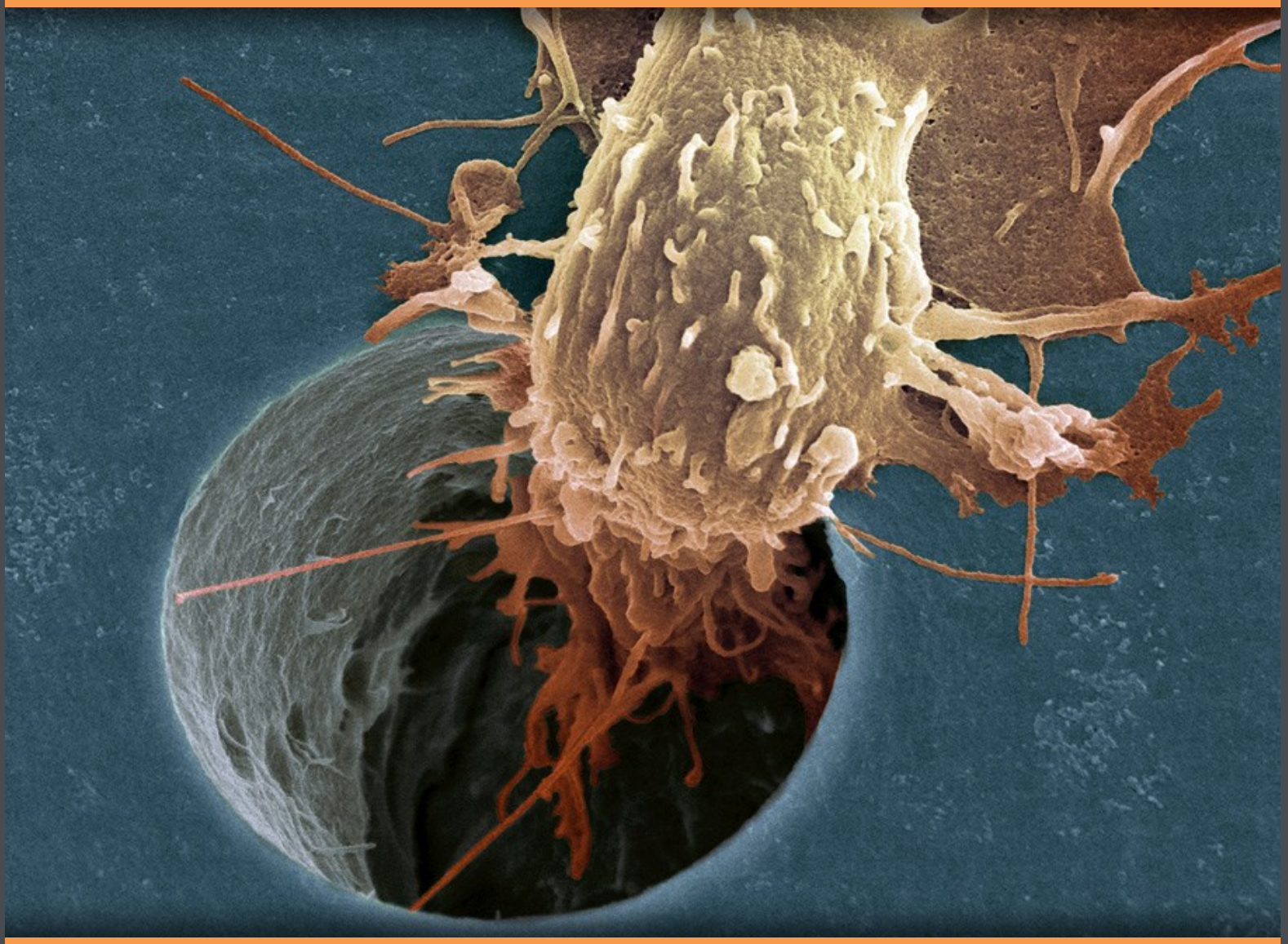
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Introduction to Cancer Biology

Momna Hejmadi



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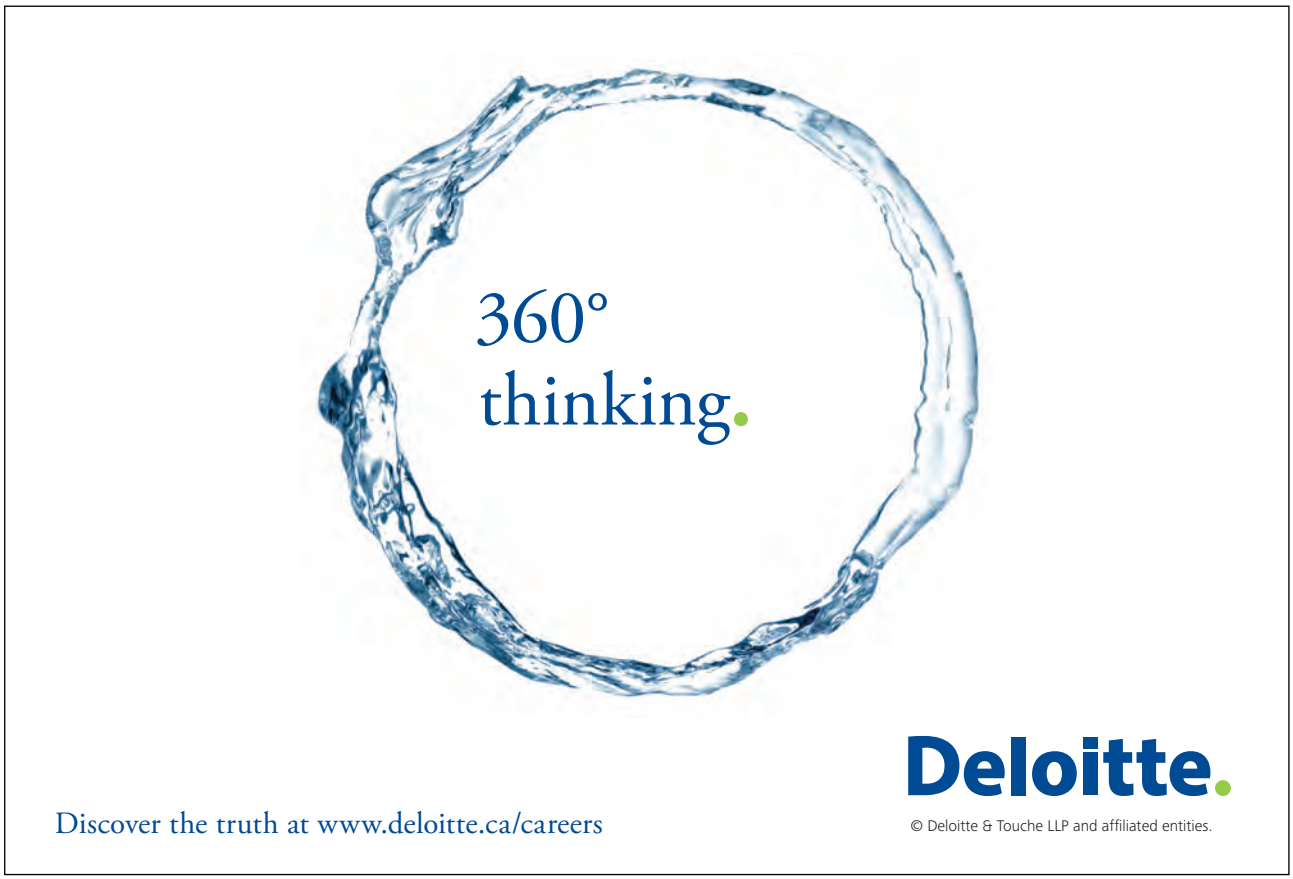
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1 How cancer arises

1.1 Defining cancer

Cancer can be defined as a disease in which a group of abnormal cells grow uncontrollably by disregarding the normal rules of cell division. Normal cells are constantly subject to signals that dictate whether the cell should divide, differentiate into another cell or die. Cancer cells develop a degree of autonomy from these signals, resulting in uncontrolled growth and proliferation. If this proliferation is allowed to continue and spread, it can be fatal. In fact, almost 90% of cancer-related deaths are due to tumour spreading – a process called metastasis.

The foundation of modern cancer biology rests on a simple principle – virtually all mammalian cells share similar molecular networks that control cell proliferation, differentiation and cell death. The prevailing theory, which underpins research into the genesis and treatment of cancer, is that normal cells are transformed into cancers as a result of changes in these networks at the molecular, biochemical and cellular level, and for each cell there is a finite number of ways this disruption can occur.

Phenomenal advances in cancer research in the past 50 years have given us an insight into how cancer cells develop this autonomy. We now define cancer as a disease that involves changes or mutations in the cell genome. These changes (DNA mutations) produce proteins that disrupt the delicate cellular balance between cell division and quiescence, resulting in cells that keep dividing to form cancers.

1.2 Cancer is clonal in origin

Current dogma states that cancer is a multi-gene, multi-step disease originating from a single abnormal cell (clonal origin) with an altered DNA sequence (mutation). Uncontrolled proliferation of these abnormal cells is followed by a second mutation leading to the mildly aberrant stage. Successive rounds of mutation and selective expansion of these cells results in the formation of a tumour mass (**FIG 1.1 a & b**).

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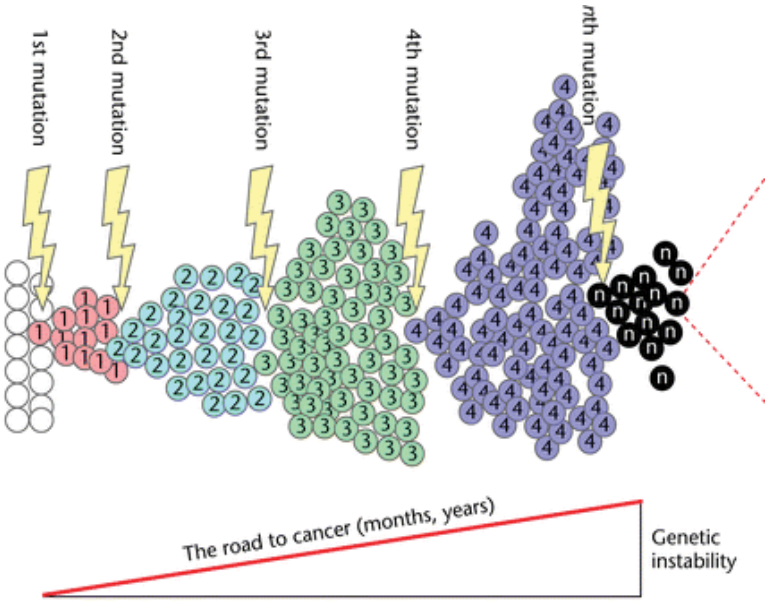


Figure 1.1 a: Clonal expansion. Cancer is a multi-gene, multi-step disease originating from single abnormal cell (clonal origin). Changes in DNA sequences result in the cell progressing slowly to the mildly aberrant stage. Successive rounds of mutation & natural selection leads to a mass of abnormal cells called tumours. Some cells in the tumour undergo further rounds of mutations leading to the formation of malignant cells which cause metastasis. (*Fig source: M Alison.* www.els.net)

Subsequent rounds of mutation and expansion leads to tumour growth and progression, which eventually breaks through the basal membrane barrier surrounding tissues and spreads to other parts of the body (metastasis). Death as a result of cancer is due to the invading, eroding and spread of tumours into normal tissues due to uncontrolled clonal expansion of these somatic cells.

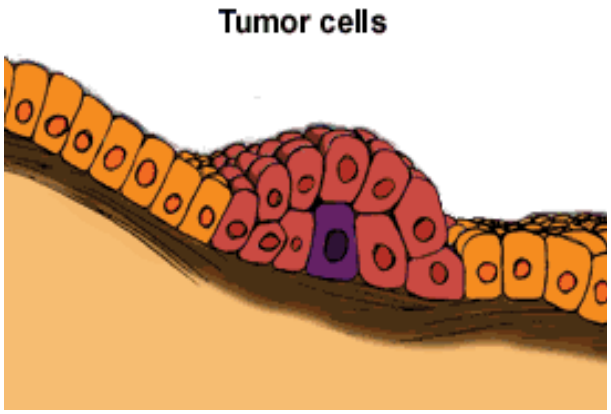


Figure 1.1b: Clonal expansion. Normal cells are subject to signals that regulate their proliferation and behaviour. All cancers disrupt normal controls of cell proliferation & for each cell there is a finite number of ways this disruption can occur. Cancer cells develop a degree of autonomy from external regulatory signals that are responsible for normal cellular homeostasis. Multiple mutations lead to a tumour mass. Subsequent mutations lead to malignant tumour which break through the basal membrane and spread to distant locations

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Evidence for the clonal expansion model can be demonstrated with a simple but striking clinical example (**FIG 1.2**). The enzyme, glucose-6-phosphate dehydrogenase (G6PD) has two forms, G6PD-A and G6PD-B, which differ from each other by 1 single amino acid. Some people have cells that contain either type A or type B but no cell contains both, hence tissues are a mosaic of cells with these two types. Individuals who develop Chronic Myeloid Leukaemia (CML – a blood cancer) contain cancerous myeloid cells which all contain only one type of the enzyme, either type A or type B, but never both, clearly demonstrating that cancers are clonal in origin.

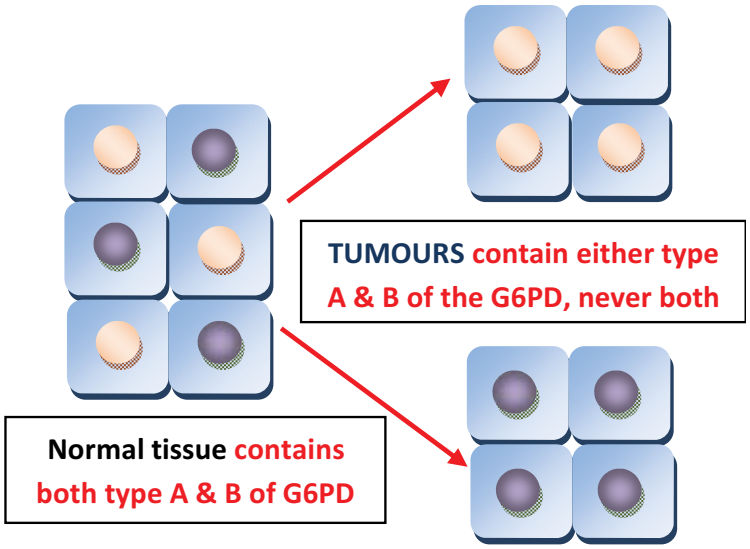


Figure 1.2: Example showing cancers are clonal in origin. Genetic analysis of myeloid cells in some patients with Chronic Myeloid Leukaemia (CML – a blood cancer) contain only one type of the enzyme, G6PD, either type A or B, but never both. Since normal tissues on the other hand, are a mosaic of cells with both type A & B, this clearly demonstrates the clonal origins of cancer.

1.3 Insights into cancer

Initiation and progression of cancer depends on both external factors in the environment (tobacco, chemicals, radiation and infectious organisms) and factors within the cell (inherited mutations, hormones, immune conditions, and mutations that occur from metabolism). These factors can act together or in sequence, resulting in abnormal cell behaviour and excessive proliferation. As a result, cell masses grow and expand, affecting surrounding normal tissues (such as in the brain), and can also spread to other locations in the body (metastasis). However, it is important to remember that most common cancers take months and years for these DNA mutations to accumulate and result in a detectable cancer.

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Cancers arise approximately in one among every 3 individuals. DNA mutations arise normally at a frequency of 1 in every 20 million per gene per cell division. The average number of cells formed in any individual during an average lifetime is 10^{16} (10 million cells being replaced every second!). It would therefore be logical to assume that human populations anywhere in the world would show similar frequencies of cancer. However, cancer incidence rates (number of individuals diagnosed) vary dramatically across countries. Evidently, some factors seem to intervene to dramatically increase cancer incidences in some populations. The obvious inference is that contributory factors that cause cancer are either hereditary or environmental. It means that either certain populations carry a large number of cancer-susceptibility genes or that the environment in which populations live largely contribute to the cancer incidence rates.

1.4 Causes of cancer (aetiology of cancer)

Which of the two – genes or the environment – play a dominant role in developing cancer? While genes are distributed unequally across populations, they do not explain the differences in cancer incidence rates in the world. This is demonstrated by most dramatically in this example. Incidences of stomach cancer are 6–8 times higher among Japanese compared to Americans. However, children of migrant Japanese settled in America show incidence rates of stomach cancer comparable to that of the American population. Therefore, the risk of developing cancer seems largely environmental, accounting for more than 90% of all cancers caused.

1.4.1 Lifestyle and Environment

The first known report linking the influence of lifestyle on cancer was by John Hill, an English physician, who noted the link between nasal cancer and the use of tobacco snuff. In the late 18th century, Sir Percival Pott reported that scrotal cancer in chimney sweeps was linked to poor hygiene and accumulation of cancer-causing agents from soot. The Danish Chimney sweeper's Guild recommended daily baths and was the most likely reason for the dramatic reduction in scrotal cancer incidence rates in Europe.

In 1950, compelling epidemiological evidence showed that heavy cigarette smokers ran a 20-fold higher risk of developing lung cancer compared to non-smokers. Since then, tobacco and alcohol consumption have been linked to almost ~170,000 mouth and throat cancer deaths per year in the US alone **(Fig 1.3)**. Over half a million deaths every year are expected to be caused by lifestyle choices such as obesity, physical inactivity, diets (low in vegetables, high in salt or nitrates are linked to stomach and oesophageal cancers whereas high fat, low fibre diets are linked to bowel, pancreatic, breast and prostate cancer).

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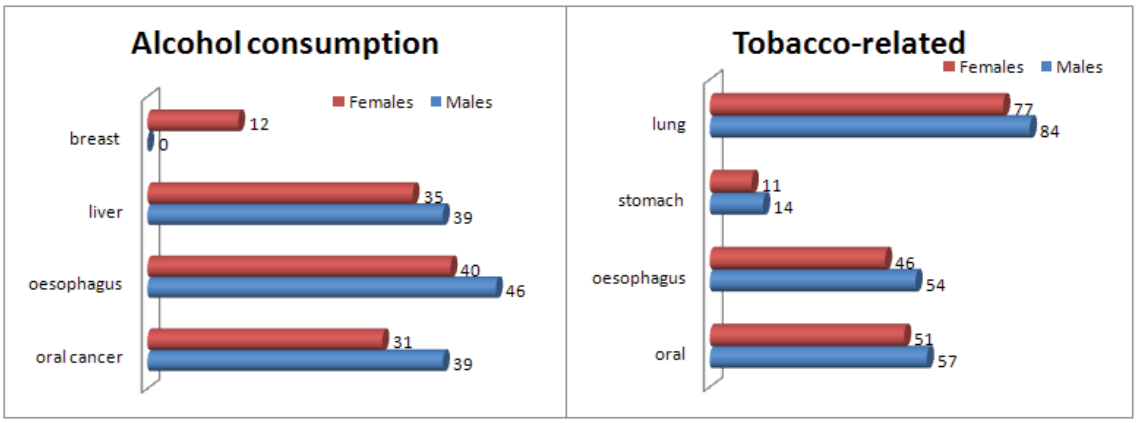


Figure 1.3: Percentage of cancers attributed to excessive alcohol consumption and tobacco use. Excessive alcohol use has been linked to liver and mouth/throat cancers in both males and females. Breast cancer risk is high in females who drink to excess. Smoking and tobacco use significantly increases the risk of lung cancers equally in males and females, and there is also a slightly higher risk of mouth/throat cancers. Source: SEER Cancer Statistics Review, NCI, USA.

Risk of cancers are also increased by infectious agents including viruses [hepatitis B virus (HBV), human papillomavirus (HPV), human immunodeficiency virus (HIV) – increase risk of nasopharyngeal, cervical carcinomas and Kaposi's sarcoma] and bacteria such as *Helicobacter pylori* (stomach cancers). Incidences of skin cancers (melanomas) are on the rise, especially in Australia, due to exposure to high levels of ultraviolet radiation in the sun's rays and popularity of tanning salons. However the risk of developing some of these cancers can be reduced by changing lifestyles and vaccines (like Gardasil© which reduces the risk of cervical carcinomas).

Initiation and progression of cancer is also due to exposure to cancer-causing agents (carcinogens, mutagens). These are present in food and water, in the air, and in chemicals and sunlight that people are exposed to. Since epithelial cells cover the skin, line the respiratory and alimentary tracts, and metabolize ingested carcinogens, it is not surprising that over 90% of cancers originate from epithelia ('carcinomas'). In less than 10% of cases, a genetic predisposition increases the risk of cancer developing a lot earlier (E.g. certain childhood leukemia's, retinal cancers etc.).

1.4.2 Age

Although cancer can occur in persons of every age, it is common among the aging population (**FIG 1.4**). Sixty percent of new cancer cases and two thirds of cancer deaths occur in persons > 65 years. The incidence of common cancers (eg, breast, colorectal, prostate, lung) increases with age.

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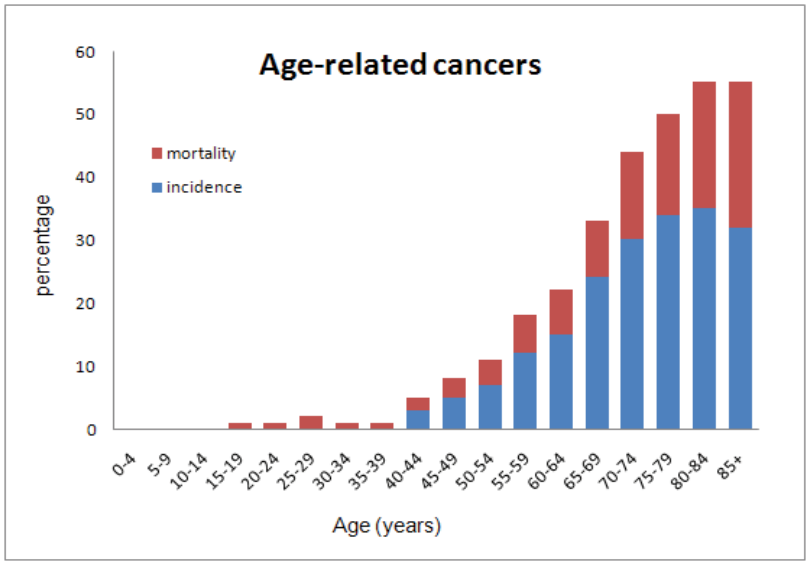


Fig 1.4: Age-related incidence and mortality for all types of cancers. *Source: SEER Cancer Statistics Review, NCI, USA*

There are several theories as to why cancer incidence increases in the elderly: age-related alterations in the immune system (decreased immune surveillance); accumulation of random genetic mutations or lifetime carcinogen exposure (especially for colorectal and lung cancers); hormonal alterations or exposure; and long lifespans.

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Multiple genetic changes are necessary for the development of cancer, most clearly exemplified by the stepwise genetic changes shown by many colon polyps progressing to cancer. The exponential rise in many cancers with age fits with an increased susceptibility to the late stages of carcinogenesis by environmental exposures. Lifetime exposure to estrogen may lead to breast or uterine cancer; exposure to testosterone leads to prostate cancer. The decline in cellular immunity may also lead to certain types of cancer that are highly immunogenic (e.g., lymphomas, melanomas). Accumulation of DNA mutations have to be amplified to constitute a cancer, therefore the longer the lifespan, the higher the risk of developing cancer.

1.5 Identification and histopathology of cancers

Why do we need to identify and classify cancers? There are several benefits to identifying and classifying cancers using histological sections and staining methodology

1) **Diagnosis:** Microscopic observation helps determine whether the tumour tissue is benign (harmless) or malignant (potentially fatal). Gross cellular morphology and tissue specific markers are used to classify cancerous cells.
2) **Therapy:** Pathology can be used as a confirmation or in prognosis E.g: has the surgeon removed the entire tumour? Or was the treatment effective in eliminating tumours? Or what is the rate of progression of the cancer? Progression can be predicted by histotyping. E.g. Patients with simple hyperplasia in the uterine epithelium have <1% chance of developing cancer compared 82% risk in patients with atypical hyperplasia.
3) **Cellular origin** (histogenesis): Determining the origins of the tumour by histopathological classification of tissue is useful in a) identifying whether the tumour is a primary or secondary tumour e.g. a liver tumour may have metastasised from elsewhere or b) source of origin of the tumour e.g. lung cancer due to smoking is epithelial (lung carcinoma) but due to asbestos exposure is mesothelial (mesothelioma* or asbestos cancer).

* What are mesotheliomas? The mesothelium is a layer of cells which cover various organs in the body protecting them and allowing organs to move against each other as the lungs expand and contract or the heart beats. The mesothelium surrounding the lungs and lining the chest cavity is called the pleura, so mesotheliomas affecting the cells lining the sacs surrounding the chest or lungs are referred to as pleural mesothelioma, whereas a cancer in the abdominal lining, or peritoneum, is called peritoneal mesothelioma.

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1.6 The 6 hallmarks of cancer

DNA mutations result in defects in the regulatory circuits of a cell, which disrupt normal cell proliferation behaviour. However the complexity of this disease is not as simple at the cellular and molecular level. Individual cell behaviour is not autonomous, and it usually relies on external signals from surrounding cells in the tissue or microenvironment. There are more than 100 distinct types of cancers and any specific organ can contain tumours of more than one subtype. This provokes several questions. How many of these regulatory circuits need to be broken to transform a normal cell into a cancerous one? Is there a common regulatory circuit that is broken among different types of cancers? Which of these circuits are broken inside a cell and which of these are linked to external signals from neighbouring cells in the tissue?

The answer to these questions can be summarised in a heterotypic model, manifested as the six common changes in cell physiology that results in cancer (proposed by Douglas Hanahan and Robert Weinberg in 2000). This model looks at tumours as complex tissues, in which cancer cells recruit and use normal cells in order to enhance their own survival and proliferation. The 6 hallmarks of this currently accepted model can be described using a traffic light analogy (**Fig 1.5**).

1) Immortality: Continuous cell division and limitless replication
2) Produce 'Go' signals (growth factors from oncogenes)
3) Override 'Stop' signals (anti-growth signals from tumour suppressor genes)
4) Resistance to cell death (apoptosis)
5) Angiogenesis: Induction of new blood vessel growth
6) Metastasis: Spread to other sites

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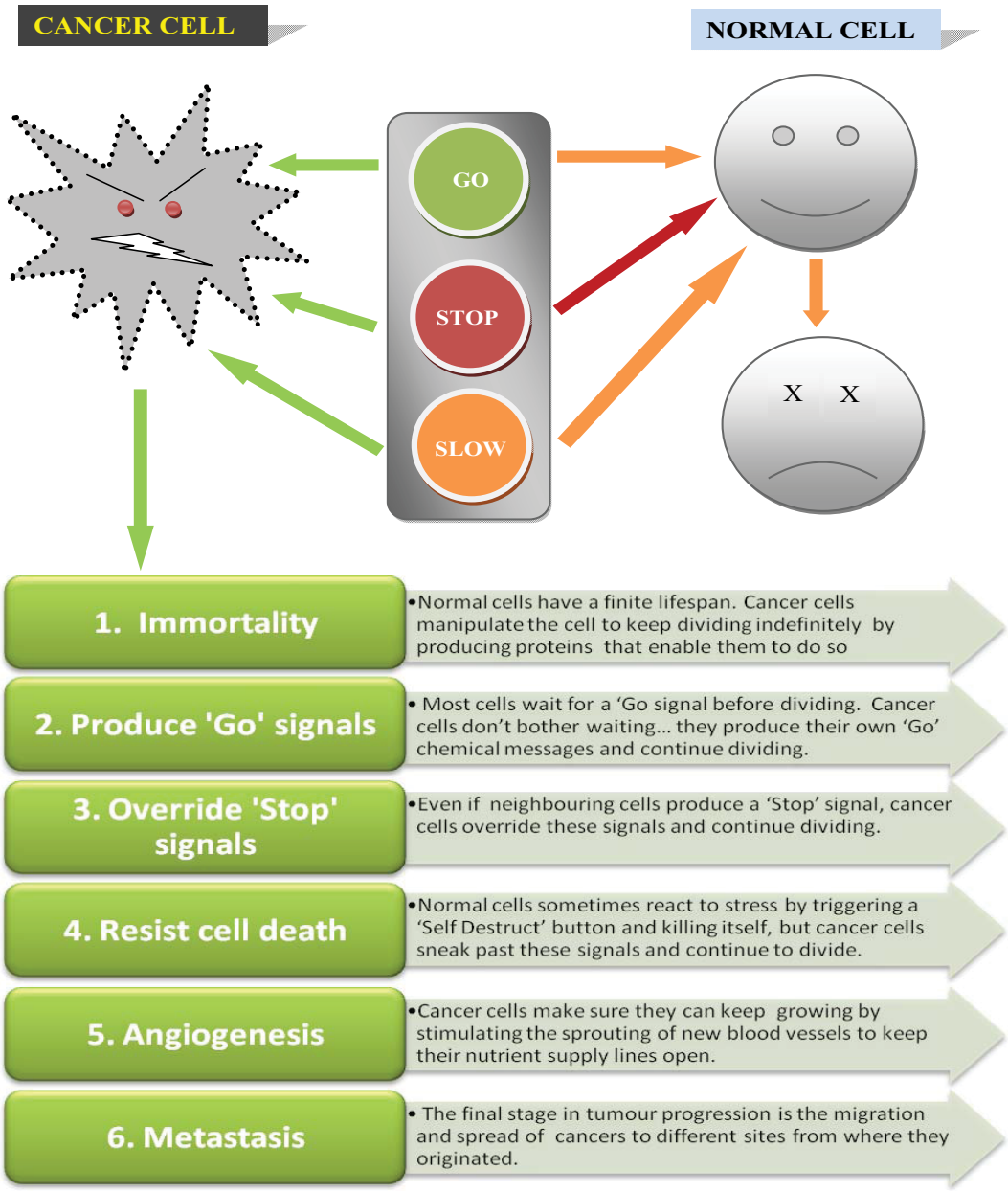


Figure 1.5: The six hallmarks of cancer. Almost all cancers share some or all of the 6 traits described below, depending on the tumour. Some tumours may show all these changes because of mutations in one key gene (e.g. the p53 gene controls at least 4 of the traits) whereas other tumours may need more than 1 mutation for progression. Arrows on the right (orange and red) show signals that regulate normal cell behaviour. The green arrows on the left indicate abnormal growth triggered by cancer cells. The green boxes outline the 6 key characteristics of cancer cells.

1.7 Bibliography

1. Chapter 2: The Biology of Cancer by Robert A Weinberg (Garland Publishing)
2. Chapters 1–4: Cancer Biology by RJB King -2e (Prentice Hall Publishing)

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1.8 Further reading

1. Hanahan, D and Weinberg, RA (2000) *Hallmarks of cancer*. Cell, Vol 100, pp. 57–70.
2. Alison, MA (2001) Cancer. www.els.net (introductory article).
3. Loechler, EL (2003) *Environmental Carcinogens and Mutagens*. www.els.net. (Standard article).
4. Turker, M (March 2009) *Ageing*. In: www.els.net Encyclopedia of Life Sciences (ELS). John Wiley & Sons, Ltd: Chichester. DOI: 10.1002/9780470015902.a0001479.pub2.
5. Doll, R (1999) *The Pierre Denoix memorial lecture: nature and nurture in the control of cancer*. European Journal of Cancer 35: 16–23.
6. Evan GI, Vousden KH.(2001) *Proliferation, cell cycle and apoptosis in cancer.* Nature. May 17;411(6835):342–8.
7. Klein, C (2008) *Cancer: The metastasis cascade.* Science, Sept 26, Vol 321, pp. 1785–87.



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2 Immortality: Continuous cell division

All organisms have a defined size and shape, both at the tissue and cellular level. Almost all types of mammalian cells carry an inbuilt circuit which controls their rate of cell division. This control is vital to maintain the integrity of the cell and the tissue. If cells continue to divide uncontrollably without any intrinsic constraint, tissues can potentially develop to enormous sizes with lethal results for the organism. For example, humans could potentially have massive hearts or enlarged lungs or livers.

Cancer cells are typically defined by their capacity to divide uncontrollably. In order for a clone of cells to expand to the size of a potentially fatal tumour, there must be a disruption in the inherent cellular circuitry controlling cell multiplication. It has long been known that normal mammalian cells grown in a petridish have a finite number of cell divisions. For example, adult fibroblast cells which have been cultured in a petridish *in vitro,* stop multiplying when the cells reach the edge of the petridish. When a small fraction of these cells are transferred to a new petridish, they start to divide again and so on – a process called passaging. However, after a certain number of transfers, the rate of cell division slows down and ultimately stops (**Fig 2.1**).

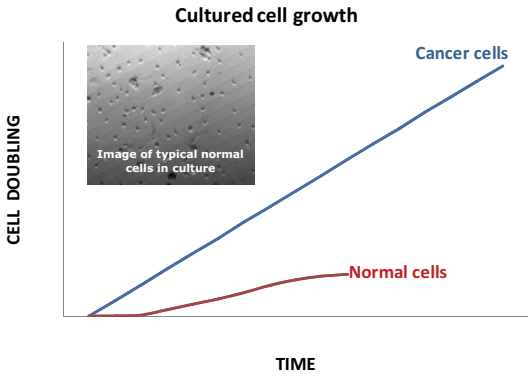


Figure 2.1: Capacity for cell division. In culture, normal cells undergo a finite number of divisions before they stop dividing completely (senescence). Cancer cells have the capacity to undergo infinite number of cell divisions. The image shows normal cells grown in a petridish.

Leonard Hayflick was the first to demonstrate that cells from rodent or human embryos have a finite number of cell divsions (replicative potential) and he called this senescence. Senescent cells are viable but have lost the capacity for cell cycling and cell division. In a petridish, these cells will take up nutrients and grow (often looking like 'fried eggs' because the nucleus and cytoplasm grow in size) but they will not divide. Typically, most normal human cell types have the capacity for 60–70 doublings.

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In contrast to normal cells, cultured cancer cells have the capacity to dramatically exceed normal doubling times to almost indefinite levels (**Fig 2.1**). A striking example of this is the HeLa cells. Originally cultured from a cervical adenocarcinoma from a cancer patient called Henrietta Lacks in 1951, these cells continue to grow and proliferate in hundreds of laboratories across the world to this day. This clearly suggests that these cancer cells have bypassed/disrupted the senescence regulators within the cell and acquired the capacity for unlimited division (replicative potential).

The cellular mechanism controlling senescence has been discovered in the past 30 years. We now know that the ticking counter which controls finite cell division lies at the end of all human chromosomes – the telomeres. Telomeres are hexanucleotide sequences of DNA (short repeats of 6 base pairs), and each end of a linear chromosome contains thousands of copies of these repeats. For example, in humans the repeat sequence is TTAGGG. These sequences are considered 'junk', i.e. they do not code for any proteins. However, they play a vital role in protecting linear chromosomes by preventing end-end fusion and also shield the DNA from degradation by nucleases. The best analogy is that telomeres are like aglets which protect the ends of shoelaces from fraying.

The importance of telomeres in cell division is defined by a unique problem of DNA replication called the 'end replication problem' (**FIG 2.2**). During cell division, DNA replicates during the S phase of the cell cycle creating twice the number of chromosomes. Each set is then divided between each daughter cell at the time of mitosis. However, after every round of DNA replication, a short sequence (50–100 base pairs) of the telomere is lost from the ends of each chromosome. This progressive shortening is because the enzyme responsible for DNA synthesis, the DNA polymerase, is unable to replicate the 50–100 base pairs on the end of one strand of the DNA (the 3' ends). As a result, every round of DNA replication results in the degradation of these sequences and shorter and shorter chromosomes. Since every chromosome has a finite number of these telomere repeats, successive cycles of replication result in a steady erosion of the telomeres until they cause genetic changes, chromosomal end-end fusions and disarray, and ultimately cell death. Thus, every normal cell has a finite lifespan, dictated by its length of telomeres.

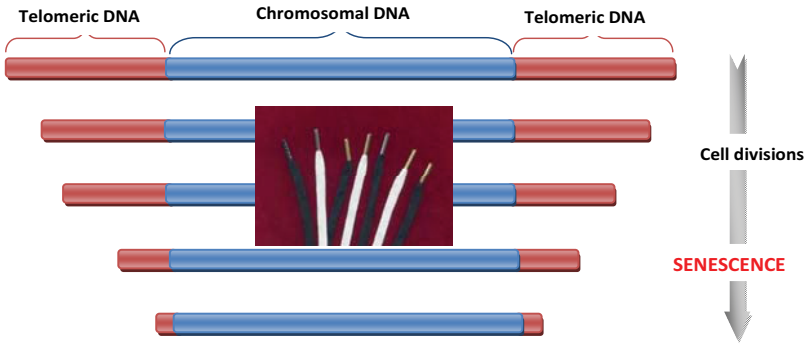


Fig2.2. Telomeres are the aglets of chromosomes. Telomeres are repeat DNA sequences that protect the linear end of chromosomes. After every round of cell division, telomere lengths get progressively shorter, until it provokes the cell to stop dividing and enter senescence. Cancer cells prevent telomere shortening by producing the enzyme, telomerase, which keeps extending telomeres, thus preventing senescence.

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Cancer cells on the other hand, maintain their telomere lengths without any loss of DNA base pairs. The main strategy used by cancer cells to maintain telomere lengths is by activating an enzyme called telomerase. Almost 85–90% of all cancers have an active telomerase. Telomerases add non-coding, hexanucleotide repeats onto the ends of telomeric DNA, thus maintaining the required lengths above the critical threshold, preventing erosion and allowing unlimited replicative capacity. Unlike cancer cells, actively dividing normal cells have levels of telomerase that are extremely low or undetectable. If telomerase is injected into these cells *in vitro*, they are transformed into cells that keep dividing limitlessly. Additional evidence on the importance of telomerases in telomere maintenance comes from tumours that have spread to distant locations in the body (metastases) which also show high levels of telomerase expression and activity.

Another interesting feature of telomerases is that they are highly expressed in germ cells (sperm and ova) and embryonic stem cells (ES cells) but gradually diminish during development into adult somatic cells. Cancer stem cells therefore, can be derived from normal stem cells, progenitor cells, or possibly somatic cells and might be immortal, having the capacity of indefinite self-renewal and proliferation.

The evidence listed above suggests that senescence is probably a protective mechanism used by cells to enter a quiescent (G0) phase to escape stress conditions and stop proliferation. Tumours circumvent senescence pathways by activating telomerases and therefore therapeutic strategies aimed at inhibiting telomerases will preferentially kill tumour cells and have no toxicity on normal cells. However, there is some debate that senescence is an artifact of cell culture conditions and not a true representation the phenotype in the body (*in vivo*). Resolution of this debate will be useful in understanding how replicative potential and tumour progression are linked.

2.1 Further reading

1. Hayflick, L (1997). Mortality and immortality at the cellular level. A review. Biochemistry 62, 1180–1190.
2. Greider CW, Blackburn EH. (1996) Telomeres, telomerase and cancer. Scientific American Feb; Vol 274(2), pp. 92–7.
3. Counter, CM, Avilion, AA, LeFeuvre, CE, Stewart, NG, Greider, Harley, CB, and Bacchetti, S. (1992). Telomere shortening-associated with chromosome instability is arrested in immortal cells which express telomerase activity. EMBO Journal. 11, 1921–1929.
4. Hiyama, E and Hiyama, K (2007) Minireview: Telomere and telomerase in stem cells. British Journal of Cancer, Vol 96, pp. 1020–1024.
5. Blasco, MA (2005) Telomeres and human disease: Ageing, cancer and beyond. Nature Reviews Genetics, Vol 6, pp. 611–22.

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3 Sustained growth signals (oncogenes*)

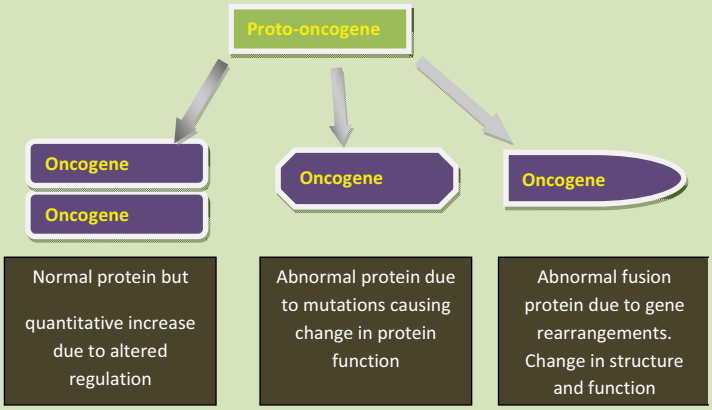
No cell can survive in isolation. Every cell is part of a community, which forms a tissue or organ. Cell behaviour is almost always dependent on growth signals from the surrounding (mitogenic), which trigger cell division. These external growth factors (or ligands) bind to membrane-bound glycoprotein receptors that transmit the message via a series of intracellular signals that promote or inhibit the expression of specific genes (**FIG 3.1**). Examples of growth signals include diffusible growth factors, extracellular matrix proteins and cell-cell adhesion/interaction molecules. If these growth signals are absent, any typical normal cell will change to a quiescent state instead of active division. This dependence on exogenous growth factors is a critical homeostatic mechanism to control cell behaviour within a tissue.



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***What are oncogenes?** An oncogene is a gene that encodes a protein capable of transforming cells in culture or inducing cancer in animals. These oncogenes are the derivatives of normal cellular genes called proto-oncogenes. Proto-oncogenes code for proteins that stimulate cell cycle division but mutated forms, called oncogenes, cause stimulatory proteins to be overactive, with the result that cells proliferate excessively. Oncogenes are dominant over proto-oncogene's.



Cancer cells, on the other hand, generate mutant proteins (oncogenic proteins) which mimic these normal growth signals (proto-oncogenic proteins). Transformation of proto-oncogenes into oncogenes is brought about by several factors such as mutations, chromosomal rearrangements, viral insertion, gene amplifications etc. The consequence of oncogenic transformation is that tumour cells become independent of these external growth signaling factors in any normal tissue microenvironment. This acquired feature by tumour cells can be demonstrated empirically *in vitro*.

Normal cells (e.g. skin fibroblast cells) that have been cultured in a petridish *in vitro*, will not divide and proliferate in the absence of growth factors found in serum. Tumour cells on the other hand can actively proliferate without depending on these growth factors. This autonomy from growth factor signaling leads to unregulated growth (such as in the absence of ideal conditions for cell division or stress) and increases the chances of acquiring further mutations in the cell genome.

There are three main cellular strategies used by cancer cells in achieving growth factor autonomy, based on the growth factor signaling pathway as shown in **FIG 3.1.**

a) Changes in extracellular growth signals
b) Changes in transcelluar mediators of those signals (receptors)
c) Changes in intracellular signaling messengers that stimulate proliferation.

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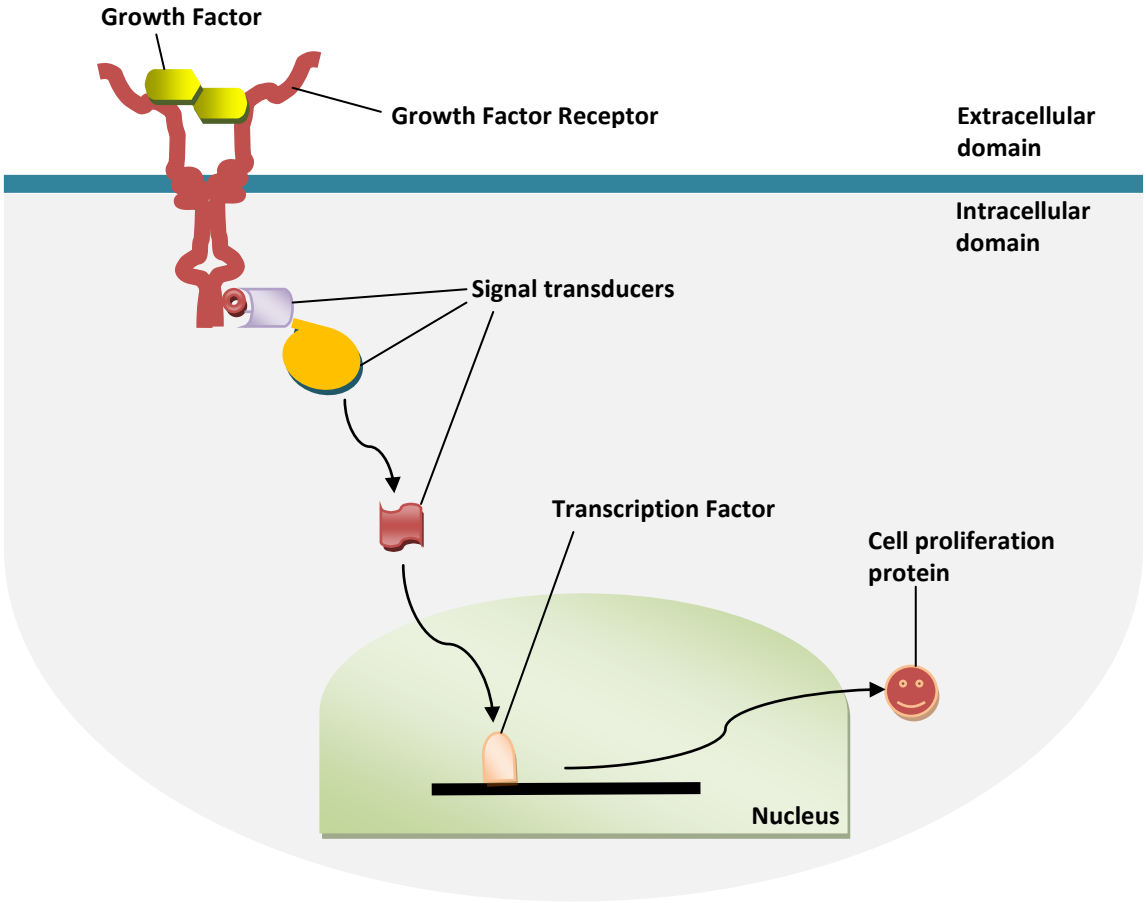


Fig 3.1: Components of a typical growth factor signaling cascade. Growth factors can be hormones or cell-bound signals that stimulate cell proliferation. Receptors are membrane bound proteins that accept signals. Signal transducers are molecules (proteins and others) that transmit the signal from the receptor to other intracellular molecules involved in cell proliferation. Transcription Factor are proteins that bind to DNA and allow expression of proteins involved in cell proliferation.

a) **Changes in extracellular growth signals**: Most soluble mitogenic growth factors (GFs) are heterotypic (made by one cell type in order to stimulate proliferation of another), but many cancer cells show autocrine stimulation (generate GF for its own cell type), thereby reducing dependence on GFs from other cells within the tissue. Many growth factor receptors, cytoplasmic and nuclear downstream effectors have been identified as oncogenes. The production of PDGF (platelet-derived growth factor) by a type of brain tumour called glioblastoma, is one example of this. Cells infected with a viral oncoprotein v-sis tend to release large amounts of PDGF-like oncoproteins (PDGF-b), which attach to PDGF receptors of the same cell. This causes an autocrine signaling loop in which the cell generates it own growth factor signal resulting in constitutive growth stimulation (**FIG 3.2**).

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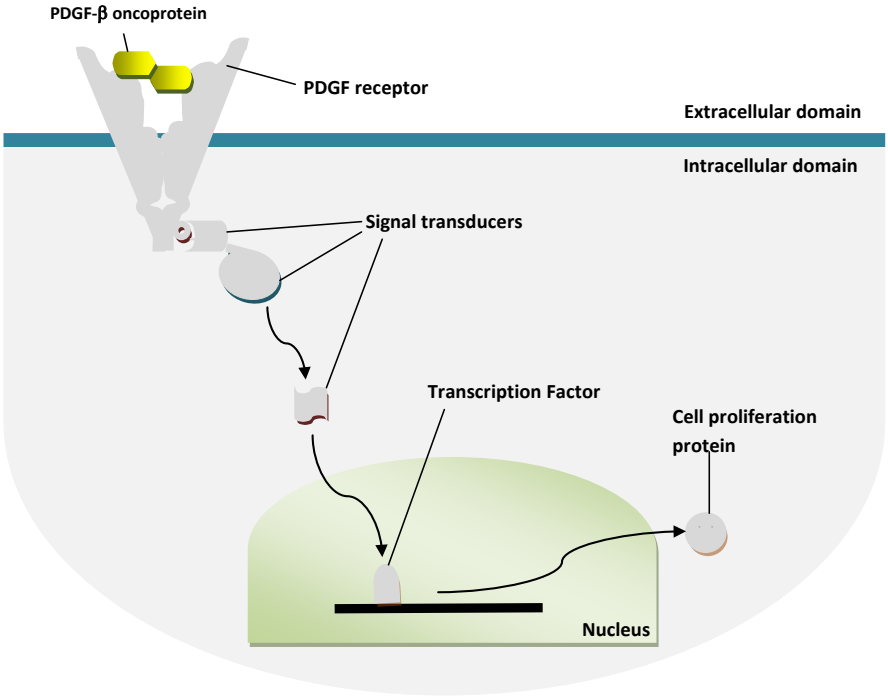


Fig 3.2: Alterations in the growth signal. Platelet-Derived Growth Factor is a protein needed by cells to stimulate cell division. PDGF normally binds to its PDGF receptor in the extracellular domain to stimulate intracellular cell proliferation pathways. Certain cancers develop by overproduction of the PDGF proteins or when cells are infected with certain viruses which overproduce a PDGF-like oncoprotein, which also binds to PDGF receptor.



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b) **Changes in transcelluar signals (receptors):** The cell surface receptors that transduce growth-stimulatory signals into the cell interior are themselves targets of deregulation during tumor pathogenesis. Mutations or changes in the number or types of receptors expressed by a cell can transform it into a cancer cell. The most common group of receptors implicated in several cancers belongs to the tyrosine kinase family. These include the EGF, FGF, IGF, PDGF receptors (Epidermal Growth Factor, Fibroblast Growth Factor, Insulin Growth Factor, Platelet-Derived Growth Factor respectively). Some examples of these changes can include

- ***Overexpression:*** GF receptors are often overexpressed in many cancers, resulting in the cancer cells becoming hyper-responsive to levels of GF that would not normally trigger cell division. For example, in certain types of breast cancer (mammary carcinomas), the HER2/neu receptor is overexpressed resulting in hyperproliferation.
- ***Ligand-independent signaling***: GF-independent signaling can be brought about either by gross overexpression of receptors or by structural changes in the receptor. For example, a truncated Epidermal Growth Factor Receptor (due to deletion in exons 2–7 of the extracellular domain) still sends growth-stimulating signals inside the cells consistently and without any EGF binding (**FIG 3.3**).

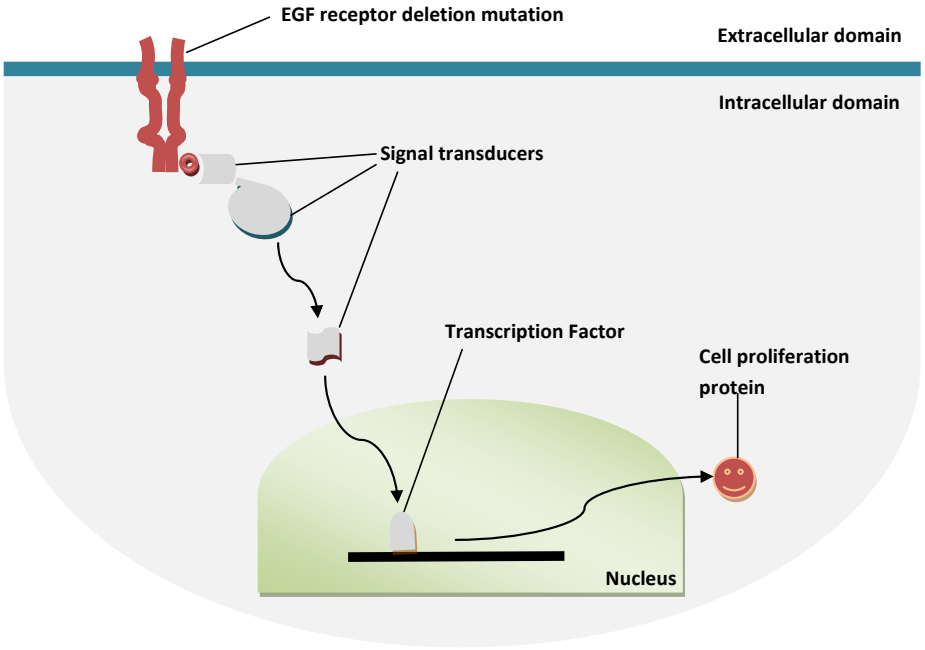


Fig 3.3: Alterations in the receptors. Mutations in the Epidermal Growth Factor Receptor (EGFR) receptors results in sustained and ligand-independent stimulation of growth-stimulating intracellular pathways. It has been implicated in the initiation and progression of certain types of breast cancers.

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- ***Receptor switching***: Cancer cells can also switch the types of receptors they express. For example – Cancer cells preferentially express extracellular matrix receptors (integrins) that favour growth. Integrins are bifunctional cell surface receptors that link cells to extracellular superstructures known as the extracellular matrix (ECM). Signaling molecules in the ECM enables the integrin receptors to transmit signals into the cytoplasm that influence cell behaviour, ranging from quiescence in normal tissue to motility, resistance to cell death, and entry into the active cell cycle in cancer cells.

c) **Changes in intracellular signaling pathways** that stimulate proliferation. The most common but complex mechanism of cancer transformation derives from changes in components of the intracellular, cytoplasmic signaling cascade, which receives input from the ligand-activated growth factor receptors on the cell membrane. Mutations in proteins belonging to the intracellular signaling cascade (adaptor proteins) are found in a majority of all human tumours. Examples of mutations in each component of this intracellular signalling pathway in human cancers are highlighted below.

Example 1 – Cell cycle related kinase (c-crk): These small adaptor proteins (~60 amino acid residues) were first identified as a conserved sequence in the non-catalytic part of several cytoplasmic tyrosine kinases such as Abl and Src and have subsequently also been identified in several other protein families such as phospholipase, PI3-kinase, ras GTPase activating protein, adaptor proteins, CDC24 and CDC25. These proteins contain only SH3 and SH2 domains, of which the SH2 domains bind phosphorylated tyrosine residues while the SH3 domains bind proline-rich sequences in ligand proteins. The main function of the C-crk proteins is to bring substrate proteins to the tyrosine kinase receptors (TKR). The viral oncoprotein, Bcr-Abl, acts as a substrate for c-crk, causing sustained activation of the tyrosine kinase receptors and resulting in cell proliferation (**Fig 3.4**). Activation of TKR by the c-crk proteins has been implicated in some human chronic myelogenous leukemias (CML).

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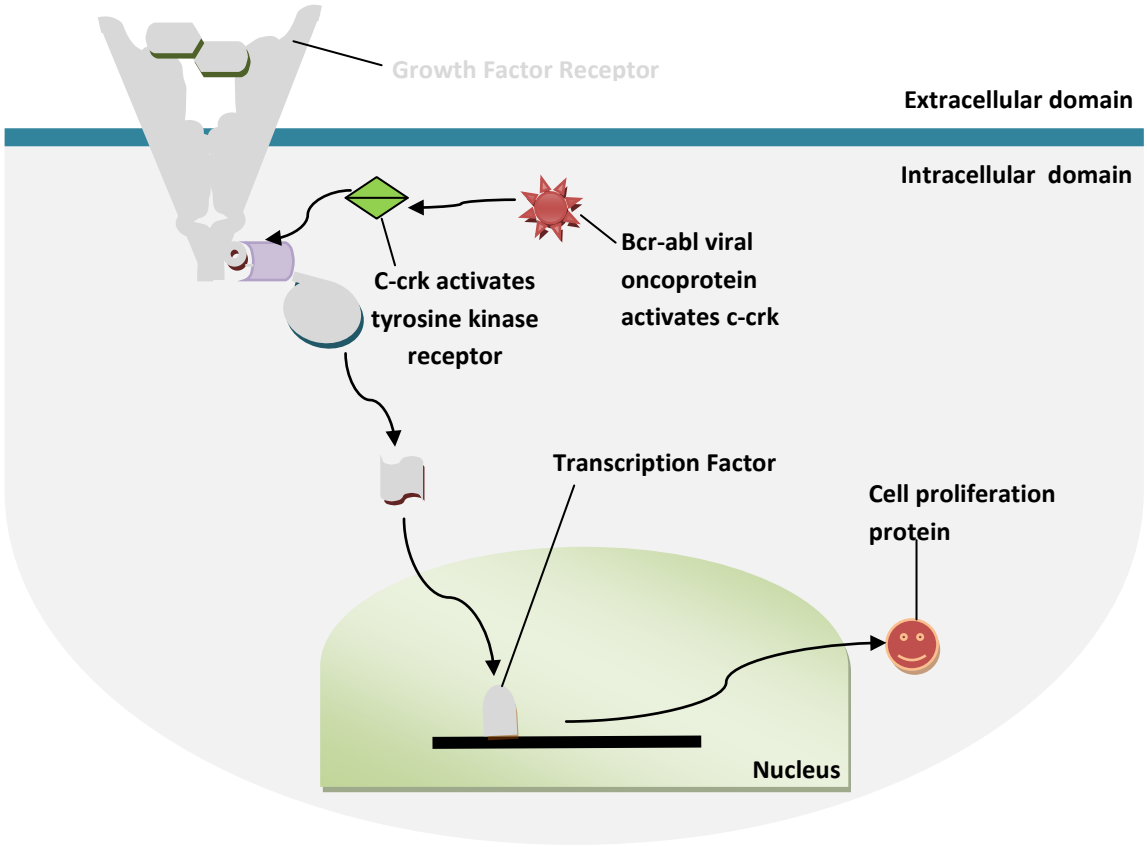


Fig 3.4: Activation of tyrosine kinase receptors by c-crk.



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Example 2 – ***Ras*** (rat sarcoma): Ras is arguably one of the most well studied oncoprotein and is a component of the SOS-Ras-Raf-MAP kinase mitogenic cascade. About 25% of human tumors and 90% of all pancreatic tumours have a mutant form of the intracellular signaling molecule Ras (a small GTP-binding signalling molecule). Ras proteins bind to GDP in an inactive state but upon stimulation through GEFs (guanine nucleotide exchange factors) are activated by binding to GTP (**Fig 3.5**). Active Ras functions as a GTPase through GAPs (GTPase-activating proteins), which hydrolyse the GTP and reach the inactive state. In tumours, typically, a single point mutation transforms the normal Ras into a Ras oncoprotein, in which the GTPase activity is lost, leading to a constantly active Ras and a cancer cell that cannot turn off its proliferation status. Alternatively, Ras activation pathways may be disrupted either due to oncoproteins or mutations in regulatory pathways (as shown in inset fig 3.5).

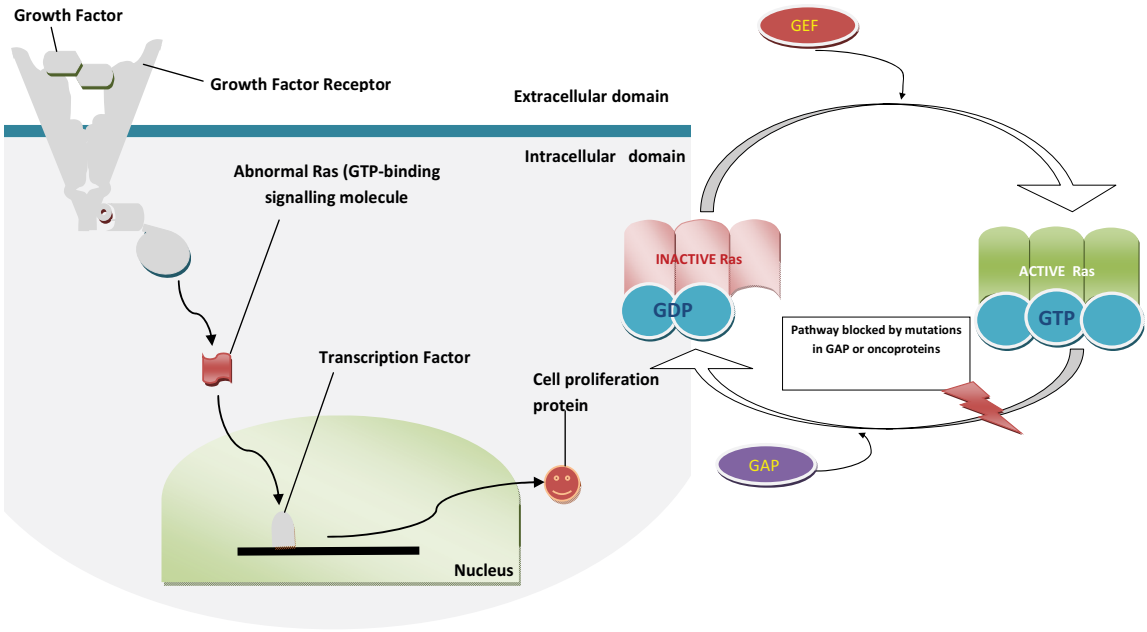


Fig 3.5: Changes in Ras signalling. Ras is vital in several signalling pathways and it acts as a binary switch. Binding GDP (**G**uanine nucleotide **DiP**hosphate) in an inactive state and GTP (**G**uanine nucleotide **TriP**hosphate) in its active state (when it stimulates GF signalling). Inactive Ras is activated by GEF (Guanine nucleotide Exchange Factor) by exhanging it's GDP for GTP. This step is short lived because Ras has an intrinsic GTPase activity, which means that it hydrolyses GTP to GDP (a phosphate group is removed), thus reverting back to the inactive state. This GTPase activity is triggered by GAP (GTPase-activating proteins). Mutations or oncoproteins that block the GTPase function restrict Ras in a permanently active, signalling state.

For the past two decades, there have been intense studies on the signaling pathways involved in cell growth and proliferation. New targets upstream and downstream of key pathways are being discovered, revised and interlinked with each other, enabling extracellular signals to elicit multiple biological effects. One way of looking at the complexity of signaling pathways is by thinking of an electronic circuit board, where transistors are replaced by key regulatory proteins which act as binary switches (such as phosphatases and kinases). Signals from either outside or inside the cell transmit messages of growth or quiescence, survival or death.

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3.1 Bibliography

1. Chapter 5: The Biology of Cancer by Robert A Weinberg (Garland Publishing)
2. Chapter 6: Cancer Biology by RJB King -2e (Prentice Hall Publishing)

3.2 Further Reading

1. Perry, AR (2001) Oncogenes www.els.net (secondary article).
2. Sharma, SV, Bell, DW, Settleman, J and Haber, DA (2003) Epidermal growth factor receptor mutations in lung cancer. Nature Reviews Cancer.
3. Karnoub, AE and Weinberg, RA (2008) *Ras oncogenes: split personalities*. Nature Reviews Molecular Cell Biology. July; Vol 9(7); pp. 517–31.



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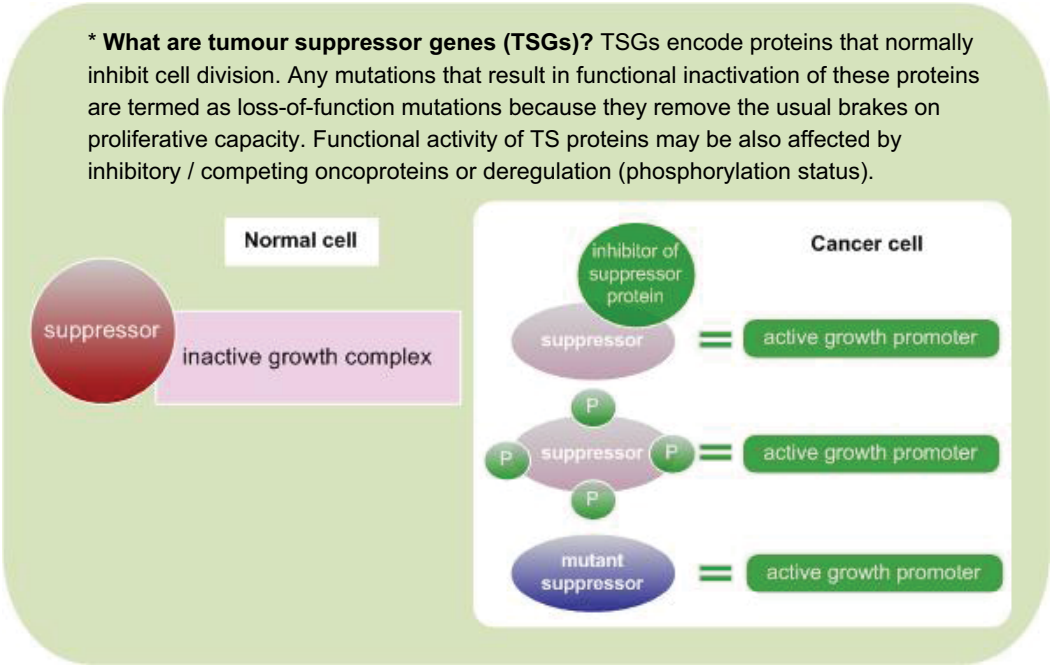
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4 Bypass anti-growth signals (Tumour Suppressor Genes*)

The balance between cell proliferation and quiescence is brought about by a complex interplay between these two signaling pathways. Chapter 3 highlights the role of positively active growth and proliferation pathways mediated by GF signaling cascades. However, normal tissues are also subjected to anti-proliferative signals which are responsible for cell quiescence, which act as brakes against proliferation signals (negative regulators of cell division). These signals can include soluble growth inhibitors as well as inhibitors present on the extracellular matrix and the cell surface.

Similar to GF signaling pathways, signals that normally suppress/block cell division are also received by cell-surface receptors that are coupled to intracellular signaling pathways. The genes that encode this class of proteins involved in restraining normal cell division are termed tumour suppressor genes. These anti-growth signals are closely linked to the cell cycle clock, which controls progressive cell division through mitosis.

Normal cells, prior to cell division, constantly monitor their internal and external environment to ensure that conditions are ideal for mitosis. Signals from the external or internal environment dictate whether the cell should divide, or undergo quiescence, or enter the post-mitotic state or destroy itself. For example, presence of proteins involved in DNA replication at the end of the G1 phase pushes the cell into the S phase, whereas severe DNA damage can trigger the cell to kill itself by apoptosis or undergo quiescence. These checks provide a critical homeostatic mechanism for the cell to progress to cell division at the right time and under optimal growth conditions.



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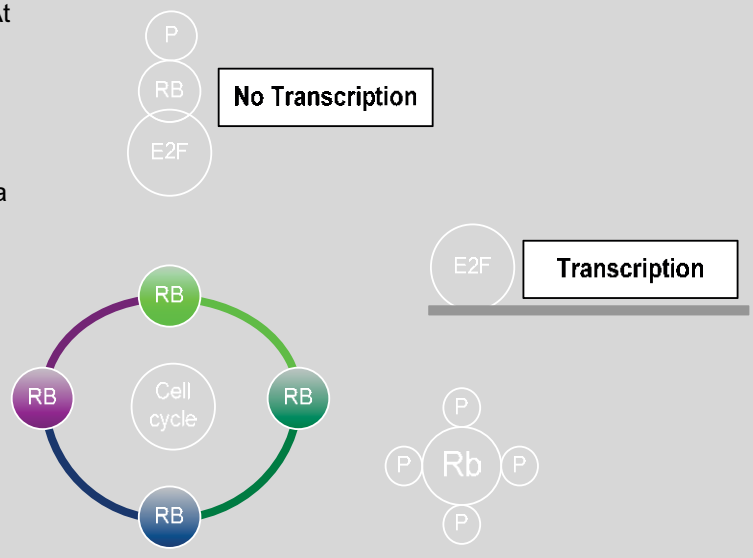
Typically, anti-growth signals work in two distinct ways

a) Forcing actively dividing cells into the quiescent (G0) phase of the cell cycle, which can be a temporary measure until there is a change in proliferative capacity (either a change in microenvironment conditions or there is a GF signal).
b) Cells may be induced into a permanent post-mitotic (non-dividing) state as a result of development. For example the specific terminal differentiation of neurons or the denucleation state of mature erythrocytes.

Cancer cells, on the other hand, bypass or evade these anti-growth signals to enable their own growth and proliferation. For example, mutations in genes that normally inhibit cell proliferation would result in increased cell division. These tumour suppressor genes (TSGs) constitute a large group of genes that encode proteins whose normal role is to restrain cell division. Mutations in these genes lead to a loss-of-function and typically, both copies (alleles) of the gene need to be altered to enable tumour formation (unlike oncogenes, which are gain-of-function mutation).

One well-studied example of a tumour suppressor protein is the retinoblastoma (Rb) protein, involved in the formation of rare paediatric tumours found in the retina of the eye. Most mutations in the Rb gene involve gross chromosomal changes in the 3kb coding region of the gene and about a third tend to be single base change mutations.

Cell cycle regulation by Rb: At the molecular level, the Rb protein (pRb) and its two relatives, p107 and p130, arguably regulate most of the anti-growth signals in a cell. In a hypophosphorylated state, pRb blocks proliferation by sequestering and altering the function of a key transcription factor called E2F, which control the expression of a multitude of genes essential for cell cycle progression from G1 into S phase.



Alteration of the pRb pathway (either due to mutations or hyperphosphorylation of pRb) releases E2F, resulting in expression of genes involved in cell proliferation. Cells can also become insensitive to anti-growth factors that normally operate along this pathway to regulate cell cycle progression.

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The pRb signaling circuit can be disrupted in a variety of ways in different types of human tumors. For example, down regulation/disruption of receptors and signaling molecules upstream of the pRb circuitry or the loss of functional pRb through mutations. Alternatively, in certain DNA virus-induced tumors, such as cervical carcinomas, viral oncoproteins, such as the E7 oncoprotein of human papilloma virus sequester pRb resulting in loss of function (**Fig 4.1**). To summarise, the anti-growth pathway which converges onto pRb is disrupted in a majority of human cancers, highlighting the concept of tumor suppressor loss in cancer.

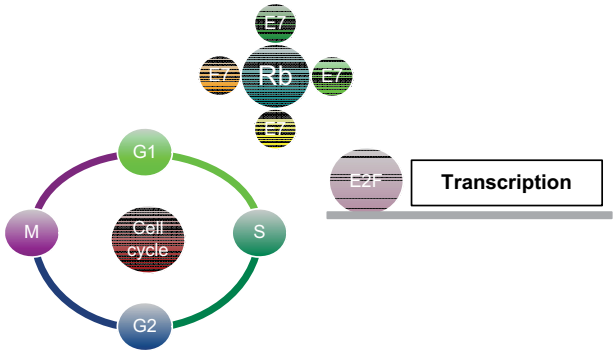


Fig 4.1 Tumour progression by sequestering the Rb protein. The human papilloma virus (HPV) is the main causative agent for cervical cancers. Long-term exposure and other lifestyle factors allow the HPV E7 oncoprotein to sequester Rb, leaving the transcription factor E2F free to transcribe growth proliferation genes.

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Another well-known example of a tumour suppressor protein is p53 (so called because it is a tumour specific nuclear antigen of 53 kD size). Originally discovered by David Lane, Arnold Levine and William Old in 1979, it has been termed 'guardian of the genome' because of its singularly critical role in the cell cycle. Almost 50% of all cancers show mutations in p53 and nearly 80% of mutations in Squamous Cell Carcinoma (SCC) involve p53. Mutations or loss of function of this vital protein leads to continued cell division despite the cell having damaged DNA (through radiation or mutagens for example).

The role of p53 as a tumour suppressor was determined by two observations

a) Mice which have both copies (alleles) of the p53 gene knocked out ($p53^{-/-}$ mice) are prone to developing tumours (although interestingly, these mice are also prone to rapid ageing!)
b) Transfection (insertion) of wild type p53 genes into tumours of $p53^{-/-}$ mice stopped tumour growth dramatically, thereby confirming the function of p53 as a tumour suppressor gene.

p53 is usually degraded in a normal cell. However, conditions of cellular stress or DNA damage (for example exposure to radiation or mutagens) result in expression of intermediate proteins which stabilize the p53 protein (**Fig 4.2**). This stable p53 protein forms a tetramer (a complex of 4 p53 proteins), which acts as a transcription factor and expresses genes involved in either halting cell cycle progression, or DNA repair (if the damage is minor) or inducing cell death (if the damage is too severe). In tumours however, the loss of function of p53 removes this essential 'guardianship', allowing the cell to continue dividing despite the DNA damaging mutations.

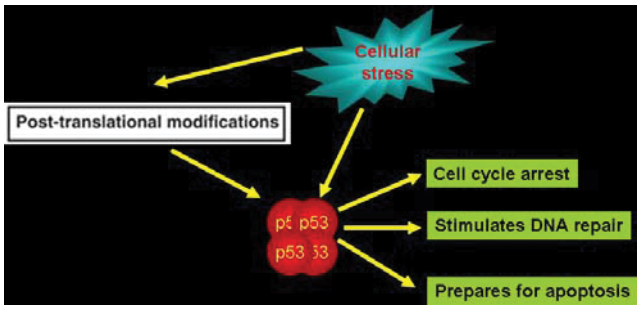


Fig 4.2. Normal function of the p53 protein. The p53 protein plays a pivotal role in regulating cell cycle progression. When a cell is exposed to stress (DNA damage, hypoxia etc), p53 forms a stable tetrameric complex and attempts to regulate the cell cycle by expressing genes involved in either cell cycle arrest, DNA repair or inducing cell death by apoptosis.

Another strategy used by cancer cells is to avoid the irreversible terminal differentiation of cells into post-mitotic states. One example of this method involves the transcription factor c-Myc, which stimulates growth during normal development by associating with another factor, Max. To induce differentiation however, Max forms complexes with Mad (Mad-Max complexes) to trigger differentiation-inducing signals. However, in certain tumours, the c-Myc oncoprotein is overexpressed, thereby shifting the balance to favour Myc-Max complexes, thereby inhibiting differentiation and promoting growth.

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Although the interlinking between the various growth and differentiation-inducing signals with the cell division cycle are still being clarified, it is clear that understanding the antigrowth signaling circuitry is vital to understanding cancer development.

4.1 Bibliography

1. Chapter 7, 8, 9: The Biology of Cancer by RA Weinberg (Garland Publishing)
2. Chapter 6: Cancer biology by RJB King -2e (Prentice Hall Publishing)

4.2 Further reading

1. Chen HZ, Tsai SY, Leone G. (2009) *Emerging roles of E2Fs in cancer: an exit from cell cycle control.* Nat Rev Cancer Nov; Vol 9(11), pp. 785–97.
2. Poznic M. (2009) *Retinoblastoma protein: a central processing unit.* J Bioscience. Jun; Vol 34(2), pp. 305–12.
3. Vousden, KH and Lane, DP. (2007) *p53 in health and disease*. Nature Reviews Molecular Cell Biology Apr; Vol 8 pp. 275–83.
4. Ferbeyre, G and Lowe, SW. (2002) *The price of tumour suppression?* Nature, January Vol 415(3), pp. 26–27.



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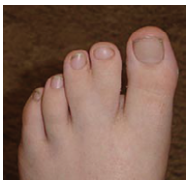
5 Avoidance of cell death (apoptosis)

So far, we have looked at cellular and molecular pathways that allow or restrict cell proliferation. The convergence of the two signaling pathways that regulate cell proliferation (proto-oncogenic and tumour suppressor), dictate whether the cell progresses through the cell cycle, diverts to quiescence or enters the post-mitotic differentiation state. This chapter will now focus on another state, wherein signaling pathways monitor the internal well-being of the cell. A cell constantly surveys its internal status including access to oxygen and nutrients, the integrity of its genome and the balance of its cell cycle regulatory pathways. If this monitoring detects damage or malfunction, backup systems are activated which in turn determine whether the cell growth should pause and repair pathways activated, or alternatively, if the damage is severe, the cell activates latent cell death (apoptotic) pathways.

The development of tumours can also be looked at as not simply excessive cell proliferation, but also as a reduction in cell death. Programmed cell death – apoptosis (from the Greek: apo – from, ptosis – falling, originally used for the falling leaves in autumn) – represents a major source of this attrition. There is increasing evidence to suggest that avoidance/resistance to apoptosis is a major hallmark of most, if not all, types of cancer.

5.1 When does apoptosis occur?

Apoptotic cell death is part of normal growth and development. For example the sculpting of human fingers or toes is due to apoptosis of the cells in between the digits. Another example is from cytotoxic T-cells (CTLs) of the immune system, which eliminate viral infected cells through apoptotic pathways thereby avoiding any messy inflammatory or infective after-effects.



Tissue homeostasis is a balance between cell division and cell death, wherein the number of cells in that tissue is relatively constant. If this equilibrium is disturbed, the cells will either a) divide faster than they can die, resulting in cancer development or b) die faster than they can divide, resulting in tissue atrophy. In terminally differentiated cells such as neurons, the induction of apoptosis can have fatal consequences, as seen in neurodegenerative conditions such as Alzheimer's disease.

Dysregulation of this complex tissue homeostasis has been implicated in many forms of cancer. For example, certain types of pancreatic adenocarcinoma show activation of antiapoptotic pathways.

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5.2 What triggers apoptosis?

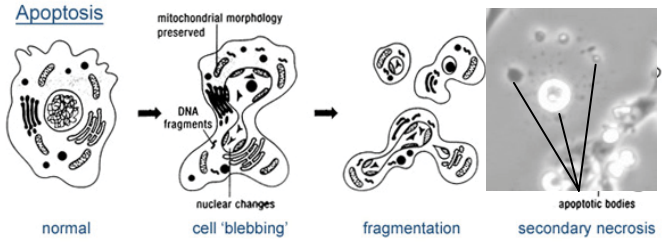
Induction of apoptosis can be simplified into 2 broad categories:

A] **Loss of positive signals:** Deprivation of growth-stimulating factors, such as growth factors, can trigger apoptosis. For example, in early development, neurons that are deprived of the Nerve Growth Factor (NGF) die due to apoptosis

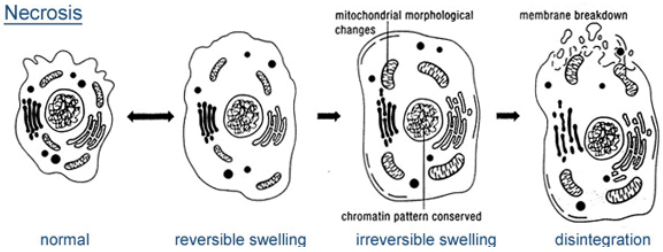
B] **Induction by negative signals**: Binding of certain external molecules (ligands) such as death ligands can trigger intracellular apoptotic signaling. Alternatively, internal signals from the cells can also activate apoptosis. For example, apoptosis usually occurs when a cell is damaged beyond repair, infected with a virus, or undergoing stressful conditions such as nutrient/oxygen deprivation.

These external or internal signals activate apoptosis in a highly specific and coordinated manner (just like a well planned military operation). Upon activation, the cell is dismantled neatly, the cell membrane undergoes blebbing, the chromosome is fragmented and packaged into discrete apoptotic bodies along with the cytosol and there is no lysis or spillage of the cell contents, which usually provoke an inflammatory response (Fig 5.1). The entire operation can occur within 30–120min! Any remaining evidence of the cell's existence is removed by neighbouring cells which engulf the apoptotic bodies and recycle the contents for its own use.

Fig 5.1: Types of cell death.



a) Apoptosis (programmed cell death) signals results in a systematic dismantling of the cell into smaller apoptotic bodies which are engulfed by phagocytes, resulting in no inflammatory response.



b) Necrosis is a messy way of cell death which results in cell swelling and lysis, leading to an inflammatory response.

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5.3 How does apoptosis occur?

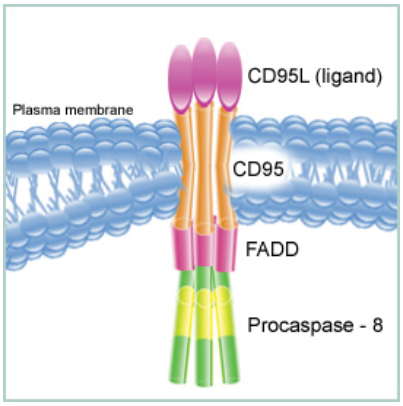
The main components of apoptotic pathways can be divided into 2 parts- sensing apoptotic signals and executing apoptosis. Sensing pathways monitor the internal and external environment of the cell to detect changes in ambient conditions that could influence cell fate (survival, division or death). The sensing pathways are closely associated with the execution pathway – the effector pathway – which carry out the task of programmed cell death by dismantling the cell.

Apoptotic sensing relies on signals either external (extrinsic induction) or internal (intrinsic induction) to the cell. External signals include exposure to toxins, certain hormones etc., which are transduced internally either through receptors or transporters on the plasma membrane. Internal signals, on the other hand, monitor response to stress or DNA damage. For example heat, radiation, hypoxia, GF insufficiency, viral infections or increase in intracellular calcium all evoke apoptosis through internal signaling pathways. Resurgent interest in apoptosis has resulted in an explosion of papers in this field, and current thinking suggests that both extrinsic and intrinsic pathways merge through common effector pathways inside the cell.



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External apoptotic signaling is typically mediated by cell surface receptors that bind death or survival factors (see Fig 5.2 extrinsic pathway). For example, death signals are activated when an endogenous ligand (CD95L) binds to CD95 receptors (also called Fas receptors) on the cell membrane. Other examples include the Tumour Necrosis Factor-α (TNF-α) binds to TNF receptors (TNFR1) or the FAS ligand binds to FAS receptors on the cell membrane. Loss of signals from extracellular matrix and cell-cell adherence proteins also stimulates apoptosis in cells.



Internal signals that elicit apoptosis converge on the mitochondria, the aerobic powerhouse of a cell (see Fig 5.2 Intrinsic pathway). Cytosolic proteins such as members of the Bcl-2 family target mitochondria causing either swelling of the organelle or make it leaky allowing release of certain apoptotic effector proteins into the cytosol. Leaky proteins include SMACs (second mitochondria-derived activator of caspases) which bind to IAPs (inhibitor of apoptosis proteins) and deactivates them, therefore allowing apoptosis to proceed.

Another important leaky protein is cytochrome c, which is released from the outer mitochondrial membrane through a channel called MAC (Mitochondrial Apoptosis-inducing Channel). In the cytosol, cytochrome c binds with Apaf-1 and ATP, which then bind to pro-caspase-9 to create a protein complex known as an apoptosome. Formation of the apoptosome is the final irreversible stage of apoptosis, wherein caspase-9 (an initiator) activates the executioners of apoptosis, effector caspase-3. These caspases (**c**ysteinyl **asp**artate-specific serine proteases) are the ultimate effectors of apoptosis that execute cell death by selective dismantling key structural proteins and DNA.

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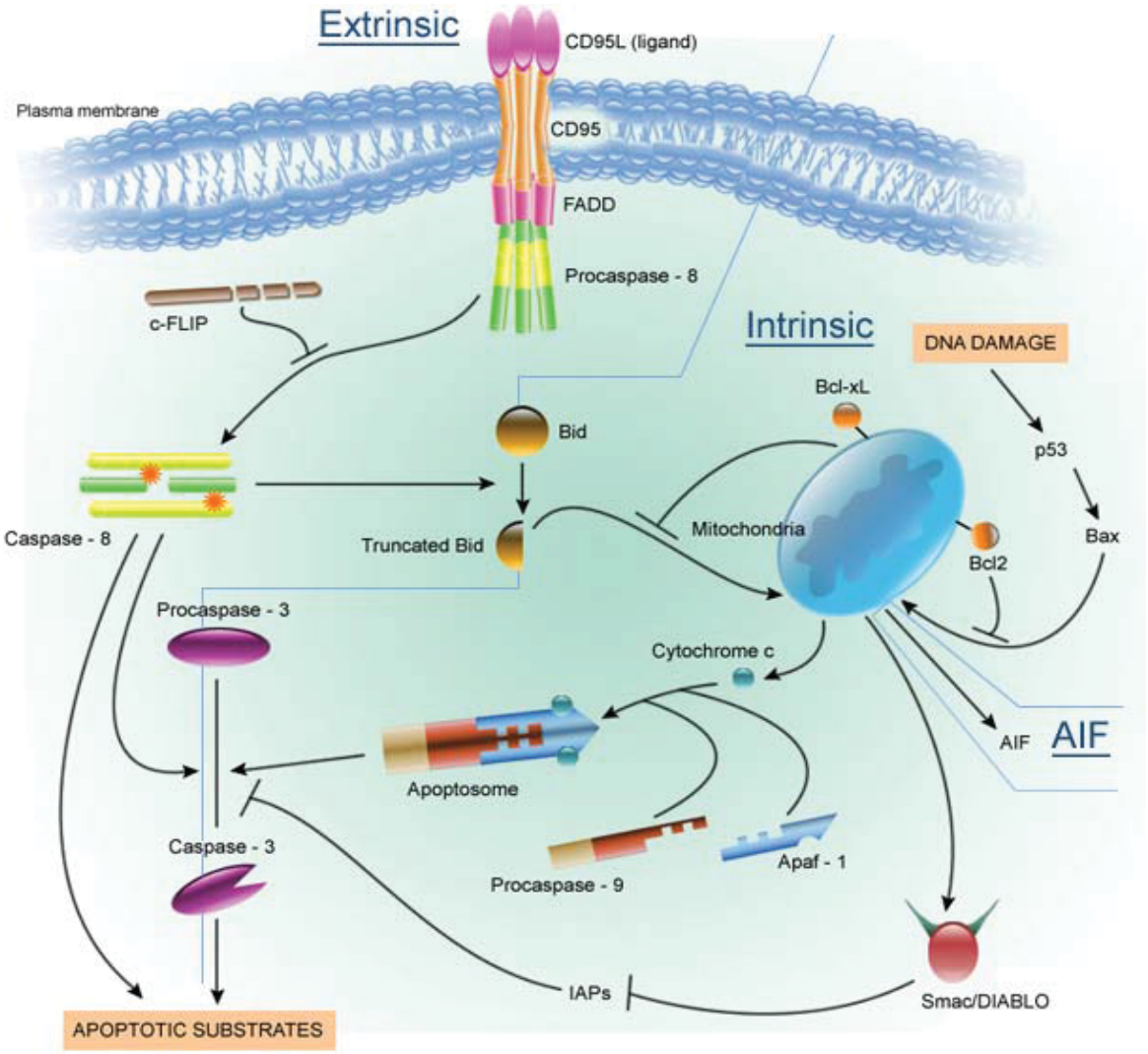


Fig 5.2: Extrinsic and intrinsic apoptotic pathways. 1) The extrinsic signals are triggered by binding of the ligand (e.g. CD95L) to its receptor (CD95). This activation of the receptor leads to the activation of FADD, which in turn activates DED. DED activation initiates apoptosis via initiator caspase 8, which leads to irreversible apoptosis either directly or through effector caspases (caspase-3). Active caspase-8 cleaves BID to tBID, which translocates to the mitochondrion to release of SMAC/DIABLO. SMAC/DIABLO sequesters IAPs resulting in apoptotic induction through caspase 3.

2) The intrinsic apoptotic pathway is initiated at the mitochondrion by diverse stimuli. a | Irreparable DNA damage signaling through the p53 proteins removes suppression of apoptosis by BCL2, leading to membrane permeabilization and the release of cytochrome c (Cyto c), SMAC/DIABLO, AIF (apoptosis-inducing factor) b | Cytochrome c interacts with APAF1 to recruit and activate caspase 9, forming the apoptosome, which activates the downstream executioner caspases 3 and 7. AIF causes DNA degradation.

FADD – Fas-associated death domain; DED – Death Effector Domain; tBID – truncated BID;

BID – [BH3 (BCL2 homology domain 3)-interacting agonist domain]; IAP – inhibitor of apoptosis; **BCL2** – B-cell leukaemia/lymphoma-2; SMAC/DIABLO – second-mitochondrial-derived activator of caspases; APAF-1 – apoptosis protease activating factor-1

Several studies using transgenic models clearly demonstrate that changing components of the apoptotic machinery can dramatically affect the dynamics of tumor progression, showing that inactivation of this machinery is a key factor during tumor development.

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Cancer cells can bypass apoptosis in many ways. The most common method involves mutations of the p53 tumor suppressor gene resulting in the loss of proapoptotic regulators. More than 50% of all human cancers (and 80% of squamous cell carcinomas) show inactivation of the p53 protein. P53 is also known as the 'guardian of the cell' because of its pivotal role in cell response to stress.

The main role of p53 is as a DNA damage sensor, and it activates either DNA repair pathways or apoptosis in response to DNA damage. Other abnormal internal signals such as hypoxia or oncogenic protein overexpression also trigger proteins involved in apoptosis, funneled in part via p53, and therefore any loss of function of p53 protein results in impaired apoptosis. Recently, a mechanism for reducing apoptosis through the FAS death signal has been demonstrated in a high fraction of lung and colon carcinoma cell lines: a non-signaling decoy receptor for the FAS ligand is upregulated, thus diluting the death-inducing signal of the FAS death receptor.

The current model of an apoptotic signaling circuitry is shown in Figure 5.2. Although it is still part of ongoing research, key regulatory and effector components have been identified. However, some questions remain which have important implications for the development of novel types of antitumor therapy. It is unlikely that all cancer types will have lost all proteins in the proapoptotic circuit; more likely is that they retain other similar proteins which activate apoptosis. The challenge lies in identifying apoptotic pathways still operative in specific types of cancer cells and designing new drugs which will switch these on in all of the tumour cell population, resulting in a substantial therapeutic benefit.

5.4 Bibliography

1. Chap 9: The Biology of Cancer by RA Weinberg (Garland Publishing)
2. Pages 160–167: Cancer Biology by RJB King (2e) (Prentice Hall Publishing)

5.5 Further reading

1. Vince, JE and Silke, J. (September 2009) Apoptosis: Regulatory Genes and Disease. In: Encyclopedia of Life Sciences (ELS www.els.net). John Wiley & Sons, Ltd: Chichester. DOI: 10.1002/9780470015902.a0006044.pub2.
2. Kaufmann SH, Hengartner MO. (2001) *Programmed cell death: alive and well in the new millennium*. Trends Cell Biol. Dec; Vol 11(12), pp. 526–34.
3. Johnstone RW, Frew AJ, Smyth MJ. (2008) *The TRAIL apoptotic pathway in cancer onset, progression and therapy.* Nat Rev Cancer. Oct; Vol 8(10), pp. 782–98.

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6 Ensuring blood vessel growth (angiogenesis)

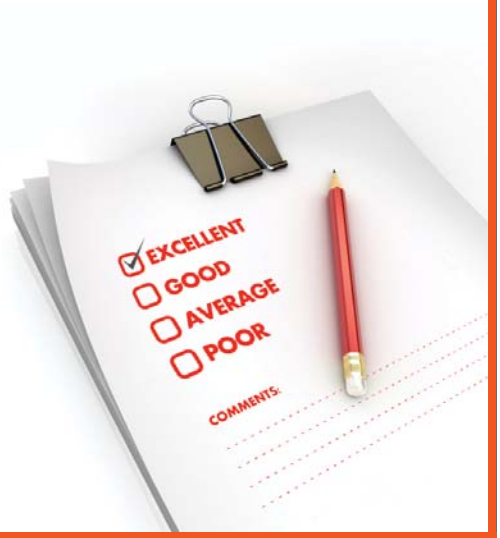
The four hallmarks of cancer progression – growth signal autonomy, insensitivity to antigrowth signals, resistance to apoptosis and unlimited replicative potential – discussed in the previous chapters looked at cancer progression at the molecular and cellular level. However, other factors that also play a major role in progression and spread of cancer need to be understood, in order to enable better strategies for cancer therapeutics.

Cell and tissues need oxygen and nutrients to survive and grow and therefore most cells lie within 100 µm of a capillary blood vessel. Under most conditions, cells that line the capillaries – the endothelial cells- do not grow and divide. However, certain conditions such as during menstruation or wound healing, trigger endothelial cell division and growth of new capillaries and this process is termed angiogenesis or neovascularisation. Tumours can also 'turn on' angiogenesis. In fact, it is a key transition step to convert a small, harmless cluster of mutant cells (an in situ tumour) into a large malignant growth, capable of spreading to other sites. Typically this transition can take many months or even years and unless angiogenesis is activated, solid tumours will grow no bigger than a pea. Therefore, understanding the process of neovascularisation in tumours is a powerful strategy for therapeutic drug design.

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As with most other cellular homeostatic mechanisms, angiogenesis also involves a delicate balance between positive and negative signals that stimulate or block this process. Judah Folkman was among the original pioneers who used in vivo bioassays to demonstrate the necessity of angiogenesis for explosive growth of tumor explants almost 30 years ago. We now know that the ability to induce and sustain angiogenesis is acquired in a series of discrete steps during tumor development, via an "angiogenic switch" from vascular quiescence.

A simplified overview of this process, shown in FIG 6.1, is given below

- Tumour cells release pro-angiogenic factors, such as vascular endothelial growth factor (VEGF), which diffuse into nearby tissues and bind to receptors on the endothelial cells of pre-existing blood vessels, leading to their activation.
- Such interactions between endothelial cells and tumour cells lead to the secretion and activation of various proteolytic enzymes, such as matrix metalloproteinases (MMPs), which degrade the basement membrane and the extracellular matrix.
- Degradation of the basement membrane allows activated endothelial cells – which are stimulated to proliferate by growth factors – to migrate towards the tumour.
- Integrin molecules, such as αvβ3-integrin, help to pull the sprouting new blood vessel forward.
- The endothelial cells deposit a new basement membrane and secrete growth factors, such as platelet-derived growth factor (PDGF), which attract supporting cells to stabilize the new vessel.

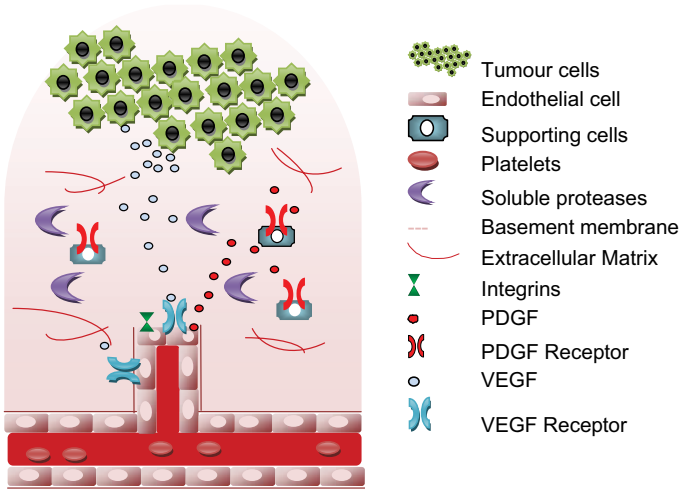


Fig 6.1. Angiogenesis in tumour growth. Tumour cells release pro-angiogenic factors, such as vascular endothelial growth factor (VEGF), which diffuse into nearby tissues and bind to receptors on the endothelial cells of pre-existing blood vessels, leading to their activation. Such interactions leads to the secretion and activation of various soluble proteases (such as matrix metalloproteinases MMPs), which degrade the basement membrane and the extracellular matrix. Degradation allows activated endothelial cells to divide and migrate towards the tumour. Integrin molecules help to pull the sprouting new blood vessel forward. The endothelial cells deposit a new basement membrane and secrete growth factors, such as platelet-derived growth factor (PDGF), which attract supporting cells to stabilize the new vessel.

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However, the question of how the delicate balancing between the various angiogenic regulators shifts to activate tumour progression is still poorly understood. The most likely scenario is that there may be an interlinking with other networks involved in cellular homeostasis. For example, an angiogenic inhibitor thrombospondin-1 has been found to be positively regulated by the p53 tumor suppressor protein in some normal cell types. Therefore, any loss of p53 function, which occurs in most human tumors, can cause thrombospondin-1 levels to fall, thereby removing the inhibitory barrier for endothelial cells proliferation.

Another emerging evidence is in the form of proteases, which can control the availability of angiogenic activators and inhibitors. Some proteases can release angiogenic factors stored in the extracellular matrix, whereas some proteases can have both proangiogenic and anti-angiogenic roles (such as plasmin). The coordinated expression of pro- and antiangiogenic signaling molecules, and their control by proteases, appear to play an important role in the complex homeostatic regulation tissue angiogenesis.

At a simplistic level, tumour angiogenesis offers a compelling and attractive therapeutic target which has a broad application for almost all solid tumours. Several anti-angiogenic drugs have shown promise in advanced clinical trials and several promising drugs targeting angiogenic regulatory molecules are in the pipeline. However, available evidence also indicates that different types of tumor cells use different molecular strategies to activate the angiogenic switch. This raises the question of whether a single antiangiogenic therapeutic will be enough to treat all tumor types, or whether selective therapeutics will need to be developed for specific types of tumours, with each targeting a specific angiogenic switch.

6.1 Bibliography

1. Chap 13: The Biology of Cancer by RA Weinberg (Garland Publishing)

6.2 Further Reading

1. Jain RK. (2008) *Taming vessels to treat cancer.* Scientific American Jan; Vol 298(1), pp. 56–63.
2. Cristofanilli, M, Charnsangavej, C and Hortobagyi, GN. (2002) *Angiogenesis modulation in cancer research: Novel clinical approaches*. Nature Reviews Drug Discovery 1, 415–426 (2002)
3. Carmeliet, P and Jain, RK. (2000) *Angiogenesis in cancer and other diseases.* Nature (Sept); Vol 407(14), pp. 249.
4. Baeriswyl V and Christofori G. (2009) *The angiogenic switch in carcinogenesis.* Seminars Cancer Biol. Oct; Vol 19(5), pp. 329–37.

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7 Spread to other sites (metastasis)

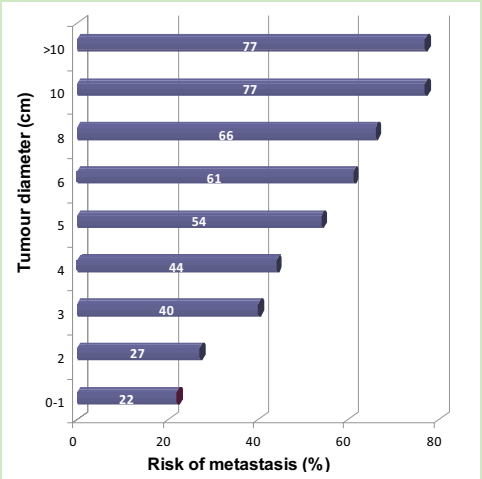
Solid tumours are usually part of normal tissues, and under optimal conditions, can invade adjacent tissues or pass out through the circulatory system to colonise distant sites in the body. These secondary tumours – metastases – are responsible for almost 90% of cancer-related deaths. This capacity of tumour cells to invade and metastasize is the final of the six hallmarks of cancer. Metastasis enables tumours to survive and grow in new environments where there are no restrictions of space or nutrients. The newly formed secondary tumours can contain cancer cells and also some normal support cells recruited from host tissue. Like any other tumour, metastatic tumours also show a disruption in signaling circuitry as described in the other 5 hallmarks.

However, metastatic tumours display additional unique cellular features, which enable them to change and adapt to their new environments. The precise mechanism of invasion and metastasis is a complex process and still the subject of intense research, but involves a common strategy to physically lodge the cancer cells in the new site by the activation of extracellular proteases.

Cancer cells are like bad magicians, when it comes to invasion and metastasis. The 'Houdini' of cellular world is the leukocyte of the immune system. In response to infection, these cells escape from the circulatory system into surrounding tissues. Leukocytes take less than a minute to persuade endothelial cells on the capillary wall to retract, allowing them make a dignified entry into the tissue to attack infectious agents (a process called dipedesis). Cancer cells however rely on brute force to enter surrounding tissue, mainly because they lack the required biochemical mechanisms needed and it can take them upto 24 hours to complete the process.

Does the risk of metastasis increase with tumour size?

A study on 1589 breast cancer patients, (figure to the right), shows an increased risk of developing distant metastases with increasing crude tumour size. For example, only 22% of women with a tumour size of less than 1cm developed metastasis compared to 77% of women with tumours of more than 10cm. However, the question whether the mutations needed for metastasis is acquired early or late in cancer progression is still unresolved.



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7.1 What are the stages of metastasis?

The migration of cancer cells from the primary location to a distant site is a complex biological process that involves changes at the molecular, cellular and physical level. Briefly, the invasion and metastasis cascade typically involves the following stages (Fig 7.1)

1. **Local invasion**: In this process, the small in situ tumour breaks through the basement membrane barrier
2. **Intravasation**: The tumour cells move through the walls of the capillaries or lymphatics into the circulatory system. This is a critical step in this pathway and it involves a complex, morphological change, wherein the cancer cell acquires properties of invasiveness and cell motility. This enables the cancer cell to push its way through the capillary wall and into the circulatory system.
3. **Transport:** Cancer cells travel through the blood or lymph until they anchor to a solid supporting tissue. Although both blood and lymph are responsible for transport, most of the distant metastases are caused by circulation through the bloodstream. At this stage, most of the cancer cells can be lost or destroyed due to hostile conditions. However, surviving cancer cells get lodged in the first set of capillaries they encounter (mainly due the large cells blocking the small passage of the capillaries) and form microthrombi.
4. **Extravasation**: This final step of migration is essentially similar to intravasation, in which eventually move into the tissues they are lodged in, typically lungs, brain or liver. The main difference is that the direction of movement is reversed. Cancer cells in the microthrombi now push through the capillary wall and into the tissue microenvironment.
5. **Formation of micrometastasis**: Upon extravasation, the cancer cells are now able to reactivate the cell proliferation pathways and form a small tumour mass which either develops in the lumen of the capillary or through the vessel wall.
6. **Colonization:** This is the most complex and challenging stage mainly because the new environment may not always provide the necessary survival and proliferation factors needed for growth. Most cancer cells usually die or survive for long periods as micrometastases (which are much harder to detect).



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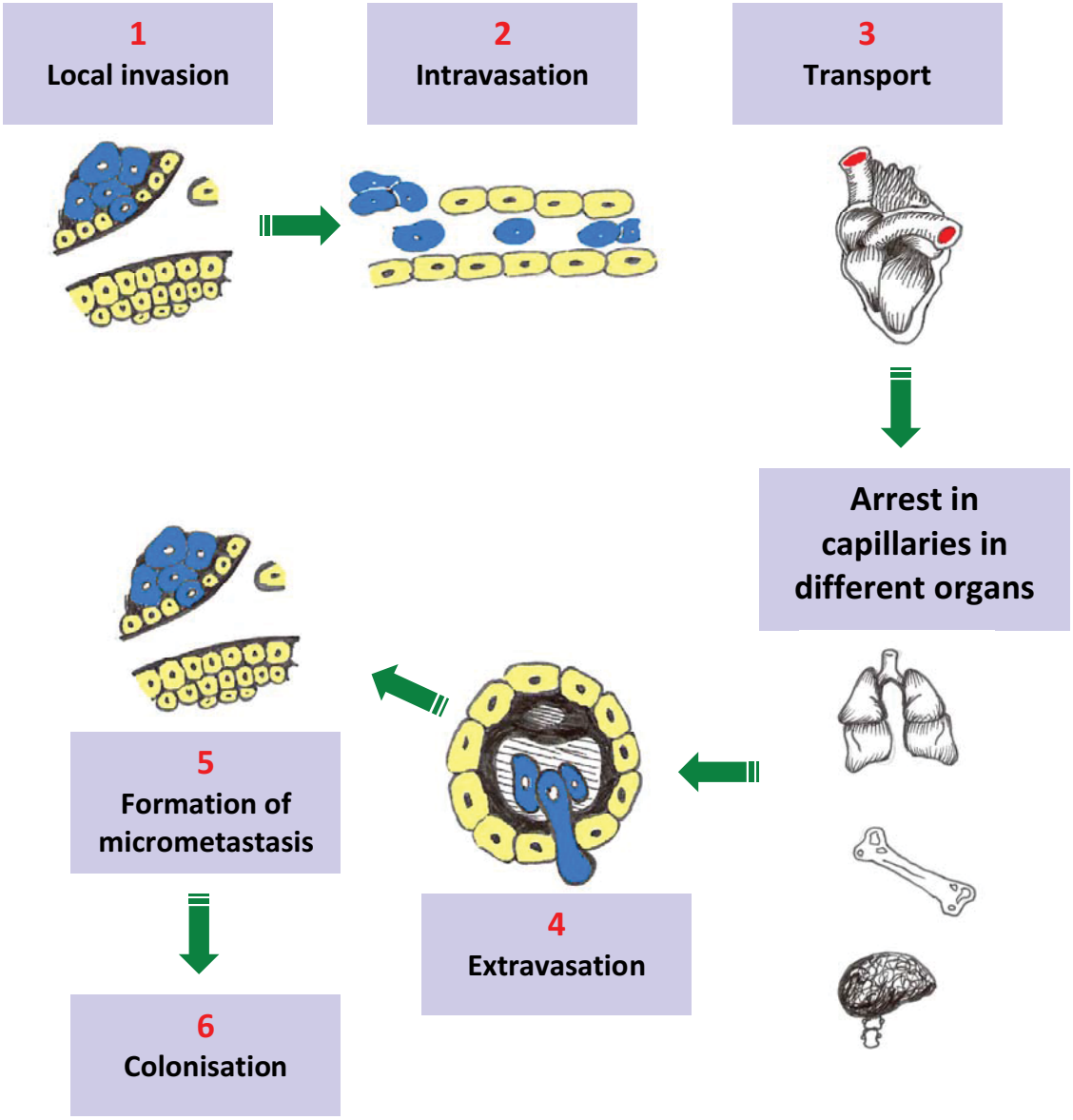


Fig 7.1. Biological pathways of cancer metastasis.

7.2 Key molecules involved in metastasis

However, the completion of all of the 6 steps outlined above does not always occur and the process of metastasis is highly inefficient, with colonization being the least efficient. In order for the tumour cell to become metastatic, several changes are needed to allow intra and extra-vasation as well as colonization. The complexity of this process is exemplified by carcinoma cells in particular. These cancer cells activate a cell differentiation circuit called EMT (Epithelial to Mesenchymal Transition) by inducing expression of several EMT-permissive transcription factors. This allows the cancer cell to acquire characteristics of mesenchymal cells such as invasiveness and motility.

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These changes in characteristics involves several proteins responsible for tissue invasion and spread, and some of the key players are outlined below

a) One of the most important proteins is the cell-cell adhesion molecules (CAMs), whose main role is to tether cells to surrounding tissue. Among the CAMs, the most common protein implicated in metastasis is E-cadherin, found in all epithelial cells. In normal cells, E-cadherin acts as a bridge between adjacent cells, enabling cytoplasmic contact and sharing intracellular signaling factors responsible for inhibiting invasion and metastatic capability. Most epithelial cancers show a loss of E-cadherin function and this elimination plays a significant role in metastatic capability.
b) Another class of proteins involved in tissue invasion are the integrins, a widely distributed family of heterodimeric transmembrane adhesion receptors, which link cells to the extracellular matrix. In addition to their role in angiogenesis, they also play a central role in cell adhesion and migration, control of cell differentiation, proliferation and survival. Changes in integrin expression are also evident in invasive and metastatic cells. Successful colonization of new sites (both local and distant) demands adaptation, which is achieved by changing integrin subunits displayed by the migrating cells. For example, carcinoma cells facilitate invasion by preferentially expressing integrin subunits needed for binding to degraded stromal components by extracellular proteases.
c) Another strategy in successful colonization is increasing expression of extracellular proteases (such as MMPs – Matrix MetalloProteinases) while decreasing levels of protease inhibitors. Cells in the stroma close to cancer cells secrete active proteases, which facilitate invasion by degrading components of the extracellular matrix. This enables cancer cells to migrate across blood vessel boundaries and through normal epithelial cell layers.

All the proteins listed above are vital for invasion and metastatic ability. However, the regulatory and molecular circuits seem to differ in various tissue environments and their precise roles in different types of tumours are highly variable. Invasion and metastasis represents the last great frontier for exploratory cancer research. The challenge is to apply the new molecular insights about tissue invasiveness and metastasis to the development of effective therapeutic strategies.

7.3 Bibliography

1. Chapter 14: Biology of Cancer by RA Weinberg (Garland Publishing)
2. Chapter 12: Cancer Biology by RJB King (2e)

7.4 Further Reading

1. Klein, C (2008) *Cancer: The metastasis cascade.* Science, Sept 26, Vol 321, pp. 1785–87.
2. Thiery JP, Acloque H, Huang RY, Nieto MA. (2009) *Epithelial-mesenchymal transitions in development and disease*. Cell. Nov 25; Vol 139(5), pp. 871–90.

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8 Summary and some thoughts for the future

The six hallmarks of cancer described so far suggests that tumour progression occurs due to DNA mutations in genes involved in regulating cellular signaling and metastatic pathways. However, genomic integrity is maintained fastidiously by an army of monitoring and repair enzymes in the cell. Order of chromosomal duplication is also zealously guarded at cell cycle checkpoints. Therefore mutations should be extremely rare events, so rare that they should not occur over a human's lifetime.

Yet cancers occur in 33% of the human population on average. Does this mean that tumours must somehow have a higher mutation rate than predicted with the signaling pathways? One school of thought argues that perhaps mutations in the 'caretaker' or guardian genes are responsible for this increased mutability. The most compelling evidence for this argument comes from mutations in the p53 tumour suppressor protein, found in the vast majority of human tumours. It plays a pivotal role in determining cell fate in response to DNA damage; should cell cycle arrest and repair pathways be switched on or should the cell be allowed to die by apoptosis? Moreover, other proteins involved in sensing and repair have also found to be functionally lost in different cancers. The genome instability generated as a result is perhaps another vital pre-requisite needed for tumour progression to all the six biological hallmarks.



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The acquisition of the various mutations in the signaling circuitry described so far, are highly variable in terms of time and tissue type. For example, mutations in p53 may be found in a subpopulation of otherwise identical tumours or some oncoproteins may be expressed early in some tumour types but late in others. Therefore, the sequence of expression of the six hallmarks of cancer can vary widely across tumours of the same type and most certainly in different tumour types. Nonetheless, almost all cancers eventually display the same six hallmarks of tumour progression.

Research into the biology of cancer have yielded a wealth of information on cancer initiation and progression, and has also resulted in dramatic new drugs in the fight against cancer. Simplifying and dissecting the pathways involved in each of these signaling pathways have been vital in understanding this process. On the other hand, the danger of simplification is that it reflects little of the biological reality of cancer progression in human patients in vivo. Empirical evidence in vitro have often failed to match the results from in vivo studies. This has led to profound rethinking and of how we study cancer experimentally. Systems level approaches (heterotypic organ cultures in vitro, refined animal models and even translational medicine) will help us define comprehensive maps in cancer signaling networks

New technologies have enabled us to identify epigenetic modifiers (agents that modify expression without altering the DNA sequence – such as microRNAs) located either within the cancer cell or elsewhere in the body. It is most likely that in the near future, diagnosis of all cancers will rely on routine expression profile analysis of the array of underlying genetic mutations and/or their epigenetic modifiers. Emerging technologies may also help us understand the complex interactions between various components of these complex signaling networks. It is only a matter of time before the 'complete integrated circuit' of the cancer cell can be completed and we understand why cancer drugs work or fail.

The goals for the future will be to detect and identify all stages of disease progression, prevent cancer from developing, while curing pre-existing cancers.

8.1 Further Reading

1. Laird, PW (2005) *Cancer epigenetics*. Human Molecular Genetics. April 15;14 Special No 1:R65–76.
2. Frank, SA (2004) *Genetic predisposition to cancer: insights from population genetics.* Nature Reviews Genetics. Oct; Vol 5(10), pp. 764–72.
3. Hatina, J and Schulz, WA. (2008) *Cancer Stem Cells – Basic Concepts*. In: www.els.net (Standard article) Encyclopedia of Life Sciences (ELS). John Wiley & Sons, Ltd: Chichester. DOI: 10.1002/9780470015902.a0021164.