.

G2 is the stage when certain protein and RNA molecules are synthesised.

M is the stage when cells, having replicated DNA and chromosomes, divide to produce two cells from one.

Mitosis is subdivided into several events:

Prophase – The cell begins to assemble the mitotic spindle, a set of microtubules extending from the centromeres which will later attach to the chromosomes.

Prometaphase – The nuclear envelope disintegrates, and the microtubules of the mitotic spindle attach to the chromosomes.

The Cell Cycle (cont)

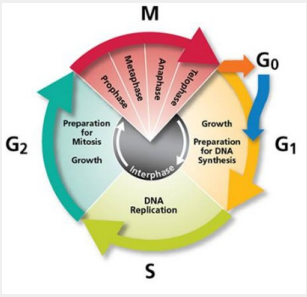
Metaphase – The chromosomes are aligned on the mitotic spindle. There is a pause here to allow all chromosomes to become attached.

Anaphase – The cohesion proteins which bind the sister chromatids together are cleaved and the chromosomes are pulled apart by the mitotic spindle.

Telophase – The nuclear membrane reconstitutes around each set of chromosomes.

The length of time required for the reproduction phases S, G2 and M does not vary very much among mammalian cells. It is the time between reproduction episodes (G1) that varies

The Cell Cycle



Variation of Radiosensitivity in the Cell Cycle

In the discussion on survival curves, we assume that the population of irradiated cells is asynchronous; that is, it consistes of cells distributed throughout all phases of the cell cycle

Techniques now make it possible to study the variation of radiosensitivity with the position or age of the cell in the cell cycle. These include: the mitotic harvest technique and the hydroxyurea technique.

Mitotic Harvest Techniquecan be used for cultures that grow in monolayers attached to the surface of the growth dish.

Cells close to mitosis round up and become loosely attached. If the culture flasks are gently shaken, the mitotic cells will detach from the surface and float in the medium.

Variation of Radiosensitivity in the Cell Cycle (cont)

If these cells are removed and incubated in new dishes, the cells will move together synchronously in step through their mitotic cycle for a few cell division cycles. By delivering a dose of radiation at various times after the initial harvestingwe can irradiate cells at various phases of the cell cycle.

Hydroxyurea Technique involves the use of the drug hydroxyurea.

Following the addition of the drug, cells that are in S phase (that is, synthesising DNA) are killedand cells that are in G2, M and G1 are halted at the end of the G1 period.

The drug is only added for a period equal to the combined G2, M and G1 time for that particular cell line. After this time, all the viable cells are poised at the end of G1 ready to enter S phase. If the drug is removed the synchronised cells proceed through the cell cycle. This technique can be used to produce synchronously dividing cell populations in tissue as well as in culture.

Irradiation of Synchronously Dividing Cell Cultures

When Chinese hamster cells, harvested at mitosis, are irradiated with a single dose of x-rays at various times afterwards, the fraction of cells surviving varies with different phases of the cell cycle.

Following a dose of 6.6 Gy of x-rays, the surviving fraction is about 13% when the cells are in G1 and increases to more than 40% near the end of S phase.

Complete survival curves for mitotic cells (M), and for cells in G1 and G2 and for cells in early and late S can be obtained by repeating the procedure for various radiation doses. The most sensitive cells are those in M and G2 indicated by a steep curve with no shoulder. Cells in late S exhibit a survival curve that is less steep with a broad shoulder, and cells in G1 and early S are intermediate in sensitivity.

Variation of Radiosensitivity in the Cell Cycle (cont)

Application For Tissues

The variation in response with the phase of the cell cycle at which the radiation is given is very similar to that observed in many cells cultured in vitro. There is a radiosensitive period between G1 and S and maximum radioresistance occurs in late S phase.

The reasons for the sensitivity changes through the cell cycle are not at all understood but a number of correlations have been observed:

1. minimum radiosensitivity coincides with DNA doubling in the S phase;

2. maximum radiosensitivity occurs just before mitosis when the chromosomes condense; and

3. radiosensitivity varies with levels of naturally occurring sulfhydryl compounds (powerful radioprotectors) which are at their highest levels in S and at their lowest near mitosis.

Radiosensitivity in Radiation Therapy

When a single dose of radiation is delivered to a population of cells that are asynchronous, the effect will be different on cells occupying different phases of the cell cycle at the time of radiation exposure.

More cells will be killed in the sensitive portion of the cell cycle, such as those at or close to mitosis, while fewer of those in the DNA synthetic phase will be killed.

The overall effect is that a dose of radiation will, to some extent, tend to synchronise the cell population leaving the majority of cells in a resistant phase of the cycle.

In the clinical situation, the radiation is delivered in many separate dose fractions. In the time between these fractions, movement of cells through the cycle into more sensitive phases may be an important factor in 'sensitising' a cycling population of tumour cells to later doses in this treatment regime.

Radiosensitivity in Radiation Therapy (cont)

This process is termed **sensitisation due to reassortment** and it is the first of what are referred to as the five Rs of radiobiology.

Cancer Cell Biology

Cancer cells are similar yet distinct to normal cells.

The features that make them different to normal cells allows them to be singled out for treatment; but these are limited by the similarities possessed between the cancer and normal cells.

Carcinomas (most common types of cancer), arise from the cells that cover external and internal body surfaces. Lung, breast, and colon are the most frequent cancers of this type.

Sarcomas are cancers arising from cells found in the supporting tissues of the body such as bone, cartilage, fat, connective tissue, and muscle.

Lymphomas are cancers that arise in the lymph nodes and tissues of the body's immune system.

Leukemias are cancers of the immature blood cells that grow in the bone marrow and tend to accumulate in large numbers in the bloodstream.

Cancer arises from a loss of normal growth control. In normal tissues, the rates of new cell growth and old cell death are kept in balance. In cancer, this balance is disrupted.

This disruption can result from uncontrolled cell growth or loss of a cell's ability to undergo cell suicide by a process called **apoptosis.**

This results in as gradual increase in the number of dividing cells, and creates a growing mass of tissue called a **tumour** or **neoplasm.**

If the rate of cell division is relatively rapid, and no "suicide" signals are in place to trigger cell death, the tumour will grow quickly in size; if the cells divide more slowly, tumour growth will be slower. As more and more of these dividing cells accumulate, the normal organisation of the tissue gradually becomes disrupted.

Cancer Cell Biology (cont)

Cancers are capable of spreading throughout the body by two mechanisms: **invasion** (the direct migration and penetration by cancer cells into neighboring tissues) and **metastasis** (penetrate into lymphatic and blood vessels, circulate through the bloodstream, and then invade normal tissues elsewhere in body).

Benign tumours cannot spread by invasion or metastasis (grow locally)

Malignant tumours ("cancer“) are capable of spreading by invasion and metastasis, making them a serious health problem.

Hallmarks of Cancer

There are six classical hallmarks of malignancy:

Self sufficiency in growth signals - malignant cells are able to grow without an external stimulus to do so.

Lack of response to growth inhibition - this is often due to loss of tumour suppressor genes, which would normally put the growth of the cell on hold.

Unlimited replicative capacity - normal cells may only multiply a set number of times before they become senescent (unable to divide further). Malignant cells circumvent this limit through activation of telomerase.

Avoidance of apoptosis - normal cells trigger apoptotic pathways in response to uncontrolled growth signalling. Apoptosis is often suppressed by malignant cells to avoid this fate.

Angiogenesis - malignant tumours must form new blood vessels in order to expand locally. Angiogenesis is also important for allowing malignant cells to metastasise.

Invasion and Metastasis - malignant tumours invade surrounding normal tissues and may also spread throughout the body.

Two more were later added:

Hallmarks of Cancer (cont)

Deregulation of Cellular Energetics: Normal cells produce energy from glucose through glycolysis to pyruvate which then enters the citric acid cycle within the mitochondria. Malignant cells upregulate glycolysis which forms an increased source of energy compared to normal aerobic cells. It can be utilised by FDG PET scanning to identify malignant cells.

Immune Avoidance: The immune system is hypothesised to provide protection again malignant transformation of cells by detecting and destroying them. Malignant cells that survive to form a tumour mass must therefore have a means of immune avoidance, either by:

And two additional 'enabling characteristics':

Genomic Instability: The ability of malignant cells to develop a wide array of mutations in multiple oncogenes and tumour suppressor genes suggests a much higher rate of mutation than is seen in normal cells. Malignant cells have been shown to downregulate the normal cellular mechanisms that detect and prevent mutation, allowing them to accelerate the rate of mutation acquisition. The cells with the ability to mutate (or with mutations that have already been acquired) their genome to avoid destruction are those which survive and reproliferate the tumour.

Tumour Promoting Inflammation: Certain autoimmune conditions, such as ulcerative colitis or Sjogren's syndrome, promote the development of malignancy in the afflicted organ. This is due to the carcinogenic effects of inflammation on the target organ. Malignant tumours are frequently infiltrated by cells of the immune system. It is thought that the inflammation caused by this infiltration, rather than helping to overcome the tumour, may in fact help to promote further mutation within the malignancy.