Understanding the genetic basis of cancer and its treatments


In the Western world one in three of us will have cancer during our lifetime and the likelihood of having a tumour significantly increases as we live longer. Older cancer treatments have drawbacks. For example, surgical removal of a tumour is not always possible — some sites are inoperable — and does not deal with metastases, and until recently chemotherapy was limited to the use of non-specific cytotoxic agents, which resulted in side effects such as sickness and hair loss.

The development of imatinib (Glivec) was a success story in the design of target-specific cancer therapy. Around 95 per cent of patients with chronic myeloid leukaemia carry a reciprocal chromosomal translocation between segments of chromosomes 9 and 22, resulting in a fusion of BCR and ABL genes on the rearranged chromosome 22 (Philadelphia chromosome). The fused gene expresses BCR-ABL protein, which drives cell proliferation and causes leukaemia. BCR-ABL protein is an ideal target for cancer treatment because it is present exclusively in aberrant cells. The search for a molecule that could inhibit it culminated in the launch of Gilvec in 2002. In early stage leukaemia imatinib has a 96 per cent remission rate (efficacy declines with the advance of the disease).

There is a growing need to understand the differences between cancer cells and normal cells at a molecular level both to appreciate how new therapies work and to help design future medicines. Awareness of the underlying causes and complex pathways that lead to an aggressive tumour can help pharmacists to:

- Educate the public on minimising exposure to environmental risk factors
- Understand the mechanism of action of cancer drugs
- Support patients in adhering to treatment

Cancer is a genetic disease in the sense that it originates from alterations in DNA. These result in the deregulation of vital proteins necessary for normal cell function. The link between DNA and cancer was supported by a number of studies demonstrating that several carcinogens damage genetic material. A correlation between the carcinogenic potential of such compounds and their mutagenicity was published in 1975. Repeated observations of chromosomal aberrations, in both numbers and structure, in cancer cells confirmed the involvement of genetic material in tumour formation.

The search for the genes involved in uncontrolled cell division was based on an observation by Rous in 1910: when the breasts of chickens with breast sarcomas were ground, liquefied, passed through a bacteria-retaining filter and injected into healthy chickens, the animals developed similar breast cancer. The conclusion was that the causative agent must be a virus, Rous sarcoma virus (RSV). Rous’s observation was largely left unexplored until the 1970s when RSV was found to contain a “transforming gene”. A normal retrovirus had infected the chickens and acquired, through its normal life cycle, a cellular gene that established itself as part of the genetic makeup of the new, transformed virus (RSV). This gene was called src. The normal function of its protein product in the chicken is to drive cell growth and division. When RSV, carrying the src gene, infects healthy chickens its DNA embeds within the genome and can be passed to the next generation.

Reflect on knowledge gaps

1. What is the significance of the p53 gene?
2. What are the major classes of cancer gene?
3. Can you give an example of a target-specific cancer medicine and outline its mode of action?

Before reading on, think about how this article may help you to do your job better.
Genes involved in cancer

In order to understand, the genetics involved in cancer, readers need to recall the cell cycle (see Panel 2, p3).

Oncogenes

Activating alterations in the proto-oncogenes leads to oncogenes and to protein products that promote cell proliferation. Amplification of a proto-oncogene through a virus is only one way of converting it to an oncogene. Mutations through chemical (eg, benzopyrines in cigarette smoke) or physical attack (eg, excessive UV radiation) or through errors of DNA replication are other important mechanisms but chromosomal translocations and epigenetic alterations can also result in oncogenes. These activating mutations lead to the gene product gaining a function and dominance so that a single copy of the mutated gene is sufficient for cell proliferation. Oncogenes encode proteins that can be classified into six subgroups, including growth factors and signal transducers.

Growth factors

Growth factors are proteins released by cells to promote growth and proliferation. Examples are platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), erythropoietin, epidermal growth factor (EGF), nerve growth factor and hepatocyte growth factor. Mutations in genes can cause overproduction of these proteins but this could be inhibited by specific antibodies or small molecules. For example, bevacizumab (Avastin) is a monoclonal antibody against VEGF. It is designed to inhibit the formation of new vasculature required by an expanding tumour mass. (The requirement for the formation of new blood vessels in a healthy adult is low but, in the presence of a growing tumour, high level of VEGF is expressed to initiate growth of new vasculature.) Bevacizumab has a cytostatic rather than cytotoxic effect on cancer cells, hence the need for repeated intravenous infusions. It is currently indicated primarily for metastatic cancers of the colorectal area, breast and kidney.

Growth factor receptors

Proteins act as receptors for the growth factors in the passage of growth signals to the cell nucleus. Various mutations have been found to activate the receptors without the need for ligand-binding. A spectrum of anti-cancer agents has been developed to inhibit over-active growth factor receptors. Trastuzumab (Herceptin) and cetuximab (Erbitux) are monoclonal antibodies against the external domain of EGF receptor.

Trastuzumab is a specific inhibitor of a subtype of EGF receptor called ERBB2 (or HER2). The gene encoding ERBB2 was found to be amplified in about 30 per cent of breast cancers, hence the need to confirm the over-expression of this subtype of protein before starting trastuzumab in a patient.

Phosphorylation is essential for signal transmission and is achieved by protein kinases, of which there are many. Tyrosine kinase, for example, phosphorylates tyrosine residue. Erlotinib (Tarceva) and gefitinib (Iressa) are both small molecules that inhibit the ability of the endoplasmic domain of EGF receptor to phosphorylate tyrosine residue. This means that they prevent signal transmission and, therefore, cell proliferation.

Panel 1: Cancer-causing viruses

Oncogenic viruses are broadly classified into two categories depending on the nature of their genomes.

RNA tumour viruses

Retroviruses have a genome made up of a single stranded RNA molecule. They can cause cancers in animals through the action of a carried mutated variant of a cellular gene controlling growth and division. Retroviruses replicate by integrating their genomes, following conversion to DNA by the action of reverse transcriptase, with the host DNA and using the host machinery to express their own genetic material and proliferate. Examples include Rous sarcoma virus and Ableson murine leukemia virus, which causes B-cell lymphoma in mice.

A few retroviruses can cause cancer not because they carry an oncogene but due to their integration site with the host DNA. If this site disrupts an important proliferation-linked regulatory gene within the host genome uncontrolled cell division could result. An example of such a retrovirus is avian leukosis virus, which causes B-cell lymphoma in chickens.

A third way in which retroviruses can cause cancer has been documented with the only cancer-causing retrovirus known to infect humans, human T-cell leukaemia type 1 virus. A protein encoded by a normal gene of this virus can stimulate proviral DNA, which can integrate with the host genome, and drive expression of a number of cellular proliferation genes.

DNA tumour viruses

The genome of DNA tumour viruses consists of a double stranded DNA molecule. These viruses can replicate extra-chromosomally within host cells and, on occasions, integrate with host DNA. Examples include human papilloma virus, which is strongly associated with cervical cancer, and Epstein-Barr virus, which causes nasopharyngeal carcinoma. Tumours, in these cases, arise from proteins encoded by the virus's own proteins interacting with products of proto-oncogenes or tumour suppressor genes residing in the host DNA. Vaccines against cervical cancer contain antigenic components of the most commonly encountered HPVs to elicit an appropriate immune response.

In order to control their replication, retroviruses have evolved two strategies to protect them against the immune system. One is to encode proteins that can mask their antigens, making them non-immunogenic. The other strategy is to render these targets as immunogenic and thus vulnerable to the host immune system. To do this, oncogenic retroviruses often insert themselves into the cellular DNA of their host as integrated DNA copies. When the host cell undergoes mitosis (cell division), the DNA copy is replicated and incorporated into each new cell's genome, where it can act in a continuous manner to cause cancer.

The integration of retrovirus DNA may occur in many different locations within the host genome. However, the integration process is not random. Human papillomaviruses (HPVs), for example, preferentially integrate into specific sites within the host genome. These sites are often associated with regions of DNA that are highly amplified or deleted in cancer cells. This finding suggests that integration at these specific locations may have a selective advantage for the virus in promoting cancer development. In fact, certain HPV integration sites have been found to be associated with increased expression of particular genes, such as those involved in cell proliferation.

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New cells go through four phases known as the cell cycle: G1 (a growth phase where enzymes are made), S (a synthesis phase where DNA replication occurs), G2 (a second growth phase, where proteins, particularly those involved in cell division, are made) and M (where the cell divides into two and the duplicated chromosomes are allocated equally between the daughter cells [mitosis]).

Non-proliferative and fully differentiated cells usually enter a quiescent state, known as G0, from the G1 phase and remain as such for long periods — sometimes indefinitely as in the case of neurons. Cells can also enter G0 phase following damage to their DNA and remain there until the error is repaired.

The cell cycle is controlled and regulated by a plethora of gene products (proteins) that help detect and repair DNA damage and provide various checks to stop uncontrolled cell division. Among these, proteins are crucial in determining the progress of the cell through the cycle. These are cyclins and cyclin-dependent kinases (CDKs).

Different cyclins and CDKs work together as complexes. To illustrate, the Figure includes a cancer gene product called retinoblastoma (RB) protein. In this pathway, cyclin D is produced in response to signals from growth factors. It binds to a subtype of CDK, CDK4, produced by the cell, forming an activated complex which, in turn, phosphorylates the RB protein. This protein is normally associated with a transcription factor called E2F and this association is facilitated by the enzyme retinoblastoma protein. In this pathway, cyclin D is produced in response to signals from growth factors. It binds to a subtype of CDK, CDK4, produced by the cell, forming an activated complex which, in turn, phosphorylates the RB protein. This protein is normally associated with a transcription factor called E2F and this association effectively blocks E2F from transcribing its target genes.

Phosphorylation of RB causes the RB-E2F complex to dissociate thus releasing E2F to transcribe its target genes, which include genes expressing cyclin A, cyclin E and DNA polymerase that play crucial roles in subsequent phases of the cell cycle and drive the cell to enter the S phase. In a similar way, a CA-CDK2 complex controls cell progression through the S phase where cyclin A and cyclin E are expressed in the S phase of the cell cycle and was found to be over-expressed and deregulated in a number of human tumours. Burkitt’s lymphoma is an often quoted example. In this disease, a chromosomal translocation fuses part of chromosome 8 with an immunoglobulin gene locus of chromosomes 2, 14 or 22. This places the MYC gene in front of a segment of DNA called immunoglobulin promoter, which normally responds to infections by eliciting immunoglobulin production. Hence there is a strong association between prior infections and the development of Burkitt’s lymphoma because MYC proteins are produced at a high rate.

**Signal transducers** Several oncogene products function as signal transducers. RAS proteins, which are associated with the cell membrane and pass signals to a number of downstream molecules, are examples. Mutations in RAS proteins are widely involved in a number of human cancers, ranging from about 30 per cent of lung cancer cases to 90 per cent of pancreatic cancer cases. Several attempts have been made to target this group of proteins in cancer therapy, particularly their association with the cell membrane, which is facilitated by the enzyme farnesyltransferase. Inhibition of farnesyltransferase can, in theory, prevent RAS protein from associating with the membrane and from picking up a growth signal. Lonafarnib and tipifarnib, are two such inhibitors that have been trialled.

**Transcription factors** Some gene products normally interact with other proteins to form complexes that initiate gene expression. An example is the FOS protein, which interacts with another transcription factor, JUN, to form a transcription complex targeting a number of genes that participate in cell growth and division.

Another important transcription factor is MYC, which functions as a heterodimer with a factor called MAX. The MYC gene is expressed in the S phase of the cell cycle and was found to be over-expressed and deregulated in a number of human tumours. Burkitt’s lymphoma is an often quoted example. In this disease, a chromosomal translocation fuses part of chromosome 8 with an immunoglobulin gene locus of chromosomes 2, 14 or 22. This places the MYC gene in front of a segment of DNA called immunoglobulin promoter, which normally responds to infections by eliciting immunoglobulin production. Hence there is a strong association between prior infections and the development of Burkitt’s lymphoma because MYC proteins are produced at a high rate.

**Apoptosis regulators** Growth and division is not only controlled by proliferative signals but also by programmed cell death signals. Apoptosis is triggered via two major pathways: extrinsic (through death receptors) and intrinsic (through the mitochondria). The extrinsic pathway is triggered by ligands such as FAS and tumour necrosis factor (TNF), while the apoptotic stimuli of the mitochondria include radiotherapy and chemotherapy.

The BCL2 family of downstream proteins have a central controlling role as proapoptotic and antiapoptotic signals, turning cell death on or off through the release of cytochrome c. Some anti...
Panel 3: p53 gene

The p53 protein is sometimes called the guardian of the genome because it plays a central role in controlling cell division. It responds to a variety of stresses, such as DNA damage, imbalances in growth signalling, absence of nucleotides (precursors for DNA synthesis) and hypoxia. It responds to DNA damage by either halting the cell cycle and repairing the damage or, if the damage is beyond repair, initiating apoptosis. It also inhibits angiogenesis.

The p53 gene is located on chromosome 17 and is mutated in up to half of the commonly occurring cancers. Most p53 mutations result in a substitution of one amino acid. In about 90 per cent of the cases the location of this substitution occurs in the part of p53 protein that binds to DNA when acting as a transcription factor. This alters the ability of p53 protein to bind to its target DNA sequences and transcribe the downstream genes dependent on p53 for their regulation.

p53 protein mutations are the most common in human cancer and restoration of p53 function is an appealing cancer strategy. Gene therapy to introduce an exogenous p53 gene through the use of a viral vector is one approach although many drawbacks have yet to be resolved. Suppressing the viral inhibitors of p53 is another possibility. In brain cancers, where the activity of p53 protein is often lost, restoring the function of p53 protein has been attempted using small molecules such as PRIMA-1. PRIMA-1 stimulates the DNA binding of the mutated p53 protein and hence induces the expression of its target genes despite the mutated nature of the protein.

Apoptotic members of BCL2 proteins were found to be upregulated in cancers such as follicular lymphomas, lung cancer and chronic lymphocytic leukaemia. The ultimate effectors of apoptosis include an array of proteases called caspases. Several investigations have been carried out to exploit the apoptotic pathways in an effort to induce cell death and to arrive at a suitable anti-cancer drug through targeting either BCL2 protein or the caspases. Bortezomib (Velcade) is a proteasome inhibitor approved for the treatment of multiple myeloma. Proteasome is a large protein complex that degrades many proteins within the cell, including those regulating proliferation through their antiapoptotic effects. Inhibiting this complex results in cell death.

Chromatin remodelers Chromatin is the composite material of DNA, RNA and proteins that makes up chromosomes. Chromatin remodelers act on chromatin epigenetically, altering its degree of compaction (rather than the DNA sequence) and, therefore, controlling gene expression, DNA replication, DNA repair and chromosome segregation. An example is the ALL1 gene. The product of this gene is a protein that forms a part of a large transcriptional complex that regulates the expression of a number of other genes through chromatin remodelling. The fusion of ALL1 protein, as seen in some leukaemias, deregulates a number of its target genes such as those encoding transcription factors. Several drugs acting on enzymes involved in remodelling the chromatin are in different stages of development, including DNA methyltransferase inhibitors, histone deacetylase inhibitors and aurora kinase inhibitors.

Tumour suppressor genes Under normal conditions cells not only receive and process growth stimulatory signals but also deal with growth inhibitory signals. Tumour suppressor genes (TSGs) play an important role in the pathways of these inhibitory signals and loss of their function could lead to tumour formation. Unlike oncogenes, for TSG products to be absent or inactive both copies of the gene must be mutated and lose their function.

The first TSG to be discovered was the RB gene — absence of a functional copy of the RB gene leads to a rare form of eye tumour. An example of a growth inhibitory molecule is transforming growth factor β (TGFβ). A variety of normal cells will stop growing when this protein is applied to them in low concentration. When cells lose RB gene function they lose responsiveness to TGFβ and continue to grow.

Usually a mutation in a copy of TSG is inherited and cells carrying that copy are said to be heterozygous for that gene variant. If another mutation hits the same gene, the loss of function gives rise to a lineage of hyperplastic cells. Such cells exhibit loss of heterozygosity and this is an indicator of the advance of the neoplastic process. Loss of heterozygosity could occur in a number of ways, including deletion, point mutations and epigenetically driven mechanisms.

Other examples of TSGs include P53 (see Panel 3) and CDK4.

Genome-stability genes Genome stability genes are DNA repair genes controlling the rate of mutations and being capable, through their influence on oncogenes and TSGs, of driving the initiation and progression of tumours. Examples include MSH2, MLH1, MSH6 and PMS2 (associated with colon and uterus cancers), ATM (associated with leukaemias and lymphomas) and BRCA1 and BRCA2 (associated with breast and ovarian cancers). MicroRNA genes MicroRNA genes do not encode proteins and their functional products consist of a single strand of RNA, usually 21 to 23 bases long, affecting the regulation and expression of other genes. They can anneal to messenger RNA blocking protein synthesis. A microRNA gene can be an oncogene or TSG depending on its complementary target sequence. Examples include miR191, miR15a, and miR155. MiR191 is found to be upregulated in many solid tumours and miR155 is also found to be over-expressed in diffuse large B-cell lymphomas, breast cancer and lung cancer. The unique patterns of expression of various microRNAs in cancers are currently being investigated as possible diagnostic and prognostic markers.

Complex pathways in context

By now, readers will appreciate the complexity of the control exerted by cells on growth and division. The neoplastic process in humans is a multi-step process involving several mutational events. Disruption of pathways involving oncogenes, TSGs and microRNAs liberates the cells from the normal controls.
mutations in malignant tumour. For example, inherited mass of growing cells to progress into a cell perspective). However, the removal of (providing a survival advantage from the other “unmutated” copy of the gene and to accumulate further mutations that could eventually lead to cancer.

Cells have a limited capacity to replicate and, in a process called senescence, will stop growing after a number of divisions (this is usually 60–70 divisions in their lifetime a limit known as the Hayflick limit). Senescence must also be disrupted if the cells are to continue dividing. The state of chromosomal ends (telomeres) determines the number of divisions a cell can undergo. Every time a cell divides a telomere loses 50–100 nucleotides and this progressive shortening will eventually lead to loss of protective function and a state of chromosomal aberrations, followed by cell death if that cell continues in that path. Cancer cells, however, have found ways to maintain their telomeres mostly through up-regulating telomerase, the enzyme responsible for repairing telomeres. This enables cells to continue to multiply. Telomerase can be considered as a potential target for cancer therapy and human trials are under way to explore telomerase inhibition and even telomerase vaccination.

In order to continue their survival, cancer cells must have required nutrients and a means of eliminating waste products. They also have to be within 0.1mm of a blood capillary vessel. Angiogenesis occurs through growth and division of endothelial cells from neighbouring blood vessels in response to upregulated proteins such as VEGF and fibroblasts growth factors 1 and 2.

Cancer cells will invade neighbouring tissues and vessels and, eventually, move out to distant tissues. Cadherin proteins play a vital role in cell-cell adhesion and the transmission of anti-growth signals. The function of cadherins is absent in many epithelial cancers. Cadherins serve as suppressors of metastasis and their elimination represents a key step in the acquisition of cancer. Integrins, another family of proteins, also appear to play an important role in invasion and metastasis.

Finally, the slow rate of mutations does not alone explain the relative prevalence of cancers. Genome instability due to malfunctioning repair genes has been suggested as a possible reason for a cell to undergo several, relatively quick, sequential mutations. Chromosomal instability, on the other hand, is more common in cancer cells, particularly solid tumours, manifesting on the molecular levels in various ways. One common manifestation is loss of heterozygosity owing to the loss of an important TSG allele. The mechanisms underlying chromosomal instability are still largely unknown but candidate genes such as CDC4 (also known as FBXW7) have been proposed.

As we live longer, the genes in our cells acquire more mutations. Most of these are either repaired or the cells containing them are eliminated. On rare occasions a progenitor cell can escape the sophisticated control system due to a particular gene defect and begin to multiply uncontrollably. On acquiring further mutations, cells of that genetic abnormality can become cancerous and spread to other parts of the body.

An emerging complex network of pathways controlling cell proliferation and tumour formation is beginning to be identified. Important elements of this network are continuously being sought and discovered with the aid of the post-genomic advances. Further target-specific products are likely to be introduced in the future, affecting pathways in angiogenesis, metastasis and the immune response, eventually replacing conventional chemotherapy. Moreover, epigenetic drugs, acting to modify the chromatin, will be refined to overcome their lack of specificity. The prospect of discovering new pathways and networks together with detailed patterns of DNA methylation and histone modification will help unravel the complex nature of cancer and will aid diagnosis and successful treatment.

References

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Act: practice points

Reading is only one way to undertake CPD and the Society will expect to see various approaches in a pharmacist’s CPD portfolio.

1. Set up a smoking cessation service and participate in sun protection campaigns.
2. Advise on the benefits of prevention programmes such as breast cancer screening, bowel cancer screening and cervical cancer vaccination.
3. Find out what regimens are used for treating different cancers in local hospitals.

Evaluate

For your work to be presented as CPD, you need to evaluate your reading and any other activities. What have you learnt? How has it added value to your practice? (Have you applied this learning or had any feedback?) What will you do now and how will this be achieved?

Record

Consider making this activity one of your nine CPD entries this year.