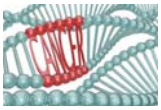
Malignant transformation

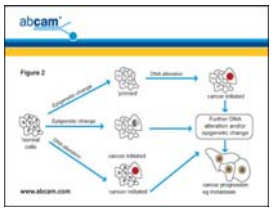
Topics

Hallmarks of cancer cells
Oncogenes and tumor suppressors
Stages of tumor development
Metastases
Interaction of tumor and organism
Cancer biomarkers



Malignant transformation

- The process of tumor formation is a **complex** involving multiple alterations of cells and their physiologic control mechanisms.
- The complexity of this process is reflected in the **long time periods** required for most human cancers to develop.
- Multi-step tumor progression can be depicted as a form of **Darwinian evolution** occurring within tissues.
- Some of the critical changes occurring during tumorigenesis are **epigenetic** and the rate of genetic diversification can occur very rapidly.



Genetic alteration can appear
– (1) due to internal errors during DNA replication and cell division
– (2) as a consequence of exposure to the external factors (**carcinogens**)
physical – e.g. UV and ionising light
chemical – organic substances, toxins, heavy metals
biologic – some RNA and DNA viruses

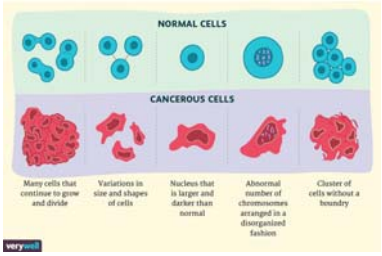
Hallmarks of cancer

- Continual unregulated proliferation of cancer cells (sustaining proliferative signaling and evading growth suppressors)
- Replicative immortality
- Genome instability
- Resisting cell death and senescence
- Inducing angiogenesis
- Inflammation
- Avoiding immune destruction
- Altered metabolism
- Invasion and metastasis

All these features do not have to be newly evolved, because they are part of physiological processes such as embryogenesis and wound healing. Cancer cells only use these processes in wrong intensity, time, and place. Cancer is a disease of regulation.

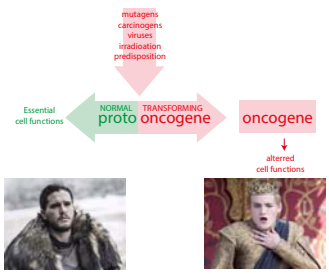
Cancer cell

- Cancer cells divide excessively - they have too many "GO" signals or not enough "STOP" signals and can also ignore "DIE", " DIFFERENTIATE ", or "GROW OLD" signals.



Oncogenes

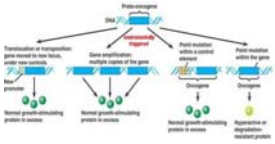
- **Proto-oncogenes** – Genes whose products encode components of the molecular cascades that mediate the "GO" response to mitogenic signals and pro-survival proteins.
- The abnormal, **mutated** form of the proto-oncogenes that lead to excessive cell proliferation and cancer are called **oncogenes**.



Oncogenes

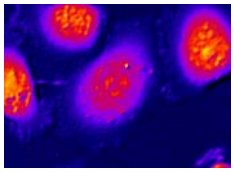
Oncogenes differ from proto-oncogenes in three basic ways:

1. timing and quality of expression
2. structure and function of protein products
3. degree to which their protein products are regulated by cellular signals



Uncontrolled growth

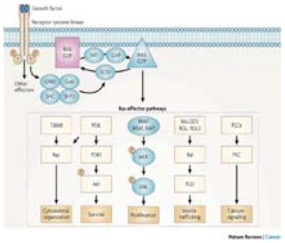
- In cancer cells, a number of alternative mechanisms operate to ensure that cell proliferation is not constrained.
- Cancer cells produce growth factors that stimulate their own proliferation (**autocrine growth stimulation**) and **hijack cellular mitogenic signals**.



Uncontrolled growth - "GO" signals

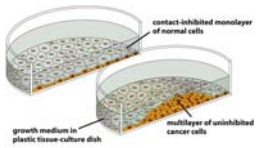
"GO" signals = main mitogenic signals include:

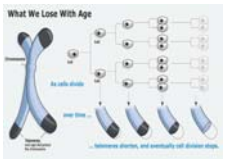
1. growth factors (e.g. EGF, VEGFA, PDGF)
2. growth factor receptors (e.g. the receptors for epidermal growth factor EGF (EGFR) and its close homologue HER2/neu (ERBB2)
3. receptor-coupled signal transduction molecules (RAS family)
4. proteinkinases (SRC,ABL)
5. transcription factors (MYC, MYB, FOS, JUN)
6. cyclins
7. cyclin-dependent kinases (cdk)



Contact inhibition and immortalization

- Proliferation of many normal cells is inhibited by cell-cell contact (**contact inhibition)** and by erosion of telomeres (**Hayflick limit)**, but cancer cells are characteristically insensitive to such inhibition of growth.
- Most pre-malignant cells escape from Hayflick limit by stabilizing their telomeres (**telomerase**, hTERT).
- Cells that have stabilized their telomeres can proliferate indefinitely and are therefore said to be **immortalized**. Immortal cells are not necessarily transformed (tumorigenic) cells.



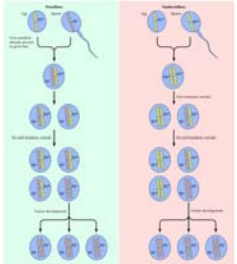


Uncontrolled growth – loss of "STOP" signals

- The critical decisions concerning growth versus quiescence are made in the G1 phase of the cell cycle.
- Growth of normal cells is controlled by signals from the external environment (extracellular matrix, surface of adjacent cells) and from the inside of the cell (DNA damage, cell damage, mitotic spindle damage).

Tumor suppressors

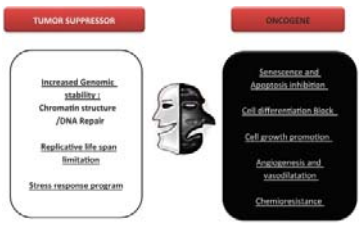
- Proteins encoded by **tumor suppressor genes** inhibit cell proliferation or survival.
- In many tumors are lost or inactivated.
- Tumor suppressor proteins inhibit the same cell regulatory pathways that are stimulated by the products of oncogenes.
- Most **familial cancer syndromes** are inherited as a recessive trait, and correspond to the constitutive inactivation of an important tumor suppressor gene
- Tumor suppressors are often named according to the type of tumor developing due to their loss of function.
  - Rb (= retinoblastoma)
  - WT (= Wilm's tumor)
  - NF1 and NF2 (= neurofibromatosis)
  - APC (= Adenomatous Polyposis Coli)
  - DCC (= Deleted in Colon Cancer)
  - VHL (= von Hippel-Lindau syndrome)



"two hits" hypothesis explain the inheritance of retinoblastoma, a rare childhood tumor type (1971, Knudsen)

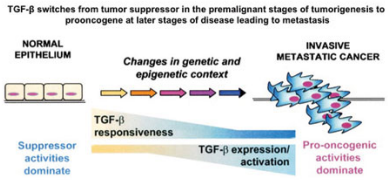
Will you be my tumor suppressor for ever?

- Tumor suppression is context dependent.
- **Sirtuins** are a class of proteins that possess deacylase aktivity.
- The evidence reported supports both an oncogenic and a tumor suppressor role for SirT1.



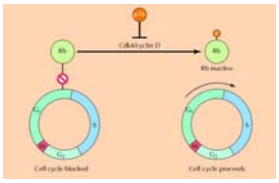
Will you be my tumor suppressor for ever?

- TGF-β (transforming growth factor-β) has antiproliferative effect and maintain genomic stability. As cancer progresses, tumor cells alter their responsiveness to TGF-β. At late-stage tumors, TGF-β promotes cell migration, promotes invasion of cancer cells, and becomes a pro-survival factor.



Rb protein - true tumor suppressor

- Rb is a main inhibitor of cell cycle and controls the transition from G1- to S-phase.
- Rb inhibits the transcription factor E2F, which upon release from Rb ↑ expression of S phase genes (e.g. DNA replication enzymes)
- Rb is present all the time, its activity is modulated by phosphorylation
  - phosphorylated Rb = inactive
  - dephosphorylated Rb = active
- *Rb* mutations are also involved in tumors of adults (bladder, breast, and lung carcinomas).
- The significance of the *Rb* tumor suppressor gene thus extends beyond retinoblastoma



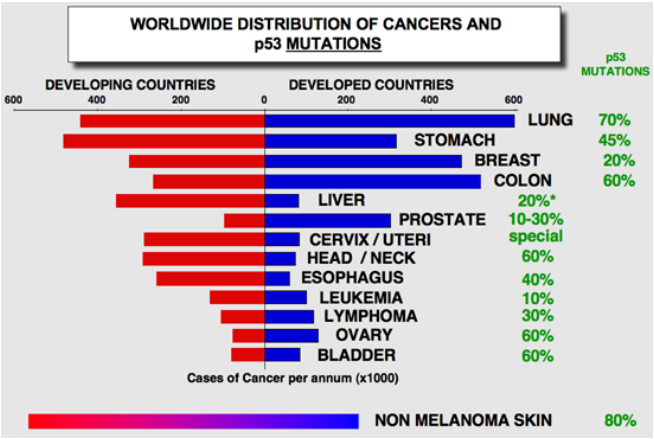
Other "STOP" signals

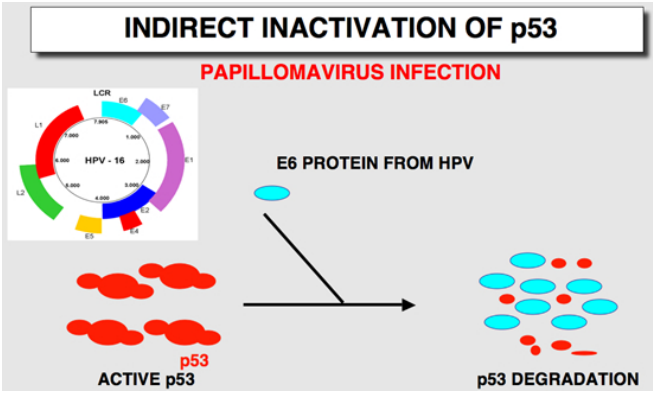
p53 protein (ch. 17p13)

- "guardian of the genome" – active in G1 and G2 checkpoints
- DNA damage increases expression of p53
- acts as a transcription factor for DNA repair and apoptosis genes

inhibitors of cyclin-dependent kinases (e.g. p21, p27, p16, etc.)

- p21 is the main target of p53 = inhibitor of Cdk – cell cycle arrest in G1 phase by inhibition of Cdk2/cyclin E complex





The E6 viral protein expressed by HPV specifically binds to the p53 protein and induces its degradation. This observation explains the rarity of p53 mutations in cervical cancers.

Genomic instability - new oportunities for evolution

- DNA damage may predispose individuals to increased tumorigenesis.
- An increase of copy number of chromosomes or genes allows cells to overexpress certain genes or mutate the extra copies to acquire growth, survival, or metastasis advantage.
- Genomic instability is a characteristic of most cancer cells due to over-replication.
- Excessive DNA damage is associated with problems in DNA replication (broken chromosomes and aneuploidy).
- Genomic integrity is closely monitored by several surveillance mechanisms, DNA damage checkpoint, DNA repair machinery and mitotic checkpoint.
- DNA methylation status is also important for genomic integrity.

DNA repair genes/proteins

MMR genes/proteins ("Mismatch repair")

- defect in respective genes leads to the microsatellite instability (MSI). Variable length of microsatellites (e.g. (CA)n repetition) leads to the DNA replication errors. MSI is most prevalent in colon cancers.

Nucleotide excision repair genes/proteins ("single strand break repair")

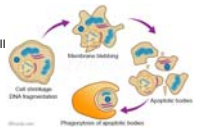
NER-defects cause xeroderma pigmentosum (XP). XP patients show severe sun sensitivity and develop skin cancers during childhood.

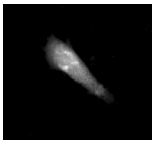
Genes/proteins of homologous recombination ("double strand break repair")

- **BRCA1** and **BRCA2** "breast cancer susceptibility genes"
- **ATM** and **ATR** (ATM-related) kinases ("mutated in ataxia-telangiectasia")

Cell death – apoptisos, necrosis, necroptosis

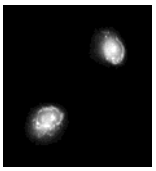
Apoptosis
Active (= energy requirement), programmed cell death. The action of caspases and other apoptotic enzymes (proteases and nucleases) leads to cell fragmentation to apoptotic bodies that are removed by macrophages.

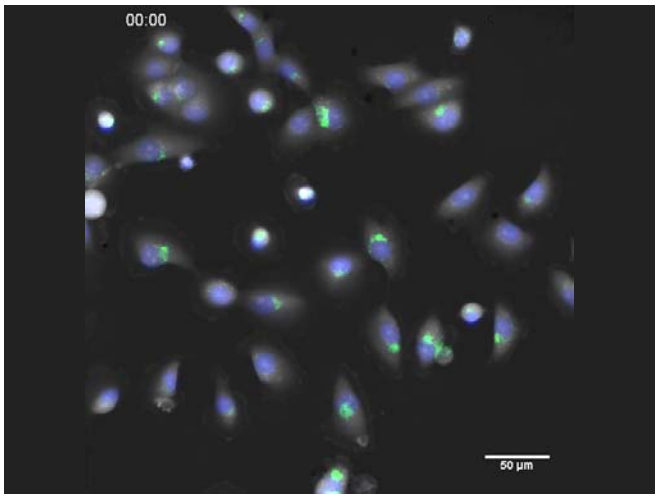




Necrosis
Accidental cell death caused mainly by external factors (infections, toxins, etc.). Cellular content is released into the environment and damage surrounding tissues. Necrosis has proinflammatory and tumor-promoting potential.

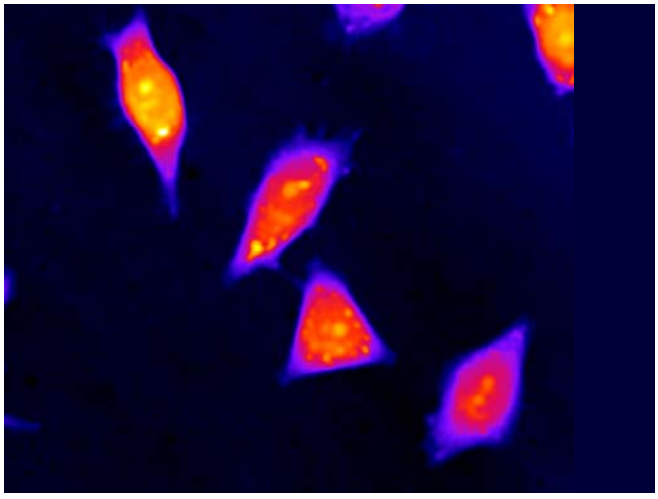
Necroptosis
Controlled form of necrosis driven by kinases RIP1 and RIP3.





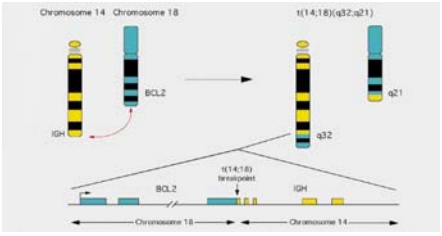
Resisting cell death

- Tumor cells evolve a variety of strategies to limit cell death. Most known are:
  - loss of **p53**
  - **increased expression of antiapoptotic** regulators (Bcl-2, Bcl-xL) and survival signals (insulin-like growth factors; Igf1/2)
  - **downregulating of proapoptotic** factors (Bax, Bim, Puma)
  - opportunistic modes of behavior (cell fusion)



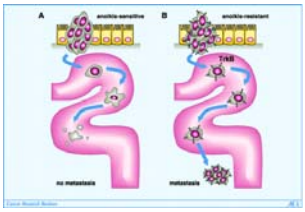
Resisting cell death

Chromosomal translocation associated with B-cell lymphomas.
The Bcl-2 gene is translocated behind a potent immunoglobulin gene promoter. Increased expression of Bcl-2 gene is associated with inhibition of apoptosis.



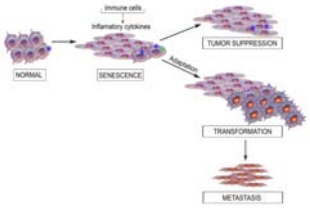
Resistence to cell death - anoikis

- **Anoikis** is a form of programmed cell death that occurs in anchorage-dependent cells when they detach from the surrounding extracellular matrix.
- barrier to metastasis
- circulating tumor cells are anoikis resistant
- TrkB (neurotrophic receptor) overexpression protects disseminated, circulating tumor cells from undergoing anoikis.



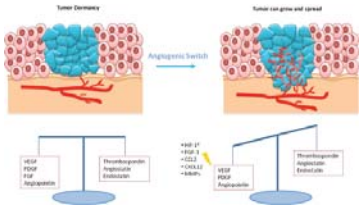
Resisting oncogene-induced senescence

- Cellular senescence is a growth-arrest program that limits the lifespan of cells and prevents unlimited cell proliferation.
- Certain mitogenic oncogenes or the loss of anti-mitogenic tumour-suppressor genes induce senescence. This is known as **oncogene-induced senescence**.
- Many cancer cells either do not have fully active senescence programs or develop bypass mechanisms to regain proliferation capabilities (c-myc overexpression).



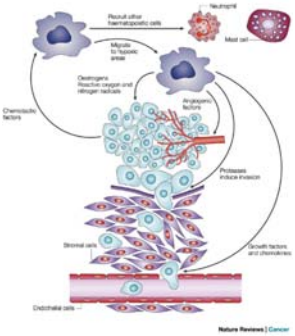
Inducing angiogenesis

- Like normal tissues, tumors need nutrients and oxygen.
- Tumor without blood circulation grew to 1–2 mm^3. In the absence of vascular support, tumors may become necrotic.
- Up-regulation of the activity of angiogenic factors is not sufficient for angiogenesis of the neoplasm. Negative regulators of vessel growth need to be downregulated.
- New vessels enable invasion of tumor cells into circulation and creation of distant metastases.



Inducing angiogenesis

Cells of the innate immune system (macrophages, neutrophils, mast cells, and myeloid progenitors) can infiltrate premalignant lesions and contribute to tumor angiogenesis.

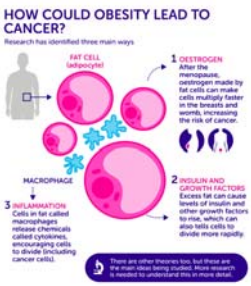


Inflammation

- There are important similarities between tumors and the inflammatory response associated with wound healing.
- "**Tumors: Wounds that do not heal**"
- Many cancers arise from sites of infection, chronic irritation and inflammation.
- Anti-inflammatory medications, such as aspirin or non-steroidal anti-inflammatory drugs, reduce the risk of cancer.

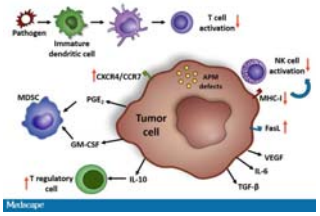
Inflammation and obesity

- As people become obese more fat cells are build up in their tissues and macrophages are recruited to clear up dead fat cells. The number of macrophages in obese fatty tissue can be substantial - four in 10 cells.
- Macrophages release cocktail of cytokines that can trigger chronic inflammation.
- Obese people tend to have higher levels of inflammatory cytokines in their blood.
- **Fat isn't just padding: it's like another organ** it is essentially a huge gland sending out biological information that affect the rest of body. Oestrogen and growth factors produced by fat cells increase the risk of cancer.



Evading immune destruction

- **Defective antigen presentation** due to down-modulating antigen presenting machinery (↓ major histocompatibility complex, MHC)
- **Immune suppression** in the tumor microenvironment, mediated by CD4+CD25+ FoxP3+ regulatory T cells (**Tregs**), or other types of suppressive cells.
- **Paralyzing cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells** via production of immune suppressive cytokines (by the cancer cells or by the non-cancerous cells in the tumor microenvironment). TGF-β is a chief mediator of this activity.
- **Down regulation of death receptors** prevents death ligand-mediated killing of tumor cells by both CTLs and NK cells.

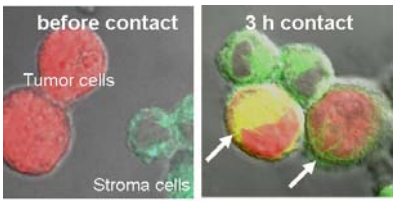


Cancer Neoantigens: A Promising Source of Immunogens for Cancer Immunotherapy

- Somatic mutations in tumor genes could be reflected in proteins. Missense or frameshift mutation has the potential to generate **tumor-specific antigens** (TSAs), which are theoretically recognized as "non-self" by the host immune system.
- TSAs, also known as "**cancer neoantigens**", have the potential to be utilized as biomarkers predicting clinical responses to immunotherapy and outcomes, as well as serving as targets for immunotherapy.
- Neoantigens are also expressed by fetal organs. Older fetal organs (21 weeks) and adult organs do not express an immunogenic neoantigens.

Oncological trogocytosis - the way how to get rid of antigens

- Intercellular exchange of intact membrane patches.
- Exchange of membrane molecules/antigens.
- Human epidermal growth factor receptor 2 (HER2) could be transferred from cancer cells to monocytes via trogocytosis

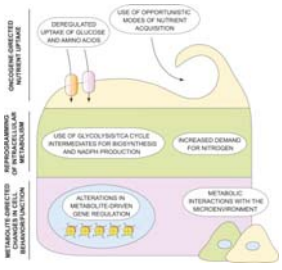


Altered metabolism

- The ability to acquire necessary nutrients from a nutrient-poor (low glucosis) and hostile (hypoxia, oxidative stress) environment and utilize these nutrients to maintain viability and build new biomass.
- Cancer-associated metabolic reprogramming have profound effects on gene expression, cellular differentiation, and the tumor microenvironment.
- These adaptations involve an ability to access normally inaccessible nutrient sources.

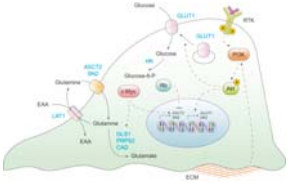
Hallmarks of cancer metabolism

(1) deregulated uptake of glucose and amino acids
(2) use of opportunistic modes of nutrient acquisition
(3) use of glycolysis/TCA cycle intermediates for biosynthesis and NADPH production
(4) increased demand for nitrogen
(5) alterations in metabolite-driven gene regulation – metabolites influence enzymes involved in deposition and removal of epigenetic marks.
(6) metabolic interactions with the microenvironment.



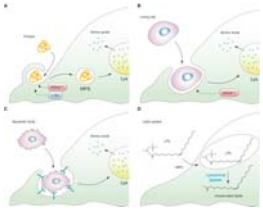
Altered metabolism

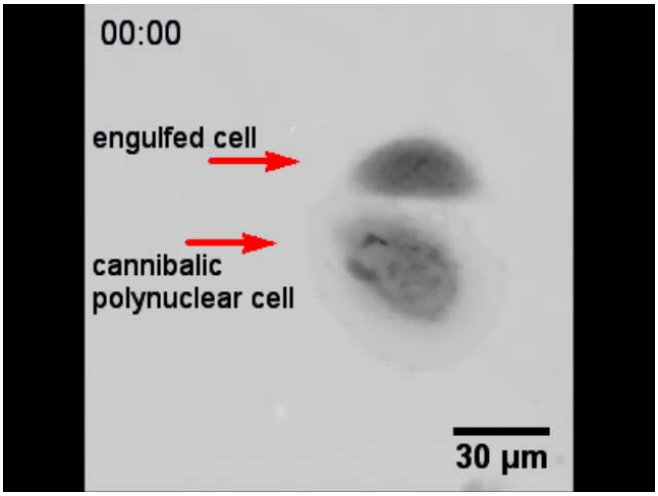
- Two principal nutrients that support survival and biosynthesis are **glucose** and **glutamine**.
- Glutamine provides the nitrogen required for the biosynthesis of purine and pyrimidine nucleotides and nonessential amino acids.
- **Warburg effect** - a markedly increased consumption of glucose by some tumors in comparison to the nonproliferating normal tissues.
- **Positron emission tomography** (PET)-based imaging of the uptake of a radioactive fluorine-labeled glucose analog, ^{18}F-fluorodeoxyglucose (^{18}F-FDG) has been successfully used in the clinic for tumor diagnosis.
- Oncogenic signaling proteins - Ras upregulate *GLUT1* mRNA expression and increase cellular glucose consumption.

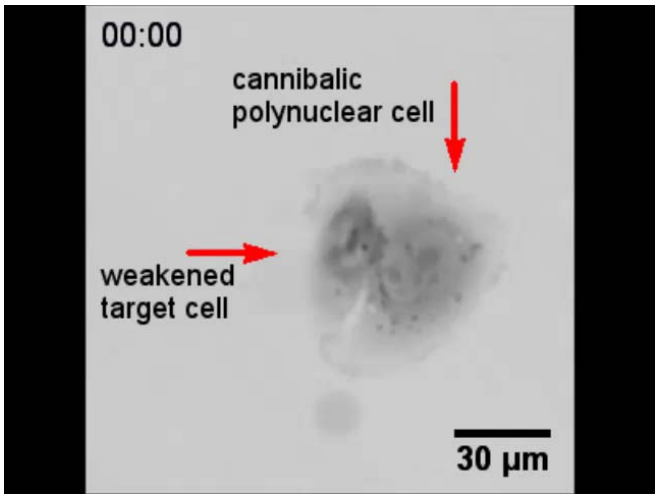


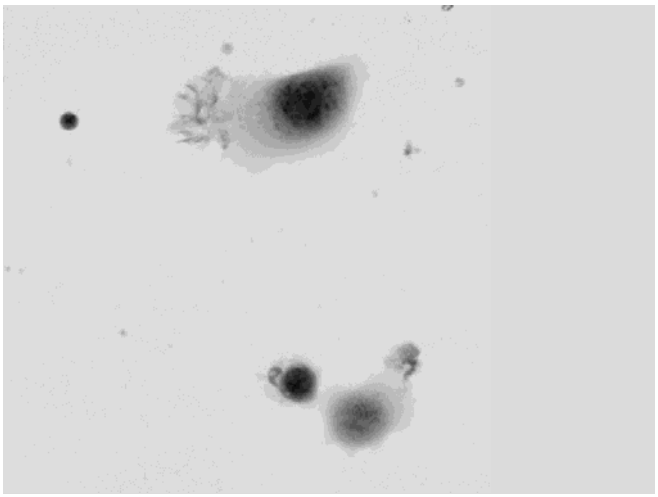
Use of opportunistic modes of nutrient acquisition

- Ras or c-Src oncogenes allow to recover free amino acids through the **lysosomal degradation of extracellular proteins**.
- **Macropinocytosis**.
- **Macroautophagy** (autophagy cannot supply cells with new biomass and thus cannot support proliferation in nutrient-poor conditions).
- **Phagocytosis** of apoptotic cellular corpses.
- **Canibalism**.



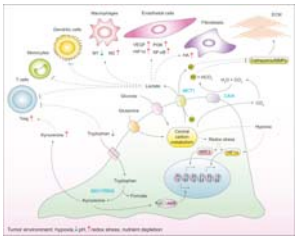






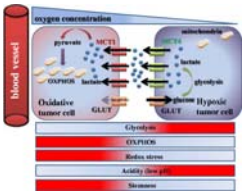
Metabolic interactions with the microenvironment

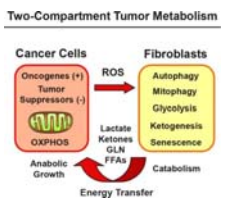
- Cancer cells alter the chemical composition of the extracellular milieu, which exerts **pleiotropic effects** on the phenotypes of normal cells that reside in the vicinity of the tumor.
- Reciprocally, the microenvironment affects the metabolism and signaling responses of cancer cells.
- The high metabolic demand of cancer cells leads to an accumulation of H+ ions in tumor microenvironment – **acidosis**.



Metabolic symbiosis

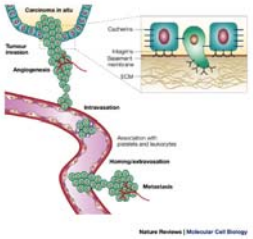
- Catabolic fibroblasts are rich source of energy and biomass for the growth and survival of anabolic cancer cells.
- A linear path of clonal succession oversimplifies the reality of cancer; number of **genetically distinct subclones** of cells coexist within a single tumor mass: intra-tumor heterogenity - oxidative and glycolytic tumor cells in one tumor.





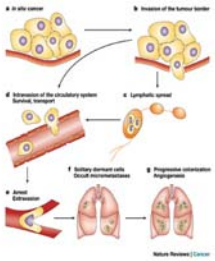
Invasion and metastasis

- Cancer cells lose **E-cadherin** dependent intercellular adhesions, acquire a **migratory phenotype** (anoikis resistence, epithelial-mesenchymal transition, EMT), penetrate the basement membrane, and invade the interstitial matrix (production of MMPs).
- Tumour angiogenesis allows cancer cells to enter the bloodstream (circulating tumor cells), either directly or through the lymphatic system, by a process called **intravasation.**
- In the circulation, tumour cells form small **aggregates with platelets and leukocytes**.
- After stopping in the microcirculation of the target organ, tumour cells exit the bloodstream, by a proces called **extravasation**, and undergo local expansion.



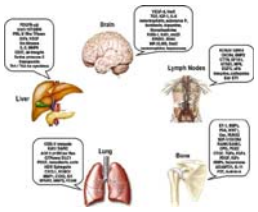
Invasion and metastasis

- Tumors that breach the basement membrane and invade underlying tissue are **malignant**. An even further degree of abnormality is metastasis, the seeding of tumor colonies to other sites in the body. Metastasis requires not only **invasiveness** but also **motility** and **adaptation** to foreign tissue environments.
- Several ways of spreading:
  - **blood** (very often in the direction of flow: from GIT to the liver, by venous blood to the lungs, from lungs by artery blood to bones and brain)
  - **lymphatic** (first neighbouring lymph nodes, than distant)



Seed and soil hypothesis – permissive microenvironment

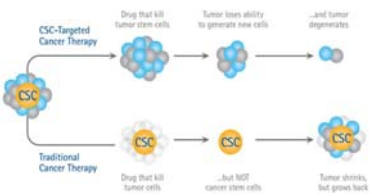
- Tumor cell-intrinsic metastatic propensities are not sufficient for metastatic seeding.
- Metastasis is dependent on the interactions between 'seeds' (the cancer cells) and the 'soil' (the host microenvironment).
- Different cancers have preferential sites of metastasis = **organotropism** (prostate cancer - the bone and the liver).
- Tumour-secreted factors and tumour-shed extracellular vesicles enable the 'soil' at distant metastatic sites to encourage the outgrowth of incoming cancer cells.
- **Pre-metastatic niches** (PMNs) are sites of immune deregulation, owing to the presence of a pro-tumorigenic, inflammatory milieu induced by tumour-secreted factors, which creates immunosuppression and coagulation disorders.



factors influencing organ-specific metastases to the liver, lung, brain, bone and lymph nodes

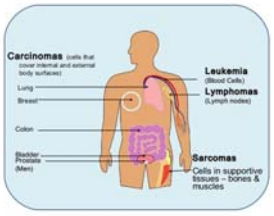
Cancer stem cell (CSC) hypothesis

- Cancer stem cells are rare **immortal** cells within a tumor that can both self-renew by dividing and give rise to many cell types that constitute the tumor.
- CSCs are **tumorigenic**, associated with metastasis and relapse.
- Enhanced resistance to therapy and cell stress.
- Such cells have been found in various types of human tumors and might be attractive targets for cancer therapy.



Tumor classification

- The most common human cancers are of epithelial origin - the **carcinomas**. two main categories: **squamous cell carcinomas** (from epithelia that form protective cell layers) and **adenocarcinomas** (from secretory epithelia).
- Nonepithelial malignant tumors include: **sarcomas** (from mesenchymal cells); **hematopoietic cancers** (from the precursors of blood cells); and **neuroectodermal tumors** (from components of the nervous system).

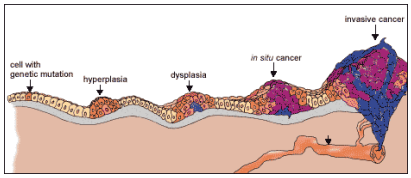


Tumor classification

- If a tumor's cells have dedifferentiated (lost all tissue-specific traits), its origin can not be readily identified; such tumors are said to be **anaplastic**.
- Benign tumors may be **hyperplastic** or **metaplastic**. Hyperplastic tissues are normal except for an excessive number of cells, whereas metaplastic tissues show displacement of normal cells by normal cell types not usually encountered at that site.
- **Dysplastic tumors** contain cells that are cytologically abnormal. Dysplasia is a transitional state between completely benign and premalignant.
- **Adenomas, polyps, papillomas, and warts** are dysplastic epithelial tumors that are considered to be benign because they respect the boundary created by the basement membrane.

Tumor classification

- **typing** = histological type
- **grading** = benign × malignant
- **staging** = TNM classification (T = tumor, N = node, M =metastasis)



Interaction of tumor with the host

local effects of tumor

- mechanical compression (eg. brain tumors)
- obstruction (e.g. carcinoma of the ductus choledochus)
- bleeding, bruising (leukaemia)
- chronic blood losses into GIT (gastric and intestinal tumors)
- oedema (e.g. lymphomas)
- coughing (lung carcinoma)
- thromboses
- difficult swallowing (oesophageal carcinoma)
- loss of vision (compression of optic nerve by hypophyseal adenoma)
- voice changes (laryngeal carcinoma)
- pathological fractures (myeloma)

Interaction of tumor with the host

systemic effects of tumor

- **anemia** (supression of bone marrow) – effect of proinflammatory cytokines
- **fever** - production of cytokines (pyrrogens) by tumor (IL-1, TNFα)
- **tumor cachexia** – anorexic mediators (TNFα)
- **paraneoplastic syndromes** – some tumors produce hormones (adenomas); important diagnostically!
  - – pigmentation
  - – endokrinopathy (Cushing sy., hypercalcaemia)

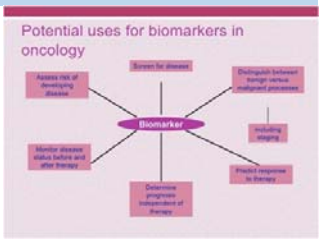
Cancer biomarkers

- Cancer biomarkers are substances that are produced in response to cancer processes.
- These substances can be found in the blood, urine, stool, tumor tissue, or bodily fluids.
- Most cancer biomarkers are proteins. However, patterns of gene expression and changes in DNA can be used.

Cancer biomarkers

Cancer biomarkers can be classified into the categories based on their usage:

- ***Predictive biomarkers*** predict response to specific therapeutic interventions (positivity/activation of *HER2* that predicts response to trastuzumab in breast cancer).
- ***Prognostic biomarkers*** aim to inform regarding the risk of clinical outcomes such as cancer recurrence or disease progression.
- ***Diagnostic biomarkers*** are used to identify whether a patient has a specific disease.



Cancer biomarkers - examples

Alpha-fetoprotein (AFP)

- Cancer types: Liver cancer and germ cell tumors
- Tissue analyzed: Blood
- How used: To help diagnose liver cancer and follow response to treatment; to assess stage, prognosis, and response to treatment of germ cell tumors

BCR-ABL fusion gene (Philadelphia chromosome)

- Cancer type: Chronic myeloid lukemia, acute lymphoblastic leukemia, and acute myelogenous leukemia
- Tissue analyzed: Blood and/or bone marrow
- How used: To confirm diagnosis, predict response to targeted therapy, and monitor disease status

Cancer antigen (CA) 15-3

- Cancer type: Breast cancer
- Tissue analyzed: Blood
- How used: To assess whether treatment is working or disease has recurred

Cancer biomarkers - examples

HER2/neu gene amplification or protein overexpression

- Cancer types: Breast cancer, gastric cancer
- Tissue analyzed: Tumor
- How used: To determine whether treatment with certain targeted therapies is appropriate

Prostate-specific antigen (PSA)

- Cancer type: Prostate cancer
- Tissue analyzed: Blood
- How used: To help in diagnosis, assess response to treatment, and look for recurrence

Carcinoembryonic antigen (CEA)

- Cancer types: Colorectal cancer and some other cancers
- Tissue analyzed: Blood
- How used: To keep track of how well cancer treatments are working or check if cancer has come back

Thank you for your attention