

Molecular Cell Biology
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Module 9
Molecular Basis of Cancer, Oncogenes and Tumor Suppressor
Genes

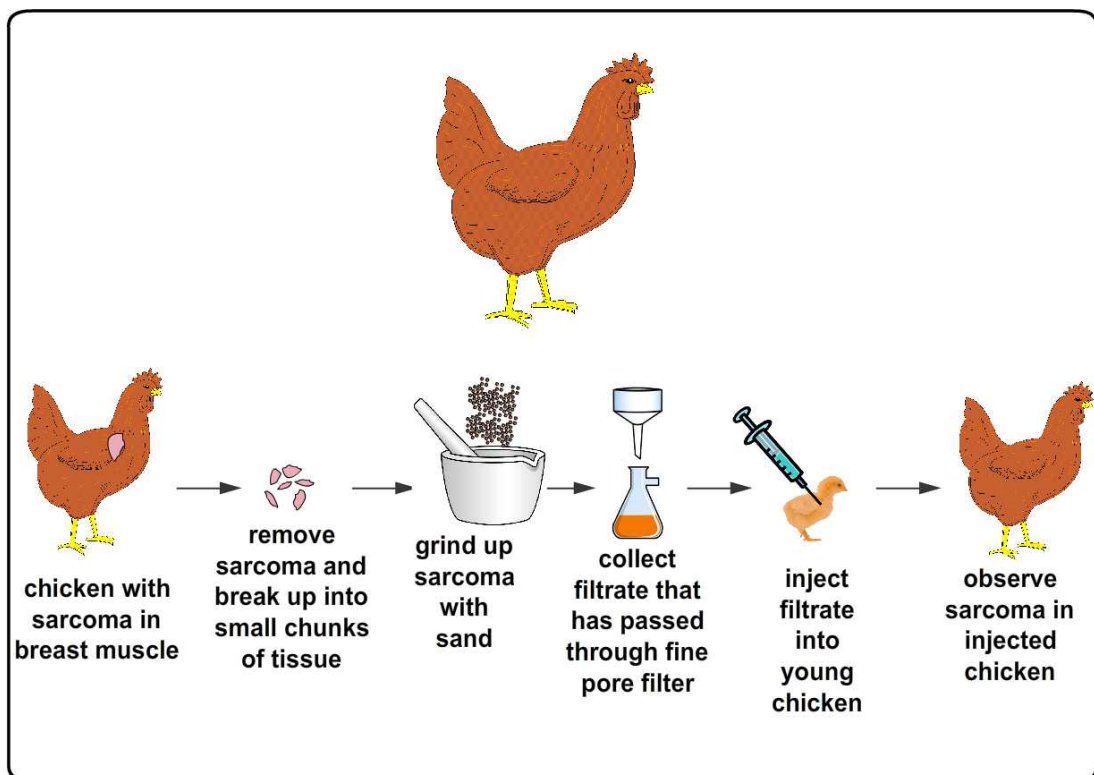
Lecture 2
Genes Associated with Cancer

- As we discussed there are about 300 genes associated with cancer.
- They can be classified into four types based on their functions.
 - Oncogenes – growth-promoting.
 - Tumor suppressor genes – growth-inhibiting/Anti oncogenes
 - Pro/anti apoptotic genes – regulate programmed cell death
 - DNA Repair genes – involved in DNA repair

Discovery of Oncogenes

Let us discuss how oncogenes were discovered

- In 1911, Peyton Rous discovered that a “cell-free” filtrate from the chickens affected by sarcomas could be used to induce tumors in normal chickens.



- The active agent was later identified as a retrovirus called “Rous sarcoma virus”
- This was the first demonstration of a virus causing cancer
- A single gene, *src*, was identified to be responsible for these cancers. The first oncogene was thus identified.
- In 1981, Michael Bishop and Harold Varmus found that normal cells contain a gene homologous to the viral *src* gene
- v-SRC and c-SRC

- *C-SRC was called a proto oncogene - potential to become activated as oncogene. An oncogene is dominant with a gain of function mutation*

Discovery of Ras oncogene

- If viruses could induce tumors by mutation of a normal cellular gene, can chemical carcinogens also do it?
- Robert Weinberg and his group have indeed shown that DNA extracted from chemically transformed Mouse fibroblasts could transform normal mouse fibroblasts.
- Identification of that DNA turned out to be the Ras oncogene which was previously known to be associated with sarcoma viruses.
- In 1982 a human oncogene derived from the bladder tumour cell line EJ was shown to be homologous to the oncogene of Harvey murine sarcoma virus and the cellular proto-oncogene was designated HRAS. The oncogene carried a point mutation—a single nucleotide substitution— (Gly-Val) in comparison to its counterpart protooncogene
- Also identified was a homologue of the Kirsten murine sarcoma virus (KRAS2) and a new member of the ras gene family NRAS, that has no viral counterpart.
- Further experiments showed that a single oncogene could not transform fully normal rat cells into cancer cells.
- Two and maybe even more oncogenes were required to effect this conversion. Ras and myc; Ras and adenoviral E1A. Cooperative nature of oncogenes was revealed by these experiments
- What do these oncogenes do? Like all the other genes, they also encode for Proteins. What kinds of proteins?
- These proteins are the work horses that promote proliferation.
- We are talking about growth and as can be expected, the oncogene products are
 - Growth factors
 - Growth factor receptors
 - Cell cycle regulators
 - Cell death regulators

- Protein kinases
- Transcription factors
- Signaling intermediates such as Ras

Oncogenes encoding growth factor

Oncogene	GF	Cancer
v-sis	PDGF	Fibrosarcoma
int-2	FGF-3	Breast
k-fgf/hst	FGF-4	gastric cancer, Kaposi sarcoma
trk	NGF	Neuroblastoma
wnt-1	WNT ligand	many carcinomas

Oncogenes encoding growth factor receptors

Oncogene	receptor	Cancer
erb-b1	EGFR	sccs of lung and many other tissues
neu	HER-2/Neu	breast, ovary
met	HGFR	Osteosarcoma
fms	CSF-1 receptor	Sarcoma
ros	Insulin receptor	Astrocytoma
mpl	thombopoietin receptor	leukemia

Cell cycle regulators

CYCD1	Cyclin	breast, lymphoma
CDK4	Cdk4	sarcoma, glioblastoma

Cell death regulators

Bcl-2	BCL-2	follicular lymphoma
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Protein kinases

Bcr-abl	Abl	CML
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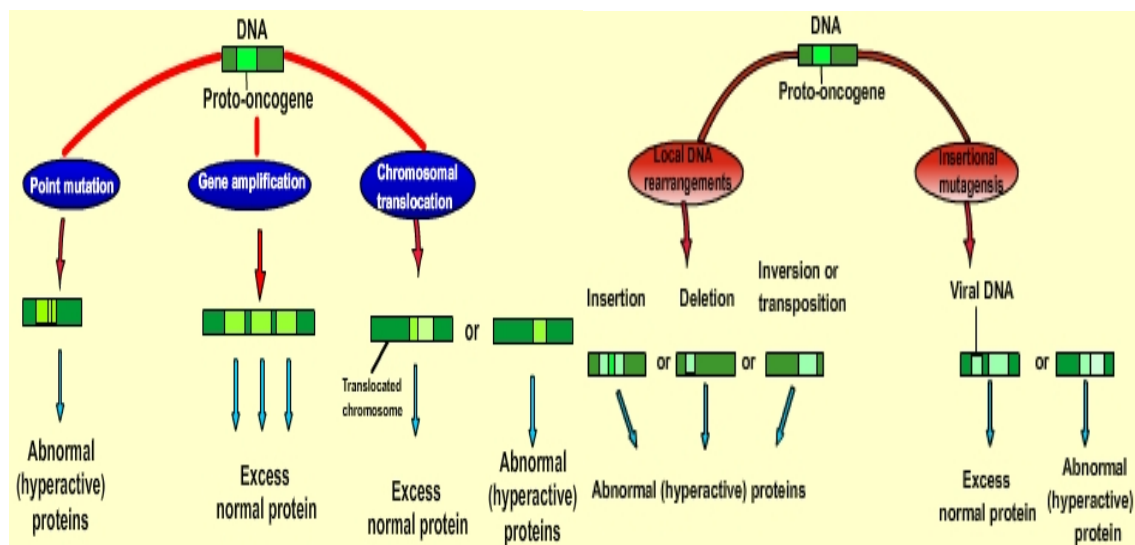
Src	SRC	Colon
Lck	lck	Colon
Jak	JAK	Leukemia
Raf	raf	Sarcoma
Mos	mos	Sarcoma
Akt	akt	breast, ovarian, pancreatic

Transcription factors

Myc	myc	Burkitt's lymphoma
Jun	JUN	Osteosarcoma
Fos	fos	Sarcoma
Myb	myb	Leukemia
Rel	NF-kB	leukemia

- We have learnt that Virus-induced alterations – may introduce their oncogenes into the cells.
- All cancers are not caused by viruses.
- What are the mechanisms by which a proto-oncogene is activated to become oncogene?

Proto-oncogenic Conversion



Molecular Mechanisms of oncogene activation

- Deletion or point mutation – may result in the synthesis of hyperactive protein without change in the normal amount of protein.
- A single point mutation in a Ras proto-oncogene can lead to altered gene function.
- Ras proteins are small (21 kDa) guanosine triphosphatases (GTPases) GTP (active) and GDP-bound (inactive) state.
- Guanine nucleotide exchange factors (GEFs), increase the amount of GTP-bound Ras.
- GTPase activating proteins (GAPs) inactivate Ras to GDP-bound form.

Molecular Mechanisms of oncogene activation

- Ras oncogenes are mutated in 30% of human cancers - colorectal and lung cancers and several forms of leukemia
- in some cancers such as pancreatic carcinoma the frequency is as high as 90% K-RAS.
- glycine to valine mutation at *residue 12* or Gly to Asp at residue 13.
- mutation of Glutamine 61 to Lysine.
- Ras is an important signaling intermediate in the growth factor signaling via receptor tyrosine kinases and its activation leads to the activation of the MAPK pathway .

Gene amplification – multiple copies of a gene - over production of normal protein

- Two distinct patterns of amplification noticed.
- high level amplification from a small region of DNA.
- In contrast, amplification may also cover many megabases of DNA and contain variations in copy number across the amplicon.
- Double minute chromosomes (DMs) and homogenously staining regions (HSRs) within chromosomes contain high-level amplification of specific genomic regions.

Gene amplification – multiple copies of a gene - over production of normal protein

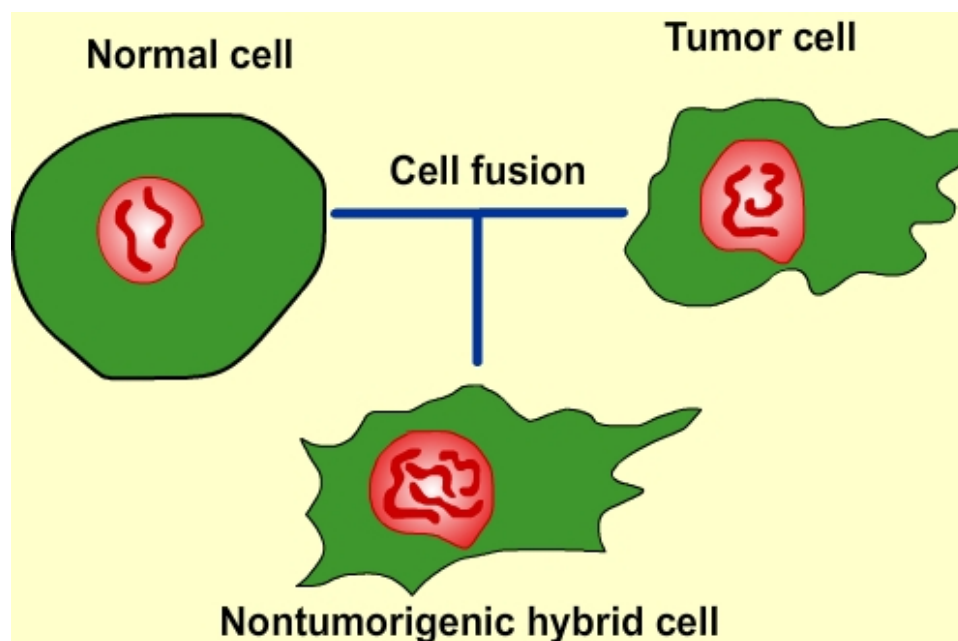
- Extra copies may be from unusual segregation at mitosis of extrachromosomal fragments.
- Amplicons are frequently found near the fragile sites in the genome.
- Amplification of MYC (8q24) was identified in the acute lymphocytic leukaemia .
- MYCN (2p24), is amplified in many neuroblastomas .
- MYCL1 (1p34), is amplified in cases of small cell lung carcinoma (SCCL).
- Amplification of ERBB2 (also known as HER2) occurs in breast cancer.
- Chromosome rearrangements may form a fusion protein that is hyperactive. This mechanism is found predominantly in leukaemias, lymphomas and sarcomas.
- Translocation of the oncogene next to the regulatory elements in immunoglobulin B- lymphocyte malignancies or T-cell receptor genes in T-lymphocyte malignancies may lead to inappropriate expression of the oncogene. This could result in the over production of normal protein

Translocations

- Chromosomal translocations are found in both chronic and acute haematopoietic malignancies and in some solid tumours of mesenchymal origin.
- a gene close to the breakpoint on one derivative chromosome is not altered in its structure but its pattern of expression is altered.
- The second is that the structure of a gene (or genes) at the breakpoints is altered. Commonly this involves the fusion of exons from two different genes to give rise to a novel transcript and fusion protein product with altered or novel function.
- Several examples of the first type of translocation involve the MYC gene Burkitt's lymphoma, t(8;14)(q24;q32), t(2;8) (p12; q24), and t(8;22)(q24;q11) in which the MYC gene is activated by translocation to the region of an immunoglobulin gene.

- Immunoglobulin and T-cell receptor genes are involved in many such translocations as they have a natural propensity to re-arrange in the generation of antibody diversity and errors in the process can occasionally lead to interchromosomal translocation.
- An example of the second type of translocation is the 9;22 translocation in chronic myelogenous leukaemia that results in the fusion of the Abelson gene (ABL) on 9q34 with the BCR gene on chromosome 22. Both genes are broken in introns resulting in the formation of an in-frame fusion mRNA and protein that is unique to these tumour cells. Almost all CML patients show this translocation and express an abnormal 210 kDa protein (normal ABL protein 145 kDa). The derivative chromosome 22 is known as the Philadelphia (Ph) chromosome.
- A Ph chromosome is also found in some cases of acute lymphoid and myelogenous leukaemia and but here the breakpoint in the BCR gene is different and the fusion protein is 190 kDa. Functional assessment of these two fusions proteins in vivo indicates that the transforming ability of the 190 kDa protein is greater, which may explain its association with acute rather than chronic disease.

Cell Fusion

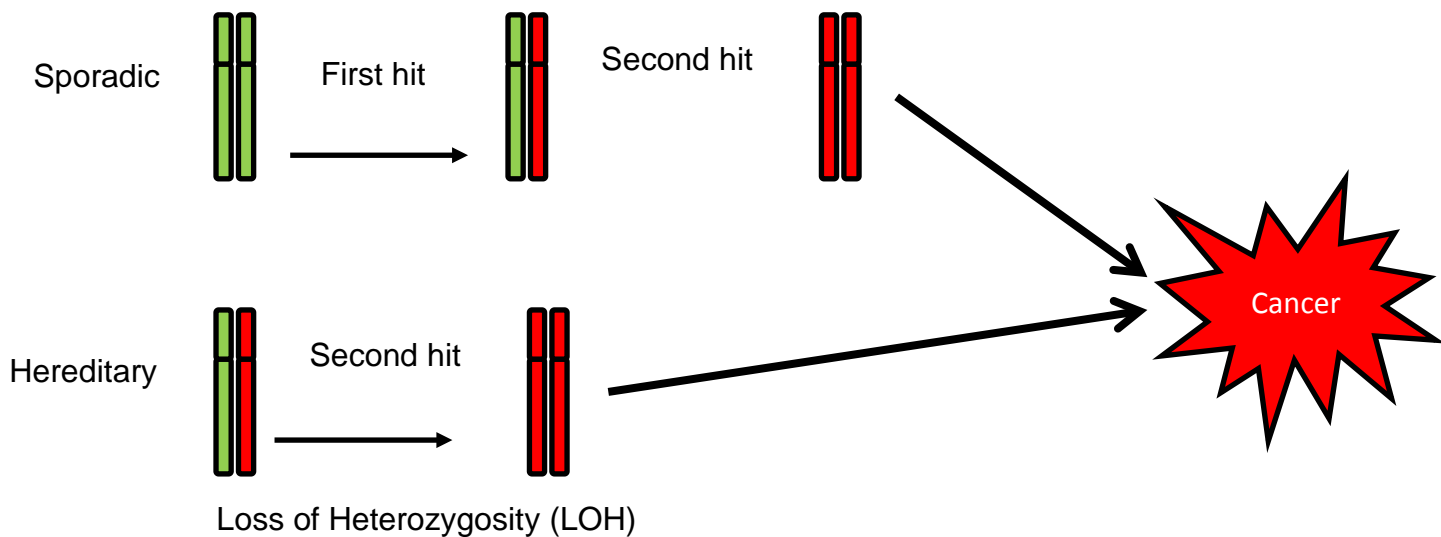


- In the case of oncogenes we mentioned that there is a gain of function as a result of a mutation whereas in the case of tumor suppressor genes mutations result in loss of function of the genes.
- Mutation is generally in the form of deletion.
- Tumor suppressor genes function by inhibiting the cell growth or inducing cell death (apoptosis).

Tumor Suppressors

Usually inactivating mutations lead to an insensitivity to growth-inhibitory signals

Knudson's Two-hit hypothesis



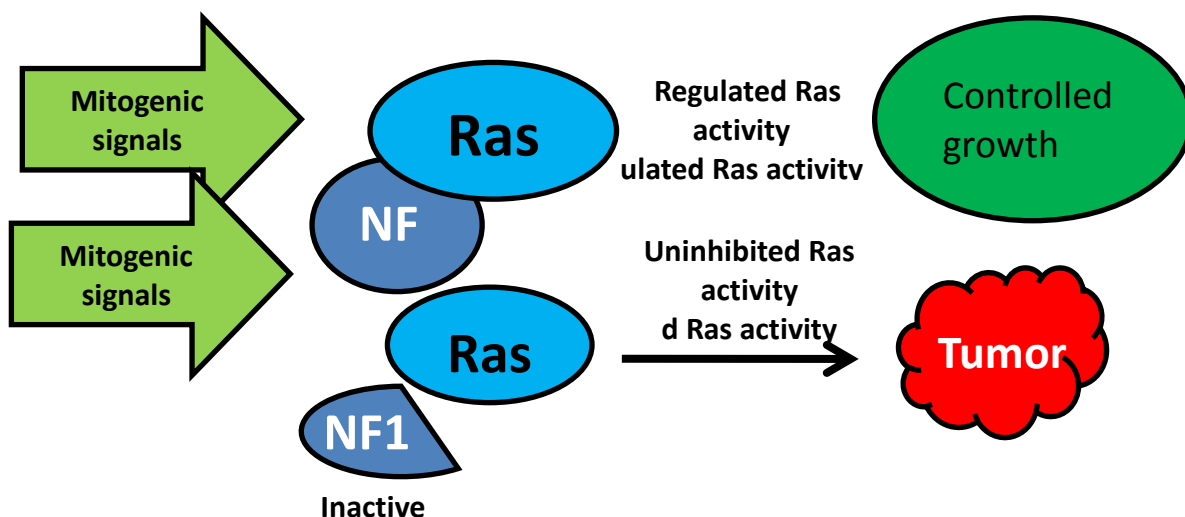
Example of Human Tumor Suppressor Genes

Gene	Inherited Syndrome	Cancer Type
APC	Familial adenomatous polyposis	Colon
BRCA1	Familial breast cancer	Breast, ovary
BRCA2	Familial breast cancer	Breast
SMADA	Colorectal cancer	Colon,rectal
NF-1	Neurofibromatosis type-1	Neurofibromas
NF-2	Neurofibromatosis type-2	Schwann cells,meninges
CDKN2A	Familial melanoma	Melenoma,others
P53	Li-Fraumeni	Bone,breast,leukemia, Brain,adrenal,others
RB	Hereditary retinoblastoma	Retina,bones,others
HL	Von Hippel-Lindau	Kidney,retina,brain
WT-1	Wilms'tumor	Kidney

Source: Becker,M.Wayne Lewis J.Kleismith and Jeff Hardin. The World of the Cell,6th ed. Pearson/Benjamin Cummings; San Francisco,2006,p784

Tumor Suppressors

- Neurofibromin 1 –inactivates Ras soon after its stimulation by growth factors, i.e., allows for only *transient* activation of Ras.



Rb

- Cell cycle control by the retinoblastoma (Rb) tumor suppressor protein.
- Unphosphorylated Rb negatively regulates progression into the S phase of the cell cycle by binding to the E2F transcription factor.
- In this complex, E2F is prevented from activating the transcription of its target genes.
- During late G1, Rb is phosphorylated by the cyclin D/Cdk4 complex and can no longer sequester the E2F transcription factor.
- E2F then binds to its target S-phase genes, promoting their transcription and allowing the cell cycle to progress.

p53

- The p53 tumor suppressor is activated in response to a wide variety of cellular stresses
 - DNA damage
 - Redox modulation
 - Hypoxia
 - Changes in cell adhesion
 - Activated oncogenes.
- The p53 protein can induce growth arrest, promote DNA repair, and stimulate cell death by apoptosis.
- Collectively these activities act to maintain genomic stability.
- Elimination of p53 function leads to increased rates of mutation and resistance to apoptosis. Thus, p53 is important for several biochemical pathways that are disrupted during tumorigenesis.
- Consequently, mutations in p53 are the most frequent genetic change encountered in human cancers.
- p53 is inactivated by several mechanisms.
- The most common event is mutation of the p53 gene, which occurs in about 50% of all sporadic human tumors.
- They can be missense, deletion of one allele, nonsense and splice mutations.
- **Mutations** can be in somatic tissues or can be inherited through the germline.

- Li-Fraumeni syndrome is a condition with inherited p53 mutations and the affected individuals develop bone or soft-tissue sarcomas at an early age.
- **Non mutational inactivation** of p53 can occur in the presence of viral transforming antigens.
- Simian virus 40 (SV40) large T antigen binds with p53 and forms an inactive complex
- HPV E6 protein eliminates p53 by causing premature degradation of the protein through the 26S proteasome.
- Another mechanism by which p53 activity can be eliminated is by **cytoplasmic sequestration**.
- p53 that is unable to enter the nucleus cannot induce the expression of downstream effector genes that are necessary for mounting the cellular response to genotoxic stress.
- Under normal conditions, levels of p53 are kept minimal by ubiquitination and proteasome-mediated degradation that contributes to the short half-life (3–20 min) of the protein.
- A key player in maintenance of low p53 levels is mdm2.
- Mdm2 performs this function by interacting with p53 at its *N*-terminus and targets p53 for proteasome-mediated degradation.
- Mdm2 and p53 function in a feedback loop where activated p53 stimulates the expression of Mdm2, which in turn reduces the duration of up-regulated p53 activity.
- Overexpression of Mdm2 suppresses p53 by preventing its accumulation in response to DNA damage.
- Consequently, Mdm2 can function as an oncogene that acts in much the same way as the papilloma virus E6 protein.
- In fact, Mdm2 is overexpressed in some tumors such as osteosarcomas.

Study Questions

1. What are the mechanisms by which a protooncogene can be activated?
2. How does p53 deregulation occur in many cancers?
3. A member of the ras family that does not have a viral counterpart is
a) Myc b) NRAS c) KRAS d) HRAS
4. Match the following

APC mutation	Breast cancer
HER2	Ras
NF1	E6
P53	Colon cancer

5. 30% of many human cancers contain-----mutations