2

The scientific basis of cancer

Learning objectives

✓ List the six hallmarks of cancer with examples
✓ Describe the enabling and emerging characteristics of cancers
✓ Classify the epigenetic changes that contribute to cancers
✓ Explain the hereditary and environmental causes of cancers with examples

The hallmarks of cancer

At a molecular level, six basic steps or "hallmarks" that turn a cell into a cancer were described in 2000. In 2011, a further two "enabling hallmarks" were added that contribute to the ability of cells to acquire the six hallmarks and a further two "emerging hallmarks" required for cancer cells to continue to survive as tumours.

The original six hallmarks of cancer are:

1. Cancer cells grow by themselves, with no need for external growth stimuli.
2. Once started, cancer cells continue to grow and will ignore any external signals telling them to stop.
3. Cancer cells bypass the normal cell mechanisms for planned cell death, known as apoptosis.
4. Cancer cells remain forever young by replenishing their own stocks of telomeres. As normal cells age they lose telomeres, leading eventually to an inability to divide.
5. Cancer cells can feed their own tumours through neoangiogenesis.

6. Cancer cells can spread to distant organs via metastases.

It is probable that all six are necessary to a greater or lesser extent. Some molecular changes may contribute to more than one of the six capabilities (e.g. a mutation on the p53 gene may contribute to both avoidance of apoptosis and insensitivity to inhibitory stimuli).

A number of mechanisms may contribute to the acquisition of these six properties, including genomic instability as a consequence of deficient DNA repair (lack of caretaking) or loss of cell cycle arrest/death in response to DNA damage (lack of gatekeeping). Many new treatment strategies for cancer aim to interrupt these steps.

The two enabling hallmarks of cancer are:

7. Cancer cells are prone to mutations due to genome instability.
8. Inflammation promotes the growth of cancers.

The two emerging hallmarks of cancer are:

9. Cancers avoid destruction by the host immune system.
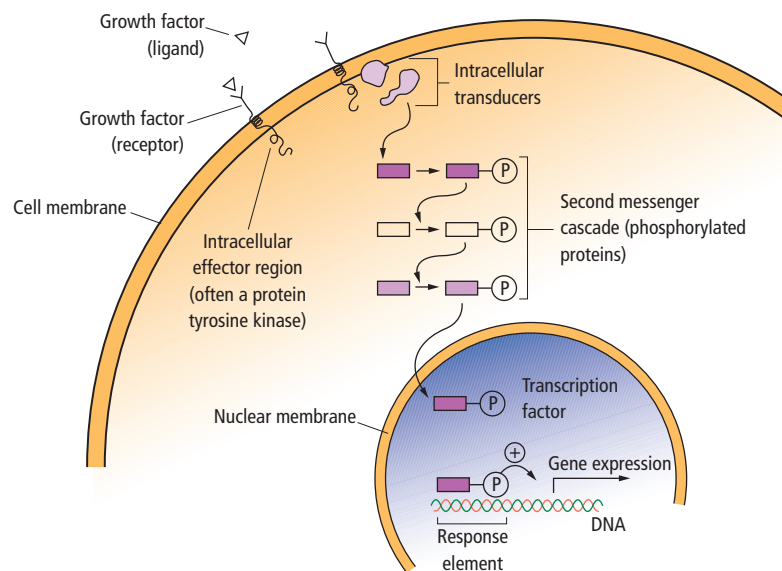10. Cancer cells deregulate cellular metabolism.

Figure 2.1 Signal transduction pathway.

1. Sustaining proliferative signalling (growing by themselves)

The instructions to cells to grow and start dividing are transmitted to cells by extracellular growth factor ligands that bind cell surface receptors. This results in the reversible phosphorylation of tyrosine, threonine or serine residues. The transfer of these molecular switches from activated receptors to downstream nuclear transcription activators is known as signal transduction (Figure 2.1). This cascade results in amplification of the initial stimulus.

Cancers achieve self-sufficiency in growth factors and are not dependent on extracellular concentrations of growth factors for continued growth. The majority of dominant oncogenes act on this mechanism by one of the following mechanisms:

- Overproducing growth factors, for example, glioblastomas produce platelet-derived growth factor (PDGF)
- Overproducing growth factor receptors, for example, epidermal growth factor receptor (EGFR/erbB) overexpression in breast cancers
- Mutations of the receptor or components of the signalling cascade, which are constitutively active, for example, mutations of Ras in lung and colonic cancers

2. Evading growth suppressors

Many normal cells grow throughout their lifespan and the co-ordination of their growth, differentiation, senescence and death is controlled by the cell cycle. Antiproliferative signals may be received by cells as soluble growth inhibitors or fixed inhibitors in the extracellular matrix. They act on the cell cycle clock (Box 2.1 and Figure 2.3), most frequently arresting transit through G1 into the S phase. Cancer cells ignore these "STOP" signals.

The co-ordination of the cell cycle and its arrest at checkpoints in response to DNA damage is achieved by sequential activation of kinase enzymes that ultimately phosphorylate and dephosphorylate the retinoblastoma protein (Rb). Periodic activation of these cyclin–cyclin-dependent kinase (CDK) complexes drives the cell cycle forward (Figure 2.2). Phosphorylation of Rb releases E2F, a transcription factor, which is then able to promote the expression of a number of target genes resulting in cell proliferation. The brakes that balance this system are CDK inhibitors (CKIs). Interference in elements of the cell cycle regulatory process is a common theme in malignancy (Table 2.1).

Examples of independence of cell cycle checkpoints:

- Cancer cells may overproduce cyclins (e.g. cyclins D and E) and CDKs (e.g. Cdk2 and Cdk4).
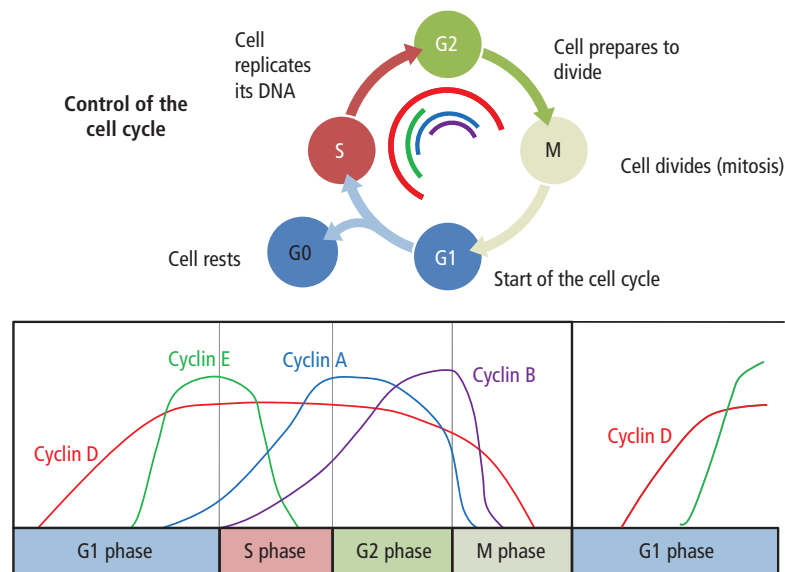
Figure 2.2 Oscillating levels of cyclins through the phases of the cell cycle.

- Cancer cells under-express or have mutations of CKIs (e.g. p16).
- Cancers have germline or somatic mutations of tumour suppressor genes (p53 and Rb).
- Human papillomavirus (HPV) oncoproteins inactivate Rb and p53.

G1/S checkpoint

An important checkpoint or restriction point in the cell cycle occurs in G1 to ensure that errors in DNA are not replicated, but instead are either repaired or that the cell dies by apoptosis. This is initiated by

Table 2.1 Examples of the six features and their molecular basis in cancers

Feature	Colorectal cancer	Glioma	Head and neck squamous cancer
1. Growth factor independence	*KRAS* mutation	EGFR amplification or mutation NF1 loss	EGFR mutation
2. Over-riding inhibitory signals	SMAD2/SMAD4 mutation	CDK4/p16 mutation	Cyclin D amplification p16 and p21 mutation
3. Evasion of apoptosis	p53 mutation	p53 mutation/MDM2 overexpression	p53 mutation
4. Immortalization	hTERT re-expression	hTERT re-expression	hTERT re-expression
5. Angiogenesis	VEGF expression	PDGF/PDGFR overexpression	Nitric oxide pathway activation of VEGF
6. Invasion and metastasis	APC, inactivate E-cadherin	Cathepsin D, MMP-2 and -9 and UPA overexpression	Cathepsin D, MMP-1, -2 and -9 overexpression

APC, adenomatous polyposis coli gene; EGFR, endothelial growth factor receptor; hTERT, human telomerase reverse transcriptase; MMP, matrix metalloproteinase; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; VEGF, vascular endothelial growth factor; UPA, urokinase-type plasminogen activator; SMAD, small mothers against decapentaplegic; CDK, cyclin-dependent kinase; *KRAS*, Kirsten rat sarcoma viral oncogene.

damaged DNA and is co-ordinated by p53, the gene that is probably most commonly mutated in cancers overall. Additional checkpoints are present in the S and G2 phases to allow cells to repair errors that occur during DNA duplication and thus prevent the propagation of these errors to daughter cells.

> ### Box 2.1 The cell cycle (Figure 2.3)
>
> There are five cell cycle phases:
>
> #### Quiescent phase (G0)
> Normal cells grown in culture will stop proliferating once they become confluent or are deprived of growth factors and enter a quiescent state called G0. Most cells in the normal tissue of adults are in a G0 state. Once the cell leaves G0, it starts the cell cycle:
>
> #### First gap phase (G1) (duration 10–14 hours)
> This occurs prior to DNA synthesis. Cells in G0 and G1 are receptive to growth signals, but once they have passed a restriction point are committed to DNA synthesis (S phase).
>
> #### Synthesis phase (S) (duration 3–6 hours)
> During this phase DNA replication occurs and the cell becomes diploid.
>
> #### Second gap phase (G2) (duration 2–4 hours)
> This occurs after DNA synthesis and before mitosis (M) and completion of the cell cycle. There is an important checkpoint at this stage to ensure there have been no duplication mistakes before mitosis and cell division.
>
> #### Mitosis (M) (duration 1 hour)
> Cell division completes the cell cycle.

3. Resisting cell death

Apoptosis is a pre-programmed sequence of cell suicide that occurs over 30–120 minutes. Apoptosis commences with condensation of cellular organelles and swelling of the endoplasmic reticulum. The plasma membrane remains intact, but the cell breaks up into several membrane-bound apoptotic bodies, which are phagocytosed. Confining the process within the cell membrane reduces activation of both inflammatory and immune responses, so that programmed cell death does not cause autoimmune disease or inflammation. Amongst the molecules that control apoptosis are the Bcl-2 family that confusingly includes both pro-apoptosis members (e.g. Bax) and anti-apoptosis members (e.g. Bcl-2).
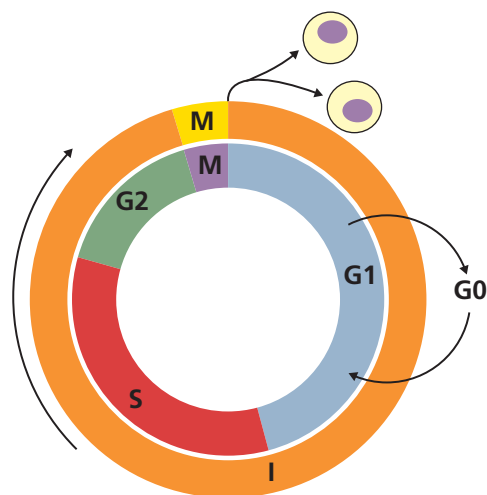

Figure 2.3 The cell cycle.

In mammalian cells two pathways initiate apoptosis (Figure 2.4):

1. **Intracellular triggers:** DNA damage leads via p53 to activation of pro-apoptotic members of the Bcl-2 family. This causes release of cytochrome c from mitochondria, which in turn activates the caspase (**c**leaves after **asp**artate prote**ase**) cascade.
2. **Extracellular triggers:** Binding of extracellular ligands to the cell surface death receptor superfamily (including CD95/Fas and tumour necrosis factor (TNF) receptors) leads to a death-inducing cytoplasmic signalling complex that activates the caspase cascade.

Ultimately both pathways activate the caspase cascade, a series of protease enzymes that result in cell apoptosis. Evasion of this pathway is a prerequisite for malignant cell proliferation and a number of strategies to this end have been identified (Table 2.1).

4. Enabling replicative immortality

In culture, cells can divide a limited number of times, up to the "Hayflick limit" (60–70 doublings in the case of human cells in culture), before the cell population enters crisis and dies off. This senescence is attributed to progressive telomere loss, which acts as a mitotic clock (Figure 2.5). Telomeres are the end segments of chromosomes and are made up of thousands of copies of a short six-base pair sequence (TTAGGG).
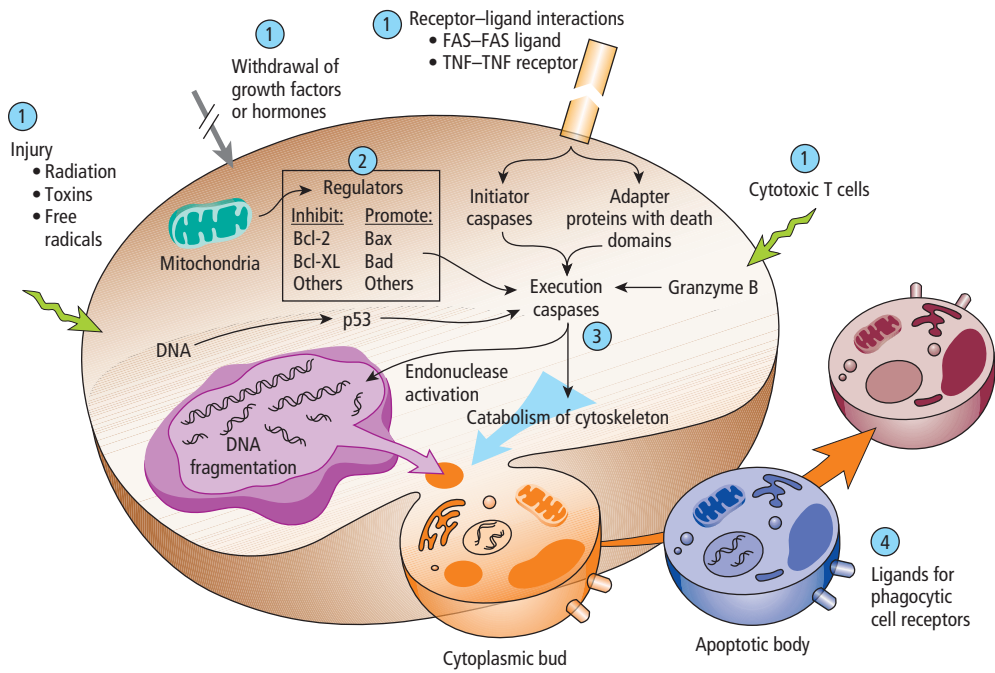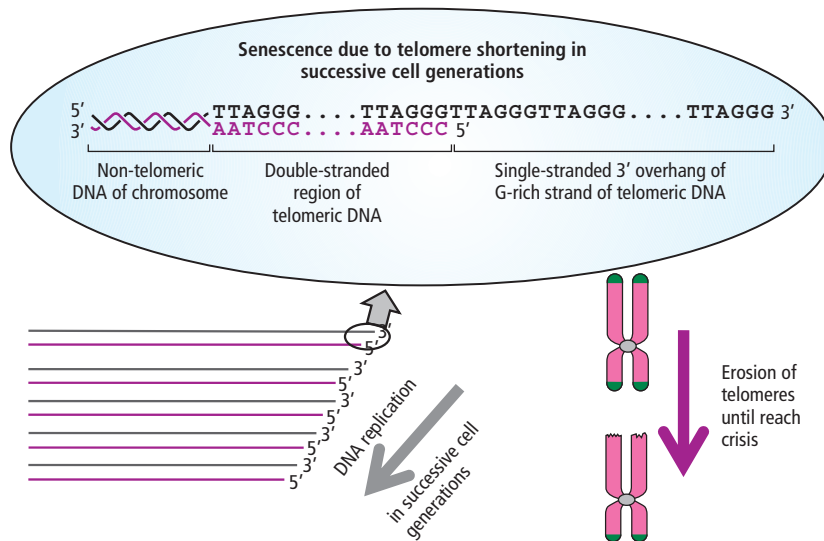
Figure 2.4 The apoptotic pathway.

Figure 2.5 Telomerase, telomere length, senescence and immortalization.

DNA replication always follows a 5′ to 3′ direction so that manufacturing the 3′ ends of the chromosomes cannot be achieved by DNA polymerases and each time a cell replicates its DNA ready for cell division, 50–100 base pairs are lost from the ends of chromosomes. Eventually the protective ends of chromosomes are eroded and end-to-end chromosomal fusions occur with karyotypic abnormalities and death of the affected cell.

Normal germ cells and cancer cells avoid this senescence, acquiring immortality in culture usually by upregulating the expression of human telomerase reverse transcriptase (hTERT) enzyme that uses an RNA template and RNA-dependent DNA polymerase to add the six-base pair sequence back onto the ends of chromosomes to compensate for the bases lost during DNA replication (Table 2.1). Dyskeratosis congenita is an inherited condition, characterized by many abnormalities, including premature ageing and an increased risk of skin and gut cancers. It is due to mutations of components of the telomerase complex including the telomerase RNA and dyskerin.

5. Angiogenesis

All tissues including cancers require a supply of oxygen and nutrients. For cancers to grow larger than about 0.4 mm in diameter, a new blood supply is needed to deliver these. The growth of new blood vessels from pre-existing vasculature is termed angiogenesis. The "angiogenic switch" denotes the ability of tumours to recruit new blood vessels by producing growth factors and is necessary for tumour growth and metastasis. Angiogenesis is determined by the balance of angiogenesis promoters and inhibitors (Figure 2.6).

Vascular endothelial growth factors (VEGF-A to -E) are a family of growth factor homodimers that act via one of three plasma membrane receptors (VEGFR-1 to -3) on endothelial cells. Overproduction of VEGF and/or FGF (fibroblast growth factor) is a common theme in many tumours (Table 2.1). Angiogenesis may be measured microscopically as microvessel density in an area of tumour or by assays of angiogenic factors. These measures are of prognostic significance in several human tumours. Angiogenesis

ON (angiogenic factors)	OFF (angiogenic inhibitors)
Vascular endothelial growth factors (VEGF-A to-E)	Tissue metalloproteinase inhibitors (TIMP 1–4)
Fibroblast growth factors (FGF) (acidic FGF and basic FGF)	Plasminogen activator inhibitor (uPAI)
Transforming growth factors (TGF-α and TGF-β)	Thrombospondin
	Angiostatin
	Endostatin

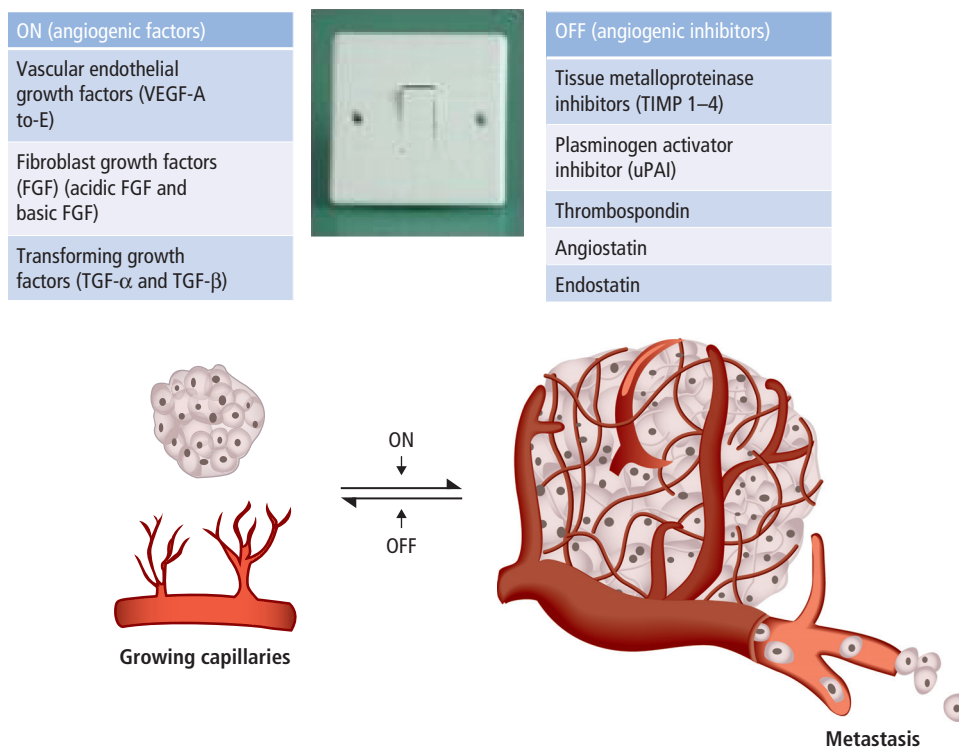**Figure 2.6** The angiogenic switch.

is becoming a major focus of anticancer drug development. It is an attractive target for several reasons. Angiogenesis is a normal process in growth and development but is quiescent in adult life except during wound healing and menstruation, so side effects are predicted to be minimal. As the target will be normal endothelial cells without any genetic instability, there should be little capacity to acquire resistance. Each capillary supplies a large number of tumour cells, so the effects should be magnified in terms of tumour cell kill. Anti-angiogenic agents should have easy access to their target through the blood stream. In combination, these elements make anti-angiogenic therapies attractive, and several pharmaceutical companies have invested heavily in attempts to develop these agents. Bevacizumab is a monoclonal antibody that binds to VEGF; it was originally licensed for use in colon cancer and is also a valuable treatment for age-related macular degeneration, which is caused by retinal vessel proliferation.

Examples of neoangiogenesis in cancers:

- Colorectal cancer cells may produce VEGF.
- Glioma cells overexpress PDGF and PDGF receptor (PDGFR).
- Head and neck cancers activate VEGF via the nitric oxide pathway.

6. Invasion and metastasis

The properties of tissue invasion and metastatic spread are the histopathological hallmarks of malignant cancers that discriminate them from benign. A number of sequential steps have been identified in the process of metastatic spread of cancers:

1. Motility and invasion from the primary site.
2. Embolism and circulation in lymph or blood.
3. Arrest in a distant vascular or lymphatic capillary and adherence to the endothelium.
4. Extravasation into the target organ parenchyma.

Central to many of these steps is the role of cell–cell adhesion that controls the contact between cells and cell–extracellular matrix connections that influence the relationship between a cell and its environment. These interactions are regulated by cell adhesion molecules. Members of the cadherin and immunoglobulin superfamilies modulate cell–cell interactions whilst integrins control cell–extracellular matrix interactions. Alterations of cadherin, adhesion molecule and integrin expression are a common feature of metastatic cancer cells (Table 2.1).

Tumours may migrate as single cells or as collections of cells. The former strategy is used by lymphoma
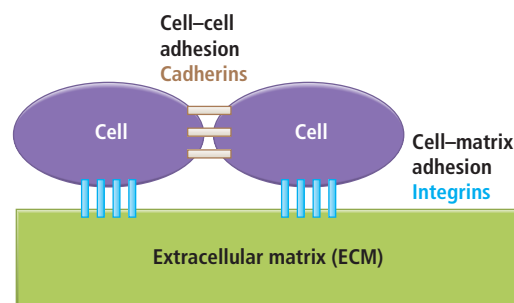
Figure 2.7 Cell–cell and cell–matrix interactions.

and small-cell lung cancer cells. It requires changes in integrins that mediate the cell–extracellular matrix interaction and matrix-degrading proteases. Metastatic migration as clumps of cancer cells is common for most epithelial tumours. In addition, this needs changes in cell–cell adhesion through cadherins and other adhesion receptors, as well as cell–cell communication via gap junctions (Figure 2.7).

Examples of invasion in cancers

- Colorectal cancer cells inactivate E-cadherin via APC and have altered expression of integrins.
- Melanoma cells overexpress MMP-2 and -9.

How to acquire the six capabilities (enabling hallmarks)

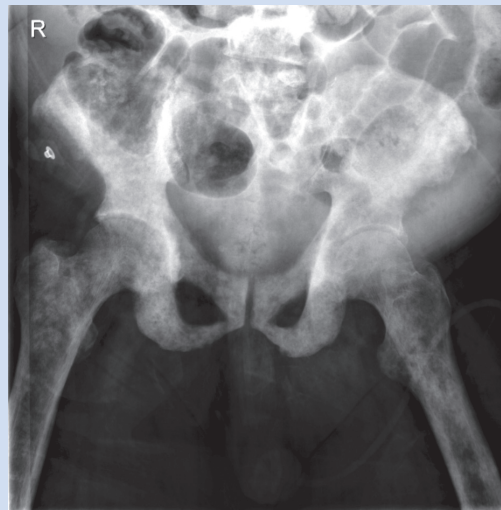## 7. Cancer cells are prone to mutations due to genome instability

Cancer is really a genetic disease caused by mutations of cellular DNA that do not occur in the germ cells (oocytes and sperm). One of our lines of defense against cancer is to repair errors in DNA or to eradicate cells that have accumulated extensive DNA damage. However, DNA mutations tend to go uncorrected in cancer cells because their DNA replication is error-prone, their DNA repair mechanisms are deficient and their DNA damage cell cycle arrest and apoptosis responses are uncoupled. Altogether cancer cells are said to have genetic instability and this enables them to acquire the six hallmarks of a cancer cell. Sometimes these mutations develop in a stepwise fashion as the cell phenotype becomes more abnormal.

Box 2.2 How cancers metastasize: routes and destinations
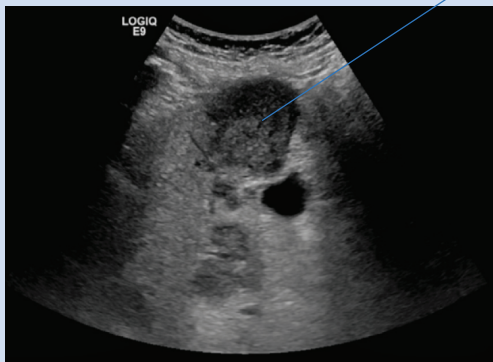
Routes of metastasis

Breast cancer cells that leave a primary tumour in blood vessels will be carried in the blood first through the heart and then to the capillary beds of the lungs. Some cancer cells might form metastases in the lung (Figure a), whilst others pass through the lung to enter the systemic arterial system, where they are transported to remote organs, such as bones (Figure b) or skin (Figure e). By contrast, colon cancer cells will be taken by the hepatoportal circulatory system first to the liver (Figures c and d). There is no direct flow from the lymphatic system to other organs, so cancer cells within it – for example, melanoma cells – must enter the venous system to be transported to distant organs. Rarely, routes other than blood and lymphatic vessels are used in metastasis. Transcoelomic spread across the abdominal cavity occurs for gastric tumours that metastasize to the ovaries (known as Krukenberg tumours). Spread within the cerebrospinal fluid is thought to be responsible for the metastasis of medulloblastoma up and down the spinal column.
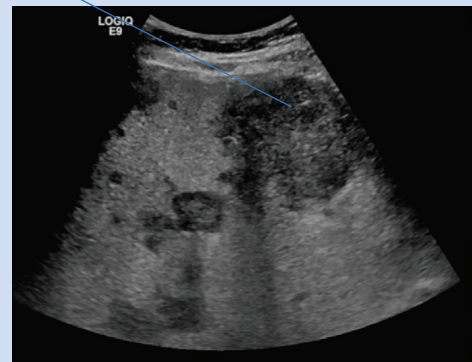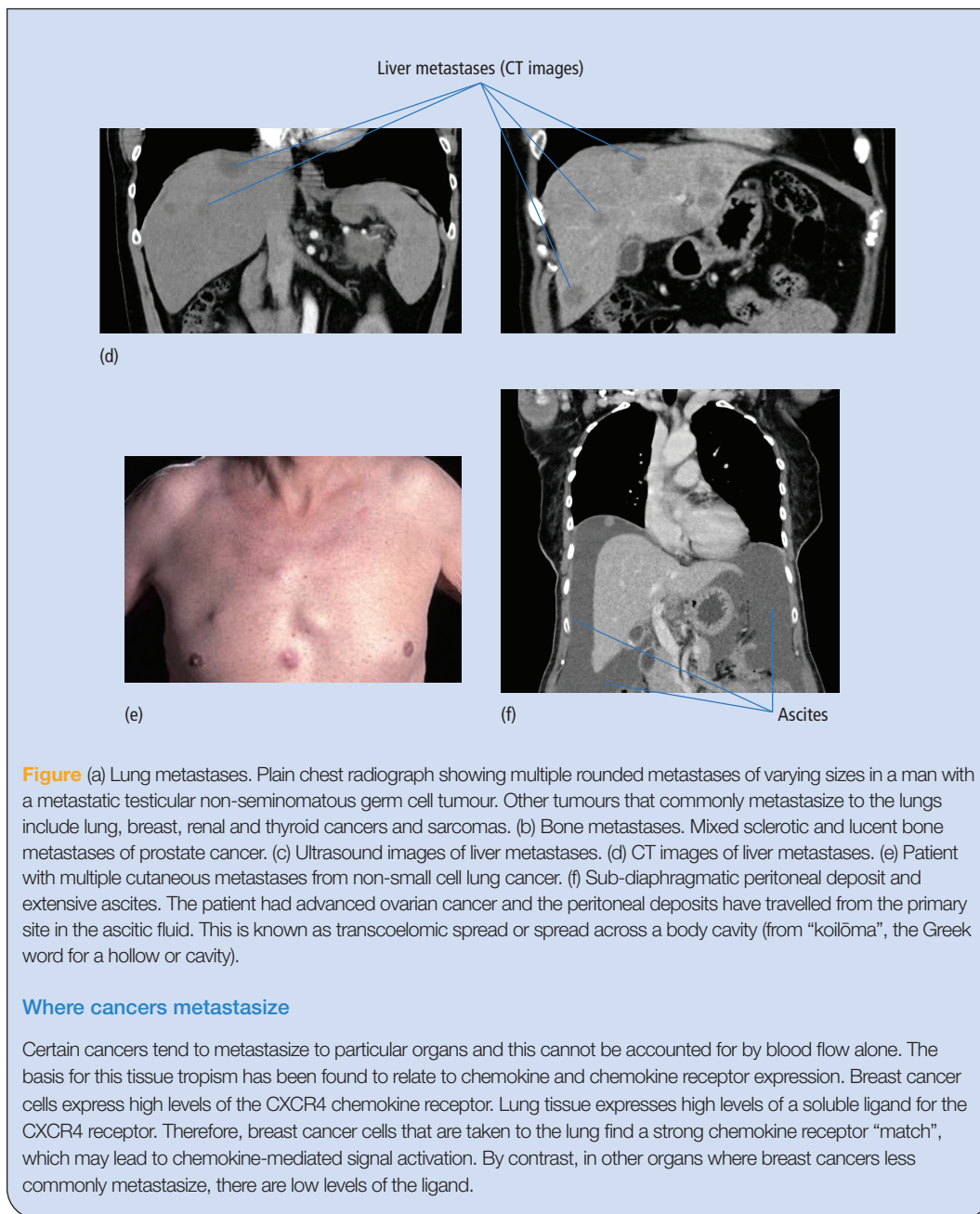
Lung metastases

Bone metastases

(a)

(b)

Liver metastases (ultrasound images)

(c)

Liver metastases (CT images)

(d)

(e)   (f)   Ascites

Figure (a) Lung metastases. Plain chest radiograph showing multiple rounded metastases of varying sizes in a man with a metastatic testicular non-seminomatous germ cell tumour. Other tumours that commonly metastasize to the lungs include lung, breast, renal and thyroid cancers and sarcomas. (b) Bone metastases. Mixed sclerotic and lucent bone metastases of prostate cancer. (c) Ultrasound images of liver metastases. (d) CT images of liver metastases. (e) Patient with multiple cutaneous metastases from non-small cell lung cancer. (f) Sub-diaphragmatic peritoneal deposit and extensive ascites. The patient had advanced ovarian cancer and the peritoneal deposits have travelled from the primary site in the ascitic fluid. This is known as transcoelomic spread or spread across a body cavity (from "koilōma", the Greek word for a hollow or cavity).

Where cancers metastasize

Certain cancers tend to metastasize to particular organs and this cannot be accounted for by blood flow alone. The basis for this tissue tropism has been found to relate to chemokine and chemokine receptor expression. Breast cancer cells express high levels of the CXCR4 chemokine receptor. Lung tissue expresses high levels of a soluble ligand for the CXCR4 receptor. Therefore, breast cancer cells that are taken to the lung find a strong chemokine receptor "match", which may lead to chemokine-mediated signal activation. By contrast, in other organs where breast cancers less commonly metastasize, there are low levels of the ligand.

8. Inflammation promotes the growth of cancers

Although cancers generally have ways of avoiding destruction by the host immune system (see hallmark 9. Cancers avoid destruction by the host immune system), they still often induce a local inflammatory response. It was thought that this inflammatory response was part of the host's attempt to eradicate the cancer cells. Instead it appears that the inflammatory cytokines actually promote tumour growth, proliferation and angiogenesis. Rather like autoimmune disease it seems that this is another own goal of the immune system.

Additional capabilities of cancers (emerging hallmarks)

9. Cancers avoid destruction by the host immune system

Cancers employ a number of strategies to escape the host immune system so that they can survive and grow. They have acquired ways of avoiding both the innate and adoptive immune systems and this is why for most cancers, immunotherapy is ineffective.

10. Cancer cells deregulate cellular metabolism

Cancer cell proliferation is enabled by changes in energy metabolism to fuel growth. In many circumstances cancer cells switch from normal aerobic glycolysis to the much less efficient anaerobic metabolism. Although the rationale behind this metabolic switch in cancer cells is unclear, it may account for the resistance of some tumours to the effects of radiotherapy.

Genome instability

DNA damage or mutation will normally result in cell cycle arrest followed by DNA repair or apoptosis. Interference in this process may occur either by deficient DNA damage recognition and repair or abnormal gatekeeping of the cell cycle arrest/apoptosis response. This will result in the uncorrected accumulation of a large number of genetic abnormalities, which is referred to as "genomic instability". It is thought that this allows cells to acquire the six capabilities that characterize the cancer cell phenotype and physiology (Figures 2.8 and 2.9).

DNA repair

Environmental damage to DNA occurs commonly and eukaryotes have developed several techniques for repairing both double strand breakages and single strand errors in DNA.

1. Repair of double strand breaks in DNA:
- homologous recombination using the sister chromatid as a template
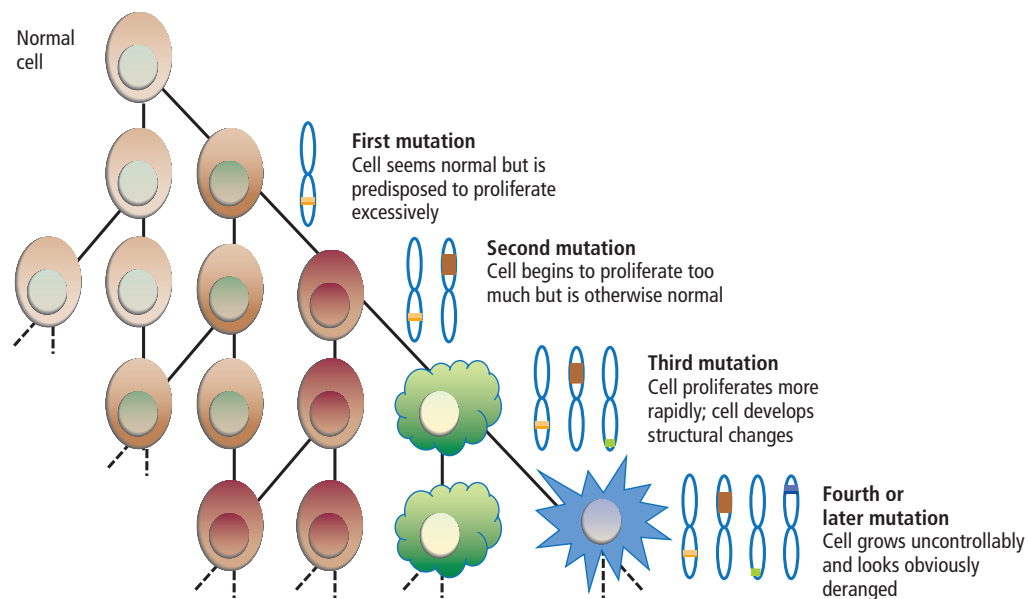- non-homologous end joining (NHEJ)

First mutation
Cell seems normal but is predisposed to proliferate excessively

Second mutation
Cell begins to proliferate too much but is otherwise normal

Third mutation
Cell proliferates more rapidly; cell develops structural changes

Fourth or later mutation
Cell grows uncontrollably and looks obviously deranged

Normal cell

Figure 2.8 Stepwise accumulation of genetic mutations contributing to oncogenic phenotype.
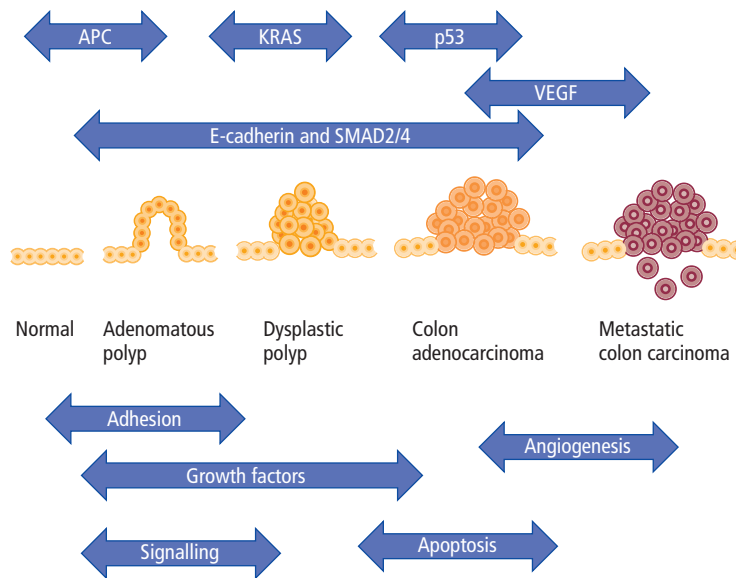
Figure 2.9 Colon cancer development from normal mucosa to metastatic carcinoma associated with stepwise acquisition of oncogenic mutations. APC, adenomatous polyposis coli gene; KRAS, Kirsten rat sarcoma viral oncogene homologue; p53, tumour protein 53 (TP53); SMAD, *Homo sapiens* homologue of *Drosophila* protein mothers against decapentaplegic (MAD); VEGF, vascular endothelial growth factor.

2. Repair of single strand mutations in DNA:
- nucleotide excision repair (NER) for bulky adducts, pyrimidine dimmers and photoproducts
- mismatch repair (MMR) for single mispaired bases and short deletions
- base excision repair (BER) for alkylated bases or loss of a single base

Hereditary mutations of the enzymes involved in DNA repair will predispose to malignancy as they confer genome instability (Table 2.2).

DNA damage recognition

Another group of enzymes are required to recognize damaged DNA, leading to cell cycle arrest to allow DNA repair to be completed before the damage is replicated and passed on to the progeny cells. A number of cancer-predisposing syndromes are associated with inherited mutations of these enzymes. Examples include p53, whose inactivation is an early step in the development of many cancers. Patients with the Li–Fraumeni syndrome carry one mutant germline p53 allele and are at high risk for the development of

Table 2.2 Hereditary DNA repair syndromes

DNA damage	DNA repair mechanism	Examples of defect of DNA repair	Examples of cancers associated with defects
Double strand DNA breakage	Homologous (sister chromatid) repair	BRCA1 (hereditary breast and ovarian cancer)	Breast and ovarian cancers
	Non-homologous end joining (NHEJ)	XRCC4 (X-ray repair complementing defect gene) (lethal)	None (lethal defect)
Single strand DNA breakage	Nucleotide excision repair (NER)	XP (xeroderma pigmentosa)	Skin cancers, leukaemia and melanoma
	Mismatch repair (MMR)	MSH and MLH (hereditary non-polyposis colon cancer)	Colon, endometrium, ovarian, pancreatic and gastric cancers
	Base excision repair (BER)	MYH (hereditary non-polyposis colon cancer)	Colon cancers

sarcomas, leukaemia and cancers of the breast, brain and adrenal glands.

Epigenetic changes

Most of the discussion above about the molecular mechanisms of malignancy has described somatic and occasional germline mutations of DNA that lead to aberrant proteins that in turn contribute to oncogenesis. This argument follows the central dogma of molecular biology introduced by Francis Crick in the late 1950s, which stated that information flows in a unidirectional course from DNA sequence via RNA sequence to protein sequence. Although there are recognized exceptions to the central dogma, such as retroviruses and prions, it remains broadly true. However, some inheritable changes in phenotype or gene expression arise by mechanisms other than changes in the sequence of DNA bases. These inheritable changes passed on from a cell to her daughters are called epigenetic changes and perhaps the most obvious of these is cell differentiation.

The term epigenetics was introduced by the British developmental biologist Conrad Hal Waddington in 1942 as a metaphor for cell differentiation and development from a progenitor stem cell. Waddington likened differentiation to a marble rolling down a landscape of hills and valleys to reach a final destination. The destination (cell fate) was determined by the landscape (epigenetics) and the marble could not

travel back to the top (terminal differentiation). Today the term refers to modification of DNA and chromatin that influences gene transcription, alteration of post-transcriptional RNA and finally to protein degradation.

DNA methylation

Perhaps the most recognized epigenetic modification of DNA is nucleotide base methylation, typically the addition of a methyl group to the cytosine pyrimidine ring. In vertebrates, DNA methylation usually occurs in a CpG dinucleotide. Unmethylated CpGs are grouped in clusters called "CpG islands" that occur in the 5′ regulatory regions of many genes. DNA methylation of CpG islands inhibits gene transcription by impeding the binding of transcriptional proteins and by binding methyl-CpG-binding domain (MBD) proteins. MBD proteins recruit additional proteins, such as histone deacetylases (HDACs), which modify histones to form compact, inactive chromatin termed silent chromatin. Since epigenetic changes such as DNA methylation are inherited during cell replication, maintenance of the pattern of DNA methylation is required following each cycle of DNA replication and this is achieved by DNA methyltransferases using the conserved DNA strand as the template (Figure 2.10). DNA methylation of tumour suppressor genes has been found to be a common mechanism of epigenetic gene silencing in cancers.
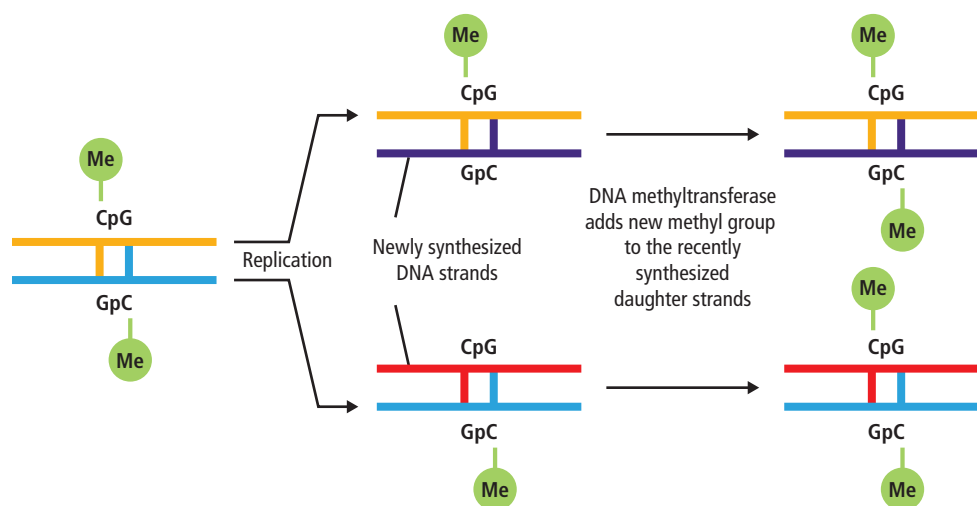

Figure 2.10 DNA methylation is passed on during cell replication to progeny cells by DNA methyltransferase enzymes that methylate CpG islands. CpG refers to the DNA dinucleotide sequence CG joined by the phosphate backbone of DNA.

Chromatin modification

Chromatin is composed of DNA and proteins, chiefly the histone proteins around which the DNA is wound. There are six classes of histones organized into core histones (H2A, H2B, H3 and H4) and linker histones (H1 and H5). The core histones, which are highly conserved through nature, share N-terminal amino acid sequences that are the sites for post-transcriptional modification, for example, acetylation and methylation. These histone modifications alter the binding of the DNA to the nucleosome and modify RNA polymerase activity and hence gene expression. In general, tightly bound DNA is less expressed. Numerous enzymes have been identified that are involved with the modification of histone protein leading to alterations of chromatin structure and regulation of gene expression. These include histone methyltransferase (HMT) and histone acetyltransferase (HAT); other enzymes catalyze the removal of these modifications including HDAC (Figure 2.11). Acetylation of histone tails reduces their binding affinity for DNA, allowing access for RNA polymerase and enhancing gene transcription. HDAC, therefore, by reversing histone tail residue acetylation suppresses gene expression, including tumour suppressor gene expression contributing to oncogenesis. A number of HDAC inhibitors have been studied including valproate and more recently vorinostat or suberoylanilide hydroxamic acid (SAHA), which is licensed for the management of cutaneous T-cell non-Hodgkin's lymphoma.

RNA interference

Post-translational interference of messenger RNA (mRNA) transcripts can also modify the expression of genes without altering the DNA sequence. Two types of small RNA molecules, microRNA (miRNA) and small interfering RNA (siRNA), can bind to specific complementary sequences of RNA or DNA and either increase or decrease their activity, for example by preventing an mRNA from producing a protein (Figure 2.12). RNA interference was originally identified in petunia plants. Botanists attempting to produce darker and darker petunia flowers inserted additional genes of an enzyme that catalyzes pigment synthesis. However, the transgenic plants produced white or variegated white flowers and this was subsequently found to be due to post-transcriptional inhibition of gene expression brought about by rapid mRNA degradation. The eventual explanation of this gene silencing phenomenon was identified in *Caenorhabditis elegans* by Craig Mello and Andrew Fire in 1998 who demonstrated that double-stranded RNA caused the gene silencing. They called this RNA interference (RNAi) and won the Nobel Prize in 2006 for this work. Both the role of RNAi in the epigenetic generation of cancers and the potential of RNAi as a therapeutic approach are the focus of fevered research.

Protein degradation

A further form of epigenetic modification that contributes to the cellular phenotype is the destruction of
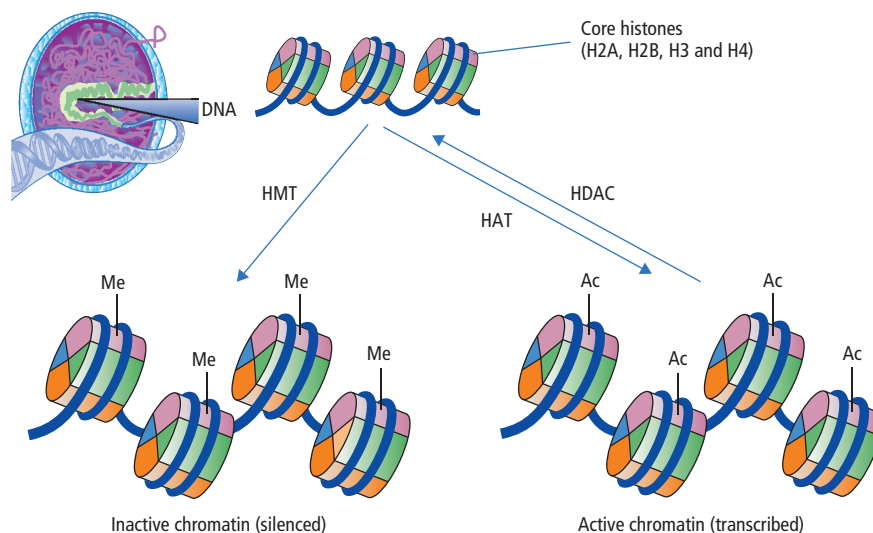

Figure 2.11 Mechanism of chromatin modification. Ac, acetyl; HAT, histone acetyltransferase; HDAC, histone deacetylase; HMT, histone methyltransferase; Me, methyl.
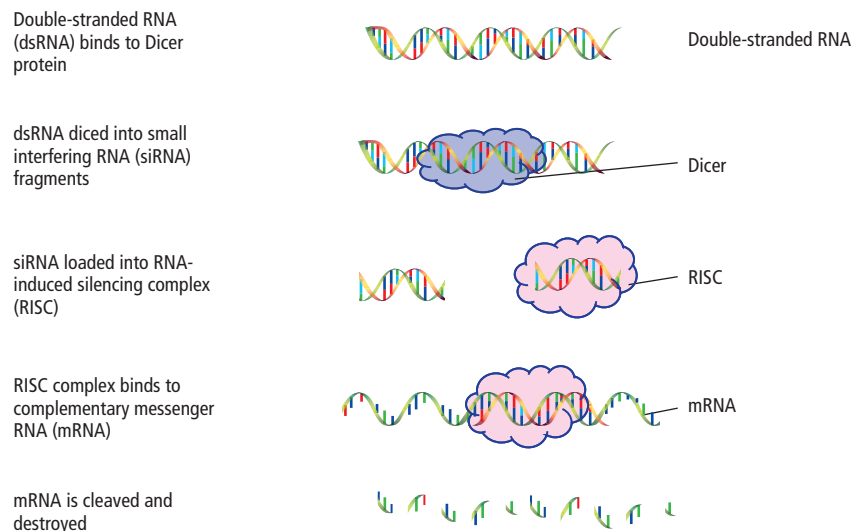
Double-stranded RNA (dsRNA) binds to Dicer protein

Double-stranded RNA

dsRNA diced into small interfering RNA (siRNA) fragments

Dicer

siRNA loaded into RNA-induced silencing complex (RISC)

RISC

RISC complex binds to complementary messenger RNA (mRNA)

mRNA

mRNA is cleaved and destroyed

Figure 2.12 Mechanism of RNA interference.

proteins chiefly by proteasomes. Proteins are tagged for degradation by a small protein called ubiquitin and this reaction is catalyzed by enzymes including the product of the gene disrupted in Von Hippel–Lindau syndrome and Fanconi's anaemia. At least four ubiquitin molecules attach to the condemned protein in a process called polyubiquitination and the protein then moves to a proteasome, where the proteolysis occurs (Figure 2.13). Epigenetic regulation of protein degradation could contribute to oncogenesis in a variety of ways. Gankyrin, a component of the proteasome, is overexpressed in hepatocellular cancers. Bortezomib, a new treatment for myeloma, acts by inhibiting proteasome function.

Genetic causes of cancer

Hereditary causes of cancer

The causes of cancer may be usefully divided into genetic and environmental factors. The genetic factors are either germline mutations that are present in every cell of the body or somatic alterations only found in the tumour cells. Germline mutations may be either inherited, in which case they follow a familial pattern or may be new sporadic mutations that neither parent has. Some of the germline mutations have been outlined as mutator phenotypes (DNA repair and damage recognition genes) above. Other germline cancer-predisposing mutations occur in tumour suppressor genes and oncogenes.

Oncogenes

The first clue to the identification of specific genes involved in the development of cancer came from the study of tumour viruses. Although cancer is generally not an infectious disease, some animal leukaemias, lymphomas and solid tumours, particularly sarcomas, can be caused by viruses. Oncogenes were identified following the discovery by Peyton Rous in 1911 that sarcomas could be induced in healthy chickens by injecting them with a cell-free extract of the tumour of a sick chicken. This was due to transmission of Rous sarcoma virus (RSV), an oncogenic retrovirus with just four genes:

- (*gag* group-specific antigen), which encodes the capsid protein
- (*pol* polymerase), which encodes the reverse transcriptase
- (*env* envelope), which encodes the envelope protein
- *src*, which encodes a tyrosine kinase

It is the *src* gene that is necessary for cell transformation and is therefore an oncogene – literally a gene capable of causing cancer. In the late 1970s Harold Varmus and Michael Bishop discovered that a homologous proto-oncogene (c-SRC) is present in the normal mammalian genome (the human *src* locus is on chromosome 20q12-q13) and has been hijacked by the retrovirus. The prefix v- denotes a viral sequence and the prefix c- a cellular sequence. In 1956, 55 years after his discovery of RSV and at the age of 87, Peyton Rous was (finally) awarded a Nobel
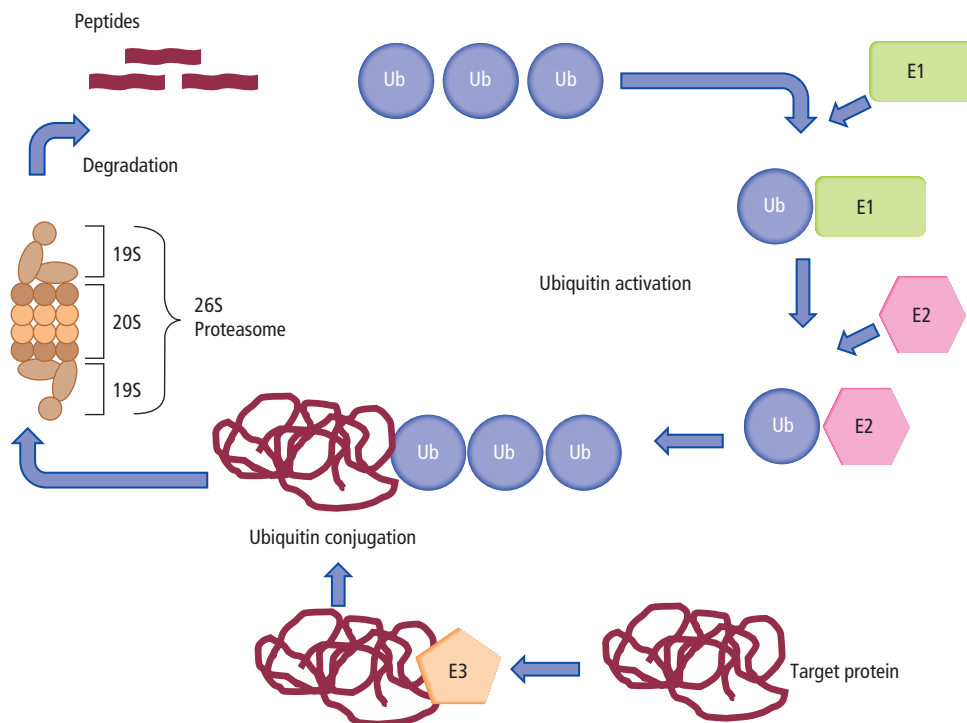
Figure 2.13 Proteasome pathway of protein ubiquitination and degradation. E, ubiquitination enzymes; Ub, ubiquitin.

Prize, whilst Bishop and Varmus only waited 10 years from their discovery to the award of their Nobel Prize in 1989. Around 50 oncogenes have been identified by their presence in transforming retroviruses (e.g. erbB, H-RAS, JUN) and further oncogenes have been discovered by positional cloning of chromosomal translocations (e.g. Bcl-2, BCR-ABL) and by transfection studies (e.g. N-RAS, RET). Most oncogenes contribute to cancer's autonomy in growth factors, either as plasma membrane receptors (e.g. RET, PTCH), signal transduction pathways (e.g. PTEN, NF1 and 2, VHL) or transcription factors (e.g. c-MYC, WT1) (Table 2.3).

Tumour suppressor genes

In contrast to oncogenes, tumour suppressor genes act as cell cycle brakes, slowing the proliferation of cells, and mutations in these genes also contribute to cancer. Germline mutations of tumour suppressor genes behave as autosomal-dominant familial cancer predispositions. Tumour suppressor genes require the loss of both functional alleles to support a cancer (unlike oncogenes where one mutant allele suffices).

In 1971 Alfred Knudson proposed the two hit model of tumour suppression to account for the differences between familial and sporadic retinoblastoma in children. In familial cases, tumours arose at a younger age and were more frequently bilateral. Knudson hypothesized that these children had inherited one defective retinoblastoma gene allele, followed by loss of the function of the second allele in the cancer cells through a somatic mutation (Figures 2.14 and 2.15). Tumour suppressor genes, like oncogenes, also involve a variety of functional categories, including cell cycle regulation (e.g. p53, Rb), DNA repair and maintenance (e.g. BRCA1 and 2, MLH1, MSH2), as well as signal transduction (e.g. NF1, PTEN) and cell adhesion (e.g. APC) (Table 2.2).

The Maths of Cancer or "How long have I had it?" (Figure 2.16)

20 doublings = 10^6 cells (million)

30 doublings = 10^9 cells (billion) weigh 1 g = earliest detectable

35 doublings = $10^{10.5}$ cells = usual number at diagnosis (3 cm diameter)

40 doublings = 10^{12} cells (trillion) weigh 1 kg = usual number at death

Table 2.3 Table of hereditary cancer predisposition syndromes

Syndrome	Malignancies	Inheritance	Gene	Function
Breast/ovarian	Breast, ovarian, colon, prostate	AD AD	BRCA1 BRCA2	Genome integrity
Cowden	Breast, thyroid, gastrointestinal, pancreas	AD	PTEN	Signal transduction (tyrosine phosphatase)
Li–Fraumeni	Sarcoma, breast, osteosarcoma, leukaemia, glioma, adrenocortical	AD	p53	Genome integrity
Familial polyposis coli	Colon, upper gastrointestinal	AD	APC	Cell adhesion
Hereditary non-polyposis colon cancer (Lynch type II)	Colon, endometrium, ovarian, pancreatic, gastric	AD AD AD AD	MSH2 MLH1 PMS1 PMS2	DNA mismatch repair
MEN 1 (multiple endocrine neoplasia 1)	Pancreatic islet cell, pituitary adenoma	AD	MEN1	Transcription repressor
MEN 2 (multiple endocrine neoplasia 2)	Medullary thyroid, phaeochromocytoma	AD	RET	Signal transduction (receptor tyrosine kinase)
Neurofibromatosis 1 (Figure 2.15)	Neurofibrosarcoma, phaeochromocytoma, optic glioma	AD	NF1	Signal transduction (regulates GTPases)
Neurofibromatosis 2	Vestibular schwannoma	AD	NF2	Cell adhesion
von Hippel–Lindau	Haemangioblastoma of retina and central nervous system, renal cell, phaeochromocytoma	AD	VHL	Ubiquination
Retinoblastoma	Retinoblastoma, osteosarcoma	AD	RB1	Cell cycle regulation
Xeroderma pigmentosa	Skin, leukaemia, melanoma	AR AR AR AR	XPA XPC XPD XPF	DNA nucleotide excision repair
Gorlin	Basal cell skin, brain	AD	PTCH	Signal transduction (repressor of hedgehog signalling)

AD, autosomal dominant; AR, autosomal recessive; GTPase, guanosine triphosphatase.

The cell cycle takes 16–24 hours to complete. For Burkitt's lymphoma the growth fraction is 0.29 (i.e. 29% of the cells are actively dividing at any one time) and hence the doubling time is about 2 days. In contrast, the growth fraction for non-small cell cancer of the lung is under 0.02 and the doubling time is around 130 days. To get to death (10^{12} cells) can take just 3 months for BL but for NSCLC to get to 10^9 (just detectable on CT) takes 10.5 years. In reality most lung cancers are found when they are 3 cm in diameter or $10^{10.5}$ cells, after 35 doublings (12.3 years).

Environmental causes of cancer

The multitude of environmental factors that are associated with the development of malignancy may be usefully divided into:

- Physical (radiation)
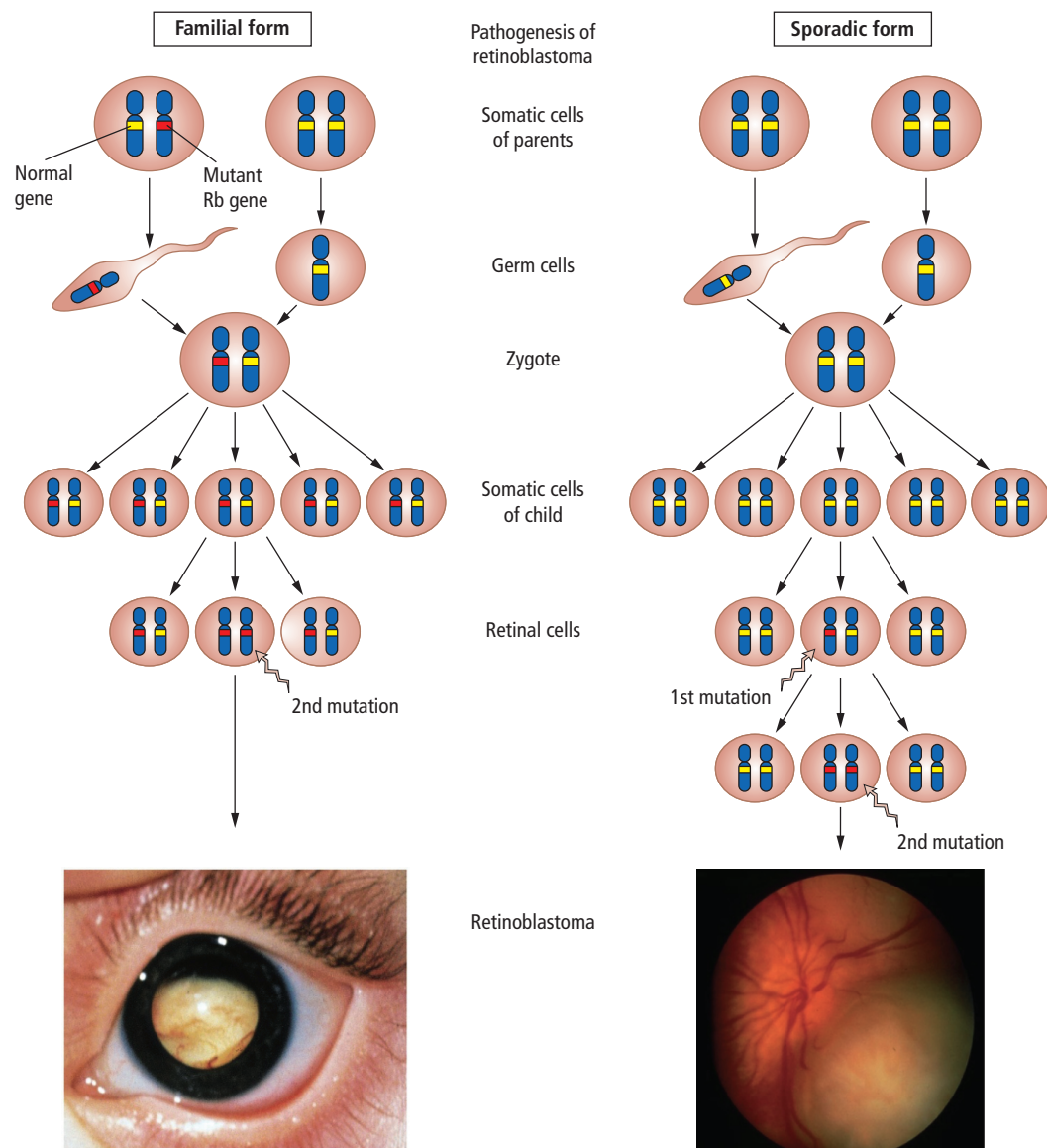- Chemical (chemical carcinogens)
- Biological (infections)

Figure 2.14 Knudson's two-hit hypotheses of familial and sporadic retinoblastoma.

Radiation

The major physical carcinogen is radiation. Radiation is ubiquitous and may either be ionizing (e.g. γ-rays from cosmic radiation and isotope decay, α-particles from radon, X-rays from medical imaging) or non-ionizing (e.g. ultraviolet (UV) light from the sun, microwave and radiofrequency radiation from mobile phones, electromagnetic fields from electricity generators and pylons, ultrasound radiation from imaging). Ionizing radiation ejects electrons from atoms yielding an ion pair and requires 10–15 eV (electronvolts). Ionizing radiation may be either electromagnetic (X-rays, γ-rays) or particulate (α-particles, neutrons). Non-ionizing radiation does not yield an ion pair but can still excite electrons resulting in chemical change.
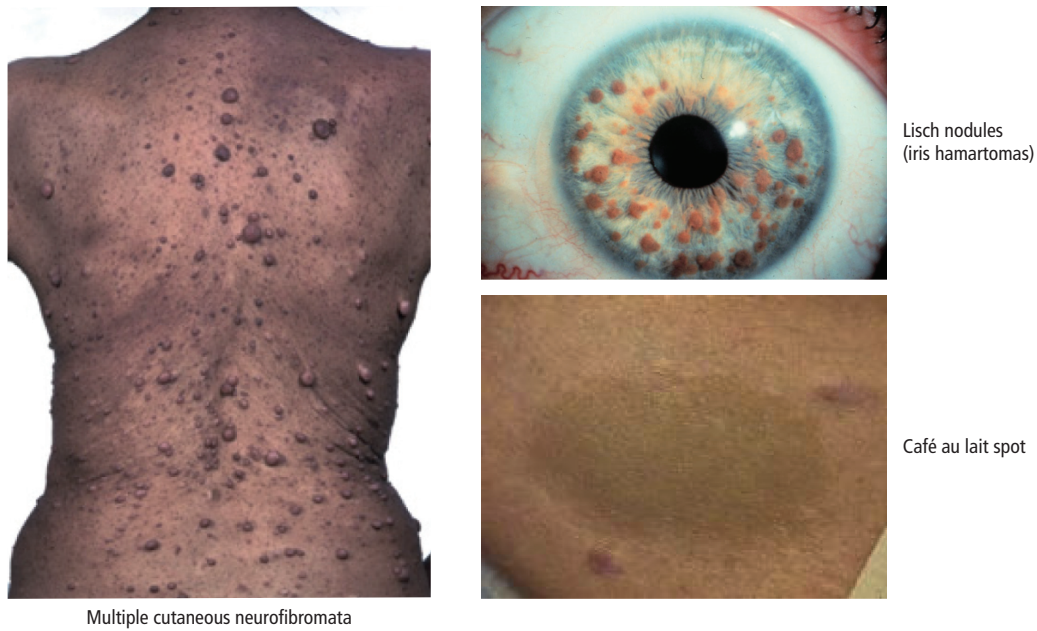
Lisch nodules
(iris hamartomas)

Café au lait spot

Multiple cutaneous neurofibromata

Figure 2.15 Multiple dermal neurofibromata typical of peripheral neurofibromatosis or type 1 NF, previously known eponymously as von Recklinghausen's disease. It is due to hereditary mutation of the NF1 neurofibromin gene on chromosome 2p22, which encodes a guanosine triphosphatase (GTPase) activating protein involved in the signal transduction cascade.

Ultraviolet radiation

UV radiation is electromagnetic radiation with a wavelength shorter than visible light (400–700 nm) but longer than X-rays (10–0.01 nm). It is subdivided into three wavelength bands:

- UVA (313–400 nm)
- UVB (290–315 nm)
- UVC (220–290 nm)

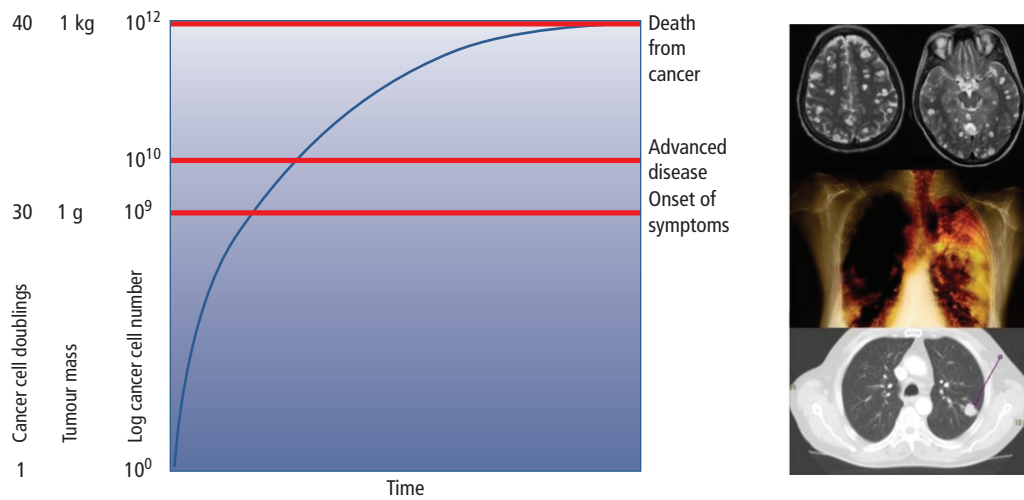UVC has the most potent effects on DNA, which absorbs most strongly at 254 nm. However, UVC is

Figure 2.16 The maths of cancer.

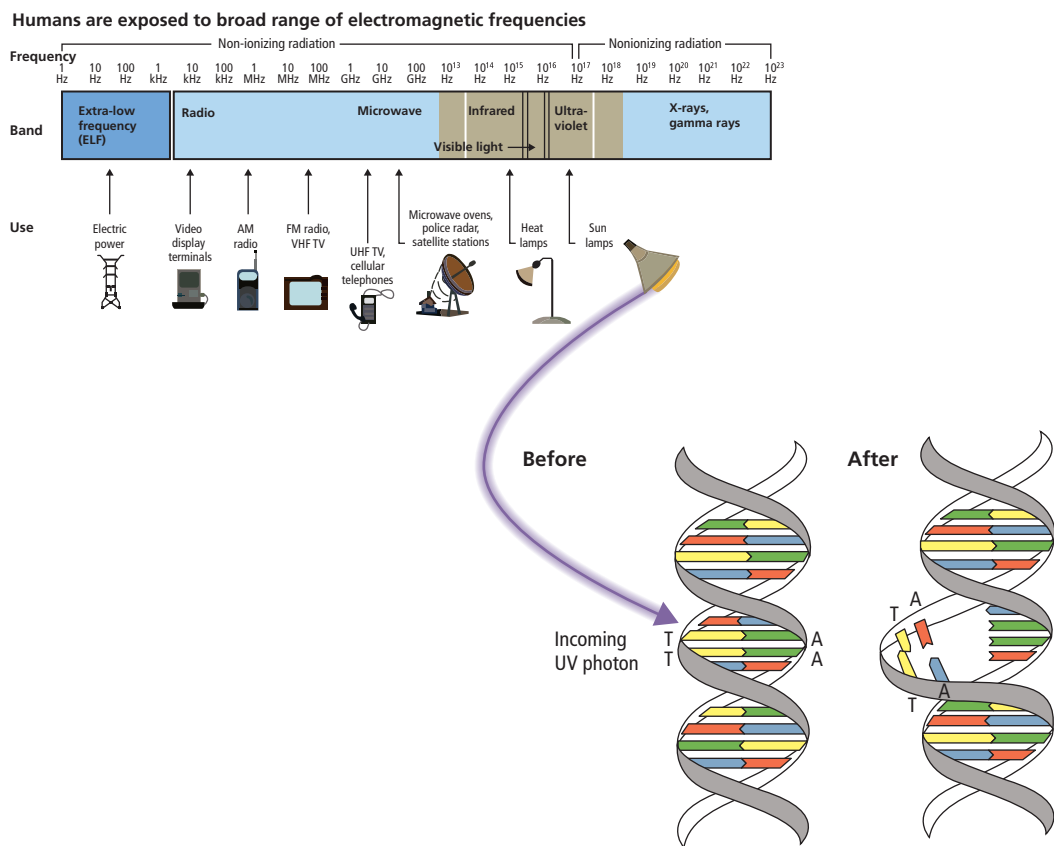**Humans are exposed to broad range of electromagnetic frequencies**

Figure 2.17 UV-light-induced thymidine dimers. T, Thymine; A, Adenine.
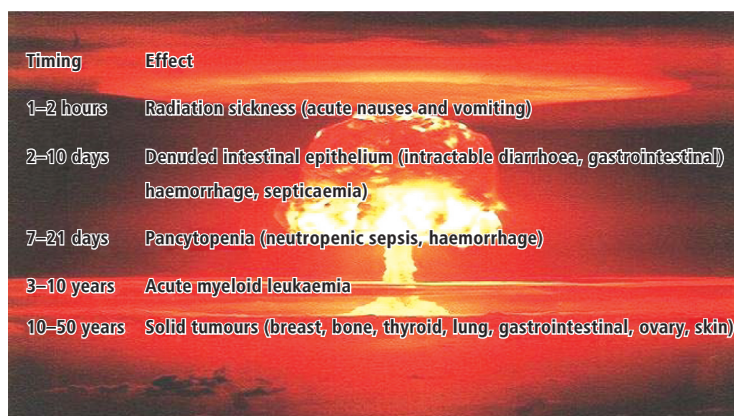
quickly absorbed by the atmosphere and hence UVB is considered to be the greater environmental hazard. Most UV radiation is absorbed by atmospheric ozone in the stratosphere and this ozone layer is being depleted in part due to chlorine in chlorofluorocarbons (CFCs), resulting in increasing UV exposure levels. Ultraviolet light is responsible for vitamin D activation and suntans. One of the major lesions induced in DNA by UV radiation is the thymidine dimer, a covalent bonding of adjacent thymidine residues on the same DNA strand (Figure 2.17). This causes local distortion of the double helix that is repaired by the NER pathway. The seven identified xeroderma pigmentosa genes encode essential components that undertake NER and hence xeroderma pigmentosa predisposes to UV-induced skin malignancies. Melanin pigment in the skin normally absorbs UV radiation, thus protecting the skin. Basal cell and squamous cell skin cancers increase with cumulative UV exposure, whilst

the relationship is less straightforward for melanoma. The evidence for an association with cancer for other forms of non-ionizing radiation (microwave, radiofrequency, ultrasound and electromagnetic radiation) is weak and inconsistent.

Ionizing radiation
Natural sources

Exposure to natural sources of ionizing radiation varies in different populations. Higher altitude and further latitude from the equator are both associated with higher cosmic radiation exposure composed of very high energy particles that are thought to originate outside our solar system including from supernovae. In addition, various regions have higher natural background levels from radon. Radon is a colourless, odourless gas formed from decay as part of the uranium-238 series. The radon-222 isotope, along

Timing	Effect
1–2 hours	Radiation sickness (acute nauses and vomiting)
2–10 days	Denuded intestinal epithelium (intractable diarrhoea, gastrointestinal) haemorrhage, septicaemia)
7–21 days	Pancytopenia (neutropenic sepsis, haemorrhage)
3–10 years	Acute myeloid leukaemia
10–50 years	Solid tumours (breast, bone, thyroid, lung, gastrointestinal, ovary, skin)

Figure 2.18 How atomic bombs kill.

with a number of its progeny, is an α-particle emitter. Radon gas levels are normally quoted in Bq/m^3 (1 becquerel (Bq) is one decay per second) and the average indoor levels in the United Kingdom are about 20 Bq/m^3. Local geology (igneous granite) with high levels of uranium produces high levels of radon in soil gas, but for it to escape to the surface the soil must be highly porous. In the United Kingdom, radon levels are particularly high in Devon and Cornwall, Derbyshire and Northamptonshire. From the results of eight case-control studies, it is believed that radon exposure accounts for a small fraction of lung cancers with a 14% increased risk for a person living for 30 years in a house with levels of 150 Bq/m^3.

Nuclear warfare

Most of the information on the induction of cancers by ionizing radiation comes from exposed populations, including Japanese people exposed to atomic bombs at Hiroshima ("Little Boy" was a uranium-235-enriched bomb dropped by Enola Gay) and Nagasaki

("Fat Man" was a plutonium-239 bomb dropped by Bockstar). The estimated populations of the two cities at the time of bombing was 560,000 and approximately 200,000 people died within the first few months of the acute effects of blast, burns and radiation exposure (Figure 2.18). The Radiation Effects Research Foundation has followed 86,000 survivors or hibakusha and, up to 1990, 7827 had died of cancer. The excess risk of leukaemia was seen especially in those exposed as children and was highest during the first 10 years after the bombing. However, the excess risk of solid tumours occurred later and still persists (Table 2.4).

Table 2.4 Cancer deaths in the hibakusha (survivors of Hiroshima and Nagasaki atomic bombs)

	Total number of deaths	Estimated number of deaths due to radiation	Percentage of deaths attributable to radiation
Leukaemia	176	89	51
Solid tumours	4687	339	7
Total	4863	428	9

Box 2.3 Chernobyl

On 26 April 1986, nuclear reactor number 4 at Chernobyl exploded in the world's worst nuclear accident. Over 10^{19} Bq of radioactive isotopes were released, including 5.2 × 10^{18} Bq of β-emitting isotopes of iodine that concentrate in the thyroid gland. An increase in thyroid cancer in children was first reported in 1990 but an excess of other tumours has not (yet?) been reported. Fallout from Chernobyl affected millions of people living within a few hundred kilometres from the reactor and caused a 30–100-fold increase in the incidence of thyroid cancer, especially in children. The younger the child at exposure, the greater the risk is. The increase so far is almost entirely papillary carcinoma of the thyroid and the dominant subtype has solid papillary morphology. At a molecular level, these tumours show rearrangement of the RET oncogene by inversion or translocation with partner genes to yield constitutively active c-RET tyrosine kinases.

Figure 2.19 Osteosarcoma of the first metacarpal 15 years after radiotherapy for arthritis (no longer used). This radiograph shows cortical destruction, a soft tissue mass with internal calcification and periosteal reaction.

Table 2.5 Diagnostic imaging radiation doses

Imaging procedure	Radiation dose	Equivalent to natural background radiation for:
Chest X-ray	0.02 mSv	3 days
Chest CT scan	8 mSv	3.6 years or one transatlantic flight
Abdominal CT scan	10 mSv	4.5 years
Intravenous urogram	2.5 mSv	14 months
Brain CT scan	2.3 mSv	1 year
Mammogram	0.7 mSv	3 months

Medical radiation

The hazards of medical ionizing radiation may be difficult to determine as ionizing radiation-induced tumours are not identifiable by a particular signature DNA mutation (unlike the thymidine dimers induced by UV radiation). Some tissues, such as breast, thyroid and bone marrow, are more susceptible to the carcinogenic effects of ionizing radiation, although tumours have been described in every organ site following radiation exposure (Figure 2.19). Well-described examples of iatrogenic tumours include acute leukaemias induced by radiation treatment for ankylosing spondylitis prescribed in the late 1930s in the United Kingdom. Similarly, 20,000 Israelis received radiation for *Tinea capitis* (ringworm) in the 1950s and by the 1980s there was a significantly increased risk of meningioma. Similar increases in tumours have been observed in patients treated with radiotherapy, including men treated for prostate cancer, women treated for cervical cancers and Hodgkin's disease survivors. Diagnostic imaging radiation doses are shown in Table 2.5.

Occupational radiation

The first victims of occupational exposure to radiation included Marie Curie (the first woman to win a Nobel Prize and the only person to win two Nobel prizes in different scientific fields: physics 1903 and chemistry 1911) and her daughter Irène Joliot-Curie (also a Nobel laureate in chemistry 1935), who both died of leukaemia. In the 1920s, watch dials were hand painted with radium-based luminous paint. The female radium dial painters often licked their paint brushes to give them a sharp point and ingested the radium. Up to 3% of these women subsequently developed osteosarcomas after a latency of 5–10 years. These "radium girls" successfully sued their employer

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Box 2.4 Units of radiotherapy

The simplest metaphor for radiation doses is rainfall.
- The amount of rain falling is the activity (Bq)
- The amount of rain hitting you is the absorbed dose (Gy)
- How wet you get is dose equivalent (Sv)

The becquerel (Bq) is the SI unit of radioactivity and 1 Bq is equivalent to one nuclear decay per second. It is named after Henri Becquerel who shared the Nobel Prize with Marie and Pierre Curie for the discovery of radioactivity. The Hiroshima bomb produced 8×10^{24} Bq.

The gray (Gy) is the SI unit of absorbed radiation dose for ionizing radiation. One gray is the absorption of 1 joule (J) of ionizing radiation by 1 kg of matter, usually human tissue. It is named after Hal Gray, a British pioneer of radiation biology and physics who also established the Gray Laboratories at Mount Vernon Hospital.

The sievert (Sv) is the SI unit of radioactive dose equivalence and reflects the biological effects in the tissue of radiation rather than its physical attributes. The equivalent dose will depend on the absorbed dose (measured in grays) as well as the type of radiation, as well as the time and volume and part of body irradiated. It is named after Rolf Sievert, a Swedish medical physicist. A dose of 3 Sv will lead to a lethal dose (LD) 50/30 or death in 50% of cases within 30 days and over 6 Sv survival is very unusual.

and this litigation resulted in the introduction of industrial safety standards and health and safety regulations at work. Similarly, pitchblende (uranium oxide) and uranium miners in Czechoslovakia, Sweden, Newfoundland and Colorado who have been exposed to radon are at increased risk of lung cancers.

Chemical carcinogenesis

Cancer is essentially a genetic disease arising from mutations of genes that affect the control of normal cell function (proto-oncogenes and tumour suppressor genes) or from polymorphic genes that govern enzyme systems that activate or detoxify environmental carcinogens (phase I and phase II enzyme reactions). Carcinogenic mutations can arise in several ways: genotoxic environmental factors (e.g. radiation and many chemical carcinogens), spontaneous DNA aberrations occurring during normal cell replication or hereditary germline mutations. Chemical carcinogenesis was shown to be a multistep process following studies in the 1940s using polycyclic aromatic hydrocarbons (PAHs) and a murine skin cancer model system. This identified three steps – initiation, promotion and progression – that involve separate biological processes. Chemical carcinogens may operate at any or all three stages. The minority of chemical carcinogens act directly on DNA (e.g. alkylating agents), whilst the majority are pro-carcinogens that require metabolic activation to the ultimate carcinogen forms. Many ultimate carcinogens are potent electrophiles, capable of accepting electrons (e.g. epoxides derived from polycyclic hydrocarbons, vinyl chloride and aflatoxins, the *N*-hydroxylated metabolites of azo dyes and the alkyldiazonium ions derived from nitrosamines).

Initiation

The key feature of initiation is the need for cell replication without repair of the chemically induced DNA damage. Initiation is irreversible, usually involves simple DNA mutations that are "fixed" by cell division and results in no morphological changes to the cells. Single exposure to a carcinogen may be sufficient for initiation. For example, aflatoxin B1 is one of a family of mycotoxin contaminants of food crops such as grain and groundnuts (peanuts). It is produced by *Aspergillus flavus*, which favours hot and humid conditions and is therefore most likely to contaminate food in Africa and Asia. Aflatoxin B1 is oxidized by hepatic P450 microsomal enzymes into aflatoxin B1 2,3-epoxide, which binds to DNA bases forming mutagenic adducts that preferentially induce GC to TA

transversions. These transversions have been identified frequently in codon 249 of the p53 gene in hepatocellular carcinomas in patients from southern Africa and China who are exposed to high levels of aflatoxin B1 and may also have hepatitis B virus (HBV) infection.

Promotion

Promotion is a reversible process requiring multiple exposures to the carcinogen, usually with a dose–response threshold. Promotion does not usually involve DNA mutations (non-genotoxic carcinogenesis) but provides a chemically mediated selective growth advantage. Thus, promotion results in the clonal expansion of cells. In the 1940s it was noted that 5% of mice treated with benzopyrene developed tumours but this figure rose to 80% when croton oil was added. Croton oil alone, however, produced no tumours. Subsequently, it was found that tetradecanoylphorbol acetate (TPA), a natural component of croton oil (from the seeds of *Croton tiglium*, a tree cultivated in India, which resemble castor seeds), interacts with the protein kinase c signal pathway, stimulating growth and thus acting as a promoter. TPA is the most widely used tumour promoter in cellular experimental models of oncogenesis. Similarly, oestrogens are believed to act as carcinogenic promoters. Indeed, transplacental diethylstilboestrol (DES) was shown to induce vaginal clear-cell adenocarcinomas in young women whose mothers had been treated with DES during pregnancy.

Progression

Progression is an irreversible step that results in morphologically identifiable cellular changes and frequently involves multiple complex DNA changes, such as chromosomal alterations. Progression and the accumulation of multiple genetic abnormalities that characterize cancer cells may occur spontaneously or may be driven by chemical carcinogens. Since individual cells may acquire these genetic changes, progression leads to heterogeneity of the cell population. Ultimately some cells will acquire a mutator phenotype and the six genetic attributes that characterize a cancer cell.

Diet and cancer

A role for dietary constituents has been described for a number of cancers and the evidence for some of these relationships is more robust than for others. Alcohol intake has been convincingly associated with

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Box 2.5 A brief epidemiological history of smoking and cancer

Tobacco was one of the "gifts" from the New World to the Old along with syphilis and potatoes. Nicotine is named after Jean Nicot, a 15th century French ambassador to Lisbon, who was a great advocate of smoking and who in 1559 sent tobacco to Catherine de Medici, the then Queen of France. Tobacco was subsequently introduced to England by Sir Walter Raleigh in 1586. Smoking was actively encouraged amongst soldiers in the Thirty Years War, Napoleonic campaigns, Crimean War and, most notably, the First World War. Smoking reduces fear and anxiety and suppresses appetite and these were deemed beneficial to soldiers.

Early epidemiological links with non-lung cancers

In 1761, John Hill, a London doctor, wrote up several cases of nasal cancer amongst heavy tobacco snuff users and, in 1795, Thomas van Soemmering suggested a link between pipe smoking and lip cancer. The American Civil War Yankee general and later US President, Ulysses S. Grant, died in 1885 of throat cancer described with some pathos by Mark Twain in the first volume of his autobiography, and this Twain attributed to excessive smoking. In an early cohort study in the 1920s, Dr R. Abbe observed that, of 90 patients with oral cancer, 89 were smokers.

Epidemiological links with lung cancer

In 1939, Dr Franz Muller of the University of Cologne performed what is generally recognized as the earliest case-control study of smoking, which showed that a very high proportion of lung cancer patients were heavy smokers. However, the results were dismissed as unreliable because Hitler was a fanatical antismoker. Shortly after the Second World War, Austin Bradford Hill, Edward Kennaway, Percy Stock and Richard Doll set out to investigate links between smoking and lung cancer, at a time when 90% of adult males in the United Kingdom smoked, using a case-control dose–response strategy. Their case-control study was performed in 1948 in 20 London hospitals, interviewing two controls with gastric or colonic cancer as controls for each lung cancer patient. In all analyses, there was a dose–response relationship between the number of cigarettes smoked and the risk of lung cancer. This was published in 1950 in the *British Medical Journal*.

In 1951, Doll and Hill set up a prospective cohort study of 60,000 doctors on the medical register who were recruited via a letter in the *BMJ*; 40,000 replies were received and, in the following 2.5 years, there were 789 deaths, including 36 from lung cancer. There was a significant increase in the risk of lung cancer with increased tobacco consumption (see table below). However, they noted that the only two doctors who definitely died of smoking had died after setting fire to their beds whilst smoking in bed! This relationship was maintained in a 1993 update of the original cohort, which now includes 20,000 deaths (883 from lung cancer) and the relative risk for smoking >25 g tobacco a day was 20-fold.

	N	**Tobacco 1 g/day**	**Tobacco 15 g/day**	**Tobacco >25 g/day**
Lung cancer deaths	36	0.4/10,000	0.6/10,000	1.1/10,000
All deaths	789	13/10,000	13/10,000	16/10,000

Similar findings were reported in the early 1950s in the United States by Ernst Wynder, a medical student, and Evarts Graham, a thoracic surgeon, who, in 1950, published "Tobacco smoking as a possible etiologic factor in bronchiogenic carcinoma: a study of 684 proven cases" in the *Journal of the American Medical Association*. Evarts, a chain smoker, did not take enough heed of his own findings and also died of lung cancer.

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an increased risk of oral, oesophageal and hepatic cancers. In contrast, dietary fat was believed to play an important role in breast cancer development based on animal studies, migrant studies and a few case-control trials. This led to great enthusiasm for reduced dietary fat intake to reduce the incidence of breast cancer. However, results from large prospective studies have failed to confirm a strong relationship between dietary fat intake and breast cancer. Two paths may contribute to dietary carcinogenesis. First, foodstuffs may include dietary genotoxins formed by contaminating moulds, products

Table 2.6 Cancers attributed to infection

Infection	Cancer	Number of cancer cases worldwide per year
RNA viruses		
Human T-cell leukaemia virus	Leukaemia	3000
Hepatitis C virus	Hepatocellular cancer	110,000
DNA viruses		
Human papillomavirus	Cervical cancer, anal cancer, oropharyngeal cancer	360,000
Hepatitis B virus	Hepatocellular cancer	230,000
Epstein–Barr virus	Burkitt's lymphoma, Hodgkin's disease, nasopharyngeal cancer	100,000
Human herpesvirus 8	Kaposi's sarcoma	45,000
Bacteria		
Helicobacter pylori	Gastric cancer, gastric lymphoma	350,000
Helminths		
Schistosoma haematobium	Bladder cancer	10,000
Liver flukes	Cholangiocarcinoma	1000

HIV, human immunodeficiency virus.

of storage or fermentation of food, products of cooking and food additives (e.g. aflatoxin contamination of food). Second, endogenous genotoxins, such as reactive oxygen species, may be formed and higher calorific intake may yield more genotoxins.

Carcinogenic infections

The association between infection and cancer is usually attributed to Peyton Rous, who described the acellular transmission of sarcoma between chickens in 1911. However, 6 years earlier, Goebel had reported a link between bladder tumours and bilharzia (schistosomiasis). It is estimated that 15% of cancers globally are attributable to infections (11% viruses, 4% bacteria and 0.1% helminths) (Table 2.6).

Oncogenic human DNA viruses
Human papillomavirus

The papillomaviruses are non-enveloped, icosahedral, double-stranded DNA viruses. Around 100 genotypes have been identified and >30 of these infect the female genital tract. Some genotypes are associated with benign lesions, such as warts (e.g. HPV-6 and -11), whilst others are known as high-risk genotypes and are associated with invasive cancer (e.g. HPV-16, -18, -31, -33, -45, -51, -52, -58 and -59) (Table 2.7). The prevalence of infection varies between populations but is 20–30% in women aged 20–25 years and

Table 2.7 Human papillomavirus (HPV) genotypes and associated conditions

Human disease	HPV genotype
Skin warts	HPV-1, -2, -3, -7 and -10
Epidermodysplasia verruciformis	HPV-5, -8, -17 and -20
Anogenital warts: exophytic condylomas	HPV-6 and -11
Anogenital warts: flat condylomas	HPV-16, -18, -31, -33, -42 and -43
Respiratory tract papillomas	HPV-6 and -11
Conjunctival papillomatosis	HPV-6 and -11
Focal epithelial hyperplasia	HPV-13 and -32

Table 2.8 Serological markers of hepatitis B virus infection

	HBsAg	HBeAg	Anti-HBe	Anti-HBs	Anti-HBc	Anti-HBc IgM
Acute infection	+	+/−	+/−	−	+	+++
Highly infectious carrier	+++	+	−	−	+	−
Low infectious carrier	+	−	+	−	+	−
Past infection	−	−	+	+	+	−
Past immunization	−	−	−	+	−	−

declines to 5–10% in women over 40 years old. HPV is sexually transmitted and the main determinant of infection is the number of sexual partners. Most infections are cleared spontaneously but a small proportion persist and are believed to be the origin of cervical dysplasia and invasive cancers. Latent infection is associated with cervical intraepithelial neoplasia (CIN), which is graded 1–3 according to the severity of cytological changes. The histological equivalents of these lesions are called squamous intraepithelial lesions, which may be low or high grade. Over 99% of invasive cervical cancers have detectable HPV DNA present and HPV can transform cells in culture. The molecular basis of papillomavirus-induced neoplasia is attributed to two viral oncogenes, E6 and E7. HPV E6 inactivates p53 and E7 degrades Rb protein. High-risk HPV genotypes have also been associated with anal, penile, vaginal and vulval cancers. In addition, HPV is thought to play a role in the development of a number of other malignancies, including head and neck cancers, conjunctival squamous cancers, oesophageal cancers and possibly cutaneous squamous cell cancers.

Studies have suggested that the detection of HPV in the cervix may be more sensitive for detecting CIN than conventional cytological screening. Prophylactic HPV vaccines that induce neutralizing antibodies may prevent infection and the associated malignancies. Most of the vaccines have used virus-like particles constructed of major capsid proteins without viral DNA or enzymes present. A nationwide HPV vaccination programme for teenage girls was started in the United Kingdom in 2008.

Hepatitis B virus

HBV is a double-stranded DNA virus that includes a single-stranded DNA region of variable length. The virus possesses a DNA-dependent DNA polymerase as well as a reverse transcriptase and replicates via an RNA intermediate. HBV has three main antigens: the "Australian antigen" is associated with the surface (HBsAg), the "core antigen" (HBcAg) is internal and the "e antigen" (HBeAg) is part of the same capsid polypeptide as HBcAg. All of these antigens elicit specific antibodies and are used diagnostically (Table 2.8).

Hepatitis B is one of the most common infections worldwide with 2 billion people having been infected and 300–350 million chronic carriers (Table 2.10). Hepatitis B is the ninth most common cause of death worldwide. Acute hepatitis B infection may be associated with extrahepatic immune-mediated manifestations and 1–4% of patients develop a fulminant form. Following acute infection, up to 10% will develop chronic hepatitis, either chronic persistent hepatitis, which is asymptomatic with modest elevation of transaminases and little fibrosis or chronic active hepatitis, which causes jaundice and cirrhosis and is associated with a 100× increased risk of hepatocellular cancer 15–60 years after infection. It is uncertain how hepatitis B leads to cancer, although the X protein of hepatitis B may interact with p53 causing disruption of the cell cycle control or the virus may act indirectly by causing increased hepatic cell turnover associated with cirrhosis.

Although treatment with α-interferon and antiviral agents (e.g. lamivudine, tenofovir, telbivudine, entecavir, adefovir) may lead to clearance of hepatitis B in chronic infection, recombinant subunit vaccines have been available since the early 1980s. The introduction of a mass immunization programme in Taiwan has been associated with a dramatic reduction in liver cancer in children.

Epstein–Barr virus

Epstein–Barr virus (EBV) (or human herpesvirus 4 (HHV-4)) is a ubiquitous double-stranded DNA gamma-herpesvirus. It was first identified by Epstein and his colleagues by electron microscopy of a cell line derived from a patient with Burkitt's lymphoma in 1964. Burkitt's lymphoma had been described only a few years earlier in 1956 by Dennis Burkitt, a surgeon working in Uganda. The subsequent finding that EBV was the cause of infectious
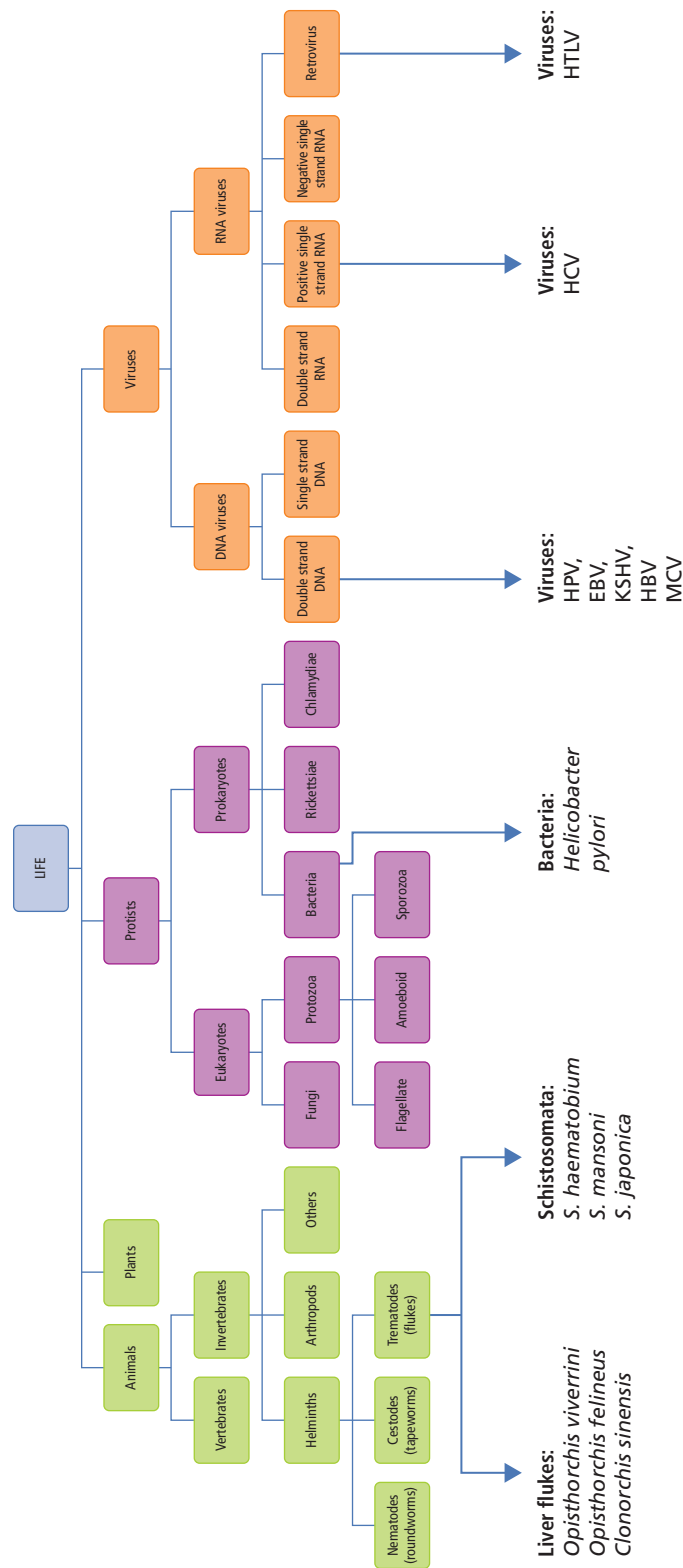
Figure 2.20 The onco tree of life. HPV, Human Papillomavirus; EBV, Epstein Barr virus; KSHV, Kaposi sarcoma herpesvirus; HBV, Hepatitis B virus; HCV, Hepatitis C virus; MCV, Merkel cell polyoma virus; HTLV, Human T cell leukemia virus.

Table 2.9 Diseases associated with Epstein–Barr virus infection

Non-malignant
Infectious mononucleosis
X-linked lymphoproliferative syndrome (Duncan's syndrome)
Oral hairy leukoplakia
Malignant
Burkitt's lymphoma
Nasopharyngeal cancer
Post-transplant lymphoproliferative disorder
Hodgkin's disease
Primary cerebral lymphoma
Primary effusion lymphoma (with HHV8)
Leiomyosarcoma in children with HIV
Nasal T/NK non-Hodgkin's lymphoma

mononucleosis arose serendipitously when a laboratory technician in Philadelphia developed mononucleosis and was found to have acquired antibodies to EBV. EBV infects over 90% of the world's population and is transmitted orally. In normal adults, from 1 to 50 per million B lymphocytes are infected by latent EBV. A carcinogenic role for EBV has been confirmed for several types of lymphoma (Burkitt's lymphoma, Hodgkin's disease and immunosuppression-associated non-Hodgkin's lymphoma) and nasopharyngeal cancer (Table 2.9). EBV is estimated to be responsible for 100,000 cancers per year in the world.

Primary infection of epithelial cells by EBV is associated with the infection of some resting B lymphocytes. The majority of infected B cells have latent virus with a small percentage undergoing spontaneous activation to lytic infection. During lytic infection EBV replicates in the cell and when the progeny virions are released the host cell is destroyed. In contrast, during latent infection there is neither virus replication nor host cell destruction. Most infected B lymphocytes have latent virus expressing at most ten of the >80 genes of EBV. The roles of these latent genes include maintenance of the episomal virus DNA, growth and transformation of B cells and evasion of the host immune system. A number of these latent genes are thought to contribute to the oncogenicity of EBV. For example, latent membrane protein 1 (LMP-1) mimics a constitutively activated receptor for TNF and BHRF1 and BALF1 are viral homologues of the anti-apoptotic protein bcl-2 that leads to evasion of programmed cell death. Thus, in contrast to retroviruses, which generally possess a single oncogene, EBV uses a number of genes that contribute to the steps towards cancer.

Human herpesvirus 8 (HHV-8/KSHV)

Kaposi's sarcoma (KS) was originally described over a century ago and four forms have subsequently been recognized. The first is classic KS and is usually found on the lower legs of elderly men of Mediterranean or Jewish descent without any immunosuppression. A second form, endemic or African KS, is found in all age groups in sub-Saharan Africa, where even before the HIV epidemic it was as common as colorectal cancer is in Europe. A third form associated with iatrogenic immunosuppression was recognized in patients who had received an allogeneic organ transplant. The fourth and most common form of the disease is associated with acquired immune deficiency syndrome (AIDS). All forms of KS are associated with HHV-8 (also known as Kaposi sarcoma herpesvirus (KSHV)), which that was identified in 1994. In addition, this virus is most prevalent in the populations at risk of KS. HHV-8 is also implicated in the pathogenesis of two rare lymphoproliferative diseases, primary effusion lymphoma and multicentric Castleman's disease (Figure 2.21). Like EBV, HHV-8
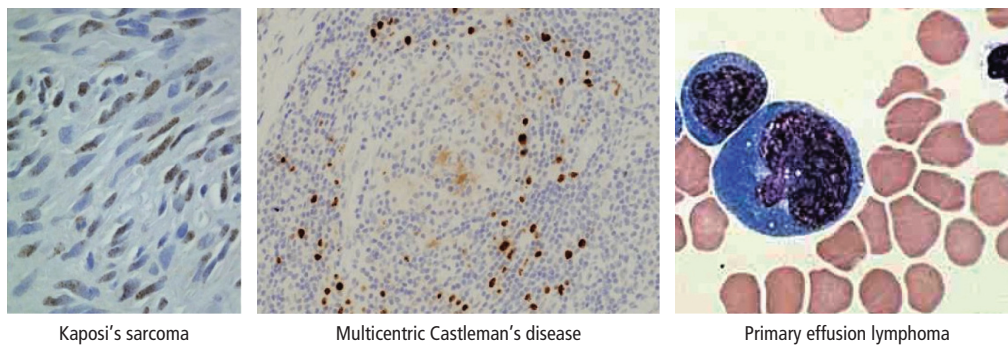

Kaposi's sarcoma   Multicentric Castleman's disease   Primary effusion lymphoma

Figure 2.21 KSHV-related tumours. Immunohistochemistry staining for KSHV latent nuclear antigen (LANA) shows the presence of virus in spindle cells of Kaposi's sarcoma and the plasmablasts in multicentric Castleman's disease. KSHV, Kaposi's sarcoma herpesvirus (also known as Human herpesvirus (HHV8)).

Table 2.10 Comparison of HIV and hepatitis B and C viruses

	HCV	HBV	HIV
Global prevalence	3%	33%	0.5%
Global prevalence	170 million	2 billion	34 million
Chronic infection	150 million	350 million	34 million
Deaths per year	350,000	1 million	1.7 million
Annual death rate	0.40%	0.49%	5%

includes a number of cellular gene homologues that are thought to contribute to its oncogenic potential.

Oncogenic human RNA viruses
Hepatitis C virus

Hepatitis C virus was identified in 1989 as the cause of transfusion-acquired non-A, non-B hepatitis by Houghton, Choo and Kuo. HCV is a single-stranded RNA virus belonging to the flavivirus genus along with yellow fever and dengue. The prevalence of HCV varies geographically from 1–1.5% in Europe and the United States to 3.5% in Africa and transmission is chiefly parenteral, particularly by blood transfusion prior to the introduction of blood product screening. In contrast to HBV, 85% develop persistent HCV and 65% progress to chronic liver disease including hepatocellular cancer for which the relative risk is 20-fold (Table 2.10). The oncogenic mechanism for HCV remains unclear. Unlike retroviruses, there is no evidence of genome integration but cancer is preceded by cirrhosis and it is hypothesized that the virus induces a cycle of inflammation, repair and regeneration and thus indirectly contributes to the formation of cancer. There are at least six genotypes of HCV and the diagnosis is usually made by enzyme immunoassay for anti-HCV antibodies and confirmed by polymerase chain reaction (PCR) for HCV RNA. Treatment with pegylated interferon, ribavarin and bocepravir or telapravir leads to clearance of the virus in 50–80% depending in part upon the HCV genotype. Promising specific protease and polymerase inhibitors are now available for HCV but not really affordable.

Human T-cell leukaemia virus type 1

Human T-cell leukaemia virus type 1 (HTLV-1) is the main cause of adult T-cell leukaemia/lymphoma, a malignancy characterized by hypercalcaemia, lymphadenopathy, hepatosplenomegaly and myelosuppression. It is associated with a particularly poor prognosis and occurs almost exclusively in areas where HTLV-1 is endemic, such as the Caribbean, Japan and West Africa or in immigrants from these regions and their offspring. HTLV-1 is also associated with tropical spastic paraparesis and uveitis. HTLV-1 is an enveloped retrovirus that integrates into the host cellular genome. The virus is able to immortalize human T lymphocytes and this property is attributable to a specific viral oncogene, *tax*. Tax is a trans-activating transcription factor that can also lead to repression of transcription. Adult T-cell leukaemia/lymphoma develops in 2–5% of HTLV-1 infected people and is more common in those infected at a younger age.

Oncogenic bacteria
Helicobacter pylori

Helicobacter pylori is a spiral, flagellated, Gram-negative bacteria that colonizes the human gastrointestinal tract. It causes gastritis leading to peptic ulceration, although many infections are asymptomatic. The discovery of *H. pylori* and the recognition of its place in the pathogenesis of peptic ulcer disease are chiefly due to Barry Marshall, who, in order to prove his point, swallowed a solution of the organism and developed acute gastritis 1 week later. It is believed that half of the world population is chronically infected with *H. pylori*. Prospective seroepidemiological data suggest that *H. pylori* infection is associated with a two fold to fourfold increase in the risk of gastric cancer as well as an increase in gastric low-grade mucosa-associated lymphoid tissue (MALT) lymphoma. As with the hepatitis viruses, the mechanism of oncogenesis is obscure but is believed to be an indirect result of chronic inflammation and consequential epithelial cell proliferation. The combination of two antibiotics with either a bismuth preparation or a proton pump inhibitor for 14 days eradicates *H. pylori* in 80% patients. However, re-infection is common. *H. pylori* is very prevalent and the time interval between *H. pylori* infection and gastric cancer is thought to be several decades. For these reasons, it may prove very difficult to assess the value of eradication interventions in reducing cancer risk.

Oncogenic helminths
Schistosomes

Schistosomes are parasitic blood flukes or flatworms (platyhelminths) belonging to the trematode class whose intermediate hosts are snails. Three species infect humans: *Schistosoma haematobium*, *Schistosoma mansoni* and *Schistosoma japonica*. Humans are infected by contact with fresh water where the parasite cercaria form penetrates the skin. It is estimated that 200 million people are infected with schistosomes

Table 2.11 Global distribution of schistosomiasis

Species	Geographical distribution	Number of humans infected
Schistosoma haematobium	North Africa, Middle East, sub-Saharan Africa	114 million
Schistosoma mansoni	Sub-Saharan Africa, Middle East, Brazil	83 million
Schistosoma japonica	China, the Philippines, Indonesia	1.5 million

(Table 2.11). Acute infection may produce swimmer's itch dermatitis and tropical pulmonary eosinophilia, although most people remain asymptomatic. The development of adult worms, days to weeks after infection, may cause Katayama fever, a systemic illness of fevers, rigors, myalgia, lymphadenopathy and hepatosplenomegaly. Chronic infection leads to granuloma formation at the sites of egg deposition, in the bladder for *S. haematobium* and in the bowel and liver for *S. mansoni* and *S. japonica*. The late sequelae include squamous cell carcinoma of the bladder in the case of *S. haematobium* and probably hepatocellular cancer with *S. japonica*. A single oral dose of praziquantel resolves the infection.

Liver flukes

Three species of food-borne liver flukes of the trematode class cause illness in humans. Infection is acquired by eating raw or undercooked freshwater fish and the flukes migrate to the biliary tree and mature in the intrahepatic bile ducts. There are two intermediate hosts in the life cycle – snails and fish. As many as 17 million people are estimated to be infected (Table 2.12). Cholangiocarcinoma has been recognized as a complication of chronic infection, and case-control studies have found a fivefold increased risk with liver fluke infection. The oncogenic mechanism is again unclear although chronic inflammation is believed to play a role. The antihelminth drug praziquantel is the treatment of choice.

Worldwide contributions to cancer

The current world population is six billion and the global burden of cancer is estimated to be 10 million new cases and 6 million deaths annually. Projections for 2020, when the global population is estimated to have risen to 8 billion are 20 million new cases and 12 million deaths annually. Tobacco contributes to 3 million cases of cancer (chiefly lung, head and neck, bladder), diet to an estimated 3 million cases (upper gastrointestinal, colorectal) and infection to a further 1.5 million cases (cervical, stomach, liver, bladder and lymphomas) globally. Prevention by tobacco control, dietary advice and affordable food and infection control and immunization could have a major impact in reducing the global burden of cancer. The differences in outcome for tumours between the developed and the developing worlds are most marked for the rare but curable cancers where access to therapy dramatically improves survival (e.g. acute leukaemias, Hodgkin's disease and testicular cancers). Small differences are recorded where screening programmes aimed at early detection are effective (e.g. cervical and breast cancers), whilst there are little differences in outcome in the common tumours where prevention has a major role (e.g. lung, stomach and liver cancers). These observations have led to a World Health Organization (WHO) list of priorities to reduce global cancer that starts not with scientific research or expensive chemotherapy, but with tobacco and infection control (Table 2.13). In an optimistic scenario the implementation of these priorities could reduce the estimated cancer incidence of 20 million in 2020 to 15 million and could reduce the expected mortality of 12 million to 6 million.

Table 2.12 Global distribution of liver fluke infection

Species	Geographical distribution	Number of humans infected
Opisthorchis viverrini	Northern Thailand, Laos	9 million
Opisthorchis felineus	Kazakhstan, Ukraine	1.5 million
Clonorchis sinensis	China, Korea, Taiwan, Vietnam	7 million

Table 2.13 WHO cancer priority ladder

1. Tobacco control
2. Infection control
3. Curable cancer programme
4. Early detection programme
5. Effective pain control
6. Sample cancer registry
7. Healthy eating programme
8. Referral guidelines
9. Clinical care guidelines
10. Nurse education
11. National cancer network
12. Clinical evaluation unit
13. Platform technology focus for region
14. Clinical research programme
15. Basic research programme
16. International aid programme

KEY POINTS

- The molecular changes of cancer are classified into six hallmarks, two enabling and two emerging characteristics
- Genetic and epigenetic modifications account for the molecular biology of cancer
- The causes of cancers can be classified into hereditary germline mutations and environmental factors including radiation, chemical and infectious agents
- Many of the causes of cancers are modifiable by lifestyle interventions
- The routes of cancer spread and the final destination of metastases varies according to the primary tumour site