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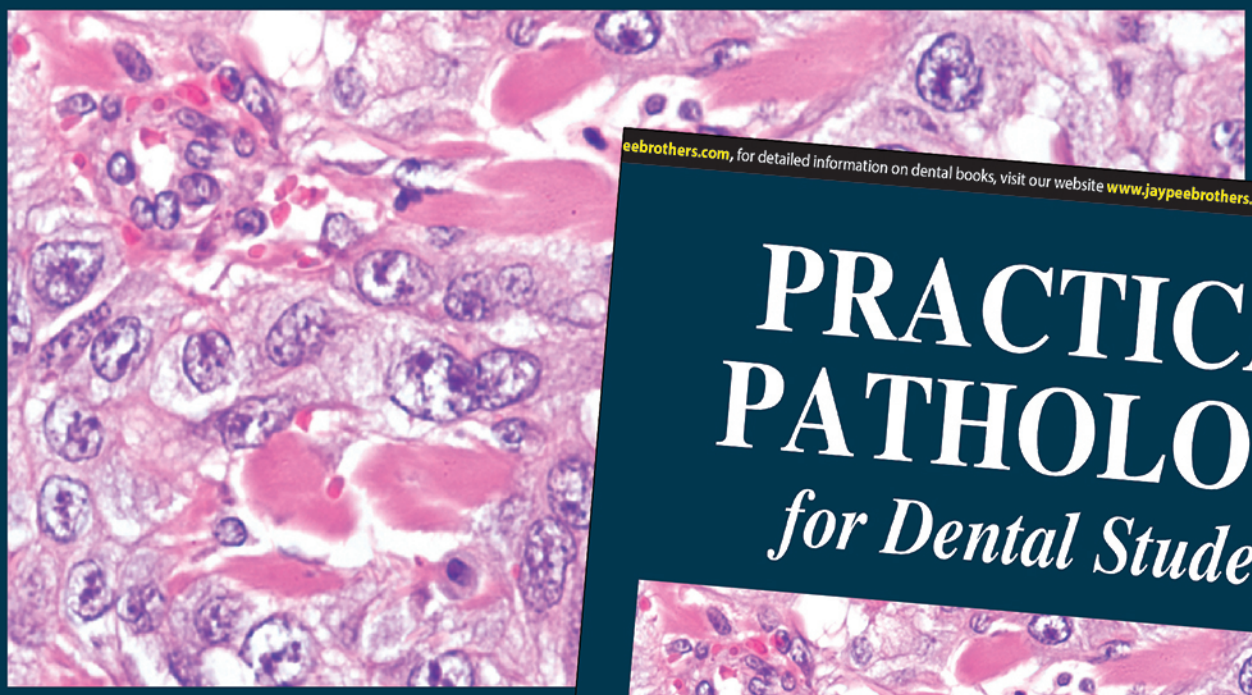

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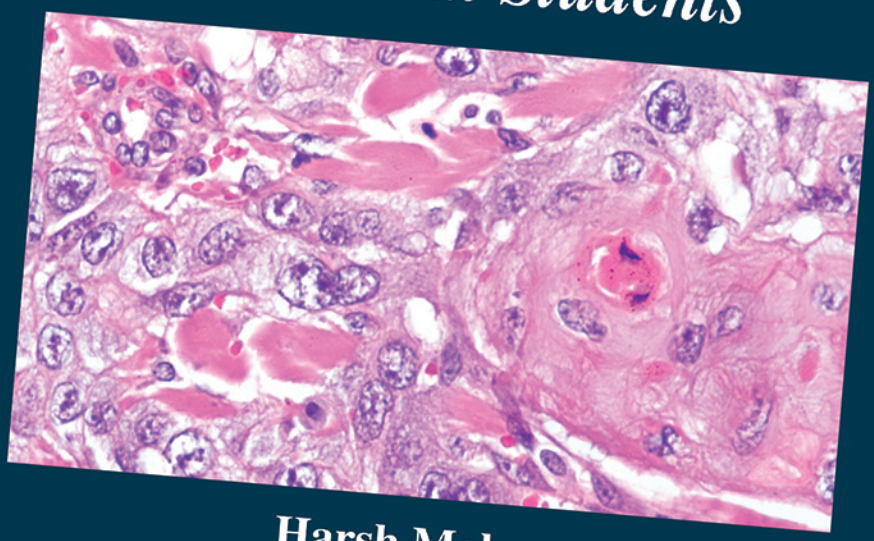

Harsh Mohan
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CHAPTER 29 Molecular Biology of Tumours

Chapter Orientation

Tumour biology, i.e. how a normal healthy cell is transformed into a precancerous and cancerous cell, is quite complex. At different times, attempts have been made to unravel this mystery by various mechanisms. Based on research done in the last few decades, biology of tumours at molecular level is discussed in this chapter under following headings: 1) theories of tumour biology, 2) basis of molecular pathogenesis of cancer, and 3) molecular hallmarks of cancer.

THEORIES OF TUMOUR BIOLOGY

A few mutually-interlinked theories which explain the driving forces behind cancer onset and proliferations are as under:

1. Monoclonal theory There is strong evidence to support that most human cancers arise by genetic transformation or mutation of a single clone of cells. For example:

i) In a case of multiple myeloma (a malignant disorder of plasma cells), there is production of a single type of immunoglobulin or its chain as seen by monoclonal spike in serum electrophoresis.

ii) Due to inactivation of one of the two X-chromosomes in females (paternal or maternal derived), normal uterine myometrial cells are mosaic having two types of cell populations for glucose-6-phosphatase dehydrogenase (G6PD) isoenzyme A and B genotypes. However, in leiomyomas (benign uterine tumour), it is observed that the tumour cells contain either A or B genotype of G6PD, i.e. the tumour cells are derived from a single progenitor clone of cell **(Fig. 29.1)**.

2. Tissue organisation field theory According to this theory, carcinogenesis is primarily a problem of tissue organisation. Disorganisation of tissue architecture by carcinogenic agents is due to disruption in cell-to-cell signalling and cell-to-stromal cell interaction that alters or mutates the genome and initiates cancer. The role of the stromal cells in tumour microenvironment is discussed later. Thus, according to this theory, DNA mutations are the outcome, not the cause, of cellular events in cancer.

3. Somatic mutation theory According to this theory, cancer is derived from a single clone of somatic cells that has undergone multiple mutations in its DNA. These mutations occur in the genes that control the cell proliferation and the cell cycle. The abnormalities in genetic composition may be from inherited mutations, or may be induced by etiologic carcinogenic agents (chemicals, viruses, radiation etc). Eventually, the mutated cells transmit their characters to the next progeny of cells and result in cancer. Thus, the cancer is the result of DNA-level events.

4. Multi-step theory of cancer phenotype and progression Genetically transformed cells having phenotypic features of malignancy—excessive growth, invasiveness and distant metastasis, is preceded by multiple mutations:

- *Driver mutations* are those which transform the target cells into phenotypic malignant cells.
- Development of cancer phenotype is a multi-step gradual process involving generations of cells beginning with *initiator mutation*. The mutated and initiated cells

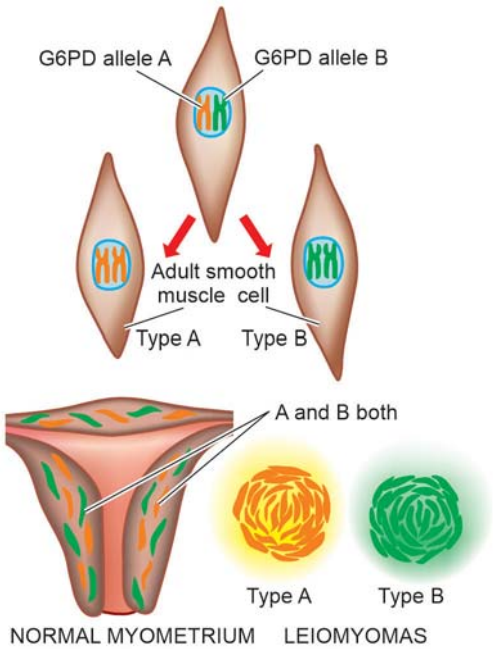

Figure 29.1 The monoclonal origin of tumour cells in uterine leiomyoma. The normal myometrial cell growth have both alleles of G6PD (A and B) (shown in orange and green colour respectively) while in leiomyoma the tumour cells are derived from either type A (shown in orange only) or type B (shown in green only) alleles, i.e. they are monoclonal.

remain in the host at pre-clinical stage as *cancer stem cells*, most evident in preclinical stage of acute leukaemias.

- Multiple driver mutations are also involved in further *progression* of the tumour. The target cell is attacked by various etiologic agents one after another (*multi-hit process*). For example, in chemical carcinogenesis, there is attack by initiator and promoter carcinogens in sequence. Evolution of colorectal cancer through adenoma-carcinoma sequence involving early *APC* mutation followed later by loss of *TP53* supports multi-step hypothesis of cancer.
- Another common type of genetic mutation in early stage of malignancy, particularly in solid tumours, is *loss-of-function mutation*. The loss-of-function mutations cause genomic instability in the target cell which may transform it into malignant phenotype due to driver mutations, or the target cell may carry passenger mutations without cancer phenotype, but it commonly accumulates several acquired mutations for cancer development later.

5. Epigenetic theory In addition to genetic mutations in DNA, abnormalities in epigenetic phenomena in cancer has attracted attention in recent times, especially because it is possible to counteract epigenetic modifications by drugs. Basic concept of epigenetics in normal cell biology is discussed on page 11. Errors in epigenetic processes which may appear in cancer are in *DNA methylation and histone modification*. These epigenetic changes are quite widespread; therefore, for successful epigenetic therapy, drugs are being developed which have to target specifically abnormal cells.

These theories stated above do not conflict with each other, but instead come into confluence and complement each other into a unifying concept of carcinogenesis which is explained on molecular basis below.

BASIS OF MOLECULAR PATHOGENESIS OF CANCER

In normal cell growth, regulatory genes control mitosis as well as cellular ageing, terminating in cell death by apoptosis.

- **In normal cell growth,** there are four regulatory genes:

i) *Proto-oncogenes* are growth-promoting genes, i.e. they encode cell proliferation pathway.

ii) *Tumour-suppressor genes or anti-oncogenes* are growth-inhibiting or negative regulators of cell proliferation.

iii) *Apoptosis regulatory genes* control the programmed cell death.

iv) *DNA repair genes* are those normal genes which regulate the repair of DNA damage that has occurred during mitosis and also control the damage to proto-oncogenes and tumour-suppressor genes.

- **In cancer,** the transformed cells are produced by abnormal cell growth due to genetic damage to these normal controlling genes. Thus, corresponding abnormalities in these four cell regulatory genes are as under:

i) *Activation of growth-promoting oncogenes* causing transformation of cell (mutant form of normal proto-oncogene in cancer is termed *oncogene*). Many of these cancer-associated genes (i.e. oncogenes) were first discovered in viruses, and hence were named as *v-onc*. Gene products of oncogenes are called *oncoproteins*. Oncogenes are considered *dominant*, i.e. mutation of single gene copy may transform the cell to cancer cell.

ii) *Inactivation of tumour-suppressor genes* permitting the cellular proliferation of transformed cells. Tumour-suppressor genes are active in *recessive* form, i.e. loss of both alleles is required for transformation of the cell to neoplastic cell.

iii) *Abnormal apoptosis regulatory genes* which may act as oncogenes or tumour-suppressor genes. Accordingly, these genes may be active in dominant or recessive form.

iv) *Failure of DNA repair genes* and, thus, their inability to repair the DNA damage resulting in mutations.

Eventually, evolution of genotypic features of malignancy in the cell shows phenotypic characteristics of cancer cell; several cycles of proliferation of such cancer cells result in formation of a mass or a growth **(Fig. 29.2)**. However, not all genotypic tumour cells survive to establish a phenotypic growth. Actually, many genotypic malignant cells die by apoptosis, or

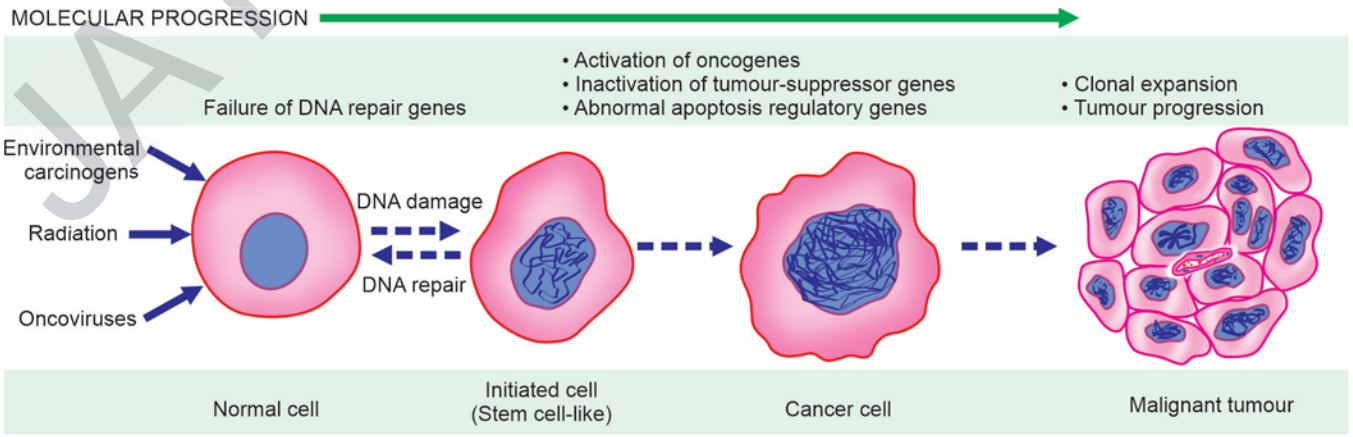

Figure 29.2 Schematic illustration to show basic concept of carcinogenesis at molecular level.

due to deprivation of nutrition, or due to unsuitable microenvironment. Thus, tumour develops by those malignant cells which have survived all odds, i.e. 'survival of the fittest' holds true for cancer cells contributing to form a tumour.

Following discussion on molecular pathogenesis is built on this basic concept.

MOLECULAR HALLMARKS OF CANCER

Cancer hallmarks are defined as *acquired characteristics which transform phenotypically normal cells into malignant cells and promote progression of malignant cells while damaging the host tissue*. In view of recent advances in our knowledge and understanding of molecular carcinogenesis having benefit of translation from research to the clinic, cancer hallmarks have been updated as listed in **Table 29.1**, schematically illustrated in **Fig. 29.3** and discussed below.

A general discussion on normal cell growth and controls in the cell cycle has been given in Chapter 2. Following discussion pertains to the pathophysiological alterations which occur in cellular growth by cancer-related genes at molecular level.

I. GROWTH AND PROLIFERATION-PERMISSIVE COMPONENTS: ALTERED CELL-SIGNALLING

Normal cell growth is regulated by growth-signalling pathways allowing the cells to proliferate in a controlled manner which gets disrupted in cancer. In cancer, 'growth-promoting' signals (oncogenes) are aberrantly produced in excess, favouring tumour progression while 'growth-inhibitory' signals (tumour-suppressor genes) are shut down or become insensitive, allowing the tumour to proliferate due to failure of brakes. The

TABLE 29.1 Molecular hallmarks of cancer.*

I.	*Growth and proliferation permissive components: Altered growth-signalling*
	1) Self-sufficiency in growth signals: Growth-promoting oncogenes 2) Refractoriness to growth-inhibitory signals: Tumour-suppressor genes
II.	*Favouring overall cell survival: Altered stress response*
	1) DNA damage and repair system: Mutator genes 2) Escaping cell death by apoptosis: Apoptosis regulatory genes 3) Evading cell senescence: Telomere and telomerase 4) Recycling intracellular components: Autophagy
III.	*Sustained perfusion of cancer: Vascularisation*
	1) Tumour angiogenesis 2) Other modes of vascularization
IV.	*Cancer dissemination: Invasion and metastasis*
	1) Invasion, intravasation and circulating tumour cells 2) Extravasation, organ predilection, micrometastasis, dormancy and colonisation
V.	*Growth-promoting metabolic changes: The Warburg effect*
	1) Excessive nutrient acquisition 2) Altered metabolic pathways 3) Oncometabolites in tumorigenesis
VI.	*Dynamic tumour microenvironment: The stromal cells*
	1) Angiogenic vascular cells 2) Cancer-associated fibroblastic cells 3) Infiltrating immune cells
VII.	*Evasion of host immunity: Immune modulation*
	1) Tumour antigens 2) Anti-tumour immune responses 3) Escape from immune surveillance

* Adapted from Fouad YA and Aanei C. Revisiting the hallmarks of cancer. *Am J Cancer Res*. 2017;7 (5):1016-1036.

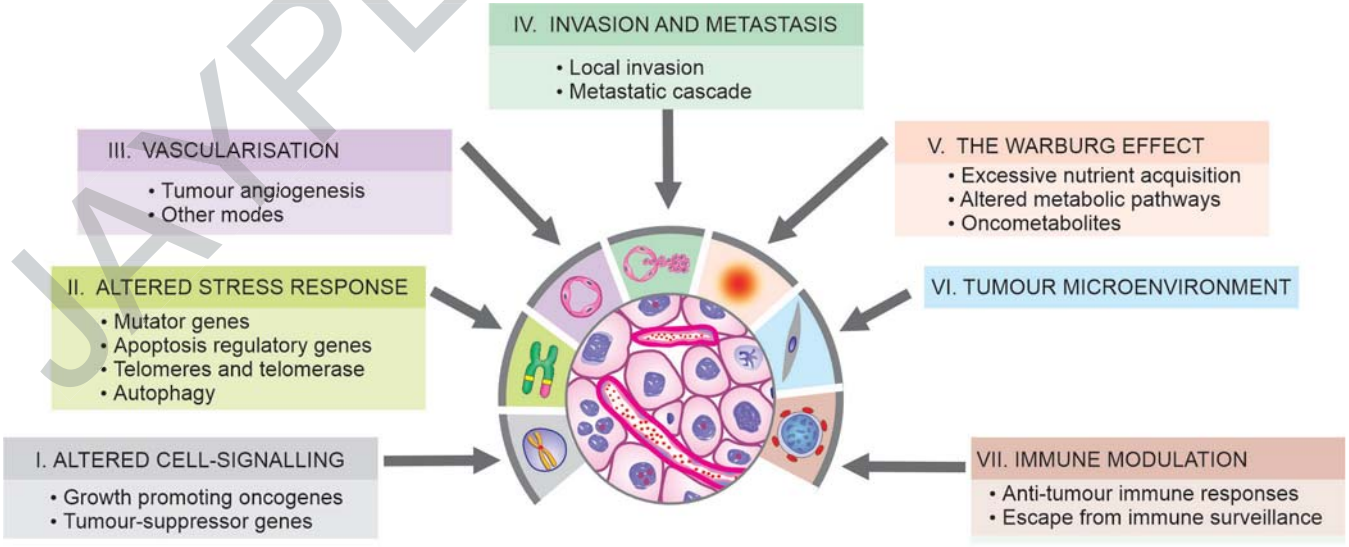

Figure 29.3 Schematic representation of revised hallmarks of cancer in terms of molecular carcinogenesis (depicted left to right clockwise).

net effect of both these signalling components is balance in favour of cell proliferation in cancer.

1. EXCESSIVE AND AUTONOMOUS GROWTH: GROWTH-PROMOTING ONCOGENES

Mutated form of normal proto-oncogenes in cancer is called oncogenes. In general, overactivity of oncogenes enhances cell proliferation and promotes development of human cancer. About 100 different oncogenes have been described in various cancers. Transformation of proto-oncogene (i.e. normal cell proliferation gene) to oncogene (i.e. cancer cell proliferation gene) may occur by one of the following three ways:

i) *Point mutations,* i.e. an alteration of a single base in the DNA chain. The most important example is *RAS* oncogene carried in many human tumours such as bladder cancer, pancreatic adenocarcinoma, cholangiocarcinoma.

ii) *Chromosomal translocations,* i.e. transfer of a portion of one chromosome carrying proto-oncogene to another chromosome and making it independent of growth controls. This is implicated in the pathogenesis of leukaemias and lymphomas; for example:

- Philadelphia chromosome is seen in 95% cases of chronic myelogenous leukaemia in which *c-ABL* proto-oncogene on chromosome 9 is translocated to BCR of chromosome 22.
- In 75% cases of Burkitt lymphoma, translocation of *c-MYC* proto-oncogene from its site on chromosome 8 to a portion on chromosome 14 is seen.

iii) *Gene amplification,* i.e. increasing the number of copies of DNA sequence in proto-oncogene leading to increased mRNA and thus increased or overexpressed gene product (i.e. oncoproteins). Examples of gene amplification are found in some solid human tumours; for example:

- Neuroblastoma having *n-MYC HSR* region
- *ERB-B2* (or HER2/neu) in breast and ovarian cancer

Most of the oncogenes encode cell signalling system for promoting cell proliferation. Since their mutated forms drive initiation and maintenance of tumour cells, these mutations are called *'driver mutations.'*

Growth-promoting oncogenes are discussed below under five groups pertaining to different components of cell proliferation signalling systems **(Table 29.2)** and are schematically shown in **Fig. 29.4**:

TABLE 29.2 Important oncogenes, their mechanism of activation and associated human tumours.

	TYPE AND ONCOGENE	PROTO-ONCOGENE	ACTIVATION MODE	ASSOCIATED HUMAN TUMOURS
1.	GROWTH FACTORS			
	i) PDGF-β chain	*SIS (PDGF-β)*	Overexpression	Gliomas, sarcoma
	ii) TGF-α	*RAS (TGF-α)*	Overexpression	Gliomas, carcinomas
	iii) FGF	*HST-1*	Overexpression	Osteosarcoma
		FGF3	Amplification	Breast cancer, stomach cancer
	iv) c-MET	*HGF*	Overexpression	Follicular carcinoma thyroid, hepatocellular Ca
2.	GROWTH FACTOR RECEPTORS			
	i) EGF receptors	*ERB B1 (HER-1, EGFR)*	Various mutations	Adenocarcinoma lung
		ERB B2 (HER-2/neu,	Amplification	Ca breast, ovary
	ii) c-KIT receptor (steel factor)	*EGFR) c-KIT*	Point mutation	Gastrointestinal stromal tumour (GIST)
	iii) RET receptor	*RET*	Point mutation	MEN type 2A and type 2B, familial medullary Ca thyroid
	iv) FMS-like tyrosine kinase receptor	*FLT-3* gene	Point mutation	Acute myeloid leukaemia
	v) PDGF receptor	*PDGFR-β*	Overexpression, translocation	Gliomas, leukaemias
	vi) ALK receptor	*ALKR*	Translocation	Adenocarcinoma lung, lymphomas
			Point mutation	Neuroblastoma
3.	CYTOPLASMIC SIGNAL TRANSDUCTION PROTEINS			
	i) GTP-bound (G) proteins	*RAS (several types)*	Point mutation	Common in 1/3rd human tumours, Ca lung, colon, pancreas
	ii) Non-receptor tyrosine kinase	*ABL-BCR*	Translocation	Chronic myeloid leukemia (CML)
			Point mutation	Acute leukaemias
	iii) JAK/STAT signal transduction	*JAK2*	Translocation	Myeloproliferative disorders, ALL
4.	NUCLEAR TRANSCRIPTION FACTORS			
	i) C-MYC	*MYC*	Translocation	Burkitt lymphoma
	ii) N-MYC	*MYC*	Amplification	Neuroblastoma, small cell Ca lung
	iii) L-MYC	*MYC*	Amplification	Small cell Ca lung
5.	CELL CYCLE REGULATORY PROTEINS			
	i) Cyclins	*Cyclin D*	Translocation	Ca breast, myeloma, mantle cell lymphoma
		Cyclin E	Overexpression	Ca breast
	ii) CDKs	*CDK4*	Amplification	Glioblastoma, melanoma, sarcomas

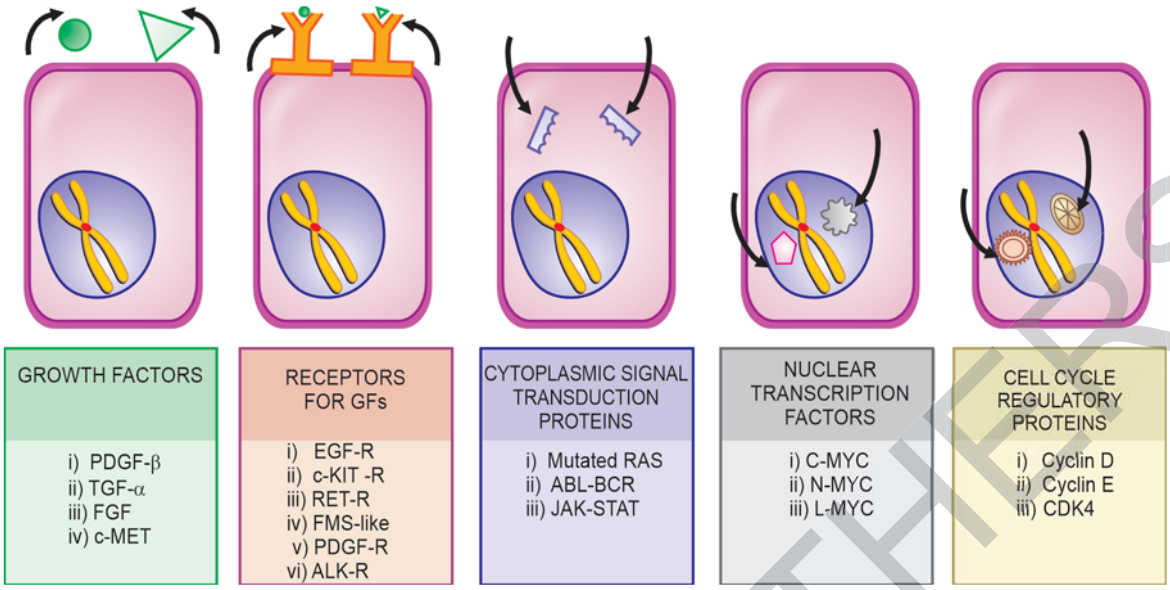

Figure 29.4 Mechanisms of activation of groups of proto-oncogenes to become growth-promoting oncogenes.

i) Growth factors
ii) Receptors of growth factors
iii) Cytoplasmic signal transduction proteins
iv) Nuclear transcription factors
v) Cell cycle regulatory proteins

i) Growth factors (GFs) GFs were the first proto-oncogenes to be discovered which encode cell proliferation cascade. They act by binding to cell surface receptors to activate cell proliferation cascade within the cell. GFs are small polypeptides secreted by many cells. To stimulate proliferation, they normally act on another cell than the one which synthesised it, i.e. *paracrine action*. However, a cancer cell may synthesise a GF as well as respond to it; this way cancer cells acquire growth self-sufficiency.

Most often, growth factor genes in cancer act by overexpression with large secretion of GFs that stimulate cell proliferation. For example:

a) *Platelet-derived growth factor-β-(PDGF-β)* Overexpression of *SIS* proto-oncogene that encodes *PDGF-β* and thus there is increased secretion of *PDGF-β*, e.g. in gliomas and sarcomas.

b) *Transforming growth factor-α (TGF-α)* Overexpression of *TGF-α* gene occurs by stimulation of *RAS* proto-oncogene and induces cell proliferation by binding to epidermal growth factor receptor (*EGFR*), e.g. in carcinoma and astrocytoma.

c) *Fibroblast growth factors (FGFs)*, e.g. overexpression of *HST-1* proto-oncogene in osteosarcoma, amplification of FGF-3 proto-oncogene in cancer of the breast and stomach.

d) Hepatocyte growth factor (HGF) Overexpression by binding to its receptor *c-MET*, e.g. familial follicular carcinoma thyroid, hepatocellular carcinoma.

ii) GF receptors Growth factors cannot penetrate the cell directly but are transported into the cell by GF-specific cell surface receptors. These receptors are transmembrane proteins, having an extracellular, intramembranous and intracellular ligand-binding domains, e.g. receptor tyrosine kinase (TK). Normally, binding of GF to the extracellular domain of the receptor TK activates the cytoplasmic component of the receptor and induces signalling. Mutated forms of growth factor receptors stimulate cell proliferation, independent of their binding to growth factors. Activation of oncogenes encoding GF receptors (i.e. receptor TK) may occur by overexpression, deletion, point mutation and gene rearrangement. Following examples of mutations in receptor TK and their associated tumours have particular clinical significance in instituting targeted therapy by way of TK inhibitors in such cases:

a) *EGF receptors (EGFR)* Normal *EGFR* gene is *ERB B1*, and hence this receptor is termed as *EGFR or HER1* (i.e. human epidermal growth factor receptor type 1). *EGFR* (or *HER1*) activation is by different types of mutations of normal GF receptor, e.g. in 80% of adenocarcinoma of lung.

Another *EGF* receptor gene called *ERB B2* (or *HER2/neu* or CD340) acts by gene amplification, e.g. in breast cancer (25% cases), and carcinoma ovary.

b) *c-KIT receptor* Gene coding for receptor for stem cell factor (steel factor) is *c-KIT* that activates tyrosine kinase pathway in cell proliferation. Mutated form of *c-KIT* by point mutation activates receptor for tyrosine kinase, e.g. in gastrointestinal stromal tumour (GIST).

c) *RET receptor RET* (abbreviation of '*re*arranged during *t*ransfection') proto-oncogene is a receptor for tyrosine kinase normally expressed in neuroendocrine cells of different tissues. Mutated form by point mutation is seen in MEN type 2A and 2B and in familial medullary carcinoma thyroid.

d) *FMS-like tyrosine kinase receptor* Point mutation of *FLT-3* gene (CD 135) that encodes for *FMS*-like tyrosine kinase receptor has been seen in acute myeloid leukaemia.
e) *PDGF-receptor* By overexpression or translocation, seen in gliomas and leukaemias.
f) *ALK receptor* This acts as oncogene by different mutations—by gene rearrangement in adenocarcinoma lung, and by point mutation in neuroblastoma.

iii) Cytoplasmic signal transduction proteins Normal signal transduction proteins in the cytoplasm transduce signal from the GF receptors present on the cell surface, to the nucleus of the cell, to activate intracellular growth signalling pathways.

There are examples of oncogenes having mutated forms of cytoplasmic signalling pathways located in the inner surface of cell membrane in some cancers. These are as under:
a) *Mutated RAS gene* This is the most common form of oncogene in human tumours, the abnormality being induced by point mutation in *RAS* gene. About a third of all human tumours carry mutated *RAS* gene (*RAS* for *Ra*t *S*arcoma gene where it was first described), notably in carcinoma colon, lung and pancreas. Normally, the inactive form of *RAS* protein is GDP (guanosine diphosphate)-bound while the activated form is bound to guanosine triphosphate (GTP). GDP/GTP is homologous to G proteins and takes part in signal transduction in a similar way just as G proteins act as 'on-off switch' for signal transduction. Normally, active *RAS* protein is inactivated by GTPase activity, while mutated form of *RAS* gene remains unaffected by GTPase, and therefore, continues to signal the cell proliferation.
b) *ABL-BCR hybrid gene ABL* (Abelson) is a non-receptor proto-oncogene having tyrosine kinase activity. *ABL* gene from its normal location on chromosome 9 is translocated to chromosome 22 where it fuses with *BCR* (breakpoint cluster region) gene and forms an *ABL-BCR* hybrid gene which is more potent in signal transduction pathway. *ABL-BCR* hybrid gene by translocation is seen in chronic myeloid leukaemia and by point mutation in acute lymphoblastic leukaemia (ALL).
c) *JAK/STAT signal transduction* Janus-kinase (*JAK*) is cytosolic non-receptor tyrosine kinase for cytokines. *STAT* (signal transducer and activator of transcription) is latent transcription factor in the cytoplasm which gets activated by *JAK*, i.e. JAK/STAT signalling pathway. This way, growth-promoting activity is stimulated by growth factor and by cytokine receptor that lacks tyrosine kinase (i.e. phosphorylation) activity. Point mutations in *JAK2* causing growth-promoting JAK/STAT signal transduction are seen in various examples of myeloproliferative disorders and in ALL.

iv) _Nuclear transcription factors Signal transduction pathway that started with GFs ultimately reaches the nucleus where it regulates DNA transcription and induces the cell to enter into S phase. Out of various nuclear regulatory transcription proteins described, the most important is *MYC* gene located on long arm of chromosome 8. Normally, *MYC* protein binds to the DNA and regulates the cell cycle by transcriptional activation and its levels fall immediately after cell enters the cell cycle.

MYC oncogene (originally isolated from myelocytomatosis virus and accordingly abbreviated) is commonly seen in human tumours. It is associated with either persistence or overexpression of *MYC* oncoproteins which, in turn, causes autonomous cell proliferation. The examples of tumours carrying *MYC* oncogene are:
a) *C-MYC oncogene* Mutated *MYC* gene due to *translocation* t(8;14) seen in Burkitt lymphoma.
b) *N-MYC oncogene* Mutated *MYC* gene due to *amplification* seen in neuroblastoma, small cell carcinoma lung.
c) *L-MYC oncogene* Mutated *MYC* gene due to *amplification* seen in small cell carcinoma lung.

v) Cell cycle regulatory proteins Normally, the cell cycle is under regulatory control of cyclins and cyclin-dependent kinases (CDKs) A, B, E and D. Cyclins activate as well as work together with CDKs, while many inhibitors of CDKs (CDKIs) are also known.

Although all steps in the cell cycle are under regulatory controls, G1 → S phase checkpoint is most significant for regulation by oncogenes as well as anti-oncogenes (discussed below). Gain-of-function mutations in cyclins (in particular cyclin D) and CDKs (in particular CDK4) are most important growth-promoting signals in cancers. The examples of tumours having such mutated oncogenes are:
a) *Mutated form of cyclin D* proto-oncogene by translocation seen in mantle cell lymphoma and myeloma.
b) *Mutated form of cyclin E* by overexpression seen in breast cancer.
c) *Mutated form of CDK4* by gene amplification seen in malignant melanoma, glioblastoma and sarcomas.

2. REFRACTORINESS TO GROWTH-INHIBITORY SIGNALS: TUMOUR-SUPPRESSOR GENES

The mutation of tumour-suppressor genes (or antioncogenes having reverse function than oncogenes) results in removal of the brakes for growth. Thus, the inhibitory influence to cell growth is removed, and thus abnormal growth continues unchecked. In other words, mutated tumour-suppressor genes behave like growth-promoting oncogenes.

As compared to the signals and signal transduction pathways for oncogenes described above, the steps in mechanisms of action by growth suppressors are not so well understood. In general, the point of action by tumour-suppressor genes is G1 → S phase transition

(page 28). Normally, tumour-suppressor genes act by either inducing the dividing cell from the cell cycle to enter into G0 (resting) phase, or by acting in a way that the cell lies in the post-mitotic pool losing its dividing capability. Just as proto-oncogenes are activated by mutations to become oncogenes, the mechanism of loss of tumour-suppressor actions of genes is by mutations, commonly chromosomal deletions, point mutations and loss-of-function mutation inhibiting G1-S phase progression.

Major tumour-suppressor genes implicated in human cancers are as under **(Table 29.3)**:

i) *RB* gene *RB* gene is located on long arm (q) of chromosome 13. This is the first ever tumour-suppressor gene identified and thus has been amply studied. *RB* gene codes for a nuclear transcription protein called pRB. *RB* gene is termed as *'master brake in the cell cycle'* and is virtually present in every human cell. It can exist in both an *active* and an *inactive* form:

- *Active form of RB gene* It blocks cell division by binding to transcription factor, E2F, and thus inhibits the cell from transcription of cell cycle-related genes, thereby *inhibiting* the cell cycle at G1 → S phase transition, i.e. cell cycle is arrested at G1 phase.
- *Inactive form of RB gene* This takes place when *RB* gene is hyperphosphorylated by CDKs and growth factors bind to their receptors. This removes *pRB* function from the cell (i.e. the 'brake' on cell division is removed). Resultantly, cell proliferation pathway is stimulated by *permitting* the cell to cross G1 → S checkpoint. Activity of CDKs is inhibited by activation of inhibitory signal, transforming growth factor-β (TGF-β), on cell through activation of inhibitory protein p16.

The mutant form of *RB* gene (i.e. inactivating mutation of *RB* gene) is involved in several human tumours, most commonly in retinoblastoma, the most common intraocular tumour in young children. The tumour occurs in two forms: sporadic and inherited/familial **(Fig. 29.5)**:

- *Sporadic retinoblastoma* constitutes about half the cases and affects one eye. These cases have acquired both the somatic mutations in the two alleles in retinal cells after birth.
- *Inherited/Familial retinoblastoma* comprises 40% of cases and may be bilateral. In these cases, all somatic cells (retinal as well as non-retinal cells) inherit one mutant *RB* gene from a carrier parent (i.e. germline mutation). Later during life, the other mutational event of second allele affecting the somatic cells occurs. This forms the basis of *two-hit hypothesis* given by Knudson in 1971. Besides retinoblastoma, children inheriting mutant *RB* gene have 200 times greater risk of development of other cancers in early adult life, notably osteosarcoma; others are cancers of breast, colon and lungs.

ii) *p53* gene *(TP53)* Located on the short arm (p) of chromosome 17, *p53* gene (also termed *TP53* because of molecular weight of 53 kD for the protein) similar to *pRB* is inhibitory to cell cycle. However, *p53* is normally present in very small amounts and accumulates only after DNA damage.

Two major functions of *p53* in the normal cell cycle are as under:

a) *In blocking mitotic activity p53* inhibits the cyclins and CDKs and prevents the cell to enter G1 phase transiently. This breathing time in the cell cycle is utilised by the cell to repair the DNA damage.

b) *In promoting apoptosis* Normally, *p53* acts together with another anti-oncogene, *RB* gene, and identifies the

TABLE 29.3 Important tumour-suppressor genes and associated human tumours.

	GENE	ACTIVATION MODE	ASSOCIATED HUMAN TUMOURS
1.	*RB*	Loss of inhibition at G1-S phase transition	Retinoblastoma, osteosarcoma
2.	*p53 (TP53)*	i) Loss of block in mitotic activity ii) Loss of promotion in apoptosis	Most human cancers, common in Ca lung, head and neck, colon, breast
3.	*TGF–β and its receptor*	Loss of inhibition in cell proliferation by activating CDKIs Loss of suppression in growth-promoting genes	Ca pancreas, colon, stomach
4.	*APC and β-catenin proteins*	Loss of inhibition in mitosis by WNT pathway and by failure to degrade β-catenin	Ca colon
5.	*Others*		
	i) BRCA 1 and 2	Loss of repair of damaged DNA	Ca breast, ovary
	ii) VHL	Prevents ubiquitination and degradation of HIF-1 and promotes angiogenesis	Renal cell carcinoma
	iii) WT 1 and 2	Transcription factor	Wilms tumour
	iv) NF 1 and 2	NF1 (neurofibromin 1) (a GTPase) NF2 (merlin protein) (a cytoskeletal protein)	Neurofibromatosis type 1 and 2
	v) PTEN	Inhibits p13K/AKT signalling	Breast cancer, other epithelial cancers
	vi) PTCH1	Inhibits Hedgehog signalling	Naevoid basal cell carcinoma
	vii) CDKN2A	Inhibitor of *p16/INK4* and *CDK4/cyclin D*	Bladder Ca, Ca cervix

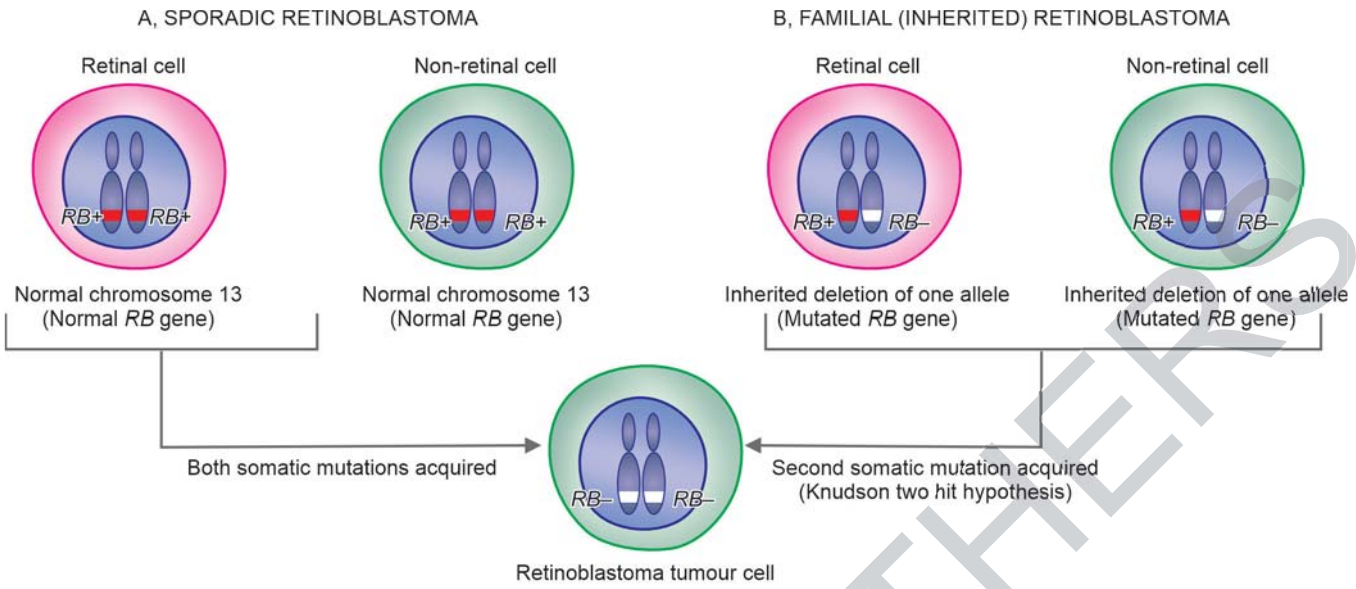

Figure 29.5 Schematic representation of role of *RB* gene in sporadic and familial retinoblastoma. A, *In sporadic form,* at birth there is no abnormality of either of two alleles of *RB* gene of retinal and non-retinal cells. Here, two mutations occur after birth involving both alleles of *RB* gene. B, *In familial/inherited retinoblastoma*, both retinal as well as non-retinal cells have one germline mutation at birth from one of the parents in one allele that encodes for *RB* protein gene. Second mutational event in these cases in the other allele occurs early during life to form homozygous mutation (two hit hypothesis of Knudson).

genes that have damaged DNA which cannot be repaired by inbuilt system. *p53* directs such cells to apoptosis by activating apoptosis-inducing *BAX* gene, and thus bringing the defective cells to an end by apoptosis. This process operates in the cell cycle at G1 and G2 phases before the cell enters the S or M phase.

Because of these significant maintenance roles in cell cycle, *p53* is called as *'guardian of the genome'*.

In its mutated form, *p53* ceases to act as protector or as growth-suppressor but instead acts like a growth-promoter or oncogene. Homozygous loss of *p53* gene allows genetically damaged and unrepaired cells to survive and proliferate resulting in malignant transformation.

More than 70% of human cancers have homozygous loss of *p53* by acquired mutations in somatic cells; some common examples are cancers of the lung, head and neck, colon and breast. Besides, mutated *p53* is also seen in the sequential development stages of cancer from hyperplasia to carcinoma *in situ* and into invasive carcinoma.

Less commonly, both alleles of *p53* gene become defective by another way: one allele of *p53* mutated by inheritance in germ cell lines rendering the individual to another hit of somatic mutation on the second allele. Just as in *RB* gene, this defect predisposes the individual to develop cancers of multiple organs (breast, bone, brain, sarcomas etc), termed *Li-Fraumeni syndrome.*

iii) Transforming growth factor-β (TGF-β) and its receptor Normally, *TGF*-β is significant inhibitor of cell proliferation, especially in epithelial, endothelial and haematopoietic cells. It acts by binding to *TGF*-β receptor and then the complex so formed acts in G1 phase of cell cycle at two levels:

a) It activates CDK inhibitors with growth inhibitory effect.

b) It suppresses the growth-promoter genes such as *MYC*, *CDKs* and cyclins.

Mutant form of *TGF*-β gene or its receptor impairs the growth inhibiting effect and thus permits cell proliferation. Examples of mutated form of *TGF*-β are seen in cancers of pancreas, colon, stomach and endometrium.

iv) Adenomatous polyposis coli (APC) gene and β-catenin protein The *APC* gene is normally inhibitory to mitosis, which takes place by a cytoplasmic protein, β-catenin. β-catenin normally has dual functions:

- Firstly, it binds to cytoplasmic E-cadherin that is involved in intercellular interactions.
- Secondly, it can activate cell proliferation signalling pathway.

APC gene is a component of *WNT* signalling pathway that signals through frizzled cell surface receptors which activate several pathways including β-catenin. In colon cancer cells, *APC* gene is lost and thus β-catenin fails to get degraded, allowing the cancer cells to undergo mitosis without the inhibitory influence of β-catenin.

Patients born with one mutant *APC* gene allele develop large number of polyps in the colon early in life,

while after the age of 20 years these cases start developing loss of second *APC* gene allele. It is then that almost all patients having both mutated alleles invariably develop malignant transformation of one or more polyps.

v) Other tumour-suppressor genes A few other tumour-suppressor genes having mutated germline in various tumours are as under:

a) *BRCA1 and BRCA2 genes* These are two breast *(BR)* cancer *(CA)* susceptibility genes: *BRCA1* located on chromosome 17q21, and *BRCA2* on chromosome 13q12-13. Women with inherited defect in *BRCA1* gene have very high-risk (85%) of developing breast cancer and ovarian cancer (40%). Inherited breast cancer constitutes about 5–10% cases, it tends to occur at a relatively younger age and more often tends to be bilateral.

b) *VHL gene* Von Hippel-Lindau (VHL) disease is a rare autosomal dominant disease characterised by benign and malignant tumours of multiple tissues. The disease is inherited as a mutation in *VHL* tumour-suppressor gene located on chromosome 3p. Product of VHL gene, VHL protein, is a component of ubiquitin ligase that acts as transcription factor HIF1α (hypoxia inducible factor), i.e. undergoes gene expression in response to hypoxia. In inactivated VHL gene, HIF1α escapes ubiquitination and degradation, even in normoxic condition and activates genes that promote angiogenesis, cell survival and proliferation. *VHL* gene is found inactivated in 60% cases of renal cell carcinoma.

c) *Wilms tumour (WT) gene WT1* and *WT2* genes are both located on chromosome 11 and normally prevent neoplastic proliferation of cells in embryonic kidney. WT1 protein acts as transcription factor. Mutant form of *WT1* and *WT2* are seen in hereditary Wilms tumour.

d) *Neurofibroma (NF) gene NF* genes normally prevent proliferation of Schwann cells. Two mutant forms are described: *NF1* and *NF2* seen in neurofibromatosis type 1 and type 2.

e) *PTEN gene* PTEN (*p*hosphatase and *ten*sin homologue) gene encodes a phosphatase on the cell membrane. It is a tumour-suppressor that acts as a brake on P13K/AKT component of receptor tyrosine kinase. Mutation of PTEN tumour-suppressor gene is by loss of function (by deletion or point mutation) in many epithelial cancers, notably breast cancer.

f) *PTCH1 gene* PTCH tumour-suppressor gene encodes PATCHED-1 transmembrane protein (PTCH = protein PATCHED homolog). It acts as a brake at the level of Hedgehog signalling pathway. Its loss-of-function in tumours permits cell proliferation by allowing pro-proliferation genes such as cyclin D, e.g. Gorlin syndrome (naevoid basal cell carcinoma).

g) *CDKN2A gene* This tumour-suppressor gene encodes inhibitors in cell cycle pathway at the level of *p16/INK4* and *CDK4/cyclin D* pathways, thereby acting as RB gene checkpoint. Deletion or point mutation in *CDKN2A* results in loss-of-function, hence removing the brakes in proliferation (particularly silencing of *p16*), e.g. in bladder cancer, carcinoma cervix.

The contrasting features of oncogenes and tumour-suppressor genes are summarised in **Table 29.4**.

TABLE 29.4 Oncogenes versus tumour-suppressor genes (anti-oncogenes).

	FEATURE	ONCOGENES	TUMOUR-SUPPRESSOR GENES
1.	*Derived from*	Mutated form of normal proto-oncogenes	Mutated form of normal growth-suppressor genes
2.	*Inheritance*	Dominant; mutation of a single copy may transform cell	Recessive; mutation of both alleles (homozygous) required for transformation
3.	*Common mutations*	Point mutation, translocation, amplification, overexpression	Deletion, point mutation, loss-of-function
4.	*Major action*	i) Allows cell proliferation by increased growth promotion pathways ii) Active action by gene products (oncoproteins)	i) Allows cell proliferation by removal of brakes in cell proliferation ii) Passive action, i.e. by loss of normal function
5.	*Level of action in cell*	At different levels (cell surface, cytoplasm, nucleus)	At different levels (cell surface, cytoplasm, nucleus)
6.	*Major types*	i) GFs *(PDGF-β, TGF-α, FGF, HGF)* ii) GF receptors *(EGFR, cKIT, RET, FMS-like TKR, PDGFR, ALKR)* iii) Cytoplasmic signal transduction proteins *(RAS, BCR-ABL, JAK)* iv) Nuclear transcription proteins *(MYC)* v) Cell cycle regulatory proteins (*CDK4*, cyclin D and E)	*i) RB* *ii) p53* *iii) TGF-β* and its receptor *iv) APC* and β-catenin v) Others *(BRCA 1 and 2, VHL, WT 1 and 2, NF 1 and 2, PTEN, PTCH1, CDKN2A)*

(GFs, growth factors)

II. FAVOURING OVERALL CELL SURVIVAL: ALTERED STRESS RESPONSE

Cancer cells are faced with stresses of a variety of types such as excessive cell-signalling, DNA damage, hypoxia and nutrient deficiency, besides facing the onslaught of anticancer therapy. Just as normal cells physiologically adapt to stresses for the larger good of the health of tissue, cancer cells respond to stresses by subverting the process in favour of cancer growth and survival. These include: 1) avoiding DNA repair, 2) escaping cell death by apoptosis, 3) avoiding cell senescence, and 4) recycling intracellular components by autophagy. These are discussed below.

1. AVOIDING DNA REPAIR SYSTEM: MUTATOR GENES

Normal cells during complex mitosis suffer from minor damage to the DNA which is detected and repaired before mitosis is completed so that integrity of the genome is maintained. Similarly, small mutational damage to the dividing cell by exogenous factors (e.g. by radiation, chemical carcinogens etc) is also repaired. In this regard, *p53* gene is considered responsible for detection and repair of DNA damage. However, if this system of DNA repair is defective, either due to germline mutations or by acquired mutations (mutator genes), the defect in unrepaired DNA is passed to the next progeny of cells and cancer results.

The examples of mutator genes exist in the following inherited disorders associated with increased propensity to cancer:

i) *Hereditary non-polyposis colon cancer* (HNPCC or Lynch syndrome) is characterised by hereditary predisposition to develop colorectal cancer. It is due to defect in genes involved in DNA mismatch repair which results in accumulation of errors in the form of mutations in many genes.

ii) *Ataxia telangiectasia (AT)* has *ATM* (*M* for mutated) gene. These patients have multiple cancers besides other features such as cerebellar degeneration, immunologic derangements and oculo-cutaneous manifestations.

iii) *Xeroderma pigmentosum* is an inherited disorder in which there is defect in DNA repair mechanism. Upon exposure to sunlight, the damage to DNA by UV radiation cannot be repaired. Thus, such patients are more prone to various forms of skin cancers.

iv) *Bloom syndrome* is an example of damage by ionising radiation which cannot be repaired due to inherited defect and the patients have increased risk to develop cancers, particularly leukaemia.

v) *Hereditary breast cancer* patients having mutated *BRCA1* and *BRCA2* genes carry inherited defect in DNA repair mechanism. These patients are not only predisposed to develop breast cancer, but also cancers of various other organs.

vi) Paradoxically, *overexpression of repair genes* by upregulation of repair pathways has also been observed as mechanism in certain tumours, e.g. leukaemias, cancer of breast and pancreas.

2. ESCAPING CELL DEATH BY APOPTOSIS: APOPTOSIS REGULATORY GENES

Besides the role of mutant forms of growth-promoting oncogenes and tumour-suppressor genes, another mechanism of tumour growth is by escaping cell death by apoptosis. Apoptosis in normal cell is guided by intrinsic and extrinsic pathways resulting in DNA damage. In this process, there is role of cell death receptor, *CD95*, some pro-apoptotic factors, apoptosis inhibitors and cell death-inducing signals (page 57).

Cancer cells escape cell death by subverting normal pathways of apoptosis at different levels:

i) By loss of *p53* leading to reduced function of proapoptotic factors such as *BAX*

ii) By reduced release of cytochrome c from mitochondria due to upregulated antiapoptotic factors such as *BCL2, BCL-XL, MCL-1*

iii) By loss of *APAF-1* (apoptotic peptidase activating factor-1)

iv) By upregulating apoptotic inhibitors

v) By reduced level of surface receptor *CD95* molecule

vi) By inactivation of cell death-inducing signals *FADD* and *FasL*

Examples of tumours by this mechanism are as under:

a) ***BCL2* gene** is seen in normal lymphocytes, but its mutant form with characteristic translocation (t14;18) (q32;q21) was first described in B-cell lymphoma, hence the name BCL. It is also seen in many other human cancers, e.g. cancers of the breast, thyroid and prostate. Mutation in *BCL2* gene removes the apoptosis-inhibitory control on cancer cells; thus more live cells undergoing mitosis are added to tumour growth. Besides, *MYC* oncogene and *p53* tumour-suppressor gene are also connected to apoptosis. While *MYC* allows cell growth, *BCL2* inhibits cell death; thus *MYC* and *BCL2* together allow cell proliferation. Normally, *p53* activates proapoptotic gene *BAX*, but mutated *p53* (i.e. absence of *p53*) reduces apoptotic activity and thus allows cell proliferation.

b) ***CD95*/*FasL*** receptors are depleted in hepatocellular carcinoma and certain lymphomas; hence the tumour cells escape apoptosis.

3. EVADING CELL SENESCENCE: TELOMERES AND TELOMERASE

As happens in cellular ageing, after each mitosis (cell doubling) there is progressive shortening of telomeres which are the terminal tips of chromosomes. Telomerase is the RNA enzyme that helps in repair of such damage to

DNA and maintains normal telomere length in successive cell divisions **(Fig. 29.6)**. However, it has been seen that after repetitive mitosis for a maximum of 60–70 times, telomeres are lost in normal cells and the cells cease to undergo mitosis. This forms the basis of cellular ageing. Telomerase is active in normal stem cells, but not in normal somatic cells.

Cancer cells in most malignancies have markedly upregulated telomerase enzyme, and thus capable of reconstituting telomere length. Certain other stresses can also induce cellular senescence in cancers by non-telomeric DNA damage such as by reactive oxygen species, anticancer therapy, continuous cell-signalling in favour of mitosis etc. Overall, cancer cells avoid ageing by disrupting tumour-suppressors such as *TP53* and INK4a/*p16* in a way that mitosis does not slow down or cease, thereby immortalising the cancer cells.

4. RECYCLING INTRACELLULAR COMPONENTS: AUTOPHAGY

Autophagy is a physiologic recycling process of intracellular components by cannibalism of misfolded proteins and long-lived organelles (page 59). The energy so produced by autophagy is utilised for cell metabolism. Autophagy is upregulated in response to a variety of stresses. In cancer, autophagy may have different stress response in different settings:

i) *Role as tumour suppressor* By eliminating damaged mitochondria, it reduces the burden of reactive oxygen species, degrades overexpressed proteins and acts as defence against oncogenic microbes.

ii) *Role in tumour survival and progression* Upregulated autophagy in cancer stress enables cancer cells to survive harsh metabolic conditions of nutrient deficiency, hypoxia or may secrete tumour microenvironment (TME)-reshaping proteins. Thus, the role of autophagy in cancer remains context-dependent.

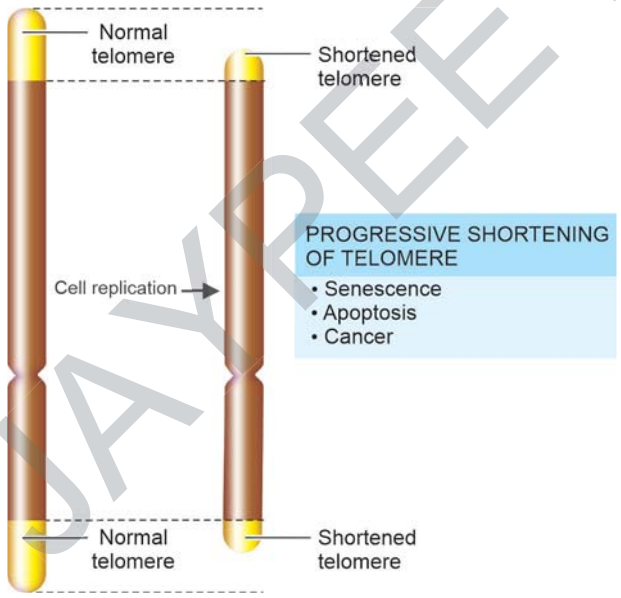

Figure 29.6 Telomeres are DNA components located on the tips of chromosomes in cells which are damaged during cell replication which are normally resynthesised by the RNA enzyme, telomerase, but are progressively shortened in senescence due to failure of telomerase for replacement synthesis. In cancer, there is upregulation of telomerase and thus cancer cells escape ageing.

III. SUSTAINED PERFUSION OF CANCER: VASCULARISATION

Cancer cells cannot thrive and metastasise without neovascularisation as it supplies them nourishment and oxygen. This process includes tumour angiogenesis as the most important mechanism, but there are a few other modes of vascularisation.

1. TUMOUR ANGIOGENESIS

Angiogenesis is the process of formation of new vessel by sprouting from the pre-existing vessels that regenerate to form new endothelial cells by mitosis and migration, e.g. in chronic inflammation, wound healing, embryogenesis (page 175). Angiogenesis is regulated by several pro- and anti-angiogenic factors:

i) Promoters of tumour angiogenesis include *vascular endothelial growth factor (VEGF)* and *basic fibroblast growth factor (bFGF)*.

ii) Anti-angiogenesis factors inhibiting angiogenesis include *thrombospondin-1, angiostatin, endostatin and vasculostatin.* Mutated form of *p53* gene in both alleles in various cancers results in removal of anti-angiogenic role of thrombospondin-1, thus favouring continued angiogenesis.

In physiologic conditions, the process is controlled and self-limited, and is switched off after its anticipated function is complete. However, in malignancies, the angiogenic process is continuously activated due to following features in cancers:

i) The major trigger for angiogenesis is hypoxia present in many cancers that is associated with raised level of HIF which activates *vascular endothelial growth factor (VEGF)* signalling pathway.

ii) Another angiogenic switch in cancers is *acidic TME* (tumour microenvironment) induced by metabolic changes in cancer (discussed later).

iii) Newly-formed vessels in cancer are *dilated, tortuous and leaky* due to loose contact of endothelial cells with each other and with basement membrane which results in accentuating hypoxia and acidosis, promoting angiogenesis.

2. OTHER MODES OF TUMOUR VASCULARISATION

Besides angiogenesis, some non-angiogenic modes of vascularisation have been described in tumours which

have clinical implications since they escape from anti-angiogenic therapy. These are as under:
i) *Vascular co-option* During early stage of tumour development (micro-tumours), tumour cells may obtain blood supply by diverting existing vasculature.
ii) *Intussusceptive microvascular growth* This is the process in which pre-existing vessels split to form daughter vessels and expand their capillary network, i.e. splitting angiogenesis.
iii) *Vascular mimicry* This is the phenomena in which aggressive cancer cells express stem cell-like phenotype that transdifferentiates to form endothelial cells and new vascular network.

IV. CANCER SPREAD: INVASION AND METASTASIS

One of the most important characteristics of cancers is local invasion and distant metastasis. The mechanism of spread involves a series of complex cell biological events called 'invasion-metastasis cascade' (page 236). It includes 1) aggressive clonal proliferation in the primary tumour, 2) loss of cell-cell and cell-ECM interaction, 3) degradation of ECM, 4) loss of basal polarity, 5) intravasation of cancer cells, 6) tumour cells in circulation, 7) extravasation of tumour cells and organ predilection, and 8) micro-metastasis, dormancy and colonisation to develop into clinically detectable metastasis.

V. PRO-GROWTH METABOLIC CHANGES: THE WARBURG EFFECT

Cancer cells demonstrate changes in metabolic pathways in such a way that promotes initiation and progression of tumours. These include the following:

1. EXCESSIVE NUTRIENT ACQUISITION

For growing cells, glucose and glutamine are essential nutrients required for several metabolic needs such as ATP generation, cell signalling, gene expression and for nitrogen donation in nucleotide biosynthesis. Cancer cells overutilise glucose in the presence of adequate oxygen, a concept that was discovered by Warburg in 1927 and called *Warburg effect* or *aerobic glycolysis*, for which he was conferred the Nobel Prize in 1931. Lately, this observation has been used by injecting a metabolite of glucose, ^{18}F-fluorodeoxyglucose, in PET (positron emission tomography) scan that shows high level of GLUT (*glu*cose *t*ransporter) protein in many cancers.

Following mechanisms may explain high glucose uptake and increased expression of GLUT by cancer cells:
i) High level of *HIF*
ii) Aberrant *P13K/AKT* signalling
iii) Mutated *K-RAS* and *BRAF*
iv) Upregulated *MYC* transcription factor
v) *RB* gene is negative regulator of glutamine synthesis

Besides glucose and glutamine, tumour cells may also adopt alternate pathways to acquire nutrients, e.g.
i) *Entosis,* i.e. engulfment and digestion of living cells
ii) *Phagocytosis* of apoptotic bodies
iii) *Macropinocytosis* of extracellular proteins.

2. ALTERED METABOLIC PATHWAYS

As a consequence of the Warburg effect, increased uptake of nutrients proceeds into different metabolic pathways in cancer cells further promoting tumorigenesis. These alterations in metabolic pathways are as under:
i) Mitochondrial oxidative phosphorylation along with glycolysis serves the higher anabolic needs of cancer cells.
ii) Higher influx into the citric acid (Krebs) cycle produces more of reactive oxygen species in a controlled manner and without toxic accumulation.
iii) Availability of higher amount of glutamine gets converted into glutamate in mitochondria which is utilised for fatty synthesis required for lipid bilayer of proliferating tumour cells.

3. ONCOMETABOLITES IN TUMORIGENESIS

In addition to growth-promoting effects of nutrients and oxygen, some intermediate metabolites too have pro-growth effects in cancer:
i) *2-HG (hydroxyglutamate),* an oncometabolite, inhibits dioxygenases with hypermethylation-silencing and thus promotes growth, e.g. in triple-negative breast cancer.
ii) Metabolites play role in *regulating epigenetic phenomena* of acetylation and methylation by addition or removal of acetyl or methyl groups respectively.

VI. DYNAMIC TUMOUR MICROENVIRONMENT: THE STROMAL CELLS

Although mutations are the driving component of tumour growth and progression, various stages of cancer need contribution from bone marrow-derived stromal cells that constitutes the tumour microenvironment (TME). Components of TME have growth-promoting function and also play a role in enabling other cancer hallmarks. There are three classes of mesenchyme-derived stromal cells that comprise TME:
i) *Angiogenic vascular cells,* e.g. endothelial cells in tumour angiogenesis
ii) *Cancer-associated fibroblastic cells,* i.e. mesenchymal stem cells and connective tissue fibroblasts in close proximity of growth that can be recruited and induced to transdifferentiate and contribute to tumour-promoting functions.
iii) *Infiltrating immune cells,* i.e. presence of cells of myeloid-lymphoid lineage which have an immunosuppressive role than acting as inflammatory cells in cancer.

The importance of each type of activated stromal cells varies in different tumours and that depends upon other

underlying oncogenic alterations in the primary tumour, invasiveness and its colonisation to develop metastasis. These components of TME are increasingly being used for developing therapeutic targets to eliminate primary and metastatic disease.

VII. EVADING IMMUNE DESTRUCTION: IMMUNE MODULATION

Existence of host immunity against cancer cells has been known for a long time, recognising them as 'non-self or foreign' and it attempts to eliminate them. Following clinical observations support the presence of host immune surveillance in cancer:

1. Certain cancers evoke significant *lymphocytic infiltrate* composed of immunocompetent cells and such tumours have somewhat better prognosis, e.g. medullary carcinoma breast (as compared with infiltrating ductal carcinoma), seminoma testis (as compared with other germ cell tumours of testis).
2. Infrequently, a cancer may *spontaneously regress* partially or completely, probably under the influence of host defence mechanism. For example, spontaneous disappearance of malignant melanoma temporarily from the primary site, although rare and it may actually reappear as metastasis later.
3. There is higher susceptibility of tumours in *immunodeficient host*, e.g. in primary immunodeficiency, HIV-infected patients, development of post-transplant lymphoproliferative disease.

However, it is also known that despite an intact immune system in the host, cancer continues to develop and progress in the body. The subject is discussed below in the context of immune surveillance in physiologic state and in cancer under three headings: tumour antigens, anti-tumour immune responses, and escape from immune surveillance.

1. TUMOUR ANTIGENS

Tumour antigens are distinguished by their molecular features and their specific recognition by host immune cells. Currently, various groups of tumour antigens are as follows:

i) Oncoproteins from mutated oncogenes Protein products derived from mutated oncogenes result in expression of cell surface antigens on tumour cells. The examples include products of *RAS*, *BCR/ABL* and CDK4.

ii) Protein products of tumour-suppressor genes In some tumours, protein products of mutated tumour-suppressor genes cause expression of tumour antigens on the cell surface. The examples are mutated proteins *p53* and β-catenin.

iii) Overexpressed cellular proteins Some tumours are associated with a normal cellular protein but is excessively expressed in tumour cells and incite host immune response. For example, in melanoma the tumour antigen is structurally normal melanocyte specific protein, tyrosinase, which is overexpressed compared with normal cells. Similarly, *HER2/neu* protein is overexpressed in many cases of breast cancer.

iv) Abnormally expressed cellular proteins In some examples, a cellular protein is present in some normal cells but is abnormally expressed on the surface of tumour cells of some cancers. The classic example is presence of *MAGE* gene silent in normal adult tissues except in male germ line but *MAGE* genes are expressed on surface of many tumours such as melanoma (abbreviation *MAGE* from 'melanoma antigen' in which it was first found), cancers of liver, lung, stomach and oesophagus. Other examples of similar aberrantly expressed gene products in cancers are *GAGE* (G antigen), *BAGE* (B melanoma antigen) and *RAGE* (renal tumour antigen).

v) Tumour antigens from viral oncoproteins Many oncogenic viruses express viral oncoproteins which result in expression of antigens on tumour cells, e.g. viral oncoproteins of HPV (E6, E7) in cervical cancer and EBNA proteins of EBV in Burkitt lymphoma.

vi) Tumour antigens from randomly mutated genes Various other carcinogens such as chemicals and radiation induce random mutations in the target cells. These mutated cells elaborate protein products targeted by the cytotoxic T-lymphocytes of the immune system causing expression of tumour antigens.

vii) Cell-specific differentiation antigens Normally, differentiated cells have cellular antigens which forms the basis of diagnostic immunohistochemistry. Cancers have varying degree of loss of differentiation, but particular lineage of the tumour cells can be identified by tumour antigens. For example, various CD markers for subtypes of lymphomas, prostate specific antigen (PSA) in carcinoma of prostate.

viii) Oncofoetal antigens Oncofoetal antigens such as α-foetoprotein (AFP) and carcinoembryonic antigen (CEA) are normally expressed in embryonic life. But these antigens appear in certain cancers—AFP in liver cancer and CEA in colon cancer, which can be detected in serum as cancer markers.

ix) Abnormal cell surface molecules Normal cell expresses surface molecules of glycolipids, glycoproteins, mucins and blood group antigens. In some cancers, there is abnormally changed expression of these molecules. For example, there may be changed blood group antigen, or abnormal expression of mucin in ovarian cancer (CA-125) and in breast cancer (MUC-1).

2. ANTI-TUMOUR IMMUNE RESPONSES

Although both cell-mediated and humoral immunity are incited against the tumour, significant anti-tumour effector mechanism is mainly cell-mediated.

i) Cell-mediated mechanism This is the main mechanism of immune surveillance that involves following immune cells in targeting the tumour cells:
a) *Specifically-sensitised cytotoxic T lymphocytes (CTL),* i.e. CD8+ T-cells are directly cytotoxic to the target cell and require contact between them and tumour cells. CTL have been found to be effective against virally-induced cancers, e.g. in Burkitt lymphoma (EBV-induced), invasive squamous cell carcinoma of cervix (HPV-induced).
b) *Natural killer (NK) cells* are lymphocytes which after activation by IL-2, destroy tumour cells without sensitisation, either directly or by antibody-dependent cellular cytotoxicity (ADCC). NK cells together with T lymphocytes are the first line of defence against tumour cells and can lyse tumour cells.
c) *Macrophages* are activated by interferon-γ secreted by T-cells and NK-cells, and therefore there is close collaboration of these two subpopulations of lymphocytes and macrophages. Activated macrophages mediate cytotoxicity by production of reactive oxygen species or by tumour necrosis factor.

The *effector cell-mediated mechanism* against cancer cells involves following four phases:
i) Recognition of tumour cells by innate immune cells and their limited killing.
ii) Maturation and migration of antigen-presenting cells and cross-priming of T lymphocytes.
iii) Generation of tumour antigen-specific T lymphocytes and activation of cytotoxic mechanism.
iv) Homing of tumour antigen-specific T lymphocytes to the tumour site to eliminate tumour cells.

ii) Humoral mechanism *In vivo* anti-tumour humoral antibodies are quite ineffective against cancer cells. However, *in vitro* humoral antibodies may kill tumour cells by complement activation or by antibody-dependent cytotoxicity.

The mechanisms of immune responses are schematically illustrated in Fig. 29.7.

3. ESCAPE FROM IMMUNE SURVEILLANCE

Most cancers grow relentlessly in spite of intact innate and adaptive host immunity. This is because the cancer cells escape the host defences by modulation of the immune system:

i) Cancer immuno-editing This is the process in which the immune system shapes the malignant disease process in one of the following ways:
a) *Eliminate* cancer cells by the mechanism explained above.
b) Develop *equilibrium* between growing cancer cells and immune competence to eliminate tumour cells. This includes tumour-dormancy stage or slow-growth stage that may last for years.
c) Last stage is *escape* of the cancer cells from the host immune attack and they continue to grow relentlessly.

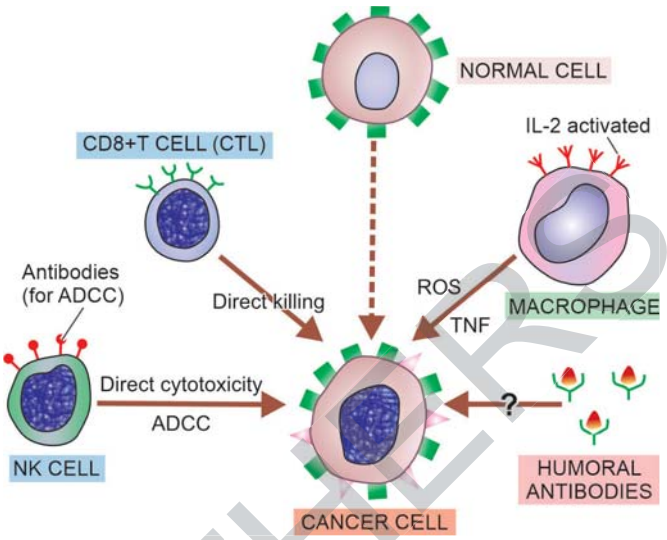

Figure 29.7 Schematic illustration of immune responses in cancer. For details see the text. (CTL, cytotoxic T-lymphocyte; NK cell, natural killer cell; ADCC, antibody-dependent cellular cytotoxicity).

ii) Escape mechanisms How cancer cells evade immune detection and elimination is explained by following mechanisms:
a) *Lack of tumour-antigen recognition* by either alteration in the tumour cells or effector immune cells.
b) *Resistance to cell death* by immune hyporesponsiveness to reactive oxygen species and resist cell death.
c) *Induction of immunologic ignorance and tolerance* through immunosuppressive factors secreted by the tumour cells and the stromal cells.

Our greater understanding of host immune mechanisms against cancer has opened possibilities of therapeutic manipulation of the immune system. While there is no magic bullet against cancer, immunotherapy protocols have been developed and increasingly being used as treatment against certain cancer in combination with other therapies such as surgery, radiation and chemotherapy.

The above properties of cancer cells are schematically illustrated in Fig. 29.8.

KEY POINTS | **Molecular Basis of Cancer**

- For understanding cancer at molecular level, mutually-interlinked theories form the basis of carcinogenesis. These are monoclonality, tissue organisation field theory, somatic mutation theory, multistep theory, and epigenetic theory.
- In cancer, normal regulatory genes of cellular growth undergo mutation in cancer. Major regulatory genes are growth promoting oncogenes, tumour-suppressor genes, apoptosis regulatory genes, and DNA repair genes.
- Following seven molecular hallmarks of cancer have been described based on their mechanisms of action:

I. *Growth and proliferation permissive components* These are oncogenes and tumour-suppressor genes.

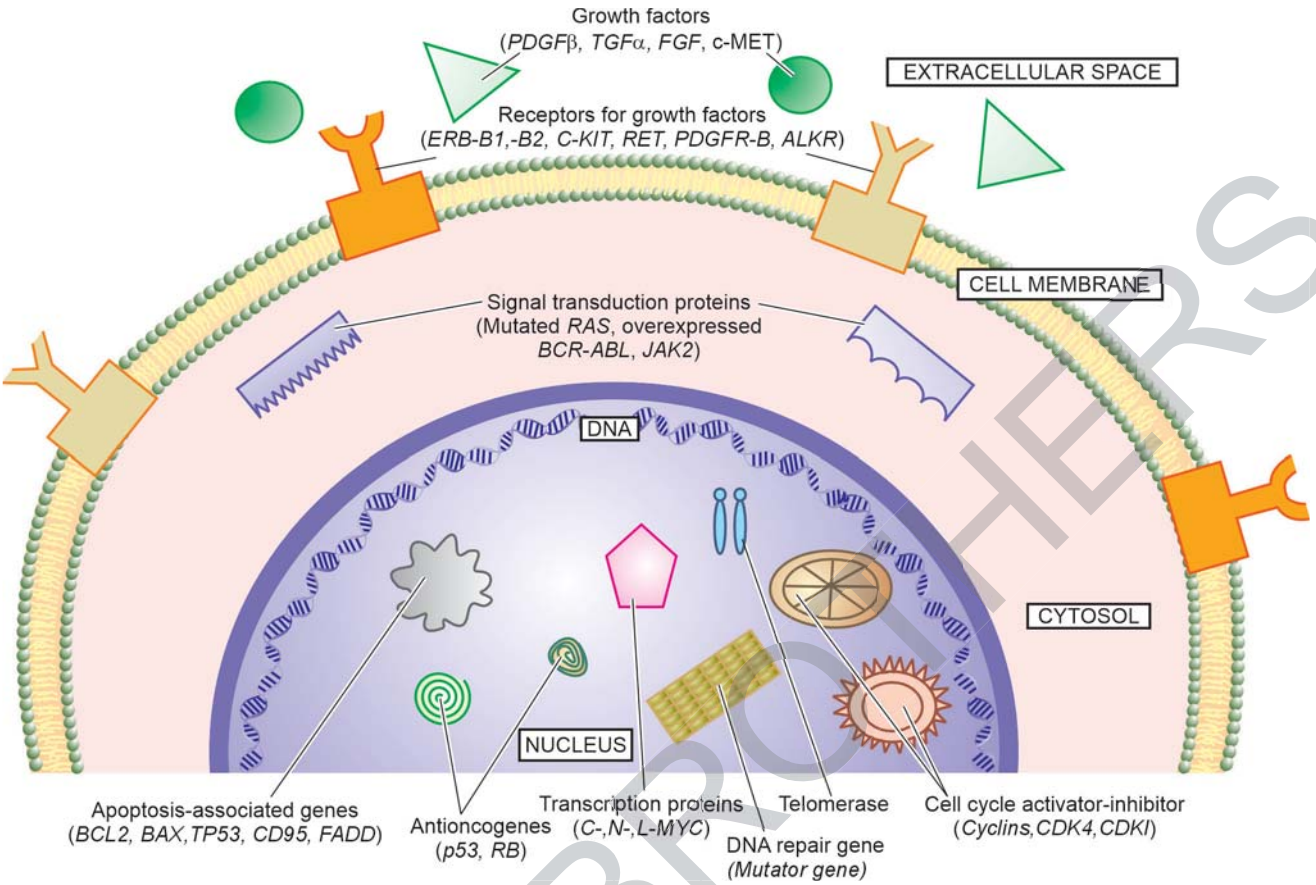

Figure 29.8 Schematic representation of activation-inactivation of cancer-associated genes in cell cycle.

1. *Oncogenes* are mutated proto-oncogenes (by point mutation, or translocation, or gene amplification). There are several classes of oncogenes having different related cancers: GFs *(PDGF-β, TGF-α, FGF, HGF)*, GF receptors *(EGFR, cKIT, RET, FMS-like TKR, PDGFR, ALKR)*, cytoplasmic signal transduction proteins *(RAS, BCR-ABL, JAK)*, nuclear transcription proteins *(MYC) and* cell cycle regular proteins (*CDK4*, cyclin D and E).
2. *Tumour-suppressor genes* (or anti-oncogenes having reverse function than oncogene) in mutated form result in removal of the brakes for growth; common mutations are loss-of-function, deletion and point mutation. Different classes of mutated tumour- suppressor genes in different cancers are: *RB* gene, *p53*, *TGF-β* and its receptor, *APC* and β-catenin, and others specific for certain tumours *(BRCA 1 and 2, VHL, WT 1 and 2, NF 1 and 2, PTEN, PTCH1, CDKN2A)*.

II. *Favouring overall cell survival by mechanisms of altered stress response* Cancer cells faced with stresses of several types respond by subverting the process in favour of cancer growth and survival. These responses include: 1) avoiding DNA repair, 2) escaping cell death by apoptosis, 3) avoiding cell senescence, and 4) recycling intracellular components by autophagy.

III. *Sustained perfusion of cancer by vascularisation* Cancer cells thrive and metastasise due to vascularisation that provides them oxygen and nourishment. Most common mode is angiogenesis under influence of VEGF. Non-angiogenic mechanisms also exist and include: co-option of blood supply, development of split vessels and stem cell-like transdifferentiation of tumour cells to form endothelial cells.

IV. *Cancer spread by local invasion and distant metastasis* It involves a series of events in cell biology called invasion-metastasis cascade.

V. *Growth-promoting metabolic changes (Warburg Effect)* Metabolic alterations in growing cancer cells are: 1) higher acquisition of nutrients (oxygen, glucose, glutamate), 2) altered metabolic pathways, and 3) oncometabolites in promoting tumour.

VI. *Dynamic tumour microenvironment by stromal components* Three types of stromal cells constitute TME having role in growth promotion and in enabling other cancer hallmarks: angiogenic vascular cells, cancer-associated fibroblasts and infiltrating immune cells.

VII. *Evasion of immune destruction by immune modulation* Host immune surveillance against cancer is exerted due to presence of tumour antigens on cancer cells and anti-cancer responses by host immune cells (CD8+ T lymphocytes, NK cells, macrophages). However, cancer cells evade immune destruction by cancer immunoediting and by various escape mechanisms.

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