

LECTURE 31

GENETICS AND CANCER [VERY BRIEF]

1. A few definitions:

Cancer: Unregulated cell growth and division, where loss of contact inhibition leads to cell-stacking, forming tumors; essentially *abnormal gene expression*

Malignant: Cells detach from tumor and invade surrounding tissues

Benign: Cells do not detach from tumor and invade surrounding tissues

Metastasis: Secondary tumors in second-site tissues formed from malignancy

Familial (hereditary) versus sporadic (spontaneous) cancers

2. Cancers are the result of a disruption of the normal restraints on cellular proliferation. Recent studies suggest there may be as few as forty cellular genes in which mutation or some other disruption of their expression leads to unrestrained cell growth.

a) There are two classes of these genes in which altered expression can lead to loss of growth control:

(i) Genes that stimulate growth and cause cancer when hyperactive. Mutations in these genes typically are dominant. These genes (alleles) are called *oncogenes*.

(ii) Genes that inhibit cell growth and cause cancer when they are turned off. Mutations in these genes typically are recessive. These genes (alleles) are called *tumor-suppressor genes*.

3. Viruses are involved in cancers because they can either carry a copy of one of these genes or can alter expression of the cell's copy of one of these genes.

4. Many tumor-causing viruses are retroviruses (RNA genomes that utilize reverse transcriptase); the initial discovery (1910) was of a retrovirus (the Rous sarcoma virus) that caused tumors (sarcomas) in the connective tissue of chickens.

a) The Rous sarcoma virus possesses four genes:

gag, a capsid protein of the virion

pol, a reverse transcriptase

env, a protein of the viral envelope, and

v-src, a protein kinase (protein phosphorylase)

- b) The *v-src* gene is responsible for the ability of the virus to cause tumors in connective tissue. This was determined from experiments that demonstrated that a virus without *v-src* was infectious but did not cause tumors. *v-src* is an oncogene.
 - c) Proteins and gene sequences of viral oncogenes are related (very similar) to cellular proteins (and gene sequences). Viral oncogenes (*v-onc*) thus arise from mutations that have occurred in a cellular gene once it was picked up by the virus.
 - d) *v-src*, for example, is similar to a 'normal' gene in chickens. The major difference is that the 'normal' chicken gene (denoted *c-src*, where *c* = cellular) possesses introns, whereas *v-src* (the homologue in the virus) does not possess introns (and is a mutant allele)
 - (i) This suggests that *v-src* (and other viral oncogenes) may originate from reverse transcription of processed *c-src* mRNA (or mRNA from any cellular oncogene). The cellular counterparts of viral oncogenes are formally called *proto-oncogenes*
 - (ii) A form of selection *at the viral level* is implicated, as viruses carrying oncogenes can transduce genes that induce cell proliferation and produce new cells for viral progeny to infect
 - (iii) Once a *v-onc* has been inserted into a host genome, it become associated with promoters and/or enhancers of the virus and can be over expressed
5. Studies to date have documented approximately 40 different viral oncogenes. Some viruses can have more than one oncogene. All known oncogenes appear associated in some fashion with regulation of gene expression.
- a) Some are related to genes that encode cell growth factors (e.g., wound healing).
 - b) Some are related to genes that encode hormone (growth factor) receptors, i.e., are associated with transcription factors.
 - c) Many encode kinases, especially tyrosine kinases that are hypothesized to play roles in regulating important effectors such as cyclic AMP (involved in gene expression).
 - d) Many directly encode transcription factors.
6. *Proto-oncogenes* -- cellular homologues to viral oncogenes ('control' cell proliferation):
- a) Numerous *c-onc* (cellular or 'normal' oncogenes) are now known, and were identified in genomic DNA by using *v-onc* (viral oncogenes) sequences as probes.
 - b) The DNA sequences of various cellular oncogenes appear highly conserved across organisms, suggesting important cellular functions. Present data indicate that virtually all proto-oncogenes are genes involved in regulation of cell growth and/or gene expression.

7. Mutated cellular oncogenes (proto-oncogenes) and cancer:

- a) Many human cancers are associated with mutations in cellular oncogenes. The first one discovered was *c-H-ras* (a proto-oncogene), the homologue of an oncogene in the Harvey strain of the rat sarcoma virus. An allele of *c-H-ras* that possessed a simple, missense base substitution (a glycine at amino-acid position 12 was changed to valine) was discovered in cells from a human bladder cancer.
- (i) Further study demonstrated that the mutant *c-H-ras* allele produced a protein that remained in an active signaling mode and stimulated cells to divide uncontrollably.
- b) Mutants of various *c-ras* proto-oncogenes are now known to be associated with a variety of human tumors in various tissues, including lung, colon, mammary, bladder, nerve cells, and connective tissue.

8. Chromosomal rearrangements and cancer

- a) Certain types of cancer are associated with chromosomal rearrangements (aberrations).
 - (i) The best known is chronic myelogenous leukemia. This leukemia is associated with a translocation involving human chromosomes #22 (the Philadelphia chromosome) and chromosome #9.
 - (ii) The translocation break point on chromosome #9 is in the *c-abl* oncogene (*v-abl* is Abelson murine leukemia virus); whereas the breakpoint in chromosome #22 is in a gene that encodes a tyrosine kinase. The *c-abl* oncogene (on chromosome #9) also encodes a tyrosine kinase.
 - (iii) Others now known include:

Burkitt's lymphoma	<i>c-myc</i>	Chromosomes 8 – 14
Acute myeloblastic leukemia	<i>c-mos</i>	Chromosomes 8 -- 21
Acute promyelocytic leukemia	<i>c-fes</i>	Chromosomes 15 -- 17
Acute lymphocytic leukemia	<i>c-myb</i>	Deletion on chromosome 6
Ovarian cancer	<i>c-myb</i>	Chromosomes 6 -- 14

9. Tumor-suppressing genes:

- a) Normal alleles of oncogenes such as *c-ras* appear to produce proteins that regulate the cell cycle. Mutant alleles at several oncogenes appear to produce defective proteins that cause over-expression of gene product.
- b) Some human cancers appear to be associated with “loss-of-function” mutations in genes whose products are involved in cell cycle regulation. Such genes (in the wild-type state) are called *tumor-suppressing genes*.

CYTOGENETICS -- THE STUDY OF CHROMOSOMES

1. Microscopic observation of chromosomes:

- a) Metaphase chromosomes are used in most cytogenetic analysis out of necessity. They are somewhat limited in terms of molecular information because of generally small size. For example, the longest chromosome in the human mid-metaphase complement is about 15 microns.
- b) With metaphase chromosomes, one can usually identify relative chromosome size, position of the centromere (primary constriction), and position(s) of secondary constriction(s) (NORs).
- c) Metaphase banding technology helps tremendously in terms of identifying homologous chromosomes and particular chromosomal regions (e.g., constitutive heterochromatin).
- d) Approaches include G- (or R-) banding, C-banding, NOR-banding, and a variety of 'banding' that employs various fluorochromes. When used in *in situ* hybridization with fluorochrome-tagged DNA (or RNA probes), the method is called FISH (fluorescent in situ hybridization).
- e) A drawback (in addition to small size) to analysis of metaphase chromosomes is that one is not examining meiotic chromosomes (i.e., not stages when homologous chromosomes are attempting to pair).

2. Polytene chromosomes in *Drosophila* overcome these two major drawbacks, in that polytene chromosomes are at interphase (and exceedingly large in comparison to metaphase chromosomes) and pairing (somatic synapsis) occurs between homologous chromosomes.

3. Reasons to study chromosomes:

- a) Visual identification of major chromosomal rearrangements (aberrations)
- b) Physical localization of genes (DNA sequences) through deletion mapping (pseudodominance) or more commonly through *in situ* hybridization

4. Chromosomal aberrations:

- a) Most arise from chromosome "breaks" where physical breakage occurs, followed by repair of chromosomal breakage but where segments of chromosomes have been rearranged. Many chromosome breaks arise spontaneously. However, the frequency of chromosome breakage (and chromosomal rearrangement) increases dramatically upon exposure to chromosome-damaging mutagens such as ionizing radiation or certain chemicals. Many chromosome breaks also arise after induction of transposable elements.
- b) We will discuss at least three types of major chromosomal aberrations.

Inversions: These are two-break events occurring on the same chromosome, followed by “inversion” of the chromosome fragment between breaks, and then by repair of the breaks.

1. There are two types of inversions: paracentric (not involving or including the centromere) and pericentric (involving or including the centromere).
 - a) For a number of reasons, inversion sequences are treated as alleles at a single locus and viewed from a population genetics perspective. With a single inversion there will thus be three inversion ‘genotypes’ in a population, i.e.,

st/st -- standard homozygote

st/in -- standard/inversion heterozygote

in/in -- inversion homozygote

- b) Inversions, when heterozygous, will form a characteristic “inversion loop” that will define the size and type of inversion. Note that both types of chromosomal homozygotes pair normally.

Translocations: These are “two-break” events that result in exchanges between non-homologous chromosomes

1. Translocation heterozygotes form a “pachytene” cross when homologous chromosomes attempt to pair. This “cross” will define the extent of a translocation. Note that both types of chromosomal homozygotes pair normally.

Robertsonian fusions/dissociations:

1. These are when two, non-homologous acrocentric/telocentric chromosomes “fuse” to form a metacentric chromosome, or where a metacentric chromosome “dissociates” to form two, non-homologous acrocentric chromosomes.
 - a) Robertsonian rearrangements may be true centric fusion/dissociation, or they may be translocations. Centromere (Cd-staining of chromosomes) suggests the former.