Oncogenes and tumour suppressor genes

Gene types

Most cancers are monoclonal, arising from a single cell that has accumulated key mutations leading to uncontrolled cell proliferation. Such mutations can cause gene function to be either enhanced (activated) or lost (inactivated).

Genes whose function becomes lost or inactivated in carcinogenesis are termed tumour suppressor genes. Both gene copies must be inactivated before the tumour suppressor function is completely lost (i.e. absence of normal protein) so they can be thought of as recessive.

Genes whose function becomes enhanced are termed proto-oncogenes and their mutated form is an oncogene. Proto-oncogenes play an essential role in controlling cell proliferation and encoding growth factors, growth factor receptors, signal transducers, cytoplasmic regulators, and transcription factors. Oncogenes generally behave in dominant fashion.

Cell mutations

DNA mutations occur with a high frequency in mammalian cells (due to radiation exposure, metabolic accidents, and exposure to environmental carcinogens). Exceptionally efficient DNA repair mechanisms normally ensure that less than 1:1000 accidental base changes in DNA causes a mutation. Mutations take a variety of forms:

- Point mutations (substitution of one base pair of a DNA sequence by another).
- Translocations (gene arrangement due to chromosomal breakage and rejoining).
- Gene amplification (multiple copies of a gene).
- Deletions (loss of genetic material—from a single base to a whole gene).

Oncogenes

A large number of oncogenes have been isolated from transformed cells, oncogenic viruses, and human and animal tumours. Mutations in *ras* genes occur in approximately 20% of human tumours—K-*ras* mutations are particularly prevalent in pancreatic cancer (75–90%).

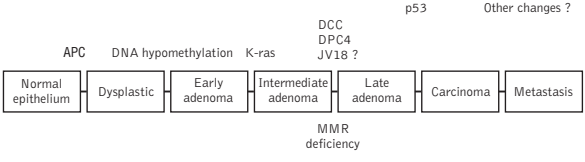
Tumour suppressor genes

Examination of familial cancer syndromes (e.g. retinoblastoma) and experimental evidence (particularly from somatic cell hybrid experiments) has demonstrated the existence of a different class of cancer gene—tumour suppressor genes. p53 is the most frequently-altered gene in human tumours. Approximately 37% of all cancers have a p53 mutation (incidence is much higher with cancers of the lung and colon). Certain specific tumour mutations are associated with particular carcinogens e.g. hepatocellular carcinoma is linked with hepatitis B and aflatoxins correlates with a high incidence of mutations in codon 249 of p53.

Products of tumour suppressor genes have a variety of functions within the cell. A large number of genes have now been isolated, as can be seen in the table.

Multi-step carcinogenesis

The development of cancer is a multi-step process characterized by repeated cellular insults resulting in the accumulation of mutations. The steps involved in the development of colorectal cancer have been particularly well characterized (see figure). Single mutations (e.g. APC) can lead to benign cellular proliferation (familial polyposis coli) that predisposes to the development of malignancy. Mutations in DNA repair genes (e.g. MMR—mis-match repair) speed up this process of mutation accumulation.



Genetic alterations associated with colorectal carcinogenesis. Mutation frequencies in MMR-deficient cells are two to three times higher than in normal cells—such MMR mutations are found in > 70% of hereditary non-polyposis colorectal cancer (HNPCC) cases and > 65% of sporadic colorectal cancers exhibiting micro-satellite instability. These cases account for 15–17% of total colorectal cancers. (Adapted from Kinzler and Vogelstein).

Cytogenetics and cancer

Cytogenetic analysis in cancer is used to confirm diagnosis, to aid differential diagnosis, and (possibly) to indicate prognosis.

Nowell and Hungerford first described a specific chromosome abnormality associated with chronic myeloid leukaemia in 1960. Since then, numbers of chromosomal abnormalities associated with cancer have grown exponentially. Abnormalities can be structural, numerical, or both; they may be restricted in distribution or found in many different tumour types. Some chromosomal alterations are useful in the diagnosis and prognosis of malignancies; the significance of others is unknown.

Nomenclature

Last revised in 1995, cytogenetic abnormalities are described by the ISCN (International System for Human Cytogenetic Nomenclature). Summary of the nomenclature used:

+ or –	Gain or loss of chromosome following the symbol.
p	Short arm of chromosome.
q	Long arm of chromosome.
del	Deletion. A deletion in the chromosome distal to breakpoint, or between two breakpoints if two are stated.
der	Derivative. A chromosome derived from a rearrangement. Rearrangement (if known) is described.
dic	Dicentric. Chromosome has two centromeres derived from the two named chromosomes.
dup	Duplication. A segment of chromosome defined by two breakpoints is duplicated.
i	Isochromosome. Chromosome is composed of two identical arms rather than a p and q arm.
ins	Insertion. Segment from one chromosome is inserted into another.
inv	Inversion. Segment of chromosome defined by two breakpoints is inverted compared to its normal orientation.
t	Translocation. Swapping of segments distal to the stated breakpoints in named chromosomes.

Chromosomal abnormalities

- structural
- numerical

Examples include:

- Chronic myeloid leukaemia
  —small chromosome 22 (Philadelphia chromosome)

- Acute myeloid leukaemia
  —many cytogenic abnormalities (80% of patients)
- Therapy-related AML
- Myelodysplastic syndromes
  —50% have chromosomal abnormalities
- Myeloproliferative disorders
- Acute lymphocytic leukaemia
  —80% have chromosomal abnormalities
- Chronic lymphoproliferative disorders
- Lymphoma
  —many structural and numerical chromosomal abnormalities
- Neuroblastoma
  —deletion of 1p and amplification of N-myc
- Wilms' tumour (nephroblastoma)
  —many chromosomal abnormalities reported

Invasion methodology

Tumour invasion and metastases is a complex, dynamic, multi-step process:

- Initial invasion of tumour through basement membrane.
- Movement into connective tissue surrounding tumour cells.
- Invasion of tumour cells into blood vessels.
- Circulating tumour cells are arrested in blood vessels of a distant organ or tissue; tumour cells invade organ from blood vessels.
- Tumour cells then grow within tissue to form a metastatic tumour that may become clinically evident.
- Process of tumour invasion and metastases results from alterations in cell-to-cell and cell-to-matrix adhesion and increased matrix degradation.

Extracellular matrix degradation

Several stages during the process of tumour invasion and metastases require increased degradation or breakdown of extracellular matrix or connective tissue surrounding tumour cells. The extracellular matrix is a complex mixture of proteins including different types of collagen, elastin, fibronectin, and laminin. Digestion of extracellular matrix is carried out by several groups of proteolytic enzymes. Major groups of enzymes implicated in tumour invasion and metastases are:

Matrix metalloproteinases (MMPs)

A family of zinc-containing enzymes involved in the degradation of the extracellular matrix; considerable evidence indicates that

individual MMPs have an important role in tumour invasion and tumour spread while expression of individual MMPs in tumours may be associated with prognosis[2]. MMPs are broadly classified into collagenases, gelatinases, stromelysins, and the recently-identified membrane-type MMPs.

MMPs are secreted proteins, produced as pro-enzymes and activated by cleavage of a N-terminal propeptide. Gelatinases, particularly MMP-2, appear to be important in initial stages of tumour invasion as they degrade components of the basement membrane, while other MMPs contribute to later stages of tumour invasion. Membrane-type MMPs that are membrane-bound appear to be involved in activation of MMP-2, which is capable of activating other MMPs.

Activity of MMPs is regulated by interaction with naturally occurring inhibitors, tissue inhibitors of metalloproteinases. Clinical interest in MMPs is considerable since the use of synthetic, low-molecular-weight, broad-spectrum inhibitors of MMPs can prevent tumour spread in human tumour xenografts. Several MMP inhibitors are being developed for clinical use.

Plasmin system

Urokinase-type plasminogen activator (uPA) is a serine protease that catalyzes the activation of plasminogen to plasmin—a broad-spectrum protease that in turn can break down a variety of extracellular matrix components. Plasmin can also promote the activation of MMP-2, thus linking plasmin system and MMPs in tumour invasion.

Cathepsins

A group of lysosomal proteolytic enzymes that also degrade many components of the extracellular matrix. Widely expressed in tumour cells, stromal cells, and endothelial cells. Cathepsins can be activated by uPA.

Cell adhesion

Tumour invasion and metastasis is also characterized by alterations in both cell-to-cell and cell-to-matrix adhesion. Cellular adhesion both to adjacent cells and surrounding extracellular matrix is mediated by a variety of molecules including:

Cadherins

Transmembrane glycoproteins involved in cell adenomatous polyposis coli (APC) gene product. The most important cadherin in relation to tumour invasion is E-cadherin whose expression is downregulated in various types of malignant tumour; loss of E-cadherin frequently appears to correlate with tumour invasion and metastasis.

Integrins

Transmembrane proteins involved in cell to matrix adhesion. Individual integrins are receptors for a variety of matrix proteins

including specific types of collagen, fibronectin, and vitronectin. Cell signalling pathways and expression of MMPs is also partially regulated by integrins. Altered regulation (often downregulation) and expression of integrins contributes to tumour cell invasion.

CD44

A cell surface glycoprotein that functions as an adhesion molecule. CD44 variants can be expressed on the surface of a variety of tumour cells. Specific splice variants of CD44 are associated with increased tumour invasion.

Angiogenesis

New blood vessel formation (angiogenesis) is an important factor for continued growth and development of both malignant tumours and metastases. Development of new blood vessels in tumours is stimulated by a wide variety of angiogenic factors produced by both tumour cells and stromal cells. In addition, several naturally occurring anti-angiogenic factors have been identified, most notably angiostatin and endostatin. New blood vessel formation in tumours is a complex and dynamic process requiring:

- Proliferation of endothelial cells from pre-existing capillaries or venules.
- Breakdown of extracellular matrix.
- Migration of endothelial cells.

Growth and development of blood vessels within tumours requires the same factors (i.e. increased matrix degradation and altered cell-to-cell and cell-to-matrix adhesion) that are crucial to tumour cell invasion.

New blood vessel formation is important in allowing tumour cells to enter the circulation and a high degree of tumour vascularity increases the likelihood of this. Newly formed blood vessels may be more permeable to tumour cells.

There is extensive interest in angiogenesis as a therapeutic target to prevent both tumour growth and metastases with both naturally occurring and synthetic anti-angiogenic compounds being intensively investigated for possible clinical use.

Formation of metastases in specific tissues

Some tissues and organs are more susceptible to the formation of metastases (e.g. liver, lung, and bone), whereas metastases are relatively uncommon in other tissues (e.g. kidney and heart). Several factors have been proposed to explain the formation of metastases in particular tissues including the expression of specific cell adhesion molecules in vascular endothelium of particular organs that are able to arrest circulating tumour cells. Another feature of metastases is the phenomenon of dormancy or latency of metastatic tumours such that

Histological identification

Histological identification of the malignant nature is the critical issue; some lesions escape the criteria mentioned previously. Examples are:

- Small-sized renal cell carcinomas (erroneously called adenomas because of their size).
- Highly differentiated 'lipoma-like' liposarcomas.
- Controversial pigmented skin lesions ('dysplastic' naevi).
- Questionable soft tissue tumours—such as atypical fibrohistiocytoma, fibromatosis, haemangiopericytoma; myelodysplasia.

While lesion classification uncertainty has decreased in recent years, 'borderline' ovarian tumours are still controversial; for some time they were classified as (cyst)adenocarcinomas of low malignant potential, but now as (cyst)adenomas with uncertain malignant potential or atypical proliferating tumours.

Lesions preceding malignant tumours are as various as the natural histories of the subsequent cancers. At some sites (e.g. uterine cervix and prostate) pre-malignant lesions may persist for a long time, others are short-lived. Among the former, some lesions permit reproducible histological typing and even grading, whereas ductal carcinoma *in situ* of the breast can be sub-typed and graded according to cell differentiation. At other sites the current criteria allow only a reproducible overall diagnosis of carcinoma *in situ*. Examples include:

- Bronchi.
- Prostate.
- Uterine cervix.
- Most organs lined by squamous epithelium (skin, upper respiratory and alimentary tract).
- Transitional epithelium (lower urinary tract).

Melanoma *in situ* is recognized as a stage in melanoma tumour progression. 'Dysplasia', though vague, is nonetheless well established and employed to define pre-malignant changes of, for example, larynx, uterine cervix, and Barrett's oesophagus.

Malignant potential without well-defined or reproducible histopathological features is attributable to hypercellular leiomyomas of the uterus, hyperplasia of plasma cells in the bone marrow accompanied by monoclonal gammopathy, nodular hyperplasia of regenerating cirrhotic liver, dysplastic naevi with random atypia, large congenital cutaneous naevi, and lymphomatoid granulomatosis.

Although clonality is a basic feature of malignancy in early phases of proliferation before multiple subclones develop due to additional

mutational events (genetic instability), it is not a synonym of malignancy as shown by the curable, occasionally self-limited, local lesions of Langerhans' cell histiocytosis (eosinophilic granuloma) whose monoclonality was recently proven.

Epithelial tumours comprise over 80% of neoplasms; adenoma being the most common epithelial benign growth and carcinoma the general term for the malignant counterpart. The latter may be additionally classified according to:

- Origin (adenocarcinoma, Merkel-cell ca.).
- Structure (papillary ca., tubular ca.).
- Extracellular matrix (desmoplastic ca.).
- Cellular content (glycogen-rich ca., lipid-rich ca.).
- Cell products (mucinous ca., keratinizing ca.).
- Cell-size (small or large cell ca.).
- Cell shape (spindle cell ca.).

The proliferating cell type is recalled in the names of tumours of soft parts and of myoid origin, both benign and malignant (e.g. fibroma vs. fibrosarcoma, leiomyoma vs. leiomyosarcoma). Most of the latter are subject to grading, and several systems have been proposed.

The central nervous system (CNS) displays an array of cell types from which several tumours may arise (astrocytes, ependymal cells, neuroblasts, etc.). A few CNS tumours are histologically benign (gangliocytoma, central neurocytoma); all others show a broad range of grades of malignancy. Progression from low to high-grade malignancy is a common event.

Mixed benign epithelial-stromal tumours are common in salivary glands (pleomorphic adenoma) and breast (fibroadenoma). Despite phenotypic multi-cellularity, clonality was recently proven for these tumours, supporting a totipotential stem-cell divergence hypothesis. Developmental errors are probably responsible for teratomas, which mostly arise along the midline of the body and in the gonads—these commonly display immature and malignant features in the testes and mature benign features in the ovaries.

Tumour-like conditions are non-neoplastic lesions that mimic neoplastic growth. Their importance lies in the differential diagnostic work-up. Chronic reactive inflammatory response may produce deceptively neoplastic-looking lesions such as nodular fasciitis of soft tissue. Other common lesions which simulate a neoplasm indicate:

- Aneurysmal bone cysts.
- Traumatic neuromas.
- Intravascular papillary endothelial hyperplasia.
- Central giant-cell granuloma of bone.
- Reactive hyperplastic nodules of mesothelium.

Tumour classification

Classification and typing of tumours still relies on histopathology and aspirated cell material. Tissue and cellular samples are submitted fresh or fixed (usually in formalin) with all relevant pieces of information (present and past clinical history, operative findings, and radiological data). Description of gross features of the organ or surgical specimen containing the tumour is essential. Size, shape, colour, consistency, appearance of the cut surface, and tumour-host interface (neoplastic pseudocapsule), presence/absence of ulceration, necrosis, and cystic spaces have to be recorded. Important histological features include:-

- Mitotic index.
- Lymphocytic infiltration (e.g. medullary carcinoma of breast, cutaneous melanoma).
- Extent and the type of necrosis (e.g. breast cancer and soft tissue tumours).
- Presence/absence of peritumoral vascular invasion (e.g. germ cell tumours of testes).
- Type of lesions adjacent to the tumour (e.g. atrophic gastritis, carcinoma *in situ*).

Grading

Grading of carcinomas is mandatory for adequate treatment (e.g. prostate, breast, endometrium, liver, kidneys) and several systems are available. Simplest consists of counting mitotic figures in defined microscopic areas. For some cancers micro-staging procedures are routinely applied (melanoma, carcinomas of organs with a cavity such as urinary bladder, gut, endometrium, uterine cervix, vulva) and consist of depth of invasion, tumour thickness, and type of margins (pushing, infiltrating).

For final diagnosis pathological reports should employ standardized nomenclature and coding using dedicated, computer-assisted reporting systems. Where appropriate, checkpoints for technical quality assurance should be incorporated. Due to rapidly expanding immunological, cytogenetic, and molecular techniques careful planning of supplementary methods is required; a tissue bank of snap-frozen samples of tumours and the corresponding normal tissues of the same patient is recommended.

Frozen section examination

Frozen section examination is still useful to establish/rule out malignancy and ascertain the status of surgical resection margins. On frozen section, tumour typing is often feasible. Enlarged non-metastatic lymph nodes and suspicious *in situ* or borderline lesions (breast nodules, polyps, ovarian cysts) are often unsuitable for frozen section procedures and require a definitive deferred diagnosis. Routine H and E staining of paraffin-embedded tissue section has to be supplemented by special stains, some of which are still used despite the advent of immunocytochemistry (ICC). Useful stains and methods are:

- Alcian blue cationic dye at pH 2.5 for intracytoplasmic epithelial acid mucin (e.g. mucin-producing carcinomas).
- Periodic acid-Schiff reaction (PAS) which visualizes glycoproteins and glycogen (e.g. Ewing's sarcoma, alveolar sarcoma, some carcinomas) and basement membranes.
- Romanowsky–Giemsa panoptical method for haematological proliferation.
- Reticulin fibre silver impregnation method.
- Stein's method for bile.
- Grimelius technique for argyrophilic substances.
- Several stains for micro-organisms.

Most enzymes are now identified by ICC, exploiting immunogenic properties of enzymatic proteins; the majority are hydrolases, oxidases, and dehydrogenases.