CELLS TO SYSTEMS - NEOPLASIA

Study Notes

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Definitions

The terminology of neoplasia is often confusing.

Mass or tumour: Any space-occupying lesion, whether neoplastic or not. The original meaning of the word "tumour" ("tumor" in Latin) is "swelling". So this can include both neoplastic growths as well as non-neoplastic growths, such hyperplastic growths and tissue malformations that can form masses. Masses may also be inflammatory (eg. abscesses, granulomas) or fluid-filled lesions (eg. cysts). As language evolves, the term tumour is now often (some may say incorrectly) used as synonymous to neoplasia.

Neoplasia: Neoplasia means "new growth" in Greek. Neoplasia is defined as the process leading to a mass resulting from unregulated growth of cells derived from normal tissue caused by irreversible genetic change. This is different from hyperplasia, which is regulated growth of cells, and from hamartoma, which is a malformation of normal tissue. A neoplastic mass is called a **neoplasm**. Neoplasia is the process, neoplasm is the product of the process.

Neoplasms may be further classified, based on their behaviour, as **benign** or **malignant** (defined later). Malignant neoplasia is also referred to as **cancer.**

The basic structure of neoplasms

All neoplasms have two basic components:

1) Neoplastic cells: These are the cells that are undergoing unregulated cell division, and are all daughter cells of the initial cells that underwent neoplastic transformation. Any cell in the body may become neoplastic, but some cell types are more susceptible than others.

2) Stroma: This is the connective tissue which supports the neoplastic cells. The stroma is non-neoplastic, and the stroma will not continue to grow if the neoplastic cells are eliminated.

Tumour stroma: function and characteristics

- The stroma is a vital component of a neoplasm. It provides the scaffold for neoplastic growth and the blood and nutrients required by the neoplastic cells. Stroma is generally composed of fibrous connective tissue and blood vessels, together with a mix of inflammatory cells such as macrophages. Sometimes the stroma may also contain bone, cartilage or mucin.
- The stroma is an extension of the adjacent normal tissue which is stimulated to proliferate and grow into the developing tumour by growth factors produced by the neoplastic cells. In turn, the stroma may produce factors to assist growth and proliferation of

the neoplastic cells. - The stromal component influences the gross and microscopic appearance of neoplasms:

- some tumours (e.g. lymphomas) have scant stroma so the neoplasm appears grossly soft and fleshy.
- some tumours (especially mesenchymal neoplasms) have a prominent stroma and are of firm consistency.
- Some tumours (especially epithelial malignancies such as squamous cell carcinoma) induce formation of an abundant, dense, collagenous stroma (**desmoplasia**); as a result, these neoplasms are very firm (scirrhous = hard) and are often described as **scirrhous carcinomas**

Benign and malignant neoplasia

The histological diagnosis of a tumour is always subjective and primarily aimed at predicting tumour behaviour (prognosis). Most neoplasms can be classified as either benign or malignant but some defy categorisation.

- Some neoplasms may fulfill morphological criteria of malignancy but behave in a benign fashion (eg. basal cell tumours of the skin typically have a high mitotic rate but are largely innocuous)
- Conversely, some tumours may appear morphologically benign but behave in a malignant fashion (eg. thyroid adenocarcinomas)

There are several characteristics of neoplastic tissues that help to distinguish benign and malignant neoplasia. The major characteristics for distinguishing benign and malignant neoplasia are:

a) Invasion into the adjacent tissue*
b) Metastasis (spread to distant sites not via direct invasion)*
c) The degree of cell differentiation
d) Growth rate

* **- These are the only definitive features that distinguish benign and malignant neoplasia.**

a) Invasion into adjacent tissue

Benign tumours typically grow by expansion as cohesive, circumscribed masses that do not infiltrate or invade, though they may compress the surrounding tissue. Histologically, benign tumours are clearly demarcated from the surrounding tissue and may be separated from them by a connective tissue **capsule**. This capsule is probably derived from the stroma of the host tissue as the parenchymal cells atrophy under the pressure of expanding tumour. Not all benign tumours are encapsulated.

Malignant tumours can grow expansively but most show progressive local infiltration, invasion and destruction of surrounding tissue. The pattern of growth is haphazard, random and uncoordinated and does not respect normal anatomic boundaries. This infiltrative behaviour makes it necessary to remove wide margins of surrounding normal tissue when surgical excision of a malignant tumour is

attempted, as the neoplasm will regrow from any neoplastic cells that are left behind. A malignant tumour that is not yet showing signs of invasion is sometimes referred to as an "*in situ*" malignancy.

b) Distant metastases

Metastasis is the process of dissemination of neoplastic cells from the primary tumour to distant sites where they establish themselves and grow. Only malignant neoplasms can metastasise. Not all cancers have the same ability to metastasise, and metastasis may be very rare with some malignancies.

Malignant neoplasms disseminate by one of the three pathways:

1) *Haematogenous spread:* Haematogenous spread is favoured by mesenchymal malignancies (sarcomas), but epithelial malignancies (carcinomas) use it as well. Veins are more readily penetrated than arteries by tumour cells, due to their thinner wall. Following venous invasion the blood-borne cells follow the venous flow draining the site of the neoplasm, with neoplastic cells stopping in the first capillary bed they encounter. This typically means the liver (as all portal circulation from the gut flows through the liver sinusoids) or lung (as all other venous drainage flows through the right side of the heart to the lungs) are the most frequently involved secondary sites in haematogenous dissemination.

2) *Lymphatic spread:* Lymphatic spread is most common with epithelial malignancies (carcinomas), whereas haematogenous spread is favoured by mesenchymal malignancies (sarcomas). However, all forms of cancer may disseminate through either or both systems. Lymphatic spread usually leads to metastasis to regional lymph nodes (e.g. oral carcinomas metastasise first to submandibular lymph nodes). A "sentinel lymph node" is the first regional lymph node that receives lymph flow from a primary tumour. Biopsy of sentinel lymph nodes allows determination of the extent of spread of tumour (and can be used to plan treatment).

3) *Implantation:* Spread by implantation occurs when neoplasm invade natural body cavities (e.g. peritoneum, pleura, bladder). The neoplastic cells separate from the main mass, float around in the cavity and then attach and grow at a new site. This is sometimes called "seeding" or "carcinomatosis". The implants literally coat the serosal surfaces without necessarily invading the underlying tissue.

c) Degree of cellular differentiation

Differentiation refers to the extent to which neoplastic parenchymal cells resemble their normal counterparts, both morphologically and functionally.

Benign neoplasms are composed of well differentiated cells that closely resemble their normal counterparts (e.g. a lipoma is made up of mature fat cells that resemble normal fat).

Malignant neoplasms are characterised by a wide range of cell differentiation, from surprisingly well differentiated to completely undifferentiated ("anaplastic").

Anaplasia, meaning "backwards formation", implies loss of functional and structural differentiation of normal cells. Although there are exceptions, the more rapidly growing and the more anaplastic the tumour, the less likely it is to have specialised functional activity (eg. hormone production). Some features that anaplastic cells may show include:

- Pleomorphism (variation in size and shape). There is often variation in cell shape, cell size (anisocytosis) and variation in nuclear size (anisokaryosis).
- The nuclear to cytoplasmic ratio is often increased (ie. the nucleus takes up much of the cell).
- Nuclei are often dark staining (hyperchromatic) due to increased amounts of nuclear chromatin. This reflects increased cellular proliferative activity.
- Giant cells with multiple nuclei may form.
- There is a lack of normal cell orientation (eg. loss of polarity of epithelial cells) and failure to form normal structures such as glands, tubules etc.
- Atypical mitoses are also frequently present

Dysplasia is a milder, reversible form of anaplasia, but the distinction is often unclear in the continuum of cancer formation. Dysplasia generally indicates a loss of uniformity in cellular features and tissue architecture, but lacks many of the bizarre features and poor differentiation that anaplastic cells display. Dysplasia does not necessarily indicate neoplasia, but dysplastic tissue may be more susceptible to neoplastic transformation, and is often found adjacent to malignancies.

d) Rate of growth

Most benign tumours grow slowly over a period of months to years; malignant tumours tend to grow rapidly. Rate of growth can be assessed grossly by determining the rate of change in tumour size and histologically by counting the number of mitoses in tissue samples. The rate of growth of malignant tumors usually correlates inversely with the level of their differentiation.

Somewhat counterintuitively, benign tumours are often larger than malignant tumours at presentation. This is because the gradual growth of benign tumours allows the host to adapt, meaning they can continue to grow over a long period. By contrast, malignant tumours often invade or metastasize and kill the host before they can become very large.

Classification of neoplasms

Most tumours are composed of a single neoplastic cell type, and the name of the neoplasm reflects the cell type from which the tumour is presumed to arise.

Why do we classify neoplasms?

The cell/tissue of origin and histological appearance is frequently indicative of the likely behaviour of the tumour, as well as the **prognosis** (prediction of the likely outcome e.g. survival time, likelihood of metastasis).

Naming conventions

The start of the name is always the type of neoplastic cell present

Benign neoplasia:

- For epithelial cells, benign glandular neoplasms end in "adenoma" and benign non-glandular neoplasms end in "papilloma"
  - Glandular example: sebaceous adenoma = benign neoplasm derived from sebaceous gland
  - Non-glandular example: squamous papilloma = benign neoplasm derived from squamous epithelium
- For mesenchymal cells, benign neoplasms end in "-oma"
  - Mesenchymal example: fibroma = benign neoplasm derived from fibrocytes

Malignant neoplasia

- For epithelial cells, malignant glandular neoplasms (usually) end in "adenocarcinoma" and malignant non-glandular neoplasms end in "carcinoma"
  - Glandular example: Sebaceous adenocarcinoma = malignant neoplasm derived from sebaceous gland
  - Non-glandular example: Squamous cell carcinoma = malignant neoplasm derived from squamous epithelium
  - Due to historical conventions, some glandular malignancies are simply referred to as "carcinomas" rather than "adenocarcinomas"
- For mesenchymal cells, malignant neoplasms end in "-sarcoma"
  - Mesenchymal example: Fibrosarcoma = malignant neoplasm derived from fibrocytes

Tumour nomenclature examples

	Cell of origin	**Benign form**	**Malignant form**
Mesenchymal	mesenchymal	-oma	-**sarcoma**
	Fibrocyte	Fibroma	Fibrosarcoma
	Adipocyte	Lipoma	Liposarcoma
	Cartilage (chondrocyte)	Chondroma	Chondrosarcoma
	Bone (osteocyte)	Osteoma	Osteosarcoma
	Smooth muscle	Leiomyoma	Leiomyosarcoma
	Striated muscle	Rhabdomyoma	Rhabdomyosarcoma
	Endothelium (vessels)	Haemangioma	Haemangiosarcoma
	Mesothelium	-	Mesothelioma
Epithelial	Glandular epithelium (e.g. mammary gland)	Adenoma	Adenocarcinoma
	Non-glandular epithelium	Papilloma, Polyp, other	**Carcinoma**

Round cells	Lymphocytes*	Lymphoma/Lymphosarcoma	
	Plasma cells*	Plasma cell tumour/Plasmacytoma	
	Mast cells*	Mast cell tumour	
	Histiocytes	Histiocytoma	Histiocytic sarcoma
	Melanocytes	Melanocytoma	Melanoma
	Circulating blood cells*	Leukaemia (prefixed by type of cell eg. lymphocytic leukaemia, erythrocytic leukaemia)	

Classifications used by pathologists are developed by the World Health Organization (WHO). *
- Neoplasms derived from these cell types are always malignant or potentially malignant

Important nomenclature exceptions

- *Lymphoma*: Lymphoma and lymphosarcoma are synonymous and both indicate malignant neoplasia of lymphoid cells. Confusingly, lymphoma is the more commonly used term, even though lymphosarcoma is technically more correct.
- *Mesothelioma*: is a malignancy of mesothelial cells (cells lining serosal surfaces such as pleura and peritoneum), and is highly malignant despite the name.
- *Melanoma*: is a malignancy of melanocytes, and is highly malignant; the benign counterpart is called melanocytoma.

Special types of tumours

- *Mixed tumours*: usually tumours arise from a single cell type (e.g squamous epithelium → squamous cell carcinoma); in some circumstances the neoplastic cells in a tumours are derived from two different lineages. The most common example occurs in dogs with mixed mammary tumours, which can be either malignant or benign. These tumours contain a neoplastic epithelial cell component, but the stromal mesenchymal cells also undergo neoplastic transformation. The cells derived from the separate lineages may be either benign or malignant (eg. malignant epithelial component and benign mesenchymal component in the same tumour).
- *Teratomas*: these are cells composed of more than one germ cell layer (usually three, endoderm, mesoderm, ectoderm). Teratomas typically display a mosaic of well-differentiated tissue of multiple lineages, often including tissues such as bone, teeth and haired skin. The tumours usually arise from totipotent cells, hence are most commonly encountered in ovaries and testis.

Lesions that can look and sound neoplastic but are not

- *Hamartoma*: This is a tissue malformation resulting in the formation of a disorganized mass; the cells are not neoplastic. It is considered a developmental anomaly.
- *Choristoma*: This term indicates a heterotopic malformation (normal tissue formed of nonneoplastic cells that develops in an abnormal location).

Effects of tumours on the host

Local effects

Compression and/or invasion of surrounding tissue: Location is crucial for both benign and malignant tumours, and both benign and malignant tumours can have devastating local effects if they occur in a critical site. A small pituitary adenoma can compress and destroy the surrounding normal gland. A benign tumour in the renal artery can obstruct the vessel leading to renal ischemia.

Restriction of movement: Some tumours may grow sufficiently large to impede movement of the host.

Ulceration and infection: Neoplasms may ulcerate, exposing raw tissue that is susceptible to infection. With intestinal malignancy, tissue invasion may also lead to intestinal perforation and secondary peritonitis.

Systemic effects

Neoplastic cells usually retain the normal function of the original cell type, but in some cases this function can also be unregulated. These effects are most apparent with endocrine neoplasms, which may secrete excessive amounts of hormones independent of normal stimulation. Examples of this include:

- Excessive growth hormone secretion by pituitary neoplasms, resulting in persistent growth and gigantism
- Excessive insulin secretion by pancreatic endocrine neoplasms (insulinomas), resulting in low blood sugar and weakness, collapse, and possibly coma and death
- Hyperthyroidism caused by excessive secretion of thyroid hormone from a thyroid neoplasm. This can result in weight loss, heart arrhythmias and hair loss.

Paraneoplastic syndromes

These are diseases or clinical signs that are the consequence of the presence of a neoplasm in the body, but are not due to the local presence of cancer cells or their normal functions (hence excessive secretion of normal hormones is NOT considered a paraneoplastic syndrome).

Neoplastic pyrexia

Many pro-inflammatory cytokines are released in response to neoplasia. These inflammatory cytokines, in particular interleukin-1, interleukin-6 and tumour necrosis factor (TNF) can lead to persistent or fluctuating fever.

Cancer cachexia

Many cancer patients suffer progressive loss of body fat and lean body mass, accompanied by profound weakness, anorexia and sometimes anaemia - a condition referred to as cachexia.

There is some correlation between the size and extent of spread of cancer and the severity of cachexia. However, cachexia is not caused by the nutritional demand of the tumour although patients with cancer often are anorexic, current evidence indicates that cachexia results from the action of soluble factors such as cytokines. Tumour necrosis factor (TNF) is the most common cytokine

produced by macrophages in response to tumour cells. TNF suppresses appetite and inhibits the action of lipoprotein lipase, inhibiting the release of free fatty acids from lipoproteins. Recent evidence suggests that neoplastic cells also secrete catabolic factors such as proteolysis-inducing factor (PIF) and lipid-mobilizing factor (LMF).

Hormone secretion

As well as unregulated growth, neoplastic cells can also display abnormal function. In some instances, neoplastic cells can secrete abnormal factors that are not normally produced by cells. The most common example of this is hypercalcaemia of malignancy. With this condition, neoplastic cells secrete a protein that is very similar to parathyroid hormone (PTH), known as parathyroid-hormone-related-protein (PTHrP), and has the same functions. This hormone stimulates bone resorption leading to increased blood calcium levels (hypercalcaemia). Typical PTHrP-secreting tumours in animals are lymphomas and adenocarcinomas of anal sac glands.

Autoimmune disease

Abnormal antigen expression by tumour cells often induces an immune response. If these antigens are cross-reactive with normal tissues, the immune system may attack normal tissues as well.
Common examples include:

- Immune-mediated haemolytic anaemia – Cross-reaction between tumour antigens and those on red blood cells causes the immune system to attack and lyse red blood cells, resulting in anaemia.
- Myaesthenia gravis – Patients with this disease have impaired nerve signalling, resulting in severe muscle weakness. This is due to antibodies against tumour antigens crossreacting with nerve signal receptors on muscles, blocking the normal signals to stimulate muscle function.

Hypertrophic osteopathy (Marie's disease)

This poorly understood condition is typically caused by a tumour developing within the chest. The mass within the chest stimulates proliferation of new periosteal bone over the distal limbs. Various theories have been suggested to explain this phenomenon, including periosteal hypoxia and vagal nerve stimulation.

ONCOGENESIS - THE MOLECULAR BASIS OF NEOPLASIA

The key to oncogenesis lies in a cell acquiring non-lethal genetic damage. Such genetic damage (mutation) may be spontaneous, may be induced by the action of environmental agents (such as chemical, radiation, viruses) or it may be inherited in the germ line. The genetic hypothesis of cancer implies that a neoplasm results from the clonal expansion of a single progenitor cell that has incurred genetic damage.

The genetic mechanism of neoplastic transformation

Initiation of neoplastic transformation usually resulting from damage to one of four classes of normal regulatory genes*:*

Proto-oncogenes: Proto-oncogenes are genes that promote cell growth and division. This includes genes for growth factors and their receptors, genes involved in intracellular growth signalling pathways, and genes involved in promoting mitosis. For neoplastic transformation a mutation must result in activation of these genes, either by increasing gene expression, increasing the activity of the gene produce (eg. increased enzyme activity), or decreasing clearance of the gene product. Activated proto-oncogenes are called oncogenes. In general, only a single oncogene copy within the cell requires activation to initiate neoplastic transformation, and thus only a single mutation event is required ("one-hit"). This means that oncogenes are essentially considered to be dominant genes.

Tumour suppressor genes are genes that normally prevent uncontrolled growth and so neoplastic transformation occurs when the gene is mutated or lost from a cell. Both normal alleles must be defective for transformation to occur ("two-hit"). Tumour suppressor genes may either be "governer" genes, which act as brakes on cellular proliferation, or "guardian" gene, which monitor for cellular changes that may promote neoplasia.

- The classic example of a "governer" gene is the retinoblastoma (RB) gene. Rb controls the movement of the cell from the G1 phase of the cell cycle (the resting point or "gap" prior to cell division) to the S phase (where synthesis of DNA occurs).
- The classic "guardian" tumour suppressor gene is p53. p53 senses genetic damage and induces growth arrest to allow DNA to repair, or in severe cases (where repair is unsuccessful) induces cellular apoptosis.

Genes involved in DNA repair: DNA repair genes correct DNA mutations that develop. Inactivation or loss of these genes allows genetic damage to accumulate and promotes progression to neoplasia.

Genes that regulate apoptosis**:** In a normal cell, once genetic mutation becomes severe, it triggers a cell to undergo apoptosis. Cells that have defective apoptotic pathways are able to survive genetic damage that would kill other cells, supporting progression to neoplasia.

Multi-step oncogenesis

Neoplastic transformation is a multi-step process. A single mutation causes pre-neoplastic transformation, but additional genetic mutations are required for a cell to become fully neoplastic. As the mutated cell proliferates, additional mutations may develop in the cell or its daughters. Some

of these new mutations make the cell better adapted to unregulated growth, resulting in greater cellular proliferation. This is essentially a form of natural selection, where the best adapted cells outgrow poorly adapted cells. Eventually, the cells accumulate sufficient genetic defects that they are fully neoplastic. Neoplasms are therefore composed of a number of distinct cell clones, each with their own mutations that they have accumulated over time.

The Hallmarks of Neoplasia

Once cells have accumulated sufficient genetic mutations, they become neoplastic, and are can undergo fully autonomous, unregulated proliferation. Neoplastic cells all share the following attributes, which are known as the hallmarks of neoplasia. Originally there were six hallmarks of neoplasia – two additional hallmarks (altered cellular metabolism and evasion of the immune system) were included in 2011.

1. Self-sufficiency in growth signals

Normal cells require growth signals (such as growth factors) to induce cellular proliferation. Neoplastic cells are able to circumvent this process and proliferate without external signals, either by directly inducing growth factor secretion (self-secretion or secretion by the stroma), or reducing/eliminating the requirement for growth signals (via oncogene activation).

2. Insensitivity to growth inhibitory signals

Neoplastic cells don't respond to the normal brakes on cell growth that are usually present. Insensitivity to inhibitory signals typically reflects inactivation of tumour suppressor genes. In addition to the types of tumour suppressor genes indicated previously, there may also be decreased sensitivity to inhibitory growth factor (eg. decreased expression of receptors for the growthinhibiting cytokine TGF-β), and insensitivity to the inhibitory signaling caused by cell crowding (contact inhibition).

3. Evasion of apoptosis

Neoplastic cells can avoid apoptosis that is typically induced by cellular injury. Apoptosis (programmed cell death) starts when there is activation of caspases (enzymes) that signal destruction the cell. Neoplastic cells can modify the balance between pro-apoptotic and anti-apoptotic molecules. For example, some neoplastic cells overexpress the anti-apoptotic factor BCL-2 (B cell lymphoma 2, named due to its high expression in B cell lymphomas).

4. Limitless proliferation

Most normal human cells have a capacity of 60 to 70 cell divisions. Thereafter, the cells lose their capacity to divide and enter senescence. This phenomenon has been ascribed to progressive shortening of telomeres (repetitive sequences of DNA situated at the end of chromosomes).

In normal cells, which lack expression of the enzyme **telomerase**, the telomere shortening that occurs with each cell division eventually activates cell cycle checkpoints, leading to senescence and placing a limit on the number of divisions the cell may undergo. Tumour cells reactivate telomerase, which restores the length of the telomeres, thus staving off mitotic catastrophe and achieving immortality.

5. Sustained angiogenesis

Tumours cannot enlarge beyond 1 or 2 mm in diameter unless they are vascularized. Like normal tissues, tumours require delivery of oxygen and nutrients and removal of waste products. Neoplastic cells can stimulate ***neoangiogenesis***, during which new vessels sprout from previously existing capillaries or can stimulate ***vasculogenesis***, in which endothelial cells newly develop from precursor cells. This allows the tumour to continue to grow in size, and also provides a route of access for haematogenous metastasis in malignancy. However, very rapidly growing tumours may still develop too fast for the blood vessels to keep up, resulting in areas of necrosis.

Tumour vasculature is abnormal. The vessels are leaky and dilated with a haphazard pattern of connection; in some tumours the pattern of blood vessels gives an important clue as to the type of neoplasm.

Angiogenesis is promoted by angiogenic factors; the most important is vascular endothelial growth factor (VEGF), which is a common target of therapeutic drugs. Hypoxia within the neoplasm is also a potent stimulator of angiogenesis. Tumours may also suppress the action of angiogenic inhibitors such as thrombospondin-1.

6. Altered cellular metabolism

Normal cells produce energy via oxidative phosphorylation under aerobic conditions, and glycolysis under anaerobic conditions. Oxidative phosphorylation is a highly efficient way of producing energy that requires oxygen, whereas glycolysis is relatively inefficient and uses glucose.

In neoplastic cells, the metabolism is altered to always use glycolysis, regardless of whether the conditions are aerobic or anaerobic. This is because oxygen levels within a tumour are often highly variable and change rapidly, and so glycolysis is preferable as glucose is always available, even when oxygen is restricted. Furthermore, while oxidative phosphorylation only produces carbon dioxide and water as byproducts, the process of glycolysis produces important precursors for biosynthetic pathways, such as acetyl-CoA (important for lipid synthesis), 5-carbon sugars (required for nucleotide synthesis) and NADPH (an important cofactor in many biosynthetic pathways). Thus, glycolysis also facilitates neoplastic cell growth.

7. Evasion of the immune system

The immune system is capable of detecting and eliminating neoplastic cells before they become established. Antigens on neoplastic cells are detected by lymphocytes (especially cytotoxic T cells), which may induce cellular apoptosis or an adaptive immune response. Antigens expressed by neoplastic cells include products of mutated genes, abnormally expressed gene products (eg. antigens that are normally only expressed during embryogenesis) and antigens produced by viruses that cause neoplasia (viral oncoproteins – see later).

Successful neoplastic cells are able to avoid stimulating an immune response. They may do this by suppressing expression of antigens, preventing presentation of antigens to lymphocytes (eg. suppressing MHC class I expression), or secreting immunosuppressive substances to impair immune function.

8. Ability to invade and metastasise

This hallmark is only found in malignant neoplasia. The spread of tumours is a complex process involving a series of sequential steps called the invasion-metastasis cascade. The steps consist of local invasion, intravasation into blood vessels and lymph vessels, transit through the vasculature, extravasation from the vessels, formation of micrometastasis, and growth of micrometastases into the macroscopic tumours.

A carcinoma first must breach the underlying basement membrane, then traverse the interstitial connective tissue and ultimately gain access to the circulation by penetrating the vascular basement membrane.

The major steps involved in this process are:

1. Loosening of cell-cell contact (loosening of intercellular connections such as E-cadherin)
2. Degradation of basement membrane and interstitial connective tissue. Tumour cells can secrete enzymes such as metalloproteinases (MMP) that degrade the extracellular matrix.
3. Migration of tumour cells though cytoplasmic movement. Recently it has become clear that stoma plays a major role in invasion, helping neoplastic cells in the process of invasion and metastasis.

Aetiology of Cancer: Carcinogenic Agents

Neoplastic transformation may occur due to spontaneous mutation. Cellular division is not a perfect process, and genetic defects may be introduced during mitosis that can lead to neoplastic transformation. For genes that require “two hits” (ie. recessive expression) it is also possible to inherit a defective gene, therefore requiring development of only a single additional defect to initiate oncogenesis.

Extrinsic agents that can induce neoplastic transformation are known as carcinogens. Three classes of carcinogens have been identified:

1. Chemicals
2. Radiant energy
3. Microbial agents

1. Chemical carcinogens

Chemical carcinogens can be classified into three different categories

- *Initiators*: These are carcinogens that can directly cause DNA damage and can therefore “initiate” the development of neoplasia. Initiators are usually electrophilic molecules (such as free radicals) that are capable of stripping electrons from DNA, which promotes cross-linkage within the DNA strands.

- *Promoters*: These carcinogens support the proliferation of mutated cells, giving rise to large numbers of clones which carry the initial mutation. This increases the likelihood that some of the cells will survive to complete neoplastic transformation. Examples of

promoters include hormonal such as oestrogens and progesterones, and this is the reason that mammary tumours are much more prevalent in intact/nonspayed bitches.

- *Complete carcinogens* are substances which can have both initiating and promoting effects. Examples include chemicals such as cantharidin and methylcholanthrene.

Examples of chemical carcinogens:

- *Tobacco smoke*: An association between cigarette smoke and lung cancer has been demonstrated in people. The same association has not been proven in animals, but appears likely to occur.

- *Asbestos*: There is striking evidence that asbestos causes mesotheliomas in people; recent evidence suggests that dogs exposed to asbestos also develop mesotheliomas.

- Other chemical compounds known to cause tumours in animals are **aflatoxin B** (from mouldy corn or peanuts) that causes hepatic neoplasia and ingestion of **bracken fern**, which predisposes to transitional cell carcinomas of the urinary bladder.

2. Ionising radiation

Ionizing radiation is a complete carcinogen in that it can both initiate and promote neoplastic transformation. Ionizing radiation can transform virtually every cell type, but dividing cells are especially prone to damage. Children and young animals are more sensitive to the effects of radiation for this reason. DNA mutations may be a direct effect of the ionizing radiation, or be induced indirectly by generation of highly charged free radicals derived from cytoplasmic water and oxygen.

- Types of carcinogenic radiation include particle radiation (*protons, neutrons, alpha particles*) and some forms of electromagnetic radiation (*X-rays, gamma radiation, ultraviolet light*).

Ultraviolet radiation: UV radiation has been incriminated as a cause of cutaneous squamous cell carcinomas, basal cell carcinomas and malignant melanomas in humans, cutaneous and conjunctival squamous cell carcinomas in animals, dermal haemangiomas in dogs and dermal haemangiosarcomas in dogs and cats. They most frequently occur in unhaired, unpigmented areas of skin, as melanin absorbs a large proportion of UV-B rays before they can cause damage.

3. Microbial agents

Viruses

Only five DNA and one RNA viral families are oncogenic and not all members of these families can cause neoplasia

- Poxviridae (poxviruses) – Smallpox
- Retroviridae (oncornaviruses, retroviruses) – Feline leukaemia virus
- Herpesviridae (herpesviruses) – Marek's disease (gallid herpesvirus 2)
- Adenoviridae (adenoviruses) – Adenovirus in rodents
- Papovaviridae (papillomaviruses) – Human papillomavirus

- Hepadnaviruses (hepatitis viruses) – Hepatitis B

Oncogenic viruses cause neoplasms by:

- Altering normal regulation and expression of proto-oncogenes and tumour suppressor genes in infected cells (eg. papillomaviruses increase degradation of products of both p53 and retinoblastoma tumour suppressor genes)
- Introducing viral-encoded homologues of proto-oncogenes (*viral oncogenes*) or strong promoter sequences into the host cell genome – this method is typical of some retroviruses (eg. feline leukaemia virus) and herpesviruses (eg. Gallid herpesvirus 2 – "Marek's disease").
- Inducing chronic inflammation which causes oxidative DNA damage (eg. human hepatitis viruses)

Bacteria

Only one bacterium has been definitively demonstrated to cause neoplasia. *Helicobacter pylori* is the first recognized bacterial carcinogen, and can cause gastric adenocarcinoma and lymphoma through chronic inflammation. This organism is a human pathogen, and species that infect animals do not appear to cause significant disease.

Parasites

Some parasitic organisms (eg. *Schistosoma, Spirocerca*) are also known to be carcinogenic.

Other causes of cancer

Chronic inflammation and tissue irritation

- *Feline post-traumatic sarcomas* are pleomorphic sarcomas which develop in eyes which have been traumatised, especially with penetrating injuries
- *Feline vaccine-associated sarcomas* (mainly fibrosarcoma) has been recognised in cats and is thought to occur in 1-2:10,000 vaccinations. Both the vaccine adjuvant and the local immune and tissue response to the injected antigens have been implicated in the pathogenesis