NORMAL TISSUE GROWTH AND CELL PROLIFERATION

- body / organ size depends on the total cell mass = number of cells (cell division - cell death) + size of cells.
- control is by intracellular programs (genes) which are regulated by the balance of extracellular signal molecules (soluble or contact dependant) that either stimulate or inhibit cell division and size.
  ① stimulatory factors - mitogens, growth factors / cytokines and survival factors (suppress apoptosis).
  ② inhibitory factors - inhibitory signal proteins and apoptosis inducers
- thus an excess of stimulators or deficiency of inhibitors, leads to net growth (uncontrolled growth in cancer).
- most adult organs contain a mixture of quiescent, permanent and continuously dividing cells.

① Continuously dividing (labile) tissues
- cells proliferate throughout life to replace differentiated cells that are continually lost, eg blood, skin, GI tract.

② Quiescent (stable) tissues
- cells have a low level of division; however can respond rapidly when stimulated, eg some parenchymal cells (liver, kidney, pancreas), fibroblasts, osteoblasts, chondroblasts and endothelial cells.
- tissue growth can be accomplished by shortening cell cycle (ie more frequent divisions of a few cells) or more importantly by recruiting more quiescent cells to proliferate.

③ Nondividing (permanent) tissues
- cells are terminally differentiated and cannot undergo mitosis (neurons, skeletal and cardiac muscle).
- note: skeletal muscle can regenerate if sarcolemmal sheaths intact and some stem cells recently discovered in the brain and heart but they have limited regenerative capacity in natural conditions.

- stem cells are undifferentiated precursor cells that retain the ability to give rise to a variety of cell types.
- to maintain self renewing capacity and also supply cells for replacement / regeneration, the stem cells must exhibit asymmetric replication; after division some of the clonal cell lines must become differentiated while others must remain as stem cells.

a) Embryonic Stem Cells
- embryonic stem (ES) cells are derived from the inner cell mass of the blastocyst.
- are the cells that form the 3 germinal layers of the embryo.
- ES cells can produce all the different cells / tissues of the body, thus called totipotent (note: pluripotent refers to cells that can become some or many different cell types).
- may be able to use ES cells in a procedure called “therapeutic cloning” to repair damaged organs.
b) Primordial Germ Cells (PGC’s)
- PGC’s are the progenitor cells that will form the haploid gametes (eggs in females and sperm in males).
- in mammals, a small group of cells in the yolk sac region of the early embryo (7 days in mouse) are induced to become PGC’s by signals from neighboring cells.
- the PGCs then move with the invaginating hindgut, migrate up the dorsal mesentery and into the genital ridges where they commit to a developmental pathway to become either eggs or sperm.
- the genital ridge becomes ovary or testis based on sex chromosomes in the somatic cells (not the PGC’s).
- after gastrulation the PGC’s and then oocytes (not sperm) are the only totipotent cells of the body.

c) Adult Stem Cells
- many adult tissues (eg marrow, skin, gut) contain stem cells within particular sites called niches.
- most adult stem cells have a more restricted differentiation capacity - often lineage specific.
- recent research suggests that some adult stem cells (eg multipotent adult progenitor cells in the bone marrow) may have pluripotent capability or can revert to a more pluripotent state.

eg’s

1. Hematopoietic stem cells (HSCs)
   - HSC’s can reconstitute the marrow after radiation, giving rise to all the progenitors of the blood and immune cell families.
   - hematopoietic tissue is very active (eg ~ 2 million rbc’s destroyed/produced per sec in 70 kg animal)

2. Bone marrow stromal stem cells
   - can generate a variety of mesenchymal cells (eg osteoblasts, chondroblasts, adipocytes, myocytes, endothelium) depending on the regulatory proteins in the microenvironment.

3. Epidermal stem cells
   - stem cells located in the basal layer; epidermis renewed every ~ 30 days as old cells are desquamated (exfoliated) from the skin surface.

4. Intestinal epithelium
   - rapid turnover of intestinal epithelium derived from stem cells near the base of the intestinal glands (each intestinal gland is the clonal product of a single stem cell).

DISTURBANCES OF GROWTH
- abnormal cell growth includes a wide spectrum of conditions varying from complete absence of tissue development (agenesis) to totally unregulated growth (neoplasia).

1) Agenesis
- refers to complete failure of an organ / tissue to develop with no associated primordium.

2) Aplasia
- refers to failure of an organ / tissue to grow, owing to failure of development of the primordium.
- this term implies the presence of a rudimentary structure, possibly represented only by connective tissue (which is often not easy to identify grossly).
- can also refer to lack of production of cells from an organ or tissue (eg aplastic anemia).
3) **Hypoplasia**
- refers to failure of an organ / tissue to reach its normal size (less severe in degree than aplasia).

4) **Dysplasia**
- in the context of organ development, dysplasia refers to abnormal organization of cells (‘abnormal growth’), eg retinal dysplasia, hip dysplasia, renal dysplasia, etc.
- during embryogenesis, development of some organs or portions of organs is dependent on induction by adjacent developing tissues, eg, the various parts of the eye are dependent on each other for their normal development.

**Causes of Developmental Anomalies**
- the cause of the above 4 types of organ / tissue anomalies must be sought in the failure of the progenitor cells to proliferate and differentiate at some stage of their development.

1) **Genetic causes:**
   i) those associated with chromosomal (karyotypic) aberrations
      - eg, a variety of reproductive anomalies associated with chromosomal abnormalities (eg XX/XO mosaicism of horses)
   ii) those arising from gene mutation.
      - eg’s, chondrodysplasia (dwarfism) in many species, collagen dysplasia in many species.

2) **Environmental causes:**
   i) *In utero* infections.
      - eg’s, cerebellar aplasia/hypoplasia caused by Bovine Virus Diarrhea virus infection of fetal calves and panleukopenia infection of fetal kittens (lesion develops when infection occurs during period of rapid growth in the developing cerebellum, since virus primarily attacks rapidly dividing cells).
   ii) *In utero* exposure to radiation and certain drugs / chemicals / toxins
      - most infamous teratogenic drug in humans is thalidomide (drug used for morning sickness).
      - eg ewes ingesting *Veratrum* type plants in early gestation have lambs with craniofacial deformities.

3) **Multifactorial causes:**
   - the cause of many anomalies are not identified and are likely due to inheritance of mutant genes and their interaction with environment factors.
   - eg testicular hypoplasia associated with cryptorchidism is due to inhibition of spermatogenesis by the high intraabdominal temperature relative to that of the scrotum; the cryptorchidism (incomplete descent of one or both testicles into the scrotum) has both genetic and environmental risk factors.

5) **ATROPHY** (see Cell Injury)

6) **HYPERTROPHY** (see Cell Injury)

7) **HYPERPLASIA**
- definition: an increase in the number of cells in an organ / tissue usually with a corresponding increase in organ / tissue volume.
- hypertrophy and hyperplasia are not mutually exclusive and are often seen together in structures which can undergo division (esp reproductive and endocrine organs).
a) Etiology

- **Physiologic Hyperplasia**
  - Physiologic hormonal hyperplasia; eg uterus and mammary gland in pregnancy / lactation.
  - Compensatory hyperplasia; eg hyperplasia of hepatocytes after partial hepatectomy.

- **Pathologic Hyperplasia**
  - Due to excessive hormonal stimulation; eg cystic endometrial hyperplasia in dogs.
  - Chronic irritation (via endogenous growth factors); eg chronic trauma, infections and wound healing.

b) Mechanisms and Biochemistry

- Cell proliferation is generally due to increased production of growth factors / hormones, increased expression of growth factor receptors or activation of specific intracellular signaling pathways.
- In contrast to neoplasia, hyperplasia remains responsive to homeostatic controls; once the causative stimulus has been removed it will regress (ie it is reversible process unlike neoplasia).
- However, pathologic hyperplasia is a "fertile soil" in which cancerous proliferation may arise.

c) Gross Appearance

- Tissue / organ is increased in volume (size & weight).

d) Microscopic Appearance

- See increased numbers of cells / cellular proliferation; eg’s:
  1. Hepatic nodular regeneration in cirrhosis
     - See hyperplasia of hepatocytes.
  2. Kidney following removal of the contralateral organ
     - See hypertrophy and hyperplasia of individual cells within glomeruli and tubules.
  3. Goitre resulting from iodine deficiency
     - See hypertrophy and hyperplasia of thyroid follicular epithelial cells in response to increased TSH.
  4. Adrenal glands stimulated by a functional (ACTH secreting) tumor of the pituitary gland
     - Hypertrophy and hyperplasia of cells in the adrenal cortex.
  5. Proliferative gill disease
     - A stereotyped response of fish gills to a wide variety of injuries: chemical, bacterial, protozoal.
     - Primarily epithelial hyperplasia (which can be massive) and also some epithelial hypertrophy.
  6. Porcine proliferative enteropathy
     - Caused by *Lawsonia intracellularis* which replicates in mucosal epithelial cells of the ileum.
     - The infected epithelial cells proliferate with the mucosal glands becoming elongated and branching, resulting in a greatly thickened mucosa.
  7. Epidermal hyperplasia
     - In response to wide range of insults / irritations of the skin, eg infections, self-trauma, etc.
- the causes of some forms of hyperplasia, specifically nodular forms, are unknown:
  1. hepatic nodular hyperplasia in old dogs.
  2. pancreatic nodular hyperplasia in old dogs, cats and cattle.
  3. adrenal cortical nodular hyperplasia in old dogs, cats and horses.
  4. thyroid nodular hyperplasia in old cats (one cause of hyperthyroidism).
- this group of hyperplastic lesions occupies a nebulous position in the spectrum of abnormal cell growth.
- these lesions are not necessarily preneoplastic, ie they do not necessarily evolve to neoplasms with time, although they can be difficult to distinguish morphologically from benign tumors.
- some morphologic features help to distinguish these hyperplastic nodules from benign neoplasms:
  - grossly: hyperplastic nodules tend to be of a smaller size and are often multiple; as opposed to a single or, at most, a few benign neoplasms within an organ.
  - microscopically: the architecture of hyperplastic nodules is more similar to that of the normal organ, has no capsule and shows no compression of the adjacent tissue.

8) METAPLASIA
- definition: a reversible change in which one adult cell type is replaced by another adult cell type (usually recognized only microscopically).

- the ability of a tissue to undergo metaplasia reflects the reprogramming of stem cells to differentiate along a new pathway; brought about by changes of soluble factors (cytokines, growth factors, ECM components) in the microenvironment that affect tissue specific (differentiation) genes.

- the most common example of metaplasia is the transformation from ciliated columnar to squamous epithelium in response to chronic irritation (eg smoking, stones in ducts of glands, etc).
- these forms of metaplasia often represents an adaptive substitution of cells sensitive to stress by other cell types better able to withstand the emergence of an adverse environment; however this is often at the expense of normal function eg loss of cilia or protective mucus with squamous metaplasia.

- possible causes of mucous or squamous metaplasia include:
  1. chronic irritation / inflammation.
     - in the habitual cigarette smoker, the normal columnar ciliated epithelial cells of the trachea and bronchi are replaced focally or widely by stratified squamous epithelial cells.
     - with abomasal nematode infection can see replacement of the normal glandular epithelium with mucous (goblet cell) epithelium.
  2. abnormal metabolism.
     - squamous metaplasia in prostate gland of dogs with estrogen-producing testicular Sertoli cell tumor.
     - squamous metaplasia of several simple epithelia in vitamin A deficiency in many species.

- metaplastic change can occur with either hyperplasia or atrophy; it is usually an orderly process and is reversible once the cause has been removed (however if the injurious stimulus persists it can promote neoplastic transformation).

- in metaplasia of mesenchymal tissue, fibrous tissue may be replaced by cartilage or bone (can be seen in areas of chronic injury or in some neoplasms).
9) Dysplasia
- in the context of mature tissues, dysplasia refers to disordered growth of cells; characterized by loss of uniformity of the individual cells and architectural disorganization.
- occurs primarily in epithelium; when severe it is an early morphologic indicator of neoplastic transformation.
- however, dysplasia is not necessarily neoplastic, ie when mild, it is reversible with removal of inciting cause.
- microscopically see cellular atypia:

  1. variation in the size and shape of the cells and their nuclei (pleomorphism).
  2. nuclei are often hyperchromatic, enlarged (high N/C ratio) and have large nucleoli.
  3. mitotic figures are more numerous and often in abnormal locations (although mostly normal patterns).
  4. tissue architecture is often disorganized.

10) Other Growth Disorders

a) Hamartoma
- a benign tumor-like mass composed of an overgrowth of mature cells and tissues normally present in the affected part, but often with one element predominating.
- they are present at birth and probably result from an overgrowth of progenitor cells in the fetus.
- they may enlarge with the growth of the animal, but should stop when animal has reached maturity.
- microscopically, they are composed of normal tissue (eg blood vessels or cartilage) with occasional admixing of other tissues (as opposed to most benign tumors which are composed of a single cell type)

b) Choristoma
- a mass of histologically normal tissue in an abnormal location (ie an ectopic rest of normal tissue) eg’s ocular dermoid, epithelial inclusions in the myocardium.

11) Cellular Aging
- aged cells have reduced proliferative and physiologic capacity (diminished ability to respond to injury).
- cell aging is the result of existing cellular programs (replicative senescence) and the accumulation of metabolic and genetic damage.

a) Structural and Biochemical changes
- morphologically see abnormally lobed nuclei, vacuolated abnormal shaped mitochondria, decreased ER, distorted Golgi, increased lipofuscin pigment.
- functional reduction in oxidative phosphorylation, protein synthesis, nucleic acid synthesis / repair, etc.
- at the molecular level see increased protein cross-linking (due to gradual nonenzymatic attachment of glucose to proteins), accumulation of oxidative damage to DNA / proteins / membranes and increased numbers of abnormally folded proteins.
b) **Accumulated metabolic / genetic damage**
- although most DNA damage is repaired by endogenous enzymes, some persists and accumulates.
- with cell aging see a steady shift in the balance of progressive metabolic damage (esp DNA damage by oxidative stress) vs repair ability.
- the life span of different animal species is generally inversely proportional to the energy expenditure relative to the body mass, ie fixed total metabolic consumption over a lifetime;
- eg → small mammals with a high metabolic rates (eg mice) tend to have a shorter life spans.
  → dietary caloric restriction increases the mean life span of animals (from fruit flies to mammals), by reducing metabolic rate, thus decreasing the production of toxic by-products of metabolism.

**c) Replicative Senescence**
- animal cells have a limited capacity for replication, eg human fibroblasts can divide 20 - 60 times, the actual number being inversely related to the age of the donor.
- two mechanisms identified that control replicative capacity:

1. **telomere length / telomerase activity**
   - telomeres are regions of highly repetitive sequences of DNA on the ends of chromosomes that prevent fusion / "end replication" / recombination / degradation of the chromosomes.
   - as somatic cells divide, the length of the telomeres progressively shortens, until they are recognized as damaged DNA and cell division is arrested.
   - telomerase is an enzyme that can maintain the length of the telomeres.
   - in most somatic cells there is no telomerase activity, but stem cells have some activity and germ cells have enough to completely maintain telomere length.
   - in cancer cells, which replicate indefinitely (cell immortalization), telomerase is reactivated.

2. **clock genes**
   - specific genes, receptors & signals appear to play a role in cellular aging (eg insulin/IGF-1 pathway).

**NEOPLASIA**

1) **Definitions**
- Neoplasia = neo ("new") + plasia ("form") = the process of new growth.
- Neoplasm = the new growth; can be benign or malignant.
- Tumor = tumere ("to swell"); historically referred to any swelling, now synonymous with neoplasm.
- Oncology = oncos ("mass, bulk") + logos ("reason"); the study of neoplasia.
- Oncogenesis = oncos + genesis ("production"); the causation or production of neoplasia.
- Cancer = karkinos ("crab")
  - the common term for all malignant neoplasms (used more frequently in human medicine).
  - origins:
    - the swollen veins sometimes seen with skin tumors suggested to Hippocrates the claws of a crab.
    - a cancer adheres to any part that it seizes upon in an obstinate manner like the crab.
    - cancer is characterized by infiltrative, erosive growth that extend crab-like feet into adjacent tissues.
A tumor is an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the normal tissue and persists in the same excessive manner after cessation of the stimuli which evoked the change." (Willis, 1952)

"Tumor cells do not necessarily proliferate at a faster rate than their normal counterparts. Tumor growth depends on other factors, such as the growth fraction (proportion of cycling cells) and the rate of cell death. The major determinant of tumor growth is clearly the fact that more cells are produced than die in a given time." (Essential Pathology: Rubin & Farber, 1995)

"the persistence of tumors... results from heritable genetic alterations that are passed down to the progeny of the tumor cells. These genetic changes allow excessive and unregulated proliferation that becomes autonomous (independent of physiologic growth stimuli) although tumors generally remain dependant on the host for their nutrition and blood supply." (Pathologic Basis of Disease, 2005)

[note: the persistence of cellular proliferation after the stimulus has been removed clearly distinguishes a neoplastic process from an inflammatory lesion or from hyperplasia]

2) Terminology

- "Despite...rules and regulations for classifying and naming neoplasms, inconsistencies persist" (Slauson&Cooper)
- the suffix -oma is almost always present (from onkoma = "swelling").
- the term ascribed to a neoplasm must convey whether it is:
  1. benign or malignant.
  2. mesenchymal versus epithelial origin (usually requires histology; sometimes IHC markers / EM).

a) Benign

1. mesenchymal (structural tissues, blood vessels, lymphatics): lipoma, fibroma, rhabdomyoma, leiomyoma
   osteoma, chondroma, hemangioma, etc

2. epithelial:
   i) papilloma, keratoma, trichoepithelioma, pilomatrixoma, etc:
      • non-glandular epithelial proliferations of stratified squamous or follicular epithelium.
   ii) adenoma: (aden, adenos = "gland")
     • the cells form recognizable glandular structures:
       (eg acini, tubules, ducts)
     • the cells are clearly derived from glandular tissue
       (but no tubular / acinar arrangements):

- polyp = hyperplastic growth or tumor (usually benign) protruding from a mucosal surface (eg intestine, bladder, nasal cavity).
b) Malignant

1. Mesenchymal sarcomas:
   - liposarcoma, fibrosarcoma, osteosarcoma, chondrosarcoma, etc.
   (sarkos = "flesh")

2. Epithelial carcinoma:
   - squamous cell carcinoma (ectoderm)
   - pituitary carcinoma (ectoderm)
   - hepatocellular carcinoma (endoderm)
   - adrenal cortical carcinoma (mesoderm)
   (karkinos = "crab")

   • adenocarcinomas:
     - bronchoalveolar adenocarcinoma (endoderm)
     - mammary adenocarcinoma (ectoderm)
     - intestinal adenocarcinoma (endoderm)
     (if glandular patterns)

- eg's of inconsistencies:
  - lymphoma (or lymphosarcoma) = a neoplastic disorder, usually malignant, of lymphoid tissue.
  - leukemia = malignant neoplasm of blood-forming tissues.
  - melanoma = a malignant tumor of melanocytes (sometimes designated “malignant melanoma”).
  - melanocytoma = a benign tumor of melanocytes.

3. Mixed Tumors
   - the majority of neoplasms are composed of cells representative of a single germ layer, and the neoplastic cells are of one type, which closely resemble each other.
   - mixed tumors contain more than one cell type, but these are all derived from one germ layer.

   • Mixed Mammary Tumor
     - one of the most common mammary neoplasms in dogs (benign forms more common than malignant).
     - this tumor contains both epithelial and mesenchymal elements; appears to originate from a pluripotential stem cell which replenishes the pool of both secretory epithelial cells and myoepithelial cells.
     - the proliferating myoepithelial cells produce a mesenchymal myxoid stroma (ie resembling mucus) that may contain cartilage and/or bone (apparent cartilagenous & osseous metaplasia).
     - in malignant mixed tumors, the epithelial and/or mesenchymal components show malignant change.

   • Embryonal Nephroma (= nephroblastoma = Wilm’s tumor of humans), benign or malignant variants:
     - the most common primary renal tumor in pigs and chickens, (also a common cancer in children <10 yrs).
     - also reported in dogs and cats, although rarely.
     - this tumor may contain multiple tissues (eg epithelium, fibrous tissue, smooth or striated muscle, cartilage, bone), but all these tissues are derived from the metanephric mesoderm;
     - the muscular, cartilaginous and osseous components are considered to represent metaplastic changes.
d) Teratomas
- are composed of a variety of neoplastic cell types representative of more than one germ layer, usually all three (ie ectoderm, mesoderm, endoderm).
- teratomas arise from totipotential embryonic cells (likely primordial germ cells) and so are primarily found in the gonads, but also rarely in ectopic primitive cell rests elsewhere.
- these totipotential germ cells can readily express various portions of their genome and therefore can differentiate into various types of tissues (eg skin, muscle, fat, gut, tooth structures, brain, eye, etc).
- testicular teratomas are the most frequently reported testicular tumors in horses, but rare in other species.
- ovarian teratomas are rare in domestic animals.
- teratomas are almost always benign in animals, in contrast to those in humans.

3) Benign vs Malignant Neoplasms

a) Benign Tumors
- most benign tumors are composed of well differentiated cells that closely resemble their normal counterparts, often including the same architectural arrangement; (occasional exceptions; eg canine cutaneous histiocytoma is anaplastic, but it is typically benign and spontaneously regresses).
- most benign tumors grow as cohesive expansile masses that remain localized to their site of origin.
- most benign tumors grow and expand slowly with compressive atrophy of adjacent parenchymal cells and many have a fibrous capsule formed from the surrounding compressed stroma.
- some benign tumors spontaneously regress (eg cutaneous papillomas, canine cutaneous histiocytoma).
- some benign tumors may slowly progress to malignancy (eg colonic adenomatous polyps in humans / dogs); while other types of tumors are malignant from the onset, eg most melanomas involving the oral mucosa in dogs.
- some tumors that are morphologically benign may have serious, if not fatal, consequences; eg's

① tumors of the brain (space-occupying lesions).

② some hormone-producing tumors, eg tumor of pancreatic islet cells $\rightarrow$ insulin $\rightarrow$ hypoglycemia.

③ in horses, pedunculated intraabdominal lipomas may become twisted around segments of intestines, thus causing intestinal obstruction or even infarction and subsequent death.

b) Malignant Tumors
- the designation of malignancy indicates an aggressive, life-threatening tumor.
- malignancy is characterized by: anaplasia, rapid rate of growth, local invasion of tissue and metastasis.

① Differentiation / Anaplasia
- differentiation refers to the extent to which neoplastic cells resemble comparable normal cells, both morphologically and functionally.
- loss of differentiation is called anaplasia (ana=backward + plasia=to form) and characterizes malignancy.
- it is now believed that most cancers don’t undergo reverse differentiation, but arise from transformed stem cells that show varying degrees of differentiation.
- morphologic features of anaplasia include:
i) **Pleomorphism**
- variation in size and shape of both the cells and their nuclei.
  [note: anisocytosis = variation in cell size and anisokaryosis = variation in nuclear size]

ii) **Abnormal nuclei**
- nuclei often large (high N/C ratio) with coarsely clumped, often marginalized chromatin.
- nucleoli often large and/or multiple.

iii) **Mitoses**
- increased numbers of mitotic figures; may reflect either a high mitotic rate (ie large proportion of cells undergoing mitosis) or an abnormally slow progression of individual cells through the mitotic cycle.
  - note: many hyperplastic or normal tissues (eg bone marrow) have high mitotic rate as well!
  - abnormal (eg tripolar) mitotic figures, when present, are a strong indicator of malignancy.

iv) **Loss of Polarity**
- normal epithelial cells have a polarized orientation, ie an apical-basal polarity with nuclei in the basal location; this polarity is lost in anaplastic cells (ie tumor cells grow in an anarchic, disorganized fashion).

v) **Other**
- tumor giant cells with single large nucleus or binucleated or multinucleated.
- rapidly growing tumors often “outgrow” their blood supply resulting in ischemic necrosis / hemorrhage.
- some malignant tumors (typically epithelial) stimulate the production of abundant collagenous stroma, called desmoplasia; eg some squamous cell or mammary carcinomas (“scirrhous carcinomas”).

- not all features of anaplasia are necessarily seen in a given malignant tumor, although the more of these features one sees, the more aggressive the tumor is likely to be.
- presence of anaplastic features usually calls for a poor or guarded prognosis; however, some well differentiated neoplasms may nonetheless be locally invasive or even have high metastatic tendencies.
- therefore, the whole clinical, gross & microscopic morphology and epidemiology of each tumor must be evaluated in order to provide an accurate prognosis.

θ **Rate of Growth (Tumor Cell Kinetics)**
- in general growth rates of neoplasms correlates with their level of differentiation, ie most malignant tumors grow more rapidly than benign ones; the rate of tumor growth is determined by:

i) **Doubling time of tumor cells**
- a transformed neoplastic cell (~10 um) needs to undergo at least 30 doublings to produce $10^6$ cells (~1 gm, smallest clinically detectable mass) and only a further 10 doublings to produce $10^{12}$ cells (~1 kg); ie by the time a solid tumor is detected it has already completed a major portion of its life cycle.
- doubling times following clinical detection are extremely variable; eg in humans, typical cancer doubling times after clinical detection are 2-3 months (but can range from 1 month to 12 months).

ii) **Fraction of tumor cells in the replicative pool** (“growth fraction”)
- in the early microscopic phase, most tumor cells are in the proliferative pool.
- at the clinically detectable phase, most cells are not in the replicative pool.
- since radiation & chemotherapy act on the cell cycle, tumors with low growth fraction (eg human breast or colon cancer) are more refractory to treatment than tumors with high growth fractions (eg some human or canine lymphomas).
iii) rate of cell loss from the tumor
   - for tumor growth, cell production must exceed cell loss (by apoptosis, cell shedding, lack of nutrients).
   - in some rapidly growing tumors cell proliferation > cell loss by up to 20%.
   - in slow growing tumors cell proliferation > cell loss by 10%.

Local Invasion of Tissue
- cancer growth is characterized by progressive infiltration (invasion) and destruction of surrounding tissues.
- next to metastasis, local invasion is the most reliable indicator of malignancy.
- local invasion can make complete surgical removal difficult (often necessitates taking wide margins).

Metastasis
- metastasis refers to tumor implants discontinuous with the primary tumor.
- unequivocally indicates malignancy, because benign neoplasms do not metastasize.
- metastasis is the ultimate hallmark of malignancy and is what kills the majority of human cancer patients.
- metastasis occurs by 3 main pathways:

i) Lymphatic spread
   - carcinomas tend to spread first via lymphatic vessels while sarcomas tend to spread mainly via blood vessels [can be misleading since the lymphatic and vascular systems have numerous connections].
   - the pattern of lymph node involvement usually reflects the natural lymphatic drainage routes.

ii) Hematogenous spread
   - hematogenous spread by veins is more common than by thicker walled arteries.
   - metastatic cells follow venous drainage, therefore the liver (portal blood) and lung (caval blood) are the most common sites of metastasis.

iii) Seeding of Body Cavities and Surfaces ("exfoliation and implantation")
   - transfer of neoplastic cells from one location to another within a body cavity, as organs rub against each other or via the small amount of fluid normally present within body cavities; also, via transplantation of neoplastic cells on surgical instruments, gloves, hypodermic needles, etc.

- in general, the more locally aggressive, the more rapidly growing, and the larger the primary neoplasm, the greater the likelihood that it will metastasize or has already done so; however some types of tumors will metastasize more easily than others with same degree of anaplasia of the cells.
  - pancreatic carcinomas in dogs tend to metastasize early, when the primary tumor is still quite small.
  - in dogs, at least 90% of the oral melanomas are malignant and metastasize rapidly and widely; while most melanocytic tumors of the skin (non-digital areas) are benign.
4) Molecular Basis of Cancer

- several fundamental principles are involved in carcinogenesis:

i) **Nonlethal genetic damage is central to neoplastic transformation**
   - genetic mutations may be inherited or acquired (eg radiation, chemical, virus, spontaneous mutation).
   - even in normal cells, DNA replication is not always a perfect process, and each time a cell undergoes DNA replication, there is a slight chance that a genomic mistake will be introduced and if it evades repair mechanisms it can subsequently become fixed as a mutation.

ii) **Tumors are formed from the clonal expansion of single, genetically damaged, precursor cell**
   - almost all tumors represent monoclonal expansion of a single transformed cell, however they generally become progressively more heterogeneous with respect to genotypic & phenotypic features.
   - ultimately, different clones of cells within a tumor may vary with respect to morphology, proliferative capacity, karyotype, metastatic capacity, expression of cell surface antigens / hormone receptors, immunogenicity, sensitivity to the defense mechanisms of the host, sensitivity to cytotoxic drugs, etc.

   “Cancer is a genetic disease, arising from an accumulation of mutations that promote clonal selection of cells with increasingly aggressive behavior.” (Science 1997; 278: 1043-5)

iii) **Normal regulatory genes are the principle targets of genetic damage**
   - genetic damage of the normal regulatory genes involves:
     - activation of the growth-promoting protooncogenes.
     - inactivation of the growth-inhibiting tumor suppressor genes.
     - alteration of the genes that regulate apoptosis.
     - alteration of the genes involved in DNA repair.

iv) **Dysfunction of DNA repair genes**
   - abnormalities in the genes involved in DNA repair can result in mutations in the normal regulatory genes.
   - cells with this tendency are said to be of the “mutator phenotype”.

v) **Neoplastic transformation is a multistep process**
   - carcinogenesis is a multistep process at both the genetic and phenotypic levels.
   - the various genomic transformations within neoplastic cells ultimately translate into the acquisition by these cells of several different properties, eg high mitotic index, ability to stimulate development of new blood supply (angiogenesis), ability to cleave various components of the extracellular matrix which thus facilitates local invasion, acquisition of metastatic properties, etc.

   “Cancer is hard to cure because it is not one disease but a hundred different diseases.” (Science 1982; 215: 275-7)
a) The Normal Cell Cycle

- The Cell-Cycle and Cellular Proliferation
  - cell division is achieved by a highly controlled sequence called the cell-cycle.
  - cell division is mostly stimulated by growth factors or ECM components (via integrins).
  - cell-cycle phases = G₀ (quiescent), G₁ (presynthetic), S (DNA synthesis), G₂ (premitotic) & M (mitotic)
    - each phase is dependant on proper activation and completion of the previous one.
    - the cell-cycle has multiple controls (both stimulatory and inhibitory) as well as checkpoint mechanisms.
  - to enter cycle, quiescent cells must go from G₀ to G₁, involving transcription of a large set of genes; ie proto-oncogenes (eg MYC, RAS) and genes for ribosomal synthesis and protein translation.
    - must then pass G₁/S restriction point, beyond which cells irreversible committed to DNA replication.
    - checkpoints are present to monitor for DNA damage, ie elimination by apoptosis if DNA damaged.
      (checkpoint defects allow replication of DNA breaks / chromosomal abnormalities → carcinogenesis)
    - progression through the cycle regulated by proteins called cyclins & cyclin-dependant kinases (CDK’s).
    - activity of cyclin-CDK complexes tightly regulated by inhibitors.
    - phosphorylation of RB by cyclin D-CDK4 complex is a molecular ON-OFF switch for the cell cycle.
    - growth factors can stimulate proliferation by upregulating cyclin expression or downregulating inhibitors.
Main Cell-Cycle Components and their Inhibitors
(Modified from Pathologic Basis of Disease, 7th ed)

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<thead>
<tr>
<th>Cell-Cycle Component</th>
<th>Main Function</th>
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<tr>
<td>• RB</td>
<td>RB (retinoblastoma susceptibility protein) is a component of the E2F/DP1/RB complex, which represses gene transcription through the recruitment of histone deacetylase, an enzyme that alters the conformation of chromatin, making it more compact. Phosphorylation of RB by cyclin D-CDK4 removes histone deacetylase from chromatin, allowing the activation of E2F transcriptional activity.</td>
</tr>
</tbody>
</table>

Cyclin-Dependant Kinases

| • CDK4 | Forms a complex with cyclin D. The complex phosphorylates RB, allowing the cell to progress through the G1 restriction point. (note opposing action of p53, p21 & p16INK4A at this point). |
| • CDK2 | Forms a complex with cyclin E in late G1, which is involved in the G1/S transition. Forms a complex with cyclin A at the S phase that facilitates the G2/M transition. |
| • CDK1 | Forms a complex with cyclin B, which acts on the G2/M transition. |

CDK Inhibitors

| • Cip/Kip family: p21, p27 | Block the cell cycle by binding to cyclin-CDK complexes. p21 is induced by the tumor suppressor p53. p27 responds to growth suppressors such as transforming growth factor-β. |
| • INK4/ARF family: p16INK4A, p14ARF | p16INK4a binds to cyclin D-CDK4 and promotes the inhibitory effects of RB. p14ARF increases p53 levels by inhibiting MDM2 activity. |

Checkpoint Components

| • p53 | Tumor suppressor altered in the majority of cancers; causes cell-cycle arrest and apoptosis. Acts mainly through p21 to cause cell-cycle arrest. Causes apoptosis by inducing the transcription of pro-apoptotic genes such as BAX. Levels of p53 are negatively regulated by MDM2 through a feedback loop. p53 is required for the G1/S checkpoint and is a main component of the G2/M checkpoint. |

Role of cyclins, CDK's and CDK inhibitors in regulating G1/S cell-cycle transition

Regulation of cell-cycle by RB - phosphorylation of RB by cyclin D-CDK4 removes histone deacetylase allowing E2F transcriptional activity.
Growth Factors / Cytokines

- **growth factors** are extracellular polypeptide signal molecules that stimulate a cell to grow or proliferate (cell biologists often restrict the term to the affect on cell mass only; however in common usage many of the stimulatory molecules - called growth factors - promote both increased cell size and proliferation).
- growth factors can also affect locomotion, contractility, differentiation and angiogenesis.
- **cytokines** are extracellular signal proteins or peptides that act as a local mediator in cell-cell communication (they usually regulate immunity, inflammation and hematopoiesis).

<table>
<thead>
<tr>
<th>Growth factor / Cytokine</th>
<th>Symbol</th>
<th>Source</th>
<th>Functions (not all functions listed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermal growth factor</td>
<td>EGF</td>
<td>Platelets, macrophages (MΦ’s), saliva, urine, milk, plasma, etc</td>
<td>Mitogenic for keratinocytes and fibroblasts; stimulates keratinocyte migration and granulation tissue formation.</td>
</tr>
<tr>
<td>Transforming growth factor alpha</td>
<td>TGF-α</td>
<td>MΦ’s, T cells, keratinocytes, others</td>
<td>Similar to EGF; stimulates replication of hepatocytes and certain epithelial cells.</td>
</tr>
<tr>
<td>Hepatocyte growth factor</td>
<td>HGF</td>
<td>Mesenchymal cells</td>
<td>Enhances proliferation of hepatocytes, epithelial &amp; endothelial cells.</td>
</tr>
<tr>
<td>Vascular endothelial cell growth factor</td>
<td>VEGF</td>
<td>Mesenchymal cells</td>
<td>Increases vascular permeability; mitogenic for endothelial cells.</td>
</tr>
<tr>
<td>Platelet-derived growth factor</td>
<td>PDGF</td>
<td>Platelets, macrophages, endothelial cells, keratinocytes, smooth muscle cells.</td>
<td>Chemotactic for and activation of PMN’s, MΦ’s, fibroblasts; mitogenic for fibroblasts, endothelial cells &amp; smooth muscle cells; stimulates angiogenesis &amp; wound contraction; etc.</td>
</tr>
<tr>
<td>Fibroblastic growth factor</td>
<td>FGF</td>
<td>MΦ’s, mast cells, T cells, endothelial cells, fibroblasts, others</td>
<td>Chemotactic for fibroblasts; mitogenic for fibroblasts/keratinocytes; angiogenesis; wound contraction, matrix deposition, etc.</td>
</tr>
<tr>
<td>Keratinocyte growth factor</td>
<td>KGF</td>
<td>Fibroblasts</td>
<td>Stimulates keratinocyte migration, proliferation and differentiation.</td>
</tr>
<tr>
<td>Insulin-like growth factor</td>
<td>IGF</td>
<td>MΦ’s, fibroblasts, etc</td>
<td>Stimulates fibroblast proliferation, keratinocyte migration, etc.</td>
</tr>
<tr>
<td>Tumor necrosis factor</td>
<td>TNF</td>
<td>MΦ’s, mast cells, T cells</td>
<td>Activates MΦ’s; regulates other cytokines, etc.</td>
</tr>
<tr>
<td>Interleukins</td>
<td>IL-1, etc</td>
<td>MΦ’s, mast cells, keratinocytes, e</td>
<td>Chemotactic for leukocytes &amp; fibroblasts; angiogenesis, etc, etc.</td>
</tr>
<tr>
<td>Interferons</td>
<td>IFN-α, etc</td>
<td>Lymphocytes and fibroblasts</td>
<td>Activates MΦ’s; regulates other cytokines, etc.</td>
</tr>
</tbody>
</table>

**Signaling Mechanisms in Cell Proliferation**

- growth factors bind to receptors (on surface or in cell) which then stimulate transcription of genes (via transcription factors), many of which are involved in regulating progression through the cell cycle.
- the general mechanism of modulating gene expression following ligand-receptor binding is called “receptor-initiated signal transduction”.
- important signal transduction mechanisms involved in normal and abnormal cell growth include:
  1. **Receptors with intrinsic tyrosine kinase activity** → for EGF, TGF-α, HGF, PDGF, VEGF, insulin, etc.
  2. **Seven transmembrane G-protein-coupled receptors** → for histamine, epinephrin, ACTH, glucagon, etc
  3. **Receptors lacking intrinsic tyrosine kinase activity** that recruit kinases → for interleukins, interferons, etc.
  4. **Steroid hormone receptors** → steroid hormones, thyroid hormone, vitamin D, etc.
- the end product of many of these pathways are transcription factors; which can activate or inhibit gene expression (including growth promoter or growth inhibitor genes).
b) Essential Alterations for Malignant Transformation
- it’s believed that the efficacy of the DNA repair mechanisms is central to the development of mutations in cancer causing genes, i.e. when genes that normally sense & repair DNA damage are altered the resultant genomic instability favors mutations in other genes that regulate the other attributes of cancer cells.
- mutations in genes that regulate the following 7 cellular traits are seen in every neoplasm; however the genetic pathways that give rise to these attributes differs among cancers.

### Self-sufficiency in Growth Signals (Activation of Oncogenes)
- tumor cells can proliferate without external stimuli; this is usually due to oncogene activation.
- **oncogenes** are altered versions of normal genes (protooncogenes) that regulate normal cell growth and proliferation; ie protooncogenes may be converted into cellular oncogenes (c-oncs) that are involved in tumor development.
- a main component of neoplastic transformation is the mutation or increased production (via overexpression, amplification or translocation) of the protein products (“oncoproteins”) of these oncogenes.
- more than 100 cellular protooncogenes have been identified.
- oncogenes generally serve similar functions as their normal counterparts, however because they are mutated or constitutively expressed, oncogenes enable the tumor cell to be self-sufficient in growth.
- oncogenes may function as growth factors, growth factor receptors, signal transducers, transcription factors (nuclear regulatory proteins), and cell cycle regulators.

### Selected Oncogenes, Mode of Activation & Associated Human Tumors

<table>
<thead>
<tr>
<th>Category</th>
<th>Protooncogene</th>
<th>Mode of Activation</th>
<th>Associated Human Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth Factors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDGF-β chain</td>
<td>SIS</td>
<td>Overexpression</td>
<td>Astrocytoma, Osteosarcoma</td>
</tr>
<tr>
<td>TGFα</td>
<td>TGFα</td>
<td>Overexpression</td>
<td>Astrocytomas, Hepatocellular carcinomas</td>
</tr>
<tr>
<td><strong>Growth Factor Receptors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGF-receptor family</td>
<td>ERB-B1 (EGFR)</td>
<td>Overexpression</td>
<td>Squamous cell carcinomas of lung, gliomas</td>
</tr>
<tr>
<td></td>
<td>ERB-B2</td>
<td>Amplification</td>
<td>Breast and ovarian cancers</td>
</tr>
<tr>
<td>Receptor for neurotrophic factors</td>
<td>RET</td>
<td>Point mutation</td>
<td>Multiple endocrine neoplasia 2A and 2B, familial medullary thyroid carcinomas</td>
</tr>
<tr>
<td>Receptor for stem cell factor</td>
<td>KIT</td>
<td>Point mutation</td>
<td>Gastrointestinal stromal tumors and other soft tissue tumors</td>
</tr>
<tr>
<td><strong>Proteins Involved in Signal Transduction</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GTP-binding</td>
<td>K-RAS</td>
<td>Point mutation</td>
<td>Colon, lung, and pancreatic tumors</td>
</tr>
<tr>
<td></td>
<td>H-RAS</td>
<td>Point mutation</td>
<td>Bladder and kidney tumors</td>
</tr>
<tr>
<td></td>
<td>N-RAS</td>
<td>Point mutation</td>
<td>Melanomas, hematologic malignancies</td>
</tr>
<tr>
<td>WNT signal transduction</td>
<td>β-catenin</td>
<td>Point mutation</td>
<td>Hepatoblastomas, hepatocellular carcinoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Overexpression</td>
<td></td>
</tr>
<tr>
<td><strong>Nuclear Regulatory Proteins (Transcriptional Factors)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transcriptional activators</td>
<td>C-MYC</td>
<td>Translocation</td>
<td>Burkitt lymphoma</td>
</tr>
<tr>
<td></td>
<td>N-MYC</td>
<td>Amplification</td>
<td>Neuroblastoma, small cell carcinoma of lung</td>
</tr>
<tr>
<td></td>
<td>L-MYC</td>
<td>Amplification</td>
<td>Small cell carcinoma of lung</td>
</tr>
<tr>
<td><strong>Cell-Cycle Regulators</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclins</td>
<td>CYCLIN D</td>
<td>Translocation</td>
<td>Mantle cell lymphoma, Breast and esophageal cancers</td>
</tr>
<tr>
<td></td>
<td>CYCLIN E</td>
<td>Amplification</td>
<td>Breast cancer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Overexpression</td>
<td></td>
</tr>
<tr>
<td>Cyclin-dependent kinase</td>
<td>CDK4</td>
<td>Amplification or</td>
<td>Glioblastoma, melanoma, sarcoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>point mutation</td>
<td></td>
</tr>
</tbody>
</table>

(modified from Pathologic Basis of Disease, 7th ed)
Gen Path I (VPM 152) Neoplasia

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**i) Growth Factors**

- many cancer cells become self-sufficient in growth by acquiring the ability to synthesize the same growth factors (eg PDGF & TGF) to which they are responsive.
- in most cases the growth factor gene is not altered or mutated, instead other oncogenes (eg RAS) cause overexpression of growth factor genes.

**ii) Growth Factor Receptors**

- growth factor receptor activation can be by mutation or overexpression.
- mutant oncogenic versions of these receptors (eg RET) can cause constitutive activation without binding to the growth factor, thus delivering continuous mitogenic signals to the cell.
- overexpression of normal growth factor receptor genes (eg ERB/EGFR) are common in many cancers.

**iii) Signal-Transducing Proteins**

- these oncoproteins often mimic the function of normal cytoplasmic signal-transducing proteins.
- mutations of RAS family genes (GTP-binding proteins) are common in many types of cancer.
- mutant RAS remains in the active state and thus stimulates cell proliferation (via MAP kinase pathway) by transducing growth factor production.

**iv) Transcriptional Factors (nuclear regulatory proteins)**

- transcriptional factors act on specific DNA sites to induce specific gene transcription, eg genes that control the entry & progression through the cell cycle.
- overexpression of MYC proteins (eg by activated transduction pathways) is the most common example in human tumors.

**v) Cell Cycle Regulators (Cyclins and Cyclin-Dependant Kinases)**

- cyclins and CDK’s are important proteins for progression through the normal cell cycle.
- overexpression of cyclins and CDK’s (esp cyclin D & CDK4) are common in neoplastic transformation.

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**Ø Insensitivity to Growth Inhibitory Signals (Inactivation of Tumor Suppressor Genes)**

- proteins that inhibit cell growth are the products of genes called “tumor suppressor genes”.
- the loss of expression and/or function of these tumor suppressor genes is present in most human tumors.

**i) RB Gene**

- RB (retinoblastoma susceptibility) protein is key in regulating cell proliferation at the G1/S transition.
- recall phosphorylation of RB by cyclin D-CDK4 removes histone deacetylase from chromatin which activates E2F transcriptional activity (cyclins E, A, CDK1, DNA polymerase, etc) allowing progression through the cell cycle independent of growth factors.
- if RB is absent (eg gene deletion) and/or mutated, molecular “brakes” are released and cell-cycle progression occurs.
- RB abnormalities are seen most frequently in retinoblastoma of children, but also seen in other tumors.

**ii) Other Genes Which Affect G1/S Cell-Cycle Transition**

- dysregulation of many other genes which control G1/S cell-cycle transition (mimics RB dysfunction).
- the vast majority of human cancers have some dysregulation at the G1/S cell-cycle transition point.
  - mutations of p16INK4a results in inability to block cyclin D/CDK4 activation.
  - several DNA viral oncogenic proteins neutralize the growth inhibitory effects of RB.
  - part of p53 activity is by upregulating the CDK inhibitor p21.
iii) **p53 Tumor Suppressor Gene**
- p53 protein acts as a critical gatekeeper against neoplastic transformation (“guardian of the genome”).
- p53 protein is activated by genes that sense DNA damage → assists DNA repair by inducing genes for G1 arrest (p21) and DNA repair (GADD45) → if DNA repair is successful p53 allows cell cycle to proceed (via MDM2) and if not successful it then induces apoptosis (via BAX).
- ~½ of human tumors have inactivating mutations of the p53 gene (most common mutation in cancer).
- most mutations (somatic or inherited) of the p53 gene affect the DNA-binding activity of p53 protein.
- p53 also inactivated by upregulation of MDM2 protein or by being bound by viral oncogenic proteins.
- other members of the p53 family with similar activity have recently been identified (eg p63, p73).
- it has been shown that many cancers that are resistant to therapies that induce DNA damage / apoptosis (eg radiation or chemotherapy) have altered p53 function (the converse is also true).

iv) **Other Genes That Function as Tumor Suppressors**
- other genes acting as tumor suppressors include: APC/β-catenin pathway, NF-1 gene, TGF-β, etc.
- fewer tumor suppressor genes have been identified than oncogenes, however “it is likely that tumor-suppressor genes play a role at least equal in importance to that of oncogenes”. (Slauson & Cooper, 2002)
Evasion of Apoptosis
- in addition to growth-promoting and growth-inhibiting genes, cell survival is affected by genes that promote or inhibit apoptosis.
- the best example of reduced apoptosis is the overexpression (due to translocation) of BCL-2 protein in most B-cell lymphomas.
  - BCL-2 protein protects against apoptosis (via the mitochondrial pathway)
  - since growth factors are not involved, this type of lymphoma is slow growing compared to other types.

Defects in DNA Repair
- cells are frequently exposed to DNA-damaging agents (eg UV radiation, chemical carcinogens, free radicals, etc) and yet because normal cells have the ability to repair DNA or eliminate cells when DNA repair fails cancer is a relatively rare event.
- inherited disorders of defective DNA repair genes (sometimes called cancer susceptibility genes) have been identified in individuals predisposed to cancer development.
- it has been proposed that defects in DNA repair genes are the main initiating events in oncogenesis, leading to widespread mutagenesis and genetic instability; ie the “mutator phenotype”.
- defects in 3 types of DNA repair systems have been identified in human cancers:
  
i) Mismatch Repair Genes
  - several genes have been identified which correct mistakes of nucleotide pairing during DNA replication, (esp G-T or A-C mismatching); eg human familial colon cancer.
  
ii) Nucleotide Excision Repair Genes
  - UV light causes cross-linking of adjacent pyrimidine nucleotides (dimer formation); results in misreading during replication, unless repaired by nucleotide excision repair (NER) genes; eg skin cancers.
  
iii) Recombination Repair Genes
  - double-strand DNA breaks (esp by radiation or oxidation) can be fixed by homologous recombination repair; the joining of the free ends is mediated by a DNA-protein kinase; eg human breast cancer.

Limitless replicative potential
- recall that in normal cells telomere shortening is an important component of replicative senescence; ie short telomeres activate cell-cycle checkpoints that result in apoptosis or cell cycle arrest.
- thus telomerase activity and maintenance of telomere length appear to be essential for the unlimited proliferation of cancer cells (perhaps this suggests that most cancer cells are derived from stem cells!).
- telomerase activity has been detected in 90% of human tumors (and also many animal tumors).
Sustained Angiogenesis
- tumors cannot grow larger than 1-2 mm without vascularization to supply O₂ and nutrients.
- tumors stimulate the growth of host vessels by a process called angiogenesis.
- angiogenic factors may be produced by tumor cells, supporting stromal cells or from inflammatory cells
  infiltrating the tumor (note - angiogenesis can stimulate tumor growth, via growth factors, eg PDGF & IGF’s).
- of the many recognized tumor-associated angiogenic factors, VEGF (vascular endothelial growth factor) and
  bFGF (basic fibroblastic growth factor) are the most common.
- in contrast to normal vessels, tumor vessels are disorganized (tortuous and irregularly shaped), unstable
  (may grow continuously) and leaky.
- new evidence suggest that many tumors can exist in situ for months to years without developing a blood
  supply and then enlarge when angiogenic phenotypes emerge (“angiogenic switch”).
- because of this central role in tumor growth, many clinical trials are being done on angiogenesis inhibitors.

Ability to Invade and Metastasize
- in a given tumor, the neoplastic cells differ widely in their ability to metastasize.
  - some malignant tumors release large numbers (eg 10⁶) of neoplastic cells in the bloodstream daily.
  - even in highly metastatic tumors, only a very small proportion (~1 in 10⁴) of the malignant cells, that are
    able to enter the bloodstream and circulate in it, survive to form distant metastases; the others die very
    quickly (within 24 hours).
- several steps are involved in the metastatic process and each of these steps is influenced by molecular
  factors (failure in any one of these factors will render a tumor incapable of metastasis).
  - metastatic properties are often acquired only late in the course of tumor progression.
  - neoplastic transformation and progression from a nonmetastatic to a metastatic tumor type are distinct
    processes that do not depend on the same oncogenes or tumor suppressor genes.
  - many of the properties that determine the metastatic potential of a tumor cell manifest themselves at the
    surface of the cell membrane and involve either increases or decreases in the ability of the cell to adhere
    to adjacent cells or to surrounding extracellular matrix.
- there are two main phases in the metastatic process:
  i) Invasion of Extracellular Matrix
    - an epithelial tumor cell must breach the subjacent BM, move through the interstitial connective tissue,
      penetrate the vascular BM and then reverse this process when tumor cells extravasate at a distant site.
    - invasion of the extracellular matrix (ECM) is an active process that has several steps:
      ① detachment of the tumor cells from each other
        - in carcinomas detachment is often due to down-regulation or mutations of cadherins / catenins.
      ② attachment to matrix components
        - many tumor cells up-regulate receptors (esp integrins) for laminin and fibronectin in BM / ECM.
      ③ degradation of the ECM (esp collagen and proteoglycans)
        - many tumor cells up-regulate MMP’s (matrix metalloproteinases) to digest a pathway in the ECM.
      ④ migration of tumor cells
        - many of the cleavage products from ECM degradation promote angiogenesis, tumor growth and
          tumor cell motility.
ii) **Vascular Dissemination and Homing of Tumor Cells**

- circulating tumor cells tend to clump with themselves and/or blood cells (esp platelets); clumping increases the survival of neoplastic cells in the bloodstream by protecting them from mechanical turbulence and from attack by the immune system (innate & adaptive).
- tumor aggregates must then arrest & adhere to vessel wall and extravasate through the BM (as above).
- the sites of metastasis are related to:

  1. the hemodynamic form of distribution stresses the correlation between the primary tumor and the site(s) of metastasis with regard to the lymph and blood flow in the target organ(s).
   - this form seems to be a satisfactory explanation for many cancers, with the regional lymph nodes and lungs (first filters) being often the first sites of metastases.
   - the liver (which drains blood from the gastrointestinal tract and spleen) may also be the first site of metastases of tumors originating in these organs.

  2. the organ tropism form ("favorable vs unfavorable soil") of distribution emphasizes some affinity between the neoplastic cells and specific organs; likely via adhesion molecules and/or chemokines.
   - in humans, renal and breast carcinomas tend to metastasize preferentially to the lungs, bones and brain; bronchogenic carcinomas tend to metastasize to the adrenals; and prostatic carcinomas tend to metastasize to the vertebral column.
   - most carcinomas in the digits of cats represent metastases of a primary pulmonary adenocarcinoma; these usually involve multiple digits of different legs, and the presenting clinical sign is typically painful digits, rather than respiratory problems.
   - in the experimentally derived B16 melanoma cell line in mice, the clonally derived cell line referred to as B16-10 metastasizes primarily to the lungs, the B16-O10 metastasizes primarily to the ovaries, and the B16-B10n metastasizes preferentially to the rhinal fissure of the brain.
   - metastases to skeletal muscles are rare.
   - brain tumors rarely metastasize outside of the nervous system.

- experimentally, damage to endothelial cells of a particular organ increases the number of metastases to that organ, possibly via formation of platelet thrombi (with tumor cells) &/or exposure of the underlying BM.
  - also sites of acute inflammation tend to favor localization and subsequent growth of tumor cells, possibly by increasing the permeability of the endothelium and/or releasing chemotactic and growth factors.
  - additionally, metastases may occur preferentially at sites of bone fracture.

- tumor metastasis must be differentiated from a true "multicentric" tumor; ie in some tumors, neoplastic transformation of cells may occur more or less simultaneously at several different sites in the body.
  - feline sarcoma virus typically causes multicentric subcutaneous fibrosarcomas in young cats.
  - avian leukosis virus most commonly causes lymphosarcoma (B-lymphocytes) in chickens, which typically starts in one location and subsequently metastasizes to other tissues; however, it can occasionally cause benign and malignant neoplasms in various types of soft tissues (eg fibrous, endothelial) that typically have a multicentric origin.
  - the multiple endocrine neoplasia syndrome, described in humans, dogs, cattle and horses, is characterized by multiple hyperplastic and/or neoplastic lesions in various endocrine organs (eg pituitary, thyroid C cells, adrenal medulla, endocrine pancreas); this syndrome may represent a simultaneous neoplastic transformation of multiple endocrine cell populations of neural crest origin.
  - neurofibromatosis type 1 is a human disease (hereditary in ~50% of the patients; inherited mutant NF-1) with development of multiple tumors of peripheral nerves dispersed anywhere in the body; it can start at an early age and malignant transformation (neurofibrosarcoma) occurs in 10 -15% of cases.
c) Dysregulation of Cancer-Associated Genes
- genetic damage that activates oncogenes or inactivates tumor suppressor genes can be due to mutations, chromosomal abnormalities (eg amplifications, translocations) or epigenetic changes.

Ø Point Mutations

i) Mutation resulting in conversion of a protooncogene into an oncogene.
Activation of the neoplastic transforming potential of the RAS gene is brought about by a simple structural change that causes one amino acid to replace another in either of two critical locations in the ras protein (ie a point mutation). The mutated ras protein has a greatly reduced ability to switch from an active GTP-bound state to an inactive GDP-bound state, and this results in continuous activation of its target proteins. The RAS oncogene is the one most commonly identified in human cancer cells, contributing to the development of ~30% of human cancers.

ii) Mutation resulting in inactivation of a tumor suppressor gene.
Retinoblastoma is a rare but highly malignant tumor of the retina of young children. In the heritable form of the tumor, which accounts for about 40% of the cases, one of the two alleles of the retinoblastoma suppressor gene (known as Rb-1) in the somatic genome is already inactivated in the fetus because of a mutation present in the germline. The remaining intact retinoblastoma gene represses neoplastic transformation until it is inactivated by a spontaneous mutation. Individuals with the heritable form of retinoblastoma also have a 200-fold elevated risk of developing mesenchymal tumors (osteosarcoma, fibrosarcoma, melanoma) in early adult life, indicating that Rb-1 is normally functional in other tissues besides the retina.

The p53 tumor suppressor gene is the most commonly mutated gene yet identified in humans cancers. Mutations of this gene have been identified in up to 50% of all human cancers. As with oncogene amplification (see below), mutation of p53 tumor suppressor gene generally occurs late in tumor progression.

Ø Translocations
- various forms of chromosomal translocations occur in up to 65% of the acute leukemias in human patients.
- translocations can activate protooncogenes in two ways:

i) Translocations resulting in overexpression of protooncogenes by removing regulatory elements
Burkitt’s lymphoma is a rapidly growing extranodal lymphosarcoma that commonly afflicts children and usually involves the jaw. It is endemic in some parts of Africa, but it also occurs sporadically in nonendemic areas, including North America. In this tumor, the most common translocation of gene sequences is between chromosomes 8 and 14 in lymphocytes. In the process, a normal cellular protooncogene (MYC, on chromosome 8) moves into a position (on chromosome 14) near the normal immunoglobulin heavy-chain gene (ie a transcriptionally active region). With the regulatory sequences left behind there is overexpression of the MYC gene, particularly with increased antibody production (especially chronic viral infection with Epstein-Barr virus).

ii) Translocations which form hybrid genes that encode growth-promoting chimeric proteins.
In chronic myelogenous leukemia in humans, there is translocation of a truncated portion of chromosome 9 (containing the protooncogene c-ABL) to a region of chromosome 22 (BCR), resulting in the hybrid fusion gene BCR-ABL which encodes a chimeric protein that has constitutive tyrosine kinase activity (which is sufficient to cause the disease). The shortened chromosome 22, referred to as the Philadelphia chromosome, can be used as a cytogenetic marker for this form of leukemia. A large proportion of patients treated orally with a synthetic inhibitor of the tyrosine kinase pathway have shown a complete hematologic response.
**Gene Amplification**
- errors in the normal DNA replication process can lead to redundant replication (gene amplification) of a DNA segment; often giving rising to karyotypic abnormalities.
- if the amplified region includes a protooncogene, there will be overexpression of the encoded protein.
- gene amplification may not be one of the initial steps by which cells become cancerous, but it may at least contribute to the progression of cancers to a more malignant form.

In neuroblastoma and in some forms of breast and lung cancers in humans, amplification of specific protooncogenes or their oncogenic equivalents (such as myc in lung cancer) carries a poor prognosis and is of greater prognostic value than the clinical stage of the disease. This finding established the first link between basic research in protooncogenes and an important clinical index (ie the biologic behavior of the tumor in vivo).

**Epigenetic Changes**
- cell regulatory systems that are not directly attributable to the DNA protein coding sequence are described as epigenetic (may affect expression of a gene or the properties of its product).
  - DNA methylation is an epigenetic mechanism which plays an important part in mammalian gene control, acting as a general method of maintaining repression of transcription (eg inactivating one allele); increased methylation of various tumor suppressor genes and decreased methylation of protooncogenes has been identified in human cancers.
  - the non-protein coding DNA regions (intrinsic or junk DNA) encode RNA’s that perform a variety of regulatory functions, eg cell growth and damage repair; recent evidence suggests these noncoding RNA’s play a role in many cancers (see “The hidden genetic program of complex organisms”, Sci Am Oct 2004)

Chronic exposure to arsenic causes cancer in humans, but not in laboratory rodents. In humans, inorganic arsenic compounds are converted into less toxic substances through addition of methyl groups. This methylation process is also one of the mechanisms (epigenetic) involved in inhibition of genomic transcription. Cells continuously adding methyl groups to arsenic because of chronic exposure, may end up depleting an enzyme used to methylate DNA and thus may be unable to use this mechanism to inhibit expression of other genes (eg protooncogenes).
d) Molecular Basis of Multistep Carcinogenesis

- carcinogenesis is a multistep process at both the phenotypic and genetic levels.

- most cancers develop late in life because several cellular changes, based primarily on genomic alterations, are required for malignant transformation.

- this need for more than one level of control to be disrupted reflects the redundancy of growth control mechanisms normally present in a cell.

- even before the discoveries of the molecular basis of cancer, the age-associated increase in cancer and the experimental models of chemical carcinogenesis suggested that several sequential or concurrent alterations were required for tumorigenesis (ie initiation and promotion).

- increased understanding of the molecular biology also supports the concept of multistep carcinogenesis.

i) DNA transfection experiments show that no single oncogene can cause neoplastic transformation, but combinations of oncogenes can do so.
   - transfection of either RAS and MYC alone cannot cause neoplastic transformation, but together they can cause neoplastic transformation of mouse fibroblasts in culture.

ii) Most human cancers show activation of several oncogenes & loss of 2 or more tumor suppressor genes.
   - incrementally acquisition of the malignant phenotype is seen in transformation of colonic carcinoma.
   - morphologically see orderly progression of epithelial hyperplasia → dysplasia → adenoma → carcinoma
   - molecularly; 1st see inactivation of APC tumor suppressor gene, followed by activation of an oncogene (eg RAS), then loss of additional tumor suppressor genes (eg p53, SMAD’s, TGF-β receptor).

- this stepwise evolution, referred to as tumor progression, continues throughout the natural history of the neoplasm and results in progressively worse malignant properties through further genomic alterations.

- even though most cancers are monoclonal in origin, by the time they are clinically evident, they consist of a heterogenous population of cells; ie the selective pressure of the microenvironment allows specific subclones to survive, grow, invade, resist chemotherapy and metastasize.
5) Carcinogenic Agents
- many agents can cause genetic damage and induce neoplastic transformation, eg oncogenic viruses, chemical carcinogens, chronic inflammation and radiation.

a) Oncogenic Viruses
- human cancers associated with papillomavirus, hepatitis B virus, Epstein-Barr (herpes) virus and human T cell leukemia-lymphoma virus infections are responsible for ~15% of the worldwide cancer incidence.
  - cancer of the cervix and hepatocellular carcinoma account for about 80% of virus-linked cancers.
  - on a worldwide basis, specific viruses are the second most important risk factor for cancer development in humans, exceeded only by tobacco consumption.
  - infections with any of the human oncogenic viruses does not lead directly to cancer development, ie additional modifications of host cell DNA result in the stepwise progression to a malignant phenotype of the infected cell. (Science 1991;254:1167-73)
- so far, the existence of oncogenic viruses has been documented much more extensively in veterinary medicine than in human medicine.

i) Oncogenic RNA viruses
- retroviruses are the only oncogenic viruses with an RNA genome.
  (with the exception of hepatitis C virus in humans, which is linked to hepatocellular carcinoma).

  i) Slowly transforming retroviruses:
  - lack a viral oncogene, do not directly transform cells in culture and typically cause cancer in persistently infected animals only after a long latent period (months to years).
  - cause cancer by inserting themselves into cellular DNA & inducing expression of protooncogenes
  - are replication-competent, ie they possess all the genes necessary for viral replication.
  - eg's of slowly transforming retroviruses:  Feline & avian leukemia viruses ("chronic leukemia viruses")
    Bovine leukemia virus and Human T-cell leukemia virus.

  ii) Rapidly transforming retroviruses:
  - carry their own oncogene, transform cells in culture and cause cancer after a short latent period.
  - retroviral oncogenes do not appear to be indigenous components of the viral genome.
    - instead, they are acquired from the genome of the vertebrate host cells in which the virus replicates.
    - occur through recombination between portions of the genome of a replication-competent retrovirus and host cell genetic sequences; all oncogenes identified in rapidly transforming retroviruses have their counterparts in protooncogenes of normal vertebrate cells.
  - if retroviral oncogenes are merely copies of genes found in normal cells, how can they transform cells?
    - mutation hypothesis: mutations were introduced into these oncogenes when the cellular genes were copied into the retroviral genome.
    - dosage hypothesis: retroviral oncogenes overburden infected cells with too much of what are essentially normal proteins carrying out normal functions (ie amplification of the protooncogene).
  - most of these retroviruses are replication-defective, having lost some of the genes required for replication at the time of recombination.
    - in order to replicate, they need the presence of the parent replication-competent retrovirus in a persistently infected animal.
    - eg
      Feline sarcoma virus (FeSV) is formed by feline leukemia virus (FeLV) genetic material combining with a specific section of the cat's genome. FeSV is always found in the presence of excess "helper" FeLV. FeSV typically causes multicentric fibrosarcomas in young cats, although this is a rare manifestation of infection by FeLV.
Oncogenic DNA viruses
- in contrast to cells transformed by RNA tumor viruses (which can produce and release viral particles), infection of a cell by a DNA tumor virus has only one of two consequences:
  - the cell is transformed, in which case the infection is non-productive and the cell is not killed (ie no new viral particles produced, although multiple copies of the viral DNA may be present in the infected cell).
  - the infection is productive, ie infected cell produces viral particles but is lysed and dies in the process.
- transforming infection and productive infection can occur in different tissue compartments in the same animal (helps to explain the epidemiology of the diseases caused by some of these viruses).
- other factors, besides these DNA tumor viruses alone, are needed for malignant transformation; eg environmental carcinogens, diet, heredity, other infections, immunodeficiency, etc.
- DNA tumor viruses produce proteins that form complexes with, and inactivate, the protein products of some of the host cell's tumor suppressor genes (eg RB, p53).
  - these viral proteins are essentially to neutralize signals in the host cell that may interfere with replication of the viral genome, eg p53 protein is normally synthesized in response to damage to the cell's DNA and disturbance of its nucleotide pools, ie just what foreign viral DNA would do when invading a new cell.
  - other viral proteins may be homologs of or enhance the activity of growth factors or the protein products of cellular protooncogenes.
- these viruses may also act as mitogens of the target cells.
  - there is often a concurrent immunodeficiency, in which the virus is kept in check but not eliminated and thus can cause gradual proliferation of cells.
  - this continuous low-grade proliferation of cells offers a good environment for specific cytogenetic rearrangements to occur, ultimately resulting in activation of oncogenes or inactivation of tumor suppressor genes in the target cells.
  - clones of genetically altered cells emerge, with a selective growth advantage over other infected cells.

i) Papilloma viruses
- a large number of host specific papilloma viruses produce papillomas (warts) in a wide variety of vertebrates; some of which may progress to carcinomas.
- approximately 90% of anogenital tract cancers and 50% of oral cavity cancers are closely linked to mucosotropic human papillomaviruses.
  - Human PV-16 or -18 → cervical carcinoma.
- papillomavirus infections are common in veterinary medicine and are mostly self-limited infections; some atypical eg's:
  - Bovine PV + bracken fern → alimentary and urinary tract carcinomas.
  - Bovine PV → cause fibropapillomas in horses (equine sarcoïds) and cats (feline sarcoïds).

ii) Herpes viruses
- Marek's disease virus + genetically susceptible strains of chickens → T-cell lymphosarcoma.
  - productive infection in this disease occurs in feather follicles, and viral particles are subsequently shed in dandruff (lesions at these sites are inflammatory rather than proliferative).
- Herpes saimiri (natural host: squirrel monkey, an Old World species) + owl monkey or marmoset (New World species which are partially immunoincompetent to this particular virus) → lymphosarcoma
- Epstein-Barr virus + immunodeficiency (eg caused by malaria) → Burkitt's lymphoma.

iii) Hepadnavirus group
- Hepatitis B virus (human, woodchuck) persistent infection → hepatocellular carcinoma.
  - accounting for an estimated million human deaths worldwide annually, hepatitis B virus is one of the most important carcinogens, second only to tobacco.
b) Chemical Carcinogens
- occupational factors / environmental pollution likely account for ≤4% of cancers in USA (Science 1992;255: 904).
- experimental models of chemical carcinogenesis demonstrate the phenomenon of initiation & promotion.
  - initiation results from exposure of cells to a carcinogen (initiator) which causes somatic mutations in DNA; it is rapid, irreversible, has “memory” and is not alone sufficient for tumor formation
  - promotors are not carcinogenic themselves, but can induce tumors in initiated cells; they don’t affect DNA directly, there affect is by cell proliferation (ie contributes to development of further mutations).
- natural or synthetic mutagenic chemicals that initiate carcinogenesis are either:
  - direct acting carcinogens do not require chemical transformation for carcinogenicity.
  - indirect-acting carcinogens (procarcinogens) require metabolic activation (esp. by P450 enzymes) to become ultimate carcinogens.

**Major Chemical Carcinogens**

<table>
<thead>
<tr>
<th><strong>Direct-acting Carcinogens</strong></th>
<th><strong>Indirect-acting Carcinogens</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkylating agents</td>
<td>Polycyclic &amp; Heterocyclic Aromatic Hydrocarbons</td>
</tr>
<tr>
<td>some anticancer drugs (eg cyclophosphamide, β-propiolactone, dimethyl sulfate, etc)</td>
<td>many produced in the combustion of tobacco and also in broiled / smoked meats, eg benzo(a)pyrene, benz(a)anthracene</td>
</tr>
<tr>
<td>Acylating agents</td>
<td>Aromatic Amines, Amides, Azo Dyes</td>
</tr>
<tr>
<td>1-acetyl-imidazole, dimethylcarbamyl chloride</td>
<td>aniline dye and rubber industries (eg β-naphthylamine), food dyes (eg dimethylaminoazobenzene)</td>
</tr>
<tr>
<td>Natural Plant and Microbial Products</td>
<td>Natural Plant and Microbial Products</td>
</tr>
<tr>
<td>Aflatoxin B., Griseofulvin, Cycasin, Safrole, Betel nuts</td>
<td></td>
</tr>
<tr>
<td>Others (often occupational exposures)</td>
<td></td>
</tr>
<tr>
<td>chromium, nickel, arsenic, asbestos, vinyl chloride, polychlorinated biphenyls (PCB’s), etc</td>
<td></td>
</tr>
</tbody>
</table>

- most direct-acting and ultimate carcinogens act by covalent bonding (ie adduct formation) to electron-rich cell molecules (esp. DNA, RNA and proteins) resulting in molecular damage.
- outcome of this cell damage can be: ① complete repair; ② cell death; ③ non-lethal DNA mutation (initiation).
- most chemically induced cancers are believed to induce mutations in oncogenes, tumor suppressor genes, apoptosis regulatory genes or DNA repair genes (both RAS & p53 have been shown to be specific targets).
- for initiation to occur the damaged DNA must be replicated, ie altered cells must undergo at least one cycle of proliferation for the mutation to become permanent; this can be done by the chemical carcinogen itself (ie the regeneration stimulus in response to adjacent cells killed by the carcinogen) or by concurrent exposure to promoting agents that cause proliferation, eg other chemicals / drugs, viruses, dietary factors, hormones.
- after initiation, continued exposure to promoting agents is often required for tumor formation; ie promoters induce proliferation and clonal expansion of initiated (mutated) cells which leads to further mutations eventually resulting in malignant transformation.
- note, individual risk of neoplasia can vary with age, sex, nutritional status and genetics; both within and among species there are differences in the metabolism of a carcinogen, different DNA repair capacities and variable genomic stability.
  In humans polymorphisms of the P450 genes are recognized as a cause of individual susceptibility to certain cancers, eg smokers with a certain genotype of the CYP1A1 gene (a P450 gene), which metabolizes benzopyrene, have a sevenfold higher risk of developing lung cancer than smokers without this permissive genotype.
- rodent cells repair DNA damage far less efficiently than do human cells.
  - “A core concept of modern theories of aging is that longer-lived animals have evolved more efficient DNA maintenance and repair capacities in order to fend off the damage that accompanies the passage of time.” (Science 2001;291:839-40)
  - thus, rodents in general, may be more sensitive than humans to carcinogens (with some exceptions, eg arsenic is carcinogenic to humans but not rodents).
c) Chronic Inflammation
- the generation of $O_2$ free radicals by inflammatory cells and the continuous regeneration of cells to replace damaged ones at a site of chronic inflammation may result in genomic damage in the local cell population.
  - this damage may, over time, progress to neoplastic transformation.
  - moreover, proliferating fibroblasts at the site of chronic inflammation can secrete some important soluble growth factors that contribute to neoplastic transformation of some of the resident cells.

① Development of sarcomas in some cats at sites of subcutaneous injection with either feline leukemia virus vaccine or rabies vaccine, usually between 3 months and 3 years after vaccination.
  - the prevalence of vaccine-associated sarcomas is approximately 5/10,000 cats vaccinated.
  - they are predominately fibrosarcomas, but also see chondrosarcomas, rhabdomyosarcomas, myxosarcomas, malignant fibrous histiocytomas and undifferentiated sarcomas.
  - these tumors are typically associated with chronic granulomatous inflammation, presumably directed at some component of the vaccine (eg aluminum hydroxide in the adjuvant, tissue culture material or preservatives); this persistent inflammatory / immunologic reaction induces continued proliferation of resident mesenchymal cells, which may in some cases, lead to neoplastic transformation.
  - ferrets may show a comparable susceptibility to the development of sarcomas at sites of previous vaccination (eg against rabies or canine distemper virus).

② Intraocular sarcomas following traumatic rupture of the lens capsule in cats (some of these tumors result from neoplastic transformation of the lens epithelium).

③ Humans with idiopathic ulcerative colitis (an autoimmune disease) have a 10-20% higher risk of developing colorectal cancer than the average population.

④ Some parasites and bacteria are known to occasionally induce tumors in chronically infected hosts.
  - Spirocerca lupi in dogs: esophageal fibrosarcoma and osteosarcoma.
  - Cysticercus fasciolaris in rats: hepatic sarcomas.
  - Helicobacter pylori in humans: gastric carcinomas and lymphomas.

d) Radiation Carcinogenesis
- ionizing radiation, either weak (eg UV rays) or strong (eg particulate radiation) can induce neoplasia.
- the latter (eg medical / occupational, nuclear power plants / bombs) is more relevant to humans.
- many skin tumors (eg squamous cell carcinoma) in humans & animals are induced by UV light exposure.

Ultraviolet (UV) Rays
- degree of risk associated with type of UV rays (esp UV-B; UV-C absorbed by ozone layer), intensity of exposure (eg equator, high altitude) and amount of protective pigmentation (eg white regions on cats/cattle).
- UV rays can damage (inhibition of cell division, inactivation of enzymes & induction of mutations) or kill cells.
- carcinogenicity is due to mutations arising from pyrimidine dimer formation.
- typically cells with DNA damage are either repaired (NER pathway) or undergo apoptosis.
- postulated that excessive sun exposure overwhelms the capacity of the NER pathway & mutations occur.
- p53 and RAS are particularly prone to mutation by UV light.

*Xeroderma pigmentosum* is a human hereditary disorder caused by mutations in one of several genes involved in NER (at least 7 variants identified). Affected people have a 2,000-fold increased risk of skin cancer in sun-exposed skin.
6) Epidemiology of Neoplasms

- due to the typically long delay between exposure to carcinogens and the development of tumors, specific causes of neoplasms can be determined accurately only through carefully designed epidemiological studies.
- in North America, a person has about a 20% chance of dying from cancer.
- almost 65% of human cancer deaths in the United States can be attributed to tobacco smoke and diet.
  - smoking causes 30% of cancer deaths, eg 90% of lung cancer is due to smoking.
  - tobacco smoke is the single most lethal carcinogen in the US (Sci Amer 1996; 275: 80-7)

<table>
<thead>
<tr>
<th>Summary of Causes of Cancer in Humans</th>
<th>(Dr. Bruce Ames, 1997)</th>
</tr>
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<tbody>
<tr>
<td>Smoking</td>
<td>30%</td>
</tr>
<tr>
<td>Diet (not enough fruits and vegetables) / obesity</td>
<td>35%</td>
</tr>
<tr>
<td>Chronic infections</td>
<td>30%</td>
</tr>
<tr>
<td>Hormonal (breast, endometrium)</td>
<td>20%</td>
</tr>
<tr>
<td>Occupational (asbestos, etc.)</td>
<td>2%</td>
</tr>
<tr>
<td>Pollution</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td>Total</td>
<td>117% (because of multiple causes)</td>
</tr>
</tbody>
</table>

- age has a strong association with particular types of cancer in animals and people.
  - most tumors occur in older age cohorts of animals and humans.
  - exceptions, eg cutaneous histiocytoma in young dogs & leukemias and brain tumors in children.

- genetic predispositions are well recognized in humans in autosomal dominant patterns (eg retinoblastomas with mutations of the RB gene), autosomal recessive patterns (eg defective DNA repair syndromes such as xeroderma pigmentosum) and less clearly defined familial patterns (eg carcinomas of breast, ovary & colon)
  - breed / familial predispositions are also recognized in animals eg's Boxer dogs predisposed to many neoplasms and osteosarcomas of the appendicular skeleton in large and giant dog breeds.

- some observations suggestive of a cause-effect relationship in animal tumors include:

  ① Squamous cell carcinoma of the bovine eye and face (ears, lips, nose, eyelids) of white cats:
    - associated with lack of pigmentation, sparse haircoat and exposure to natural ultraviolet light.

  ② Cutaneous melanomas:
    - higher incidence in dogs with greater skin pigmentation; high incidence in gray horses >6 yrs old.

  ③ Transitional cell carcinoma of the bladder in dogs:
    - associated with exposure to flea and tick insecticide dips, particularly in overweight dogs.

  ④ Intestinal adenocarcinoma in sheep (and humans) in New Zealand:
    - associated with exposure to phenoxy and picolinic acid herbicides.

  ⑤ Alimentary and Urinary tract cancers in cattle ingesting bracken fern
    - high rates of bladder and alimentary tract cancers seen in cattle grazing bracken fern infested land.
8) Systemic Effects of Neoplasms

- both benign and malignant neoplasms can cause problems by a variety of mechanisms.

a) Local effects and compression of adjacent structures

- expansile growth of benign pituitary or brain tumors can compress adjacent vital structures.
- benign or malignant tumors can cause tubular organ obstruction (eg intestinal or urinary tract obstruction).
- tumors on organ surfaces (eg skin, gut lumen, bladder lumen) can have ulceration, bleeding, 2° infections.

b) Rupture or Infarction of Tumor

- rapid tumor growth can lead to rupture / hemorrhage or they can outgrow their blood supply (infarction).

c) Hormonal effects

- well-differentiated tumor cells of endocrine glands can produce hormones in an uncontrolled manner.
  - ACTH secreting pituitary adenoma,
  - corticosteroid secreting tumors of the adrenal cortex,
  - thyroid adenoma secreting thyroxine,
  - feminization syndrome associated with testicular tumors (eg estrogen from sertoli cells),
  - pancreatic islet tumors (eg insulinoma, glucagonoma)

d) Cachexia (Emaciation)

- cachexia designates a progressive weight loss due to loss of muscle mass and fat stores.
  - it can be the first sign noticed in an affected animal, and its degree does not necessarily correlate with the total mass of the neoplastic tissue.
  - in humans, some types of cancer (eg pancreatic, lung) are more prone than others to induce cachexia.
- it results from the action of cytokines produced by tumor cells or by host cells in response to the tumor.

1 Tumor Necrosis Factor (TNF)

- TNF produced by macrophages or in some cases by the tumor cells themselves, is an important mediator of wasting in malignancies and chronic infectious diseases of animals.
- TNF-α induces a net catabolic state by increased catabolism of specific tissues such as muscle and fat.
  In adipocytes, TNF-α induces catabolism and lipolysis by suppressing the synthesis of several lipogenic enzymes. Unfortunately the amount of acetyl-CoA produced as a result of the increased availability of fatty acids may exceed the capacity of the citric acid cycle to oxidize it and it will be converted to ketone bodies which can be lost in the urine.

2 Other Cytokines

- other cytokines produced by macrophages, eg interleukin-1 & interferon-γ can act synergistically with TNF.
- other soluble factors from tumor cells (eg proteolysis-inducing factor) can directly catabolize fat and muscle. Muscle protein degradation can increase the supply of amino acids available for metabolism. However, as with surplus acetyl-CoA (see above), these excess amino acids may eventually exceed the body’s capacity to utilize it, leading to catabolism and excretion of the excess amino acids instead of their incorporation into newly synthetized proteins.

3 Cancer Cells as Metabolic Parasites

- cancer cells tend to revert to anaerobic metabolism (converting glucose to lactate) even in the presence of oxygen; this is a general property of rapidly dividing cells, either neoplastic or non-neoplastic.
  The production of ATP through lactate formation yields energy at a much faster rate than aerobic metabolism, but it is a wasteful form of energy production (2 vs 34 ATP). Because of this, malignant cells tend to utilize anywhere from 5 to10 times as much glucose as normal tissues. Lactate can be recycled in the liver to produce glucose (gluconeogenesis); again however, the formation of 1 molecule of glucose from lactate requires the input of 6 molecules of high-energy phosphate, whereas conversion of 1 molecule of glucose to lactate generates only 2 molecules of ATP. This results in a negative energy balance.
e) Anemia
- anemia is another common manifestation of chronic diseases, including infections and malignancies.
- decreased production of erythrocytes by the bone marrow appears to be the main cause of this anemia, and this seems to result from a decreased availability of iron at the cellular level.
  - iron may be sequestered within macrophages as a result of the inflammatory process accompanying malignant neoplasia (this is a good strategy in cases of bacterial infections, since macrophages are attempting to starve bacteria of one of their essential elements for growth and proliferation).
- a mild decrease in the life-span of erythrocytes also occurs.
  - abnormal tissue proteins may cause subtle injury to erythrocytes, increasing their removal by the mononuclear phagocyte system, and there may also be mechanical fragmentation.
- chronic blood loss from hemorrhages within the tumor or within adjacent normal tissues eroded by the invading neoplastic cells may also contribute to anemia.

f) Paraneoplastic Syndromes
- in human medicine, paraneoplastic syndromes are defined as "symptom complexes in cancer-bearing patients that cannot readily be explained, either by the local or distant spread of the tumor or by the elaboration of hormones indigenous to the tissue from which the tumor arose" (Pathologic Basis of Disease, 2005)
- these syndromes occur in about 15% of human patients with advanced malignant disease; however, they can occasionally appear as a manifestation of an occult, small neoplasm.
- these syndromes are not as prevalent in veterinary medicine, since an animals life is rarely maintained for as long a period as that of humans; however, in some cases, they may represent the main presenting sign.

Paraneoplastic Endocrine Syndromes
- refer to systemic effects that in some way mimic an endocrinopathy.
- as mentioned previously, benign and malignant tumors of endocrine glands may produce excessive amounts of the corresponding hormones, however these do not qualify as paraneoplastic syndromes.
- they occur when non-endocrine tumors secrete hormones or hormone-like substances that are not normally associated in physiological amounts with the organ / tissue of origin.
  - some of these hormone-like substances may actually represent regulatory molecules with an autocrine or paracrine function that are normally synthesized in the fetus during development of the organ, are repressed to small or negligible amounts in the adult, and then are re-expressed in the tumor.

Humoral Hypercalcemia of Malignancy (HHM) (Pseudohyperparathyroidism)
- HHM is the most common example of a paraneoplastic syndrome and is a serious complication of several types of human and animal tumors.
- tumors can induce hypercalcemia through the release of humoral factors that act systemically, mainly to increase osteoclastic bone resorption, but also to increase Ca\(^{2+}\) reabsorption from the kidneys or, occasionally, increase Ca\(^{2+}\) absorption from the intestinal tract.
  - production of parathyroid hormone-related protein (PTHrP) by tumor cells is one of the major factors that induce hypercalcemia in human and animal patients.
  - PTHrP has some sequence homology with parathyroid hormone (PTH), shares the same receptor on target cells in bone and kidneys, and induces many of the same biological effects, however, it is immunologically distinct from PTH and is the product of a separate gene.
  - PTHrP has a normal paracrine function in adults and is the major Ca\(^{2+}\)-regulating hormone in fetuses.
- HHM can be more immediately life-threatening than the neoplasm itself, since it may cause severe gastrointestinal and central nervous system disturbances, cardiac arrhythmias and nephropathy.
- it is normally the ionized Ca\(^{2+}\) that is biologically active (about ½ total Ca\(^{2+}\) bound to plasma proteins); cancer patients with a low serum albumin, resulting from cachexia, malnutrition or liver dysfunction, may have fatal complications of hypercalcemia with only slight increases in serum Ca\(^{2+}\).
- note: hypercalcemia can also be induced through local bone resorption by neoplasms that have metastasized to bone or by hematopoietic bone marrow tumors (not part of the paraneoplastic syndrome).
- some neoplastic disease which have HHM:
  
dog:  - adenocarcinoma of apocrine glands of the anal sac (hypercalcemia in 90% of cases).
  - lymphosarcoma; usually of T-cell origin, with a mediastinal predilection (in 10-40% of cases).

  cat:  - squamous cell carcinoma (especially those involving the head and neck).
  - lymphoproliferative disorders.

  horse: - gastric carcinoma.

  human: - squamous cell bronchogenic carcinoma (25-30% of patients).

ii) Other paraneoplastic endocrine syndromes.
- in humans (and to a lesser extent animals), other hormone-like factors have been found to be produced by tumors, eg ACTH-like substance, TSH-like substance, insulin-like substance, erythropoietin, etc.

Some Other Paraneoplastic Syndromes Reported in the Veterinary Literature

i) Cutaneous paraneoplastic syndromes
- a variet of paraneoplastic dermatosis, with obscure pathogenesis, have been identified primarily in dogs and cats, eg feline paraneoplastic alopecia, feline thymoma-associated exfoliative dermatitis, nodular dermatofibrosis, superficial necrolytic dermatitis and paraneoplastic pemphigus.

ii) Paraneoplastic neurologic syndromes
- peripheral neuropathies associated with malignant neoplasms has been described in dogs.
- many of these syndromes are believed to have an autoimmune basis: antibodies produced against tumor antigens cross-react with various neurons of the CNS or PNS.

iii) Coagulation abnormalities associated with thrombocytopenia
- thrombocytopenia is a result of decreased platelet production, increased platelet destruction or consumption, platelet loss through hemorrhage, platelet sequestration or a combination of these factors.
- thrombocytopenia is reported most frequently in dogs with lymphosarcoma, hemangiosarcoma, and some carcinomas (Comp Cont Educ 2000; 22:1006-17)

iv) Myasthenia gravis in cats with thymoma.
- probable autoimmune pathogenesis which results in loss of self-tolerance and production of autoantibodies to acetylcholine receptors on muscle end-plate membrane.

v) Hypoglycemia associated with intraabdominal leiomyomas and leiomyosarcomas in dogs
- possibly caused by the production of insulin-like growth factor.

vi) Persistent leukocytosis (often neutrophilia) associated with carcinomas in dog and cats
- likely due to the production of a granulocyte colony-stimulating factor.
9) Grading and Staging of Tumors

a) Clinical staging
- attempts to classify tumors according to their progression.
- the TNM system is based on:
  - assessment of the size of the primary tumor (T)
  - involvement of regional lymph nodes (N)
  - presence or absence of distant metastasis (M)

b) Histological grading
- represents an attempt to quantify characteristics of tumors as a means of predicting their outcome.
  - degree of differentiation of neoplastic cells.
  - the size of nuclei.
  - mitotic activity (may use specific nuclear markers of the mitotic cycle).
  - degree of vascularization.
  - presence or absence of necrosis.

c) Analysis of specific genomic alterations
- in human medicine it has become an important method of evaluating the degree of malignancy of some tumors and of distinguishing between morphologically closely related types of tumors.
  - identification of RAS oncogene in stool samples may help in the early detection of human colon cancers.
  - overexpression of the protein product of the mutated p53 tumor suppressor gene has been correlated with higher grade malignancies of various types of tumors in humans.
  - some specific types of mutations in oncogenes or tumor suppressor genes may reflect exposure to particular carcinogens, eg UV light, aflatoxins, polycyclic aromatic hydrocarbons in cigarette smoke.