Cancer biology: Molecular and genetic basis

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Cellular basis of carcinogenesis

Cancer is a disease of uncontrolled growth and proliferation whereby cells have escaped the body's normal growth control mechanisms and have gained the ability to divide indefinitely. It is a multi-step process that requires the accumulation of many genetic changes over time (Figure 1). These genetic alterations involve activation of proto-oncogenes to oncogenes, deregulation of tumour suppressor genes and DNA repair genes and 'immortalisation' which will be discussed in this chapter.

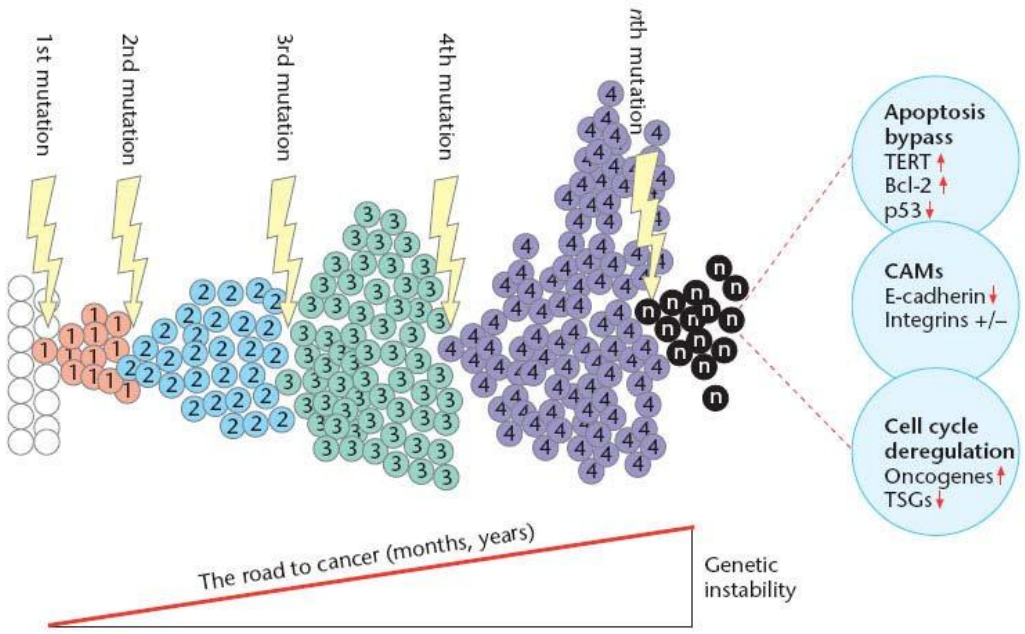

Figure 1: Overview of the road to cancer. Cells may acquire mutations in genes that control proliferation, such as proto-oncogenes and/or tumour suppressor genes. Each new mutation may provide a selective advantage for this cell, leading to 'clonal expansion'. Cellular properties changed in this process include cell cycle deregulation, apoptosis prevention and cell adhesion properties (CAMs – Cellular adhesion molecules).

Source: Alison MR. Cancer. Encyclopedia of Life Sciences, 2001[1] Reproduced with permission from John Wiley & Sons.

Cell cycle regulation and the importance of apoptosis

In normal cells, proliferation and progression through the cell cycle is strictly regulated by groups of proteins that interact with each other in a specific sequence of events (Figure 2). Checkpoints ascertain that individual stages of the cell cycle are completed correctly and ensure that incompletely replicated DNA is not passed onto daughter cells. Core to this control system are cyclin-dependent kinases (CDKs). CDKs are 'master protein kinases' that drive progression through the different phases of the cell cycle by phosphorylating and activating other downstream kinases. CDK activity is dependent on the presence of activating subunits called cyclins which are synthesised and degraded in a cell cycle-dependent manner. Cyclin-CDK complexes are further tightly regulated by CDK inhibitors.

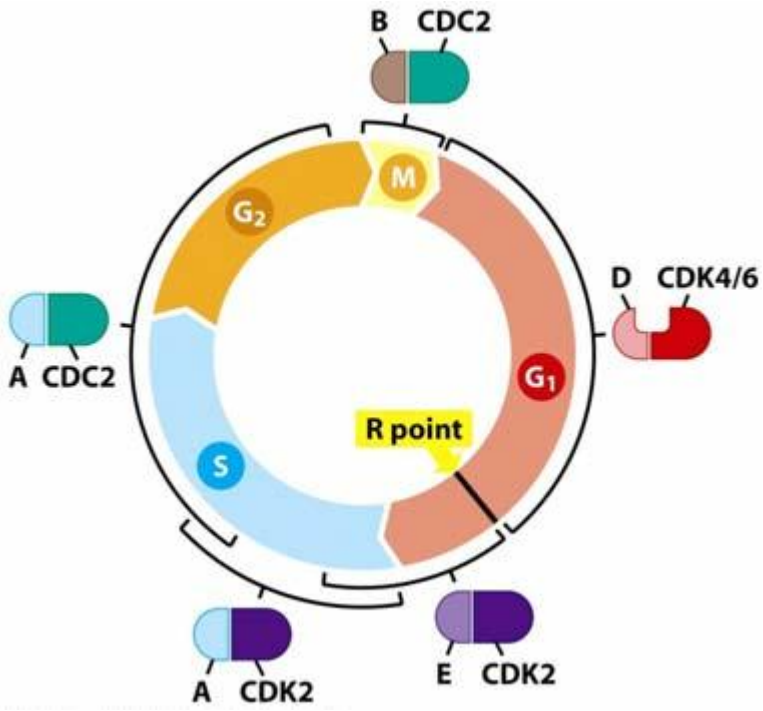

Figure 2: Cyclins and cyclin-dependent kinases (CDKs) regulate the cell cycle. CDK's and their regulatory subunits, cyclins (A, B, D & E) tightly control transition through the cell cycle. The brackets indicate the periods in which the cyclin-CDK complexes are active and orchestrate all events necessary in this period. The **restriction point** (R point) is a point in G1 at which the cell becomes 'committed' to the cell cycle and after which extracellular proliferation signals are no longer required.

Source: Weinberg RA. The biology of cancer 1st ed. Garland Science, 2007[2] Reproduced with permission of Garland Science/Taylor & Francis LLC.

The re-entry of cells into the cell cycle is decided at the **restriction point (R point)**. This decision is influenced by extracellular mitogenic signals which are transmitted via signalling pathways to key regulatory proteins, such as transcription factors (e.g. E2F) in the nucleus (refer to Figure 3, Section 2). These regulatory proteins ultimately activate the S-phase CDKs, which trigger the start of DNA synthesis.

In normal cells, activation of another transcription factor, p53, often referred to as the 'guardian of the genome', can impose cell cycle arrest and induce apoptosis (programmed cell death) through its ability to:

- induce the expression of cell cycle inhibitors to prevent proliferation of a cell until any damage has been repaired or
- initiate apoptosis, if the genomic damage is too great and cannot be repaired.

In >50% of all human tumours the p53 pathway is aberrant. Inactivation of the p53 protein renders it unable to signal and activate the cell's apoptotic machinery resulting in increased survival of cancer cells.

Cell immortalisation and tumourigenesis

Immortalisation is defined as the acquisition of an infinite lifespan. Normal mammalian **somatic** cells proliferate a limited number of times before undergoing senescence. Senescent cells may remain metabolically active even though they have permanently ceased proliferation. Immortalisation is an essential step in the malignant transformation of normal cells and can be attributed, in part, to the presence of **telomerase**, the enzyme responsible for maintaining telomeres at the ends of chromosomes. By extending telomeric DNA, telomerase is able to counter the progressive telomere shortening that would otherwise lead to cell death. Unlike normal cells that lack detectable levels of telomerase activity, approximately 90% of human tumours consist of cells that contain an active telomerase enzyme.

Cell signalling in carcinogenesis

Growth factors and their receptors

Growth factors (GFs) play an important physiological role in the normal process of growth control aimed at maintaining tissue homeostasis. They transmit growth signals from one cell to another. These signals are sensed on the cell surface by specific growth factor receptors (GFRs). GFRs transfer the growth signal via signalling pathways to activate target molecules that promote proliferation (Figure 3).

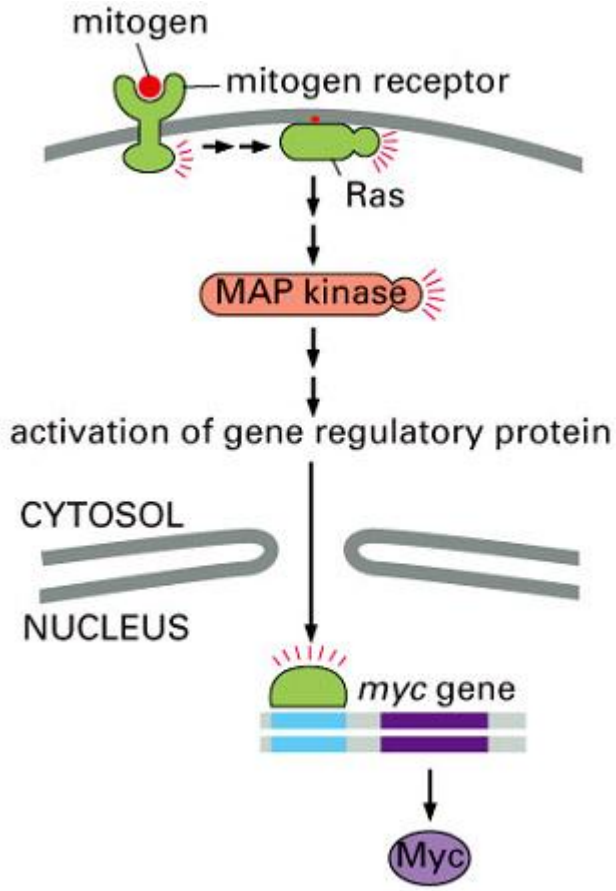

Figure 3: The MAP kinase pathway as an example of a growth signalling pathway. The mitogen (or growth factor) binds to its receptor, a receptor tyrosine kinase. Tyrosine phosphorylation of the receptor leads to activation of several docking proteins, and eventually to the activation Ras, bound to the inside of the cell membrane. Active Ras in turn activates the MAP kinase signalling cascade, beginning with Raf (not shown here). The final MAP kinase in this sequence activates several target proteins, for example a transcription factor that activates expression of the Myc gene. Myc itself is a transcription factor that activates the expression of cell cycle regulatory genes. Source: Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. Molecular biology of the cell, 4th ed. Garland Science/Taylor & Francis LLC; 2002[3] Reproduced with permission of Garland Science/Taylor & Francis LLC.

Steps that characterise normal cell proliferation include:

- the binding of a GF to its specific receptor on the cell membrane
- transient and limited activation of the GFR, which, activates several signal-transducing proteins (e.g. Ras) on the inner leaflet of the plasma membrane
- transmission of the signal by signal transduction molecules, either to cytosolic targets or to the nucleus where they activate transcription of specific genes
- entry of the cell into the cell cycle, ultimately resulting in cell division.

This pathway is often derailed in cancer and allows wayward cells to generate their own internal signals that stimulate proliferation and become independent of their environments. Cancer cells are able to induce their own growth stimulatory signals when mutations in the GFR gene occur, which facilitates activation in the absence of GFs or when overproduction of GFs results in an autocrine signalling loop.

Other elements of cell signalling

An alternative strategy by which cancer cells can become GF independent involves constitutive activation of internal signalling components. For example, the Ras protein in normal cells is switched off and does not signal unless a GFR becomes activated, which through a series of intermediaries, is able to activate the Ras protein, converting it from its quiescent state to an active, signal-emitting state. Thereafter, the Ras protein is able to release further downstream signals that are capable of inducing proliferation. In cancer cells, this signalling pathway is deregulated because structurally altered Ras proteins are able to continuously send growth stimulatory signals into the interior of the cell in the absence of GFs.

Genes frequently mutated in cancer

The genes that have been implicated in carcinogenesis are divided into two broad categories oncogenes ('cell accelerators') and tumour suppressor genes ('cell brakes') but also include DNA repair genes (see The importance of DNA repair systems for further detail).

Cellular oncogenes

Genes that promote autonomous cell growth in cancer cells are called **oncogenes**, and their normal cellular counterparts are called **proto-oncogenes**. Proto-oncogenes are physiologic regulators of cell proliferation and differentiation while oncogenes are characterised by the ability to promote cell growth in the absence of normal mitogenic signals. Their products, oncoproteins, resemble the normal products of proto-oncogenes with the exception that oncoproteins are devoid of important regulatory elements. Their production in the transformed cells becomes constitutive, that is, not dependent on growth factors or other external signals. Proto-oncogenes can be converted to oncogenes by several mechanisms including point mutation and gene amplification resulting in:

- Overproduction of growth factors
- Flooding of the cell with replication signals
- Uncontrolled stimulation in the intermediary pathways
- Cell growth by elevated levels of transcription factors

The RAS oncogene is the most frequently mutated oncogene in human cancer. It encodes a GTP-binding protein Ras that functions as an on-off 'switch' for a number of key signalling pathways controlling cellular proliferation. In a normal cell, Ras is transiently activated and recruits Raf, to activate the MAP-kinase pathway to transmit growth-promoting signals to the nucleus. The mutant Ras protein is permanently activated leading to continuous stimulation of cells without any external trigger. Other oncogenes frequently mutated in cancer are listed in Table 1.

Table 1. Selected oncogenes and associated cancers

Category / Protein Function	Proto-oncogene	Mode of Activation	Associated Cancer
Growth Factors			
PDGF (β chain)	SIS	Overexpression	Astrocytoma, osteosarcoma
Fibroblast growth factors	HST-1	Overexpression	Stomach cancer
	INT-2	Amplification	Bladder & breast cancer
			Melanoma
Transforming growth factor α	TGFα	Overexpression	Astrocytomas
			Hepatocellular carcinomas
Growth Factors Receptors			
EGF-receptor family	ERB-B1	Overexpression	SCC of the lung, gliomas
	ERB-B2	Amplification	Breast and ovarian cancers
PDGF receptor	PDGF-R	Overexpression	Gliomas
Receptor for stem cell (steel) factor	KIT	Point Mutation	Gastrointestinal stromal tumours
Proteins Involved in Signal Transduction			
GTP-binding	K-RAS	Point mutation	Colon, lung, pancreatic tumours
	H-RAS	Point mutation	Bladder & kidney tumours
	N-RAS	Point mutation	Melanoma, leukaemia, lymphoma
Non-receptor tyrosine kinase	ABL	Translocation	CML, ALL
RAS signal transduction	BRAF	Point mutation	Melanomas
WNT signal transduction	β-catenin	Point mutation /Overexpression	Hepatoblastomas & HCC
Nuclear Regulatory Proteins	C-MYC	Translocation	Burkitt lymphoma
Transcriptional activators			Neuroblastoma, small cell
	N-MYC	Amplification	carcinoma of lung
	L-MYC	Amplification	SCC of the lung
Cell-Cycle Regulators			
Cyclins	CYCLIN D	Translocation	Mantle cell lymphoma
		Amplification	Breast & oesophageal cancers
	CYCLIN E	Overexpression	Breast cancer
		Amplification or	Glioblastoma, melanoma,
Cyclin-dependent kinase	CDK4	Point mutation	sarcoma

Adapted from Table 7-6, Kumar V, Abbas AK, Fausto N, Aster J. Robbins & Cotran pathologic basis of disease, 8th edition. Elsevier; 2010

Tumour suppressor genes

Tumour suppressor genes (Table 2) encode proteins that are:

- receptors for secreted hormones that function to inhibit cell proliferation
- negative regulators of cell cycle entry or progression
- negative regulators of growth signalling pathways (e.g. APC or PTEN)
- checkpoint-control proteins that arrest the cell cycle if DNA is damaged or chromosomes are abnormal
- proteins that promote apoptosis DNA repair enzymes.

The transformation of a normal cell to a cancer cell is accompanied by the loss of function of one or more tumour suppressor genes and both gene copies must be defective in order to promote tumour development (see Alteration of genetic mechanisms in cancer).

Table 2. Examples of tumour suppressor genes

Gene	Protein function	Inherited Disease	Spontaneous Tumours
APC	Negative regulator of the signalling pathway	Adenomatous polyposis coli (APC)	Most colon cancers
BRCA1, BRCA2	Components of DNA repair systems	Familial breast and ovarian cancer	Spontaneous breast cancers
CDH1	E-cadherin, a cell adhesion molecule	Hereditary diffuse gastric cancer	Many epithelial cancers
CDKN2A	INK4a, inhibitor of cyclin-dependent kinase Cdk4	Some familial melanomas	Some esophageal and pancreatic cancers
MEN1	Transcription factor and protein kinase	Multiple endocrine neoplasia	Many metastatic cancers
NF1	Neurofibromin, Ras-GTPase activation	Neurofibromatosis type 1	Some tumours of neural crest origin
PTEN	Negative regulator of PI3K growth signalling pathway	Cowden disease	30%-50% of spontaneous cancers
RB	Repression of transcription factor E2F	Retinoblastoma, osteosarcoma	Retinoblastoma, sarcomas, several carcinomas
SMAD4	Signal transducer in TGF-signalling	Juvenile polyposis	Colon and pancreatic cancers
TP53	Transcription factor; guardian of the genome'	Li-Fraumeni syndrome	Most frequently mutated in human cancers
TSC1,			

Gene	Protein function	Inherited Disease	Spontaneous Tumours
TSC2	Inhibitor of mTOR	Tuberous sclerosis	Rare
VHL	Ubiquitin ligase	von Hippel-Lindau disease	Many renal cell carcinomas
WT1	Transcription factor	Wilms tumour	Some leukaemias

Adapted from Table 7.1 Weinberg RA. Biology of cancer, 1st ed. Garland Science, 2007[2]

The retinoblastoma (Rb) protein is a tumour suppressor gene that controls the cell cycle transition from G1 to S Phase. Rb protein binds regulatory transcription factor E2F which is required for the synthesis of DNA replication enzymes. When Rb is bound to E2F, transcription/replication is blocked. The presence of growth factors (via the Ras pathway) activates cyclin-dependent kinase 4/6 (Figure 2) Active CDK4/6- phosphorylates and inhibits Rb, taking the brakes off E2F, and transition to S phase occurs. Disruption/deletion of the Rb gene therefore leads to uncontrolled cell proliferation.

Causes of cancer

Mutations and cancer

Cancer development is based on the accumulation of somatic mutations over lifetime. Germ line mutations are typically not involved, but in very rare cases of inherited cancer predisposition, they are contributing to disease progression.

Typically the basal mutation rate is low in humans, but it may be enhanced through one of the three following groups of environmental carcinogens: **chemical mutagens, radiation and tumour viruses**. Exposure to mutagens or radiation greatly increases the mutation rate and thus the probability of developing cancer.

Chemical mutagens comprise a quite disparate group of chemicals that modify DNA through a range of mechanisms, such as alkylation or deamination of DNA bases, or through intercalation between base pairs and formation of DNA adducts (e.g. aromatic hydrocarbons). Oxidative damage may also affect DNA integrity.

X-rays and radioactive radiation tend to induce DNA double-strand breaks, whereas **UV radiation** results in the formation of pyrimidine dimers, by cross-linking of adjacent pyrimidine bases.

Viral causes of cancer

Certain viruses, derived from quite different taxonomic groups (Table 3), are able to induce cancer development. We distinguish the highly **oncogenic viruses**, which contain **viral oncogenes** in their genomes that are in most cases derived from cellular proto-oncogenes, whereas **slowly transforming viruses** do not contain such genes. They tend to use one of the following mechanisms to stimulate proliferation of their host cells:

- Insertion of a strong promoter in the vicinity of a host cell proto-oncogene
- Expression of proteins that neutralise host cell tumour suppressor proteins

- Expression of proteins that prevent or delay apoptosis

Characteristics of viral carcinogenesis include:

- Tumour viruses often establish persistent infections in the human host
- Host factors are important determinants of virus-induced carcinogenesis
- Viruses are rarely complete carcinogens; they require additional factors to fully activate carcinogenesis.

Table 3. Human tumour viruses

Virus (Group)	Associated Human Cancer
DNA VIRUSES	
Papilloma virus family Human papilloma virus (HPV) (various subtypes)	Genital tumours, squamous cell carcinoma
Herpes virus family Human herpes virus 8 (HHV8) Epstein-Barr virus (EBV)	 Kaposi sarcoma Burkitt's lymphoma, Hodgkin's disease, Nasopharyngeal carcinoma
Hepadnavirus family Hepatitis B virus	 Hepatocellular carcinoma
RNA VIRUSES	
Retrovirus family Human T-cell leukaemia virus Human immunodeficiency virus	 Adult T-cell leukaemia AIDS-related malignancies
Flavivirus family Hepatitis C virus	Hepatocellular carcinoma

Adapted from Table 43-1, Brooks GF, Carroll KC, Butel JS, Morse SA. Jawetz, Melnick & Adelberg's medical microbiology, 24th ed. McGraw-Hill, 2007[4])

The importance of DNA repair systems

Sophisticated DNA repair systems have evolved in order to maintain the human genome, by fixing damage that may have occurred to the DNA. Principal DNA repair mechanisms include: **mismatch repair, base and nucleotide excision repair, repair of depurinated sites and repair of double-strand breaks.**

The importance of these repair systems for protection against accelerated mutagenesis and the development of cancer is impressively demonstrated through rare inherited **cancer predisposition syndromes** based on mutations in DNA repair enzyme systems (Table 4).

Table 4. Inherited diseases caused by DNA repair defects

Disease	Protein Affected	Affected Function	Manifestation
Bloom syndrome	13 different proteins	Recombination repair?	Immunodeficiency, cancer susceptibility, chromosome breaks
Breast cancer susceptibility	BRCA1, BRCA2; proteins of DNA repair complexes	Homology-directed DNA repair	Breast and ovarian cancer
Cockayne syndrome	Nucleotide excision repair protein	Transcription-coupled nucleotide excision repair	Poor growth, early senility, neurological degeneration
Fanconi anemia	8 different proteins	Repair of DNA cross-links?	Anaemia, leukaemia, chromosome breakage
Hereditary nonpolyposis colon cancer (HNPCC)	Proteins of mismatch repair	Post-replication mismatch repair	Cancer susceptibility
Nijmegen breakage syndrome	Activator of nuclear protein kinases	Signalling for DNA double-strand break repair	Growth retardation, immunodeficiency, cancers
Werner syndrome	DNA helicase and exonuclease	Unknown	Premature aging, short telomeres
Xeroderma pigmentosum	Nucleotide excision repair proteins	Genome-wide nucleotide excision repair	Cutaneous photosensitivity

(Adapted from Table 9.1, Meisenberg G, Simmons WH. Principles of medical biochemistry, 3rd ed. Mosby/Elsevier, 2012[5])

Multistep carcinogenesis

Carcinogenesis can be considered as a complex micro-evolutionary process, which requires the accumulation of a range of (somatic) genetic mutations (Figure 1). Under selection pressure and through these mutations, cells acquire new characteristics, which provide them with an advantage in growth behaviour and other cellular properties, such as enhanced survival and invasiveness. This process is in most cases drawn out over many years and requires a series of individual steps.

The main stages of carcinogenesis – an overview

There are three major qualitative changes, which cells have to undergo in order to successfully proceed through the complete process of carcinogenesis, **malignant transformation, invasion** of neighbouring tissues and **metastasis**. Each one of these major stages comprises a series of genetic alteration of cells affecting specific genes that are involved in regulating cell properties relevant for the individual stage, i.e. growth behaviour (for malignant transformation), invasive properties and metastatic potential.

Early steps characterised in colon cancer

The best characterised example supporting the theory of multi-step carcinogenesis is colorectal cancer. This is largely due to the relative accessibility of colon cancer samples and due to the availability of the distinct histo-morphological description of early stages of cancer development. Genetic characterisation of a large number of early, intermediate and late adenomas and frank carcinomas led to the establishment of a 'preferred' sequence of genetic alterations during the adenoma-adenocarcinoma pathway of colorectal cancer (Figure 4). These include the activation of the K-ras oncogene from its cellular proto-oncogene (pink letters) and the loss for three tumour suppressor genes (blue letters), where loss of APC (adenomatous polyposis coli) is an early event, whereas loss of p53 is normally a late event.

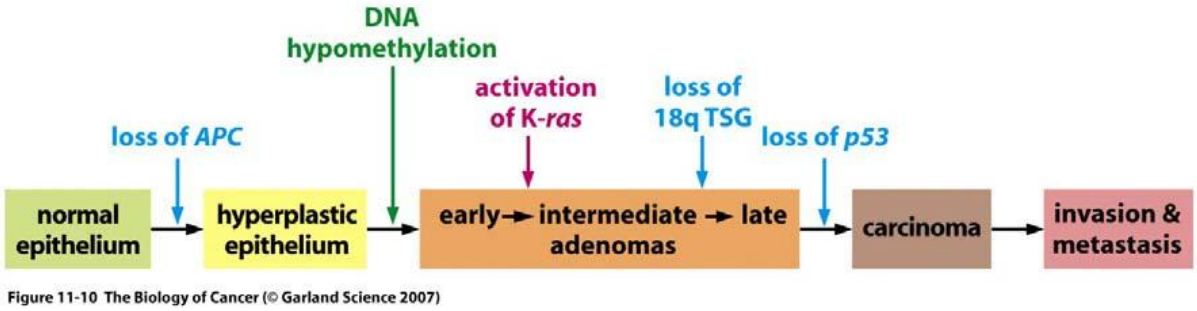

Figure 4: Genetic events in early colon carcinoma progression. Approximate correlation of early genetic events in the development of colon carcinoma (the adenoma-adenocarcinoma pathway) with histopathological features. Note that clinical staging typically refers to the later observations and cannot be correlated with the genetic events. Genetic events are indicated by vertical arrows and colour-coded as follows: Blue: loss of tumour suppressor gene (TSG) function, red: activation of oncogenes, green: epigenetic events. The sequence of genetic events is not necessarily obligatory, but loss of APC is typically the first event and loss of p53 typically the last one.

Source: Weinberg RA. The biology of cancer, 1st ed. Garland Science, 2007[2] Reproduced with permission of Garland Science/Taylor & Francis LLC.

Cellular principles of invasion and metastasis

The spread of cancer cells to distant sites in the body via the blood stream/lymphatics is known as **metastasis** and is the most lethal form of the disease (Figure 5). Metastatic cells are less adhesive than normal cells and are able to degrade and penetrate tissue barriers such as the extracellular matrix (ECM) of surrounding connective tissue and the basement membrane of blood vessels. After gaining access to the systemic circulation they can invade normal tissue at various sites in the body forming secondary colonies. The **invasion - metastasis cascade** involves:

1. Acquisition of local invasiveness
2. Invasion of the cell into blood/ lymph vessels (intravasation)
3. Transport through the blood/lymph vessels to distant tissue sites
4. Escape of the cancer cells from circulation (extravasation)
5. Ability to adapt to the local tissue environment and to proliferate

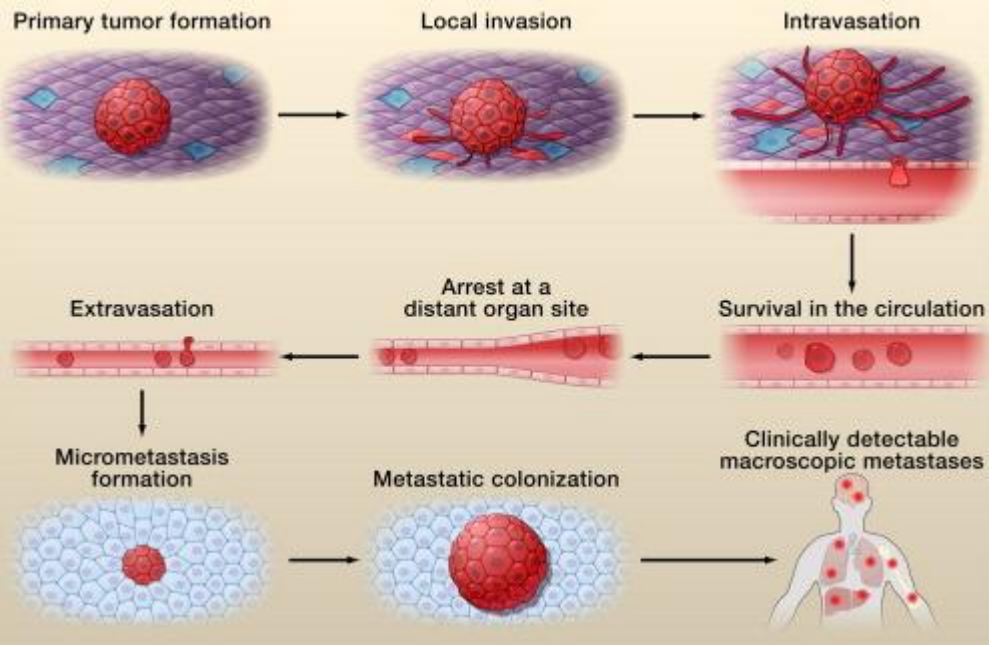

Figure 5: Steps involved in the metastatic cascade. During metastatic progression, tumour cells exit their primary sites of growth (local invasion, intravasation; **1** & **2**), translocate systemically (survival in the circulation, arrest at a distant organ site, extravasation; **3** & **4**), and adapt to survive and thrive in foreign microenvironments (**5**).

Source: Valastyan S, Weinberg RA. Tumor metastasis: molecular insights and evolving paradigms. Cell 2011[6]

Epithelial-mesenchymal transition (EMT) is a key transition enabling cancer cells to become motile and invasive, and ultimately form metastases in distant tissues.

Cell motility is regulated by small G proteins that are activated by cytoplasmic signalling pathways controlling the assembly of new actin cytoskeleton.

Cell invasiveness is enhanced through overexpression of various matrix metalloproteinases (MMPs) that degrade components of the ECM.

Angiogenesis, the growth of the new blood vessels, is necessary for solid tumours to continue growing beyond a certain size. More than a dozen different proteins and several small molecules are released by tumours as signals for angiogenesis. Two proteins most important for sustaining tumour growth are vascular endothelial growth factor (**VEGF**) and basic fibroblast growth factor (**bFGF**).

Stromal microenvironment and carcinogenesis

Cross talk between stromal cells within the ECM and tumour cells is also vital for carcinogenesis. The following factors are thought to contribute to malignant transformation:

- Cleavage of matrix components releases angiogenic factors (VEGF) promoting new vessel growth and proteolytic fragments that favour cancer cell motility.
- The ECM stores GFs in inactive forms, which are released by active matrix proteases and stimulate the growth of tumour cells in a paracrine manner.
- Stromal cells within the ECM may directly transmit oncogenic signals to tumour cells.

Other genetic aspects of cancers

Apart from the three major types of genes frequently altered in cancer, i.e. tumour suppressor genes, proto-oncogenes and DNA repair genes, there are several other genetic alterations observed in tumours, which will be briefly described here.

Genetic instability of tumour cells

Genetic analysis of solid tumours revealed the presence of a high degree of genetic abnormalities, such as aneuploidy, chromosome translocations etc. This is likely due to the lack of active p53 protein, and the ability of cancer cells to avoid cell death through apoptosis. Other mechanisms may also play a part here, e.g. mitotic defects that result in chromosome miss-segregation. Chromosomal instability (CIN) is widespread in cancer cells from epithelial origin, but much rarer in haematopoietic tumours.

Alteration of genetic mechanisms in cancer

Three different alterations of genetic mechanisms often observed in cancer will be briefly explained below.

Loss of heterozygosity (LOH): This describes a genetic phenomenon often seen with tumour suppressor genes in cancer. Since the human karyotype is diploid, mutation of one allele of a tumour suppressor gene is not sufficient to cause cancer. In heterozygous individuals, the wildtype allele will provide for a functional phenotype. However, when a 'second hit' occurs, e.g. through missegregation of chromosomes, this individual (or cell) may lose its 'heterozygosity', leading to a full cancerous phenotype. Genetic analyses of LOH helped to identify the chromosomal location of many tumour suppressor genes.

Microsatellite instability (MIN): This is a phenomenon often seen in colorectal cancer cells with defective DNA mismatch repair system, e.g. in hereditary nonpolyposis colorectal cancer (HNPCC). Microsatellites are regions of repetitive DNA sequences in the genome that are prone to shortening or extension if the mismatch repair enzymes are defective. Genetic analysis of these regions can be used to identify such defects.

DNA hyper- or hypomethylation: DNA methylation of gene promoter regions on CpG (cytosine-phosphate-guanine) sequences is an important epigenetic control mechanism to silence specific genes. In cancer, DNA hypermethylation is often involved in the silencing of tumour suppressor genes. Conversely, DNA hypomethylation may contribute to the activation of oncogenes, although the former occurs much more commonly.

Inherited predisposition to cancer

Whilst cancer as such is not inherited, there are a wide range of rare familial syndromes that predispose affected family members to cancer development. We mentioned above cancer predisposition syndromes that are based on mutations in DNA repair enzyme systems (Table 4, in The importance of DNA repair systems). A by far larger number of familial cancer syndromes is based on mutations of tumour suppressor genes, of which a selection is shown in Table 2. It is interesting to note that germ line mutations of activated oncogenes are normally not inherited. They may arise during gametogenesis, but the mutant alleles are typically dominant at the cellular level, which results in disturbance of normal embryonic development, and reduced viability of these embryos. Fortunately, the inherited cancer predisposition syndromes listed in Tables 2 and 4 are extremely rare diseases, but they represent powerful illustrations for the importance of DNA repair and tumour suppressor genes for maintaining body homeostasis.

Principal applications of genetic testing in cancer

As an increasing number of cancer-related genes or gene mutations is characterised, the potential of DNA and RNA expression testing for cancer-related applications is being explored. Principal applications include:

Gene mutation screening in families with inherited cancer predisposition syndromes, which identifies at- risk individuals in such families and allows for decisions to be made about early disease monitoring, aggressive treatment regimens and prophylactic surgery (e.g. mastectomy in familial breast cancer).

Gene expression microarray analysis can be used for classification of cancer subtypes, e.g. in breast cancer or for the distinction between acute lymphoblastic and acute myeloid leukaemia. Other applications include the diagnosis of benign vs. malignant tumours or the monitoring of response to therapies.

Modern treatment modalities arising from cancer cell biology

Tumour immunology and immunotherapy

The immune system is able to launch attacks not only against foreign invaders, but also against body cells that may display 'foreign' antigens, such as cancer cells. The 'immune surveillance theory' is supported by the observation that the incidence of certain cancers is drastically increased in immune-compromised patients. Tumour cells may be recognised by the immune system through the expression of tumour-associated antigens, but the antigenicity varies considerably between different types of antigens.

In order to avoid an attack by the immune system, tumour cells use a range of strategies, such as suppression of expression of tumour-associated antigens or of MHC class 1 molecules, or even counterattack against immune cells.

Research into **immunotherapy of cancers** aims to devise novel strategies to support the anti-cancer immune response; principal approaches include:

- Antigen-independent cytokine therapy (e.g. interleukins or interferons)
- Stimulating cell-mediated immune responses (adoptive T-cell transfer, vaccines)
- Passive immunotherapy using monoclonal antibodies (e.g. Herceptin, Rituxan).

Novel approaches arising from cancer cell biology

The progress in our knowledge about gene mutations frequently occurring in cancers, combined with the development of modern molecular biology methods has led to both new diagnostic tools (see Principal applications of genetic testing in cancer) and new treatment modalities that have shown some success in the management of selected types of cancers. The knowledge about cancer–associated genes and their role in cellular growth signalling pathways has led to the development of a considerable number of anti-cancer drugs targeting such signalling pathways: 1) **monoclonal antibodies** that target the extracellular domains of growth factor receptors and 2) **small-molecule inhibitors**, targeting either receptor tyrosine kinases or other components of growth signalling pathways, such as Ras, b-Raf or mTOR (Figure. 6). Two examples of such successful anti-cancer agents are the monoclonal antibody **Herceptin** for the treatment of a specific subtype of breast cancer, and the small-molecule inhibitor **Gleevec** targeting the fusion protein Bcr-abl, a mutant tyrosine kinase, involved in the development of chronic myeloic leukaemia (CML). A third group of potential drug targets are some anti-apoptotic proteins that are frequently overexpressed in cancer cells.

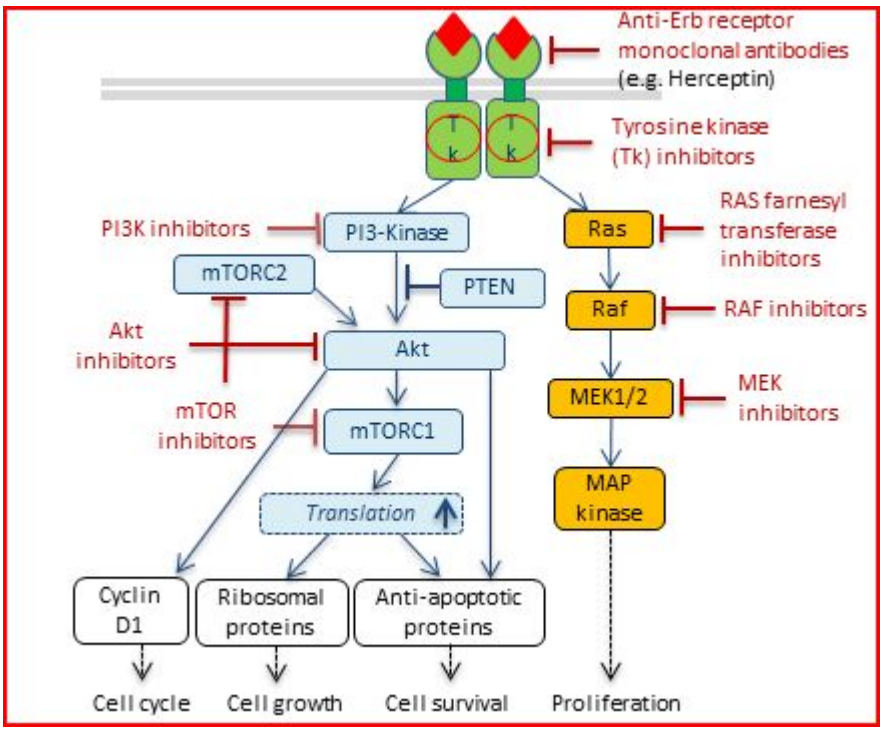

Figure 6. Targets of novel anti-cancer drugs in cellular growth signalling pathways. The cell membrane is indicated in light grey, red diamonds represent growth factors, green shows the growth factor receptor with the intracellular tyrosine kinase domain (Tk) indicated by the red circle. Coloured rectangles symbolise signalling components belonging to specific pathways (Blue: PI3K/Akt pathway; ochre: Ras/MAP kinase pathway). Dotted (black) arrows point to cell biological outcomes of these pathways. Groups of novel anticancer drugs and their targets are shown in red.

Source: Weinberg RA. The biology of cancer. Garland Science, 2007[2]

Summary: The hallmarks of cancer

To summarise the core points, we are listing the 'hallmarks of cancer', which describe the biological capabilities acquired by cells during the multistep development of human tumours (Figure 7):

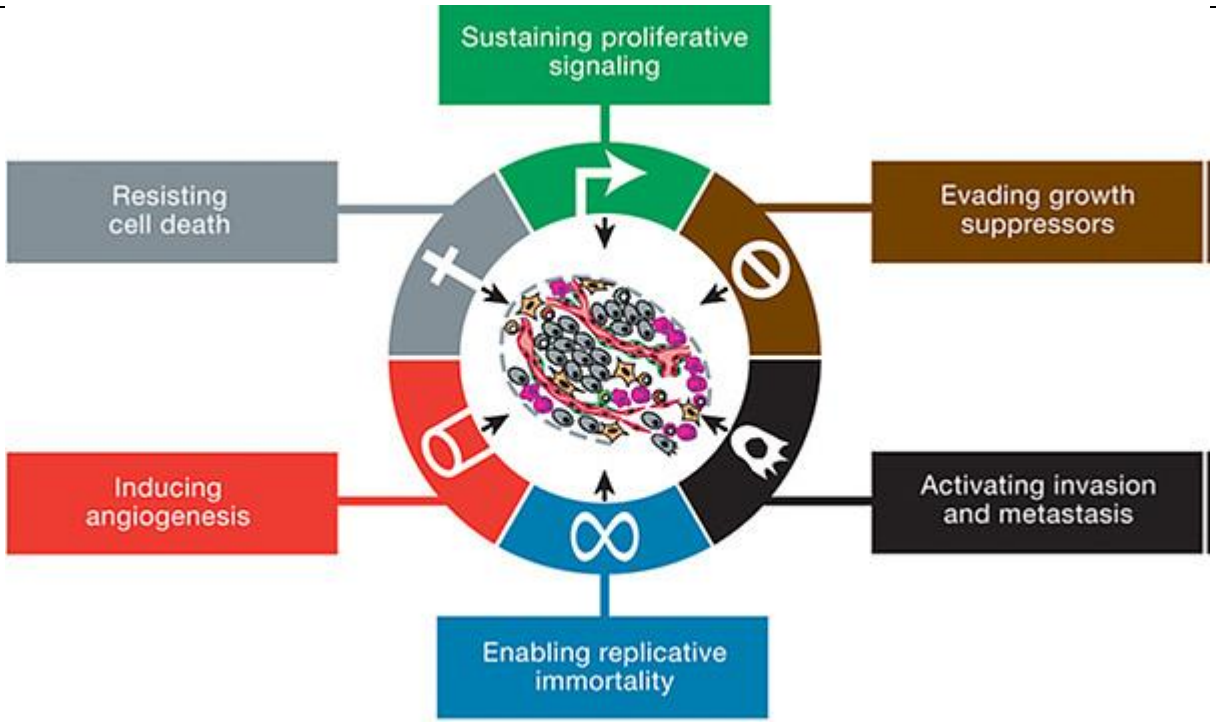

Figure 7: A summary of the 6 hallmarks of cancer. Additional capabilities crucial to cancer phenotypes that are not shown here include defects in DNA repair mechanisms and signalling interactions of the tumour microenvironment.

Source: Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell, 2011[7]

Self-sufficiency in growth signals: Tumours have the capacity to proliferate without external stimuli, usually as a consequence of oncogene activation.

Insensitivity to growth-inhibitory signals: Tumour cells may not respond to molecules that are inhibitory to the proliferation of normal cells.

Evasion of apoptosis: Tumours may be resistant to programmed cell death, as a consequence of inactivation of p53 or overexpression of anti-apoptotic proteins.

Defects in DNA repair: Tumours may fail to repair DNA damage caused by carcinogens or unregulated cellular proliferation.

Limitless replicative potential: Tumour cells have unrestricted proliferative capacity, associated with maintenance of telomere length and function.

Sustained angiogenesis: Tumours are not able to grow without formation of a vascular supply, which is induced by various factors, the most important being vascular endothelial growth factor (VEGF).

Ability to invade and metastasise: Tumour metastases are the cause of the vast majority of cancer deaths and depend on processes that are intrinsic to the cell or are initiated by signals from the tissue microenvironment.

Further reading

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