Cancer
When good cells go bad

What is cancer?

- Cancer is defined as the continuous uncontrolled growth of cells.
- A tumor is any abnormal proliferation of cells.
- Benign tumors stay confined to its original location.
- Malignant tumors are capable of invading surrounding tissue or invading the entire body.
- Tumors are classified as to their cell type.
- Tumors can arise from any cell type in the body.

Cancer is an umbrella term covering a plethora of conditions characterized by unscheduled and uncontrolled cellular proliferation.

- Almost any mammalian organ and cell type can succumb to oncogenic transformation, giving rise to a bewildering array of clinical outcomes.
- The causes of cancer are many and varied, and include genetic predisposition, environmental influences, infectious agents and ageing. These transform normal cells into cancerous ones by derailing a wide spectrum of regulatory and downstream effector pathways. It is just this complexity that has hampered the development of effective and specific cancer therapies.
- Any attempt to provide a comprehensive overview of cancer-related knowledge would be futile — therefore the next two lectures will focus on topics undergoing particularly rapid progress.

Cancer continued; three cancer types

- Carcinomas; constitute 90% of cancers, are cancers of epithelial cells.
- Sarcomas; are rare and consist of tumors of connective tissues (connective tissue, muscle, bone etc.).
- Leukemias and lymphomas; constitute 8% of tumors. Sometimes referred to as liquid tumors. Leukemias arise from blood forming cells and lymphomas arise from cells of the immune system (T and B cells).

Properties of cancer cells

Normal cells show contact inhibition
Cancer cells lack contact inhibition
Cancer Incidence and Death Rate

Fig. 16.2

Cancer Fig 16.3

- Cells in culture and in vivo exhibit contact-inhibition
- Cancer cells lack contact inhibition feedback mechanisms. Clumps or foci develop.

Early detection is the key!

What causes Cancer?
Genetic mutations

Cancer: Benign

- **Benign**: localized and of small size
- Cells that closely resemble, and may function, like normal cells
- May be delineated by a fibrous (Basal lamina) capsule
- Become problems due to sheer bulk or due to secretions (e.g. hormones)

Cancer: Malignant

**Malignant tumors**: high rate of division, properties may vary compared to cells of origin. Most malignant cells become metastatic. Invade surrounding tissue and establishment of secondary areas of growth: **Metastasis**
Metastasis
Carcinoma: derived from endoderm or ectoderm

ASSOCIATION WITH HUMAN CANCERS
1. Growth Factor Receptor
   Increased numbers in 20 percent of breast cancers
2. Ras Protein
   Activated by mutations in 20 to 30 percent of cancers
3. Abl Kinase
   Activated by abnormal chromosomes in chronic myelogenous leukemia
4. Src Kinase
   Activated by mutations in 2 to 5 percent of cancers
5. p53 Protein
   Mutated or deleted in 50 percent of cancers

Cancer has a lot to do with cell signaling for growth
ErbB is mutant EGFR

It takes two or more

Pathways leading to cancer

Cloning human ras

Cancer is a multi-step process

Loss of Rb and cancer
B. Virology

DNA tumor viruses- subvert cellular machinery for replication

Adenovirus:
Early: dedicated to replication of genome.
Triggered by E1A
Need host cell to be in S-phase, and E1A does the job
Uses host cell factors to activate transcription of essential early viral genes.
(late: viral capsid/packaging proteins)

Immortalization characterized by increased S-phase entry-
overcome a G1 block

How DNA TV cause cancer

What cellular proteins bind E1A and SV40 Large T??
(Harlow, Livingston)
Objective: provide clues into the cellular pathway.
What kinds of proteins co-IP's with E1A?
(anti E1A IP from 35S-cells)

RB Family
: p105, p107, p130
Cell Cycle
: Cyclin A, CDK2

Model
E1A neutralized RB growth arrest to enhance S-phase.

RB PATHWAY

The Retinoblastoma Family: pRB, p107, p130

Focus mainly on RB
(Merger of virology, genetics, and cell biology)

A. Genetics/Tumor Suppressors

The concept of tumor suppressor protein came from studies of retinoblastomas—tumors of the eye.

Found loss of heterozygosity in a particular position in the chromosome.

When gene was cloned-p105-110
A. highly mutated in retinoblastomas
B. many other tumors have mutations.

Mutations in tumors: in a pocket region.
Led to the idea that the normal function is the suppression of cell growth.

Over expression leads to suppression of growth.

Nuclear phosphoprotein
Mitogenic Stimuli (e.g. GF, Ras) → RB, E2F

**RB Pathway**

- D-Cyclin
- CDK 4/6
- CDK 4/6
- pRB
- E2F
- DNA Pol
- Cyclin E, p19
- DHFR, MYB

**Tumor Suppressor Genes**

- RB, p16
- Inhibits cell proliferation

**Oncogenes**

- Cyclin D1
- CDK 4/6
- P16
- Ink4a

From Sharpless and DePinho (1999). Current Opinions in Genetics and Dev. 9:22

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**Viral Oncogenes Induce Proliferation and Suppress Apoptosis**

- Adenovirus E1A
- HPV E7
- SV40 Lg T

**p53**

- Adenovirus E1B (55K)
- HPV E6
- SV40 Lg T

**APOPTOSIS**

- Adenovirus E1B (19K)
  (Bcl2-like)

**Apoptosis Vs Programmed Cell Death**

- Apoptosis is a morphological description of dying cells which contrast with necrosis.
- Programmed Cell Death (PCD) is a term originally used to describe cells that die at predictable time and places during development.
- Since nearly all PCD is apoptotic these terms are sometimes used interchangeably.

**Features of Apoptosis Vs Necrosis**

<table>
<thead>
<tr>
<th>Apoptosis</th>
<th>Necrosis</th>
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<td>Nuclear swelling</td>
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<tr>
<td>Cell Shrinkage</td>
<td>Cell Swelling</td>
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<tr>
<td>Preservation of Organelles and cell membrane</td>
<td>Disruption of Organelles</td>
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<tr>
<td>Rapid engulfment by neighboring cells preventing inflammation</td>
<td>Rupture of cell and release of cellular contents</td>
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<td>Inflammatory response</td>
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**2 Pathways for p53 Induced Apoptosis**

**A) Bax Independent**

- Thymocytes
  - Irradiation → p53 → Not Bax → Apoptosis
  - Bax Independent 40-50% "X+"

**B) Bax Dependent**

- MEFS
  - Bax Dependent → Apoptosis

- Bel-2

- Bel-2
p53 in apoptosis

Following DNA damage, e.g. by radiation, p53 levels rise, and proliferating cells arrest in G1. This allows time for DNA repair prior to the next round of replication. This arrest is mediated by stimulation of expression of p21CIP1, the cyclin kinase inhibitor. Very high p53 levels, or susceptible cell types, e.g. lymphocytes, are triggered to undergo apoptosis. Bel-2 acts between p53 and the caspase:

Apoptosis

- Apoptosis is a tightly regulated form of cell death, also called the programmed cell death. Morphologically, it is characterized by chromatin condensation and cell shrinkage in the early stage. Then the nucleus and cytoplasm fragment, forming membrane-bound apoptotic bodies which can be engulfed by phagocytes. In contrast, cells undergo another form of cell death, necrosis, swell and rupture. The released intracellular contents can damage surrounding cells and often cause inflammation.

The role of caspase

- During apoptosis, the cell is killed by a class of proteases called caspases. More than 10 caspases have been identified. Some of them (e.g., caspase 8 and 10) are involved in the initiation of apoptosis, others (caspase 3, 6, and 7) execute the death order by destroying essential proteins in the cell. The apoptotic process can be summarized as follows:
  1. Activation of initiating caspases by specific signals
  2. Activation of executing caspases by the initiating caspases which can cleave inactive caspases at specific sites.
  3. Degradation of essential cellular proteins by the executing caspases with their protease activity.

Capsase activation

- Comparison between active and inactive forms of caspases. Newly produced caspases are inactive. Specifically cleaved caspases will dimerize and become active.

Mechanisms: What are the cellular targets?

E1A: CR1 and CR2; In IPs
p105, p107, p130—all pocket proteins
RB protein.
Binds in pocket
Model: neutralization of RB leads to G1/S progression.
(Aside p300 family of co-activators-through CR1; CBP, p300)
P53 as a transcription factor which exerts its effect by regulating other genes

Kinases in checkpoints must be activated for cell to proceed through cell cycle

Cell enlarges

Cell divides

G1 Prepares for division

M Cell divides

G2 Cell replicates

S DNA

Cell Cycle

P53 can bind to DNA!

Stabilized by Zn$^{2+}$.

DNA

p53

P53 and the cell cycle

P53 arrests the cell cycle primarily by upregulating p21 (Cip1/Waf-1), which inactivates CDK/cyclin

P53 can also activate apoptosis

P21 is a kinase inhibitor

What does p53 do?

- Suppresses tumors in response to DNA by inducing cell cycle arrest or apoptosis

How can you inhibit gene expression?

How is p53 Activated?

1) Regulation of p53 by MDM2
   P53 tumor suppressor protein can be stabilized and activated by two separate mechanisms in response to DNA-damage-induced phosphorylation.

2) p53 nuclear export is inhibited, to ensure that it is activated in response to DNA damage.

Cancer Fig. 16.14
p53 and tumor formation

- The P53 tumor suppressor gene is the most frequently mutated gene in human cancer
Mouse double minute 2

- The mdm2 gene encodes a zinc finger protein that negatively regulates p53 function by binding and masking the p53 transcriptional activation domain. Two different promoters control expression of mdm2, one of which is also transactivated by p53.

- What does negative regulation mean? MDM2 protein inhibits p53 activity during normal cell growth.

- How: Inhibits p53 transcriptional activity

- Targets p53 for ubiquitination and degradation.

- This inhibition is inhibited by p53 is phosphorylated.

- MDM2 has been shown to be overexpressed in sarcomas and more recently was implicated in the pathogenesis of carcinomas.

The discovery of p53

- Studies of SV40-transformed cells show that a 55-kDa protein is essential for transformation. In 1979, Linzer and Levine showed that a cellular 54-kDa protein is similar to the middle-T antigen of SV40. Linzer and Levine (1979) showed that the p53 gene product binds specifically to DNA and represses the expression of viral late genes. This observation was confirmed by the work of Chang et al. (1979) and Kress et al. (1979). It was then postulated that this cellular 54-kDa protein (p53) is the cellular homologue of the SV40 middle-T antigen.

- Wild type p53 gene seems to confer a selectivity in cell transformation. Mouse double minute 2 (mdm2) gene recently was implicated in the pathogenesis of carcinomas. MDM2 protein inhibits p53 activity during normal cell growth.

- In 1984 two groups reported that cotransfection of murine p53 with plasmids encoding an activated mouse ras oncogene could transform NIH3T3 cells in a manner similar to that observed with wild type p53. Similar experiments performed with wild type p53 and murine ras oncogenes were performed with NIH3T3 cells and NIH3T3 cells transformed with murine ras oncogenes (Jenkins et al. 1984). Furthermore, wild type p53 reduced the transformation potential of plasmids encoding p53 and an activated ras oncogene (Finlay et al. 1989). In 1984, Jenkins et al. (1984) performed a cotransfection of murine p53 with plasmids encoding murine ras oncogenes and found that wild type p53 reduced the transformation potential of plasmids encoding murine ras oncogenes. These results suggested that p53 may play a role in the prevention of cell transformation.

53 cooperates with Ha-ras

- In 1986 a set of experiments has shown that cotransformation of T antigen was prevented by p53 but this was not observed with wild type p53. These results suggested that p53 may be a tumor suppressor gene.

Inactivation of p53 in Friend murine erythroleukemia

- In these tumors induced by the Friend virus, the p52 gene found in the tumor cells is very often rearranged, leading to an absence of expression of the p53 gene. The mutation often affects one of the conserved blocks of the protein (Murane et al. 1988). In all cases studied, the second allele is either lost through loss of the chromosome, or inactivated by deletion. In this tumor model, functional inactivation of the p52 gene seems to confer a selective growth advantage to erythroid cells during the development of Friend leukemia in vivo.

Wild type p53 has antiproliferative properties and does not cooperate with Ha-ras

- A new set of experiments has shown that wild type p53 reduced the transformation potential of plasmids encoding wild type p53 but not an activated ras oncogene (Finlay et al. 1989). These results suggested that wild type p53 could be prevented by a repressor of ras oncogenes. In these experiments, wild type p53 reduced the transformation potential of plasmids encoding wild type p53 and an activated ras oncogene (Finlay et al. 1989). This observation was confirmed by the work of Chang et al. (1979) and Kress et al. (1979). It was then postulated that this cellular 54-kDa protein (p53) is the cellular homologue of the SV40 middle-T antigen. A third group, demonstrated that p53 could prevent transformation when cotransfected with ras oncogenes leading to cells sensitive to the transformation (Jenkins et al. 1986).
The expression of p53 in different human cancers or in normal cell lines has long been under study by several different research teams. This expression is often high, indicating that the p53 gene is often mutated in these cancers. Some of these p53 mutants have been shown to be oncogenic. Genetic analysis of human cancer has revealed a very high rate of heterozygous loss of the short arm of chromosome 17, which contains the p53 gene. However, these observations have been made in the case of lung cancer (Mang et al., 1989). So far, no evidence has been found that there is a direct correlation between the loss of the p53 gene and its expression in the normal human cell.

In several families with Li-Fraumeni syndrome, a high proportion of sarcomas including osteosarcoma, breast cancer, soft tissue sarcoma, and leukemia, appearing at a very early age. Statistical analysis predicts that 50% of these families will develop a sarcoma before the age of 30, and 85% before the age of 70. Germline mutations in the p53 gene have been found in several families with this syndrome (Marks et al., 1990; Shih et al., 1990). In these families, there is an increased risk of other forms of cancer, which could mimic the carboxy-terminus of a normal p53 protein.

Taken together, these data made it possible to define the p53 gene as a tumor suppressor gene. Yet unlike the Rb gene, which is involved in the regulation of the cell cycle, the p53 gene is involved in the transformation of cells. The p53 gene has some original features. In particular, many point mutations of the p53 gene can be found in all cases of this syndrome. These observations led to the proposal that several classes of p53 mutants exist, according to the point of mutation and its consequences. The synthesis of these mutant p53 proteins is not harmless for the cell. In particular, it has been shown that some p53 mutants that do not bind to DNA have also been shown to be oncogenic. These observations suggest that monoclonal antibodies can induce a growth arrest (Krupp et al., 1990). The current hypothesis is that the expression of p53 in different human cancers or in normal cell lines has long been under study by several different research teams.

The p53 gene is mutated in a wide variety of human cancer. To suppress oncogene expression:

(1) transrectional level: deliver a transcriptional repressor acting on the promoter of oncogene, e.g. adenovirus E1A gene products can repress the ne promoter or truncation protein of SV40 Large T antigen

(2) post-transectional level: deliver ribozyme, antisense, dominant negative molecule, e.g. ribozyme for activated ras (point mutation)

Germline mutation of the p53 gene are found in Li-Fraumeni patients

Wild type p53 as a tumor suppressor gene and mutant p53 as a dominant oncogene

Suppression of Oncogene

Why p53 micro-injection of monoclonal antibodies induces a growth arrest?

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Steps in the activation of Ras by RTKs.

Fig. 15.24

Raf is a PK that triggers MAP-K pathway

- Raf-GEF
- Ras-GTP
- Cell proliferation

Raf is a PK that triggers MAP-K pathway

- Raf
- SH2 binds RTK, SH3 binds SOS
- c-fos, c-jun

Cell proliferation

- Raf is a PK that triggers MAP-K pathway
- G1
- S
- G2
- M
- Tissue differentiation
- G0
- Jun, FOS
- p53

- Cyclin A
- CDK2
- G2
- M
- Cyclin D
- CDK4
- Cyclin E
- CDK2
- G0
- Jun, FOS
- p53

- Tissue differentiation
- G0