Cancer Genetics
What is Cancer?

• Cancer is a term used for diseases in which abnormal cells divide without control and are able to invade other tissues. Cancer cells can spread to other parts of the body through the blood and lymph systems.

• Cancer is not just one disease but many diseases. There are more than 100 different types of cancer. Most cancers are named for the organ or type of cell in which they start - for example, cancer that begins in the colon is called colon cancer; cancer that begins in basal cells of the skin is called basal cell carcinoma.
Cancer types can be grouped into broader categories. The main categories of cancer include:

- **Carcinoma** - cancer that begins in the skin or in tissues that line or cover internal organs.
- **Sarcoma** - cancer that begins in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue.
- **Leukemia** - cancer that starts in blood-forming tissue such as the bone marrow and causes large numbers of abnormal blood cells to be produced and enter the blood.
- **Lymphoma and myeloma** - cancers that begin in the cells of the immune system
- **Central nervous system cancers** - cancers that begin in the tissues of the brain and spinal cord.

http://www.cancer.gov
Basic terminologies

Oncogenesis = process of initiation of tumours (cancer) in an organism (onkos = mass; genesis = birth)

Tumour = tissue composed of cells that deviate from normal program of cell division and differentiation.

Benign tumour = tumour cells remain together in a single mass and do not invade or disrupt surrounding tissues

Malignant tumour = tumour cells invade and disrupt surrounding tissues (and are diagnosed as cancer).

Metastasis = spread of malignant tumour cells throughout the body (typically through the blood and lymphatic system)
Carcinogens-chemicals

Carcinogen = natural or artificial agents that increases the frequency of mutations and cancerous cells.

- Sir Percival Pott first correlated scrotal cancer with exposure to coal soot in chimney sweeps in 18th century Britain.
- Many present day cancers arise from occupational exposure risks to chemicals: asbestos, PVCs.
- Tobacco smoke and diet implicated in ~50-60% of U.S. cancers.
- Two types of chemical carcinogens cause point mutations:
  - Direct acting carcinogens mutate DNA directly (e.g., alkylating agents)
  - Procarcinogens are metabolically converted to carcinogens (e.g., cigarette smoke, aflatoxins [fungi], nitrosamines)
Carcinogens-radiation:

Sources of radiation include the sun, cell phones, radon gas, electric power lines, and household appliances.

• ~2% of cancers deaths are caused by radiation.

• Ionizing radiation (x-rays, radon gas, radioactive material) can cause leukemia and thyroid cancer.

• Ultraviolet light:
  
  • **UV-B (290-320 nm)** is the main cause of sunburn and is directly mutagenic.
  
  • **UV-A (320-400 nm)** increases the effects of UV-B on skin.
Cancer Arises From Gene Mutations

Germline mutations

- Present in egg or sperm
- Are heritable
- Cause cancer family syndromes

Somatic mutations

- Occur in non-germline tissues
- Are non-heritable

Mutation in egg or sperm → All cells affected in offspring

Somatic mutation (e.g. breast)
• Usually these mutations are **acquired**, but occasional tumors arise because of **inherited** mutations passed on through the germ line from one or both parents.

• The mutations can be thought of as broadly affecting genes that regulate the growth, differentiation and normal turn-over of a variety of cells.

• Collectively, these genes are referred to as **cancer-associated genes**.
Single Cell Origin

• Most cancers are believed to originate from a single parental cell that undergoes a mutational event leading to an altered pattern of growth. All of the progeny of this cell are said to be clonal (or monoclonal) in origin.

• The initial cellular mutation is usually not sufficient for the development of full malignant potential.

• Rather the mutated clone of cells acquires a growth advantage over its neighboring cells and is typically “immortalized”.
Cancer Associated Genes

These include a very diverse group of genetic elements, which when over-expressed, under-expressed or mutated result in a cell with abnormal growth characteristics.

1. Proto-oncogenes
2. Tumor-suppressor genes
3. Apoptosis genes
4. DNA repair genes
Types of Genetic Alterations in Cancer

• Subtle alterations
• Chromosome number changes
• Chromosomal translocation
• Amplifications
• Exogenous sequences
Subtle Alterations

- Small deletions
- Insertions
- Single base pair substitutions
  - (Point mutations)
Point Mutations

Normal       THE BIG RED DOG RAN OUT.
Missense     THE BIG RAD DOG RAN OUT.
Nonsense     THE BIG RED.
Frameshift   THE BRE DDO GRA.
Frameshift   THE BIG RED ZDO GRA.

Point mutation: a change in a single base pair
Chromosome Number Changes

• Aneuploidy
  – somatic losses or gains

• Whole chromosome losses often are associated with a duplication of the remaining chromosome.

• LOH
  – loss of heterozygosity
Chromosome Translocations

- **Random translocations**
  - breast, colon, prostate (common epithelial tumors)
- **Non-random translocations**
  - leukemia, lymphoma
Amplifications

• Seen only in cancer cells
  – 5 to 100-fold multiplication of a small region of a chromosome
• “Amplicons”
  – contain one or more genes that enhance proliferation
• Generally in advanced tumors
Exogenous Sequences

• Tumor viruses
  – contribute genes resulting in abnormal cell growth

• Cervical cancer
  – HPV (human papilloma viruses)

• Burkitt’s lymphoma
  – EBV (Epstein-Barr virus)

• Hepatocellular carcinoma
  – hepatitis viruses
Avoid Cancer Viruses

The most common cancer-causing virus in the United States is the human papillomavirus (HPV), which is involved in the transmission of cervical cancer. Since this virus is sexually transmitted, its spread can be combatted using the same "safe sex" approaches that are recommended to prevent the spread of HIV—e.g., limiting exposure to multiple sexual partners.
Cell Cycle Regulation

Cancer results from alterations in critical regulatory pathways that control cell proliferation, differentiation and survival.

Two classes of genes frequently are mutated in cancer:

- Proto-oncogenes (⇒ oncogenes) – promote cell growth
- Tumour suppressor genes – inhibit cell growth
Oncogenes

- Oncogenes are genes whose presence in certain forms and/or overactivity can stimulate the development of cancer.

- Oncogenes arise from the mutation of proto-oncogenes.

- When oncogenes arise in normal cells, they can cause the cells to become malignant.

- Since they are mutant forms of proto-oncogenes, oncogenes resemble proto-oncogenes in that they code for the production of proteins involved in growth control.

- However, oncogenes code for an altered version (or excessive quantities) of these growth-control proteins, thereby disrupting a cell's growth-signaling pathway.
Oncogenes Act Like an Accelerator

• By producing abnormal versions or quantities of cellular growth-control proteins, oncogenes cause a cell's growth-signaling pathway to become hyperactive.

• To use a simple metaphor, the growth control pathway is like the gas pedal on the automobile, controls how fast cells grow and divide.

• The presence of an oncogene is like having a gas pedal that is stuck to the floorboard, causing the cell to continually grow and divide.

• Aurora kinase A, H-Ras, Myc, EGFR etc.
Oncogenes

Normal genes (regulate cell growth)

1st mutation (leads to accelerated cell division)

1 mutation sufficient for role in cancer development
Many cancer cells display abnormalities in chromosome structure: translocations, duplications and deletions.

These gene rearrangements frequently lead to the generation of oncogenes.

The first characterized example of oncogene activation by chromosome translocation was the involvement of c-myc oncogene in Burkitt’s lymphoma- Chr 8 to IgH on Chr 14.
Tumor Suppressor Genes

- Normal genes (prevent cancer)
- 1st mutation (susceptible carrier)
- 2nd mutation or loss (leads to cancer)
Tumor Suppressor Genes

Key Attributes

- Familial Cancer Syndromes
- Inactivation in Common Human Cancers
  - Loss of Heterozygosity
- “Recessive” at a cellular level
- Two-hit hypothesis
Tumor Suppressor Genes

Familial Cancer Syndromes

• Most familial cancer syndromes are related to Tumor Suppressor Genes
  – Retinoblastoma, Li-Fraumeni, Familial Breast-Ovarian, Melanoma, Tuberous Sclerosis...

• Only 3 known syndromes related to Oncogenes
  – RET, MET, CDK4

• Few DNA repair syndromes
  – XP, AT, Bloom, Fanconi, Werner, HNPCC
Tumor Suppressor Genes

• Loss of Heterozygosity (LOH)
• 2 copies of each gene
• 1 is lost or inactivated
• Only 1 remains...
  – no longer heterozygous
  – one copy of a defective gene, same as no gene
Mechanisms Leading to Loss of Heterozygosity

Loss of normal allele

Chromosome loss
Deletion
Unbalanced translocation
Loss and reduplication
Mitotic recombination
Point mutation
The Two-Hit Hypothesis

First hit

First hit in germline of child

Second hit (tumor)
TSG: TP53

- The **TP53** gene may have more to do with the development of human cancer than any other component of the genome.

- Encodes p53, which is a polypeptide having a MW of 53,000 daltons.

- Recognized as a TSG in 1990 - when absent, is responsible for a rare inherited disorder called **Li-Fraumeni syndrome**.

- **Anti-tumour weapon** - approximately 50% of all human cancers contain cells with point mutations or deletions in both copies of the p53 gene.

- **Indicator of tumour’s virulence**; cells with p53 mutations tend to be more invasive and more likely to metastasize and also correlated with a poorer survival rate.

- referred to as the “**guardian of the genome**”.

• The main functions of the Tp53 protein are cell cycle arrest through transactivation of p21$^{Cip1/waf1}$; genome stability through transactivation of GADD45; and apoptosis through transactivation of the proapoptotic Bax and suppression of transcription of antiapoptotic Bcl-2.

• The main regulators of Tp53 are Mdm2, p14$^{ARF}$ and a relatively newly identified protein, Mdm4.
Retinoblastoma

- Retinoblastoma alleles are recessive; only homozygotes (RB/RB) develop tumours.

- Retinoblastoma appears as dominant in pedigree analysis:
  - RB/RB+ individuals are predisposed and have a significant incidence of the disease.
  - Homozygous dominant individuals (RB+/RB+) require two mutations in the same cell to develop the cancer.

- Retinoblastoma was mapped to the long arm of chromosome 13 (13q14.1-q14.2).

- Retinoblastoma is rare among cancers; most cancers result from a series of mutations in many different genes.
TSG: Retinoblastoma

• Role of the product of the RB gene

• Cell cycle importance in cell growth and proliferation

• Primary role of pRB is in regulating the passage of cells from G₁ stage into S phase, during which DNA synthesis occurs.

• Pivotal role in cancer initiation (classical model)
Features of Retinoblastoma

- 1 in 20,000 children
- Most common eye tumour in children
- Occurs in heritable and nonheritable forms
- Identifying at-risk infants substantially reduces morbidity and mortality
The **RB1** Gene

- Large gene spanning 27 exons, with more than 100 known mutations
- Gene encodes Rb protein which is involved in cell cycle regulation
Alfred Knudson’s (1971) model for retinoblastoma

- Two mutations are required for the development of retinoblastoma.

**Sporadic retinoblastoma**

- Child starts with two wild type alleles (RB+/RB+).
- Both alleles must mutate to produce the disease (RB/RB).
- Probability of both mutations occurring in the same cell is low; only one tumour forms (e.g., one eye).

**Hereditary retinoblastoma**

- Child starts with heterozygous alleles (RB/RB+).
- Only one mutation is required to produce disease (RB/RB).
- Mutations resulting in loss of heterozygosity (LOH) are more probable in rapidly dividing cells, and multiple tumours occur (e.g., both eyes).
Presentations of Retinoblastoma

- Nonheritable: ~60%
- Heritable: ~40%

All Retinoblastoma

- Bilateral: ~80%
- Unilateral: ~20%

Heritable Retinoblastoma (rare)

Trilateral (rare)
Sporadic retinoblastoma

- 60% of retinoblastoma cases.
- Develops in children with no family history.
- Occurs in one eye.

Hereditary retinoblastoma

- 40% of retinoblastoma cases.
- Onset typically is earlier than sporadic cases.
- Multiple tumours involving both eyes.
- Consistent pedigrees; siblings and offspring develop the same type of tumours.
Genetic Features of Heritable Retinoblastoma

- Autosomal dominant transmission
- \textit{RB1} gene on chr 13 (first tumor suppressor gene discovered)
- Penetrance >90%

Bilateral RB, 1 yr d. 78

Bilateral RB, 6 mo

Bilateral RB, 1 yr osteosarcoma, 16

Bilateral RB, 1 mo
Applied Question

1. A woman goes to see a genetic counselor, terribly distressed. Her sister developed breast cancer at age 52, and her husband’s sister at age 34. The woman’s mother was diagnosed with breast cancer at age 68. With these three cases in her family, she is convinced she has a mutant *BRCA1* gene and will suffer the same fate.

   a. What questions should the counselor ask her?
   
   b. Do you think she would benefit from a *BRCA1* gene test?
   
   c. What complications might arise from such testing?
Changes in Cell Growth and Behaviour

- Characteristics distinguishing cancer cells from their normal counterparts
- Provide description of malignancy
- Abnormalities in the mechanisms that are involve in the regulation of
  - normal cell proliferation
  - differentiation
  - survival
Density-dependent inhibition

- Uncontrolled proliferation *in vivo* is mimicked in culture
- Normal cells reach quiescent stage ($G_0$)
- Cancer cells have reduced dependent for extracellular growth factors
Limited proliferation capacity:

Vertebrate somatic cells divide a limited number of times (ca. 50-70 divisions for human cells) before the cells enter a senescent state that maintains metabolic activity but stops all further division.

This is known as the Hayflick limit (Hayflick, 1965).
Contact Inhibition

Normal cells

Tumor cells
Such abnormal production of a growth factor by a responsive cell leads to continuous autostimulation of cell division.
Additional Properties

• Affecting interactions with other tissue components -- roles in invasion and metastasis
  • Secretion of proteases that digest extracellular matrix components - invade adjacent normal tissues
    – e.g Secretion of collagenases
  • Secretion of growth factors that promote the formation of new blood vessels (angiogenesis)
    – support growth of a tumor beyond the size of about a million cells--also important in metastasis.
Other Properties Contd.

• Failure to differentiate normally
  – closely coupled to abnormal proliferation
  – blocked at an early stage of differentiation

  – e.g. leukemias -- relationship between defective differentiation and malignancy
  
  • From stem cells - become committed to specific differentiation pathways - form erythrocytes, lymphocytes, granulocytes or macrophages.
Detected differentiation and leukemia. Different types of blood cells develop from a multipotential (pluripotent) stem cell in the bone marrow. The precursors of differentiated cells undergo several rounds of cell division as they mature, but cell division ceases at the terminal stages of differentiation. The differentiation of leukemic cells is blocked at early stages of maturation, consistent with their continued proliferation.
Other Properties Contd.

- Failure to undergo apoptosis
  - programmed cell death
  - exhibit increased life spans
  - contributes substantially to tumor development
  - resistance to irradiation and many chemo-therapeutic drugs
  - unrelenting growth of tumor cells
Transformation of Cells in Culture

- *In vitro* assays to detect conversion of normal to tumor cells (cell transformation) -- focus assay
  - altered morphology
  - loss of contact inhibition
  - loss of density dependent growth
Examples of transformed cells

**3T3**: A minimally transformed mouse embryo fibroblast line, selected by plating frequently and at medium cell density. Aneuploid and immortal, grows on calf serum rather than fetal calf serum. Contact inhibited and anchorage dependent.

**3T12**: A related mouse embryo fibroblast cell line, selected by plating at low cell density. Not contact inhibited, but won't grow in suspension or on soft agar.

**K-RAS 3T3**: Properties similar to 3T12, but a little more so. Tumorigenic in when injected in nude mice.

**HeLa**: Human carcinoma cells; able to grow in suspension, highly tumorigenic.
Transformation and tumorigenicity

While transformation refers to the behaviour of cells grown in culture, there is a correlation in the degree of transformation \textit{in vitro} and the behaviour of such cells \textit{in vivo}, which can range from dysplasia, to hyperplasia, to full blown malignancy.

Tumorigenicity is not solely loss of proliferation control; the organism protects itself by directing cells with aberrant growth into the cell death pathways of apoptosis. Cancer results from cells which have regressed from the controlled phenotype in most or all of the regulatory mechanisms, and this is an indication that cancer involves the cumulative effect of multiple defects in the control pathways.
"Hallmarks" of tumorigenicity and cancer

Disregard of signals to stop proliferating and to differentiate

Autonomous generation of signals that promote growth

Capacity for sustained proliferation

Evasion of apoptosis

Motility and invasiveness

Angiogenesis
This theory focuses on the pH differences between tumors and normal tissue. Experiments have shown that tumors are more acidic than normal tissue.

These findings refer to the environment outside the cell, the extracellular pH. Despite this more acidic extracellular tumor environment, the level of pH inside tumor cells has been shown to be similar to normal cells (pH 7.0), or even slightly alkaline (pH 7.1-7.2).

This gives rise to a reversed pH gradient between tumors and normal tissue, which implies that cells within solid tumors are capable of maintaining their level of intracellular pH at normal levels, despite lower than normal levels of extracellular pH. These findings have important implications to anti-cancer therapies.
Tumour-Immune cell interactions

In response to potentially harmful challenges to the body, such as viruses and cancer cells, the body has evolved active defenses that compose the immune system.

It has been known for many years that immune cells can kill cancer cells by a diversity of mechanisms. However, new experimental evidence suggests that cancer cells also express these cell killing mechanisms.

This enables the tumor to mount a counterattack against the anti-cancer immune cells.
The photo shows an illustration of a macrophage (immune cell) engulfing two cells, detected as being foreign.
Skin cancer

• A simple molecular change can make normal skin cells behave just like cancer cells shedding light on how tumors develop.
• Scientists altered just one molecule which normally plays a role in how skin cells join up with other skin cells.
• But this produced huge shifts in the behaviour, spread and appearance of the resulting cells.
• In this case, the scientists at University of Chicago bred mice missing the gene which produces alpha-catenin, a molecule with a role in causing cells to stick together and form part of the normal structure of the skin.

• The mice suffered severe changes in the skin, which was thick and "disorganised" - the different cell layer types were distorted.
• The skin changes observed were very similar to those normally seen in squamous cell carcinoma, a pre-cancerous condition associated with overexposure to sunlight.

• Looking closer into how alpha-catenin loss could have this effect, the team found that system which regulates cell growth had been activated.

• Missing this molecule could lead to unregulated cell growth which characterizes many forms of skin cancer.
Most cancer cells are known to have numerous changes in their chromosomes that lead to such mutated genes. Comparative genomic hybridisation (CGH) is a technique that allows the identification of some of these changes.

This method involves the detection of chromosomal regions that have been amplified or deleted in tumour DNA (labelled green) by mixing it with normal DNA (labelled red). Regions of amplification in tumour DNA will be detected by an increased green signal and may indicate the presence of an oncogene.

Alternatively, deleted regions will be detected by an increased red signal and may contain a tumour suppressor gene.
CGH analysis detects amplification (green) and deletion (loss) of genetic material in a thyroid tumour.
Colon cancer as a model of carcinogenesis

Colon cancer is one of the common forms of the disease. It is estimated that by age 70, 50% of the population at large have acquired pre-cancerous adenomas; 10% of this group will progress to malignancy in the following 10 years. Two heritable genes predispose toward this class of cancer.
Familial Adenomatous Polyposis (FAP) is linked to the APC gene whose protein is involved in β-catenin signaling. The gene acts as a tumor suppressor, and the loss of function mutation causes development of hundreds to thousands of adenomas, with a consequent high risk of progression to malignancy.

Hereditary Non-Polyposis Colon Carcinoma (HNPCC) is a hereditary predisposition to carcinoma without the prior accumulation of adenoma.
APC protein, (Adenomatous Polyposis Coli) is normally expressed in colorectal epithelial cells, a site of relatively high natural proliferation rates. The epithelium is convoluted into deep recesses called crypts and projections called villi. Crypts contain stem cells for tissue replacement, and the base of the crypt is a site of high mitotic activity. As cells age, they progress up the villus to the tip. Aged cells may be resorbed by apoptosis or sloughed off.

APC concentration increases with cell age, hence correlates with height in the villus. A high concentration of APC induces apoptosis, due to negative effects on survival factors, and this can be demonstrated by ectopic expression of APC.
Loss of function mutations frequently appear as truncations to the first third of the normal polypeptide (about 80% of cases of FAP).

Experimental truncations that retain the central catenin/axin binding region maintain tumor the suppressive effect. The mutation itself appears as dominant in its tendency to FAP.

The early frequency of advance to adenoma suggests that the first step in manifestation of FAP is a somatic mutation affecting the wild-type allele. Large numbers of polyps appear by late teenage, and if untreated, cancer is a high risk by age 40.

Thus FAP represents a gross acceleration of the preliminary stages of cancer.
<table>
<thead>
<tr>
<th></th>
<th>Polyps</th>
<th>Adenomas</th>
<th>Progression to cancer</th>
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<tbody>
<tr>
<td><strong>Germline APC+/- FAP</strong></td>
<td>&gt;90% by age 20</td>
<td>&gt;90% by age 30</td>
<td>50% by age 40</td>
</tr>
<tr>
<td><strong>Germline APC++ normal</strong></td>
<td>50% by age 70</td>
<td></td>
<td>5%</td>
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<td></td>
<td>Cell accumulation and dysplasia</td>
<td>Hyperplasia, aneuploidy</td>
<td>Proliferating, anti-apoptotic, metastatic, angiogenic</td>
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Cancer invasion

• Solid tumors develop initially as a single mass of cells. Cancer invasion is the process in which cells break away from this primary tumor and crawl through surrounding tissue.

• This enables the cells to move into a blood vessel and be transported through the body, possibly establishing a secondary tumor (metastasis) at another site.
Cancer invasion involves a number of changes in cell behaviour, in particular the production of enzymes, called proteases, that will break down surrounding tissue.
Conclusion

• Types of Carcinogenes
• Types of Genetic Alterations in Cancer
• Oncogenes
• Tumor Suppressor Genes – Tp53 & pRB
• Knudson’s Two Hit Model