Multistep nature of cancer development

• Phenotypic progression
  – loss of control over cell growth/death (neoplasm)
  – invasiveness (carcinoma)
  – distal spread (metastatic tumor)

• Genetic progression
  – multiple genetic lesions required for cancer
  – normal cell → malignant cell
  – how many independent genetic lesions are necessary for development of a clinically obvious cancer?
  – mutation rates, rate-limiting steps, genomic instability

Cancer genes

• What genes, when altered, promote cancer?
  – tumor suppressor genes and proto-oncogenes

• Some genes are altered in a restricted set of tumor types
  – e.g., APC in colorectal carcinoma

• Others are altered in a broad spectrum of tumor types
  – e.g., p53 and the Ras genes

• The importance of tumor gene “pathways”
  – the p53 and Rb tumor suppressor pathways
Proto-oncogenes vs. tumor suppressor genes

Proto-oncogenes promote cancer when malignantly activated

– An activated proto-oncogene contributes to tumorigenesis by "gain-of-function"

– Thus, an activated proto-oncogene is genetically dominant at the cellular level
  • an activated oncogene can elicit a new phenotype (tumorigenesis) even in the presence of the corresponding wildtype allele

Proto-oncogenes vs. tumor suppressor genes

Tumor suppressor genes promote cancer when malignantly inactivated

– A tumor suppressor contributes to tumorigenesis by "loss-of-function"

– In most instances, an inactivated tumor suppressor gene is genetically recessive at the cellular level.
  • it will not promote tumorigenesis in diploid cells unless the other (wildtype) allele is also lost or inactivated
  • some exceptions (e.g., dominant-negative p53 mutations)
Tumor suppressor genes

• in this lecture we will focus on…
  – the retinoblastoma susceptibility (Rb) gene
  – the p53 tumor suppressor gene

• genetic properties

• biochemical functions of their protein products

• the p53 and Rb tumor suppressor “pathways”

Cancer susceptibility syndromes

• What proportion of human cancers are heritable?

• Hereditary syndromes of cancer susceptibility are usually caused by germline mutations of tumor suppressor genes.
  – familial retinoblastoma: Rb
  – Li-Fraumeni syndrome: p53
  – familial adenomatous polyposis coli: APC
  – hereditary non-adenomatous c.c.: MLH1, MSH2

• Penetrance: fully penetrant mutations segregate as dominant traits in a Mendelian fashion
Sporadic and Heritable forms of Retinoblastoma

- age of tumor onset
  - sporadic (~60% of cases): ~ 6 years
  - heritable (~40% ““ ): ~ 2 years

- number of independent tumors
  - sporadic: single tumor (only one eye is affected)
  - heritable: multiple tumors (both eyes are affected)

- tumor frequencies in children of patients
  - sporadic: 1 in 10^5
  - heritable: 1 in 2

patients with heritable retinoblastoma transmit a “Rb susceptibility gene” to their children in a dominant Mendelian fashion

Knudson's hypothesis (1970s)

- Two (rate-limiting) genetic lesions required…

- Sporadic retinoblastoma:
  - both alterations are acquired somatically
  - incidence: (10^-6)(10^-6)(10^7 cells) = 10^-5 tumors/person
  - very rare; involves only one eye

- Heritable retinoblastoma:
  - one alteration is inherited in the germline (i.e., the “Rb susceptibility gene”)
  - the second alteration is acquired somatically
  - incidence: (1)(10^-6)(10^7 cells) = 10 tumors/person
  - all carriers affected; involves both eyes!!
Hereditary Retinoblastoma

- one mutation is inherited in the germline
- second mutation is acquired somatically
- incidence: \((1)(10^{-8})(10^7 \text{ cells}) = 10 \text{ tumors/person}\)

• All mutation carriers are affected
  - Rb susceptibility is a highly penetrant trait
  - tumor susceptibility is transmitted as a dominant trait in family pedigrees (despite the fact that tumor suppression genes function recessively at the cellular level).

The Retinoblastoma (Rb) gene

• What are the two rate-limiting genetic alterations?

• Cytogenetic abnormalities of chromosome 13:
  - interstitial deletions of variable length
  - always involve material from chromosome band 13q14
  - sporadic patients: deletions in tumor cells only
  - heritable patients: deletions in both normal & tumor cells

• Is Rb susceptibility due to genetic loss at 13q14?

⇒ If so, then the two mutations required for retinoblastoma might represent inactivation of both alleles of a single gene at 13q14
Sporadic Retinoblastoma  |  Familial Retinoblastoma
---|---
retinal cells:  |  tumor cells:  
Rb, Rb (normal)  |  Rb\textsuperscript{m1}, Rb (normal)
  \(\downarrow\) m1  |  \(\downarrow\) m2
  \(\downarrow\) m2  |  Rb\textsuperscript{m1}, Rb\textsuperscript{m2} (neoplastic)

Important: genetic lesions in the same gene are responsible for both the familial and sporadic forms of retinoblastoma!!

**The Retinoblastoma gene (Rb)**

- 1988: isolation of the Rb gene on 13q14

**Familial retinoblastoma**
- one Rb gene lesion in germline of familial patients
- other (normal) Rb allele lost or inactivated in tumors

**Sporadic retinoblastoma**
- both alleles of Rb are normal in germline
- both Rb alleles lost or inactivated in tumors

\(\uparrow\) Important: genetic lesions in the same gene are responsible for both the familial and sporadic forms of retinoblastoma!!
Inactivation of the second Rb allele (in somatic cells)

Rb\textsuperscript{m1} × Rb\textsuperscript{m2}  
\textit{de novo} mutation

Rb\textsuperscript{m1} × Rb\textsuperscript{m1}  
chromosome loss

Rb\textsuperscript{m1} × Rb\textsuperscript{m1}  
chromosome loss & reduplication

Rb\textsuperscript{m1} × Rb\textsuperscript{m1}  
gene conversion

chromosome 13  
maternal & paternal homologues

The penetrance of germline Rb mutations

• Almost all carriers will develop \textbf{retinoblastoma}  
  – high penetrance
    • (mutation rate)(target cells) = (10\textsuperscript{6})(10\textsuperscript{7} cells) = 10
    • retinoblastoma susceptibility is transmitted as a dominant Mendelian trait

• Some carriers will also develop \textbf{osteosarcoma}  
  – low penetrance
    • (mutation rate)(target cells) < 1
    • osteosarcoma susceptibility is not transmitted as a dominant Mendelian trait
Tumor Suppressors: the p53 gene

- p53 encodes a transcription factor
- the p53 gene is altered in many human tumors (usually by missense mutation).
- *in vitro* cell transformation by mutant p53 genes.

Is p53 a proto-oncogene?

- murine erythroleukemias induced by Friend Leukemia Virus: a natural knockout of the p53 gene by proviral insertion!
- suppression of cell transformation by the wild-type p53 gene.

Is p53 a tumor suppressor gene?

Dominant-negative mutations of a tumor suppressor gene

- **dominant-positive** mutation (e.g., missense mutations in the Ras proto-oncogenes).
- **recessive-negative** mutation (e.g., Rb loss).
- **dominant-negative** mutation (e.g., many p53 missense mutations).

- note: dominant-negative mutations result in functional inactivation of the protein products of both alleles (including the normal allele).
Dominant-negative mutations of p53

- how do dominant-negative mutations work?
- p53 normally functions as a homo-tetramer
- consider p53 function in a cell with one wildtype and one mutant p53 allele:

```
wildtype p53
mutant p53

functional p53 tetramer?
yes
yes
no
no
```

- mutant p53 is more stable than wildtype p53
p53 mutations in hereditary and sporadic cancer

• Li-Fraumeni Syndrome (LFS)
  – caused by germline mutations of p53
  – LFS carriers develop many different forms of cancer

• sporadic cancer
  – often caused by somatic mutations of p53
  – very common in human cancer
  – found in many different forms of cancer

Tumor suppressor proteins

• proteins encoded by Rb and p53
• the normal functions of these proteins
• mechanisms of tumor suppression
• the Rb and p53 tumor suppressor pathways
Phosphorylation of the Rb protein

- The phosphorylation state of Rb changes during normal cell cycle progression.
  - Rb is hypophosphorylated in:
    - G0 (resting cells)
    - early G1 (cycling cells)
  - Rb is hyperphosphorylated in:
    - S phase
    - G2 phase
  - Rb is phosphorylated before the G1/S transition…
    - by an enzymatic complex: CDK4 / cyclin D
The restriction point (in late G1)

- the major control point of cell cycle progression
- G1/S transition is mediated by the E2F family of transcription factors
- E2F binds the promoters of genes required for cell cycle progression (G1/S transition and S phase).

Some S phase genes regulated by E2F:

<table>
<thead>
<tr>
<th>S phase gene</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>thymidine kinase</td>
<td>nucleotide synthesis</td>
</tr>
<tr>
<td>DHFR (dihydrofolate reductase)</td>
<td>“” “”</td>
</tr>
<tr>
<td>DNA polymerase α</td>
<td>DNA synthesis</td>
</tr>
<tr>
<td>ORC1</td>
<td>“” “”</td>
</tr>
<tr>
<td>histone H2A</td>
<td>chromosome assembly</td>
</tr>
<tr>
<td>cyclin E</td>
<td>cell cycle progression</td>
</tr>
<tr>
<td>cyclin A</td>
<td>“” “” “”</td>
</tr>
</tbody>
</table>
Resting cells and early G1 phase cells

- hypophosphorylated Rb binds promoter-bound E2F
- Rb inactivates transcription by E2F
- S phase genes are repressed
- G1/S transition is blocked

restriction point

- CDK4/cyclin D phosphorylates Rb in the “pocket”
- hyperphosphorylated Rb dissociates from E2F
- E2F activates transcription of S phase genes
- cells enter S phase
- In normal cells, phosphorylation of Rb by the CDK4/cyclin D kinase is a highly regulated process.
- Focal point for the major signal transduction pathways that control normal cell growth.

![Diagram showing regulation of Rb and E2F](image)

- Diverse signaling pathways influence the phosphorylation status of Rb and the expression of S-phase genes.
The function of Rb

• hypophosphorylated Rb serves to restrain the proliferation of normal cells.
• regulated phosphorylation of Rb allows normal cells to proliferate at the correct time and place.
• therefore, imagine the consequences of losing normal Rb function…
  – deregulation of E2F (and the G1/S transition)!
• how might Rb become inactivated in cancer?

Inactivation of Rb function in tumors (leaving E2F unregulated)

• Direct inactivation:
  – Rb gene deletion (occurs in retinoblastoma)
  – point mutations in the Rb pocket (in retinoblastoma)
  – occupancy of the Rb pocket by early proteins of DNA tumor viruses
    • human papilloma virus (HPV), an etiological agent in human cervical carcinomas
    • HPV encodes two proteins required for tumorigenesis
    • E7 binds the pocket of hypophosphorylated Rb
    • Deregulation of E2F (and the G1/S transition)
The Rb tumor suppressor pathway

Indirect inactivation of Rb function in tumors

- overexpression of cyclin D1
  - breast cancer, B cell lymphoma
- loss of p16, an inhibitor of Cdk4
  - many human cancers
- inherited point mutation in Cdk4 that renders it insensitive to inhibition by p16
  - familial melanoma

» Inactivation of the Rb pathway occurs in most, if not all, human tumors!
Normal functions of the p53 protein

- p53 polypeptides are very unstable in normal cells (1/2 life of ~30 minutes)
- the cellular response to genotoxic stress
  - DNA damage by UV light, ionizing radiation, chemical carcinogens, errors in replication, etc.
  - induction of certain signal transduction pathways
- post-translational modifications of p53 polypeptides:
  - especially, phosphorylation and acetylation
  - stabilize p53 (1/2 life of ~150 min.), leading to higher steady-state levels
  - increase the transcriptional activity of p53

consequences of p53 activation

- The transcriptional activity of p53 induces a cellular response, the nature of which is dependent on various factors, including the cell type.

  - p53 induces G1 arrest (and DNA repair) in:
    - normal fibroblasts
    - certain epithelial cells

  - p53 induces apoptosis in:
    - thymocytes
p53 induction of cell cycle arrest or apoptosis

- in either case, replication of damaged DNA ceases
- prevents accumulation of oncogenic mutations
- In essence, p53 suppresses tumor formation by maintaining the integrity of the genetic material in cells subjected to genotoxic stress.

Transcriptional targets of p53

- p21 CDK inhibitor
  - G1 and G2 arrest in fibroblasts
- 14-3-3σ
  - G2 arrest in epithelial cells
- PUMA
  - promotes apoptosis in thymocytes, fibroblasts, neurons
- p53R2 nuclear ribonucleotide reductase
  - required for DNA repair
- p48 subunit of the XPA complex
  - required for nucleotide excision repair
- etc…
Normal cell

p53 protein is activated
p53 induces target genes
growth arrest
DNA repair
Normal cell

Apoptosis

p53 mutant cell

No response
DNA damage persists
- proto-oncogenes activated
- tumor suppressors inactivated
- cancer -

The p53 tumor suppressor pathway

ATM

mdm2

p53

p21

Inactivation of the p53 pathway occurs in most, if not all, human tumors!
Sporadic colorectal carcinoma

• Accounts for >80% of colorectal carcinomas.

• Natural history of the sporadic disease (viewed by colonoscopy):
  – normal epithelium
  – hyperproliferative epithelium (APC*)
  – early adenoma (APC*)
  – intermediate adenoma (APC*, Ras*)
  – late adenoma (APC*, Ras*, SMAD2/4*)
  – carcinoma (APC*, Ras*, SMAD2/4*, p53*)
  – metastasis
Hereditary colorectal carcinoma

• A predisposition to colorectal carcinoma is associated with two distinct syndromes.
  – Familial adenomatous polyposis coli (FAP)
  – Heritable non-polyposis colorectal carcinoma (HNPCC)

Familial adenomatous polyposis coli (FAP)

• FAP: rare, autosomal, dominantly-inherited
• Natural history of FAP:
  – multiple polyps (hundreds or thousands) develop throughout the colon by early adulthood.
  – inevitably, one or more of these polyps will progress into an invasive carcinoma.
• FAP results from inherited lesions in the APC gene (which behaves as conventional tumor suppressor)
• FAP accounts for less than 1% of all colorectal carcinomas. However, most sporadic cases of colorectal carcinoma have somatic mutations of APC.
Hereditary non-polyposis colorectal carcinoma (HNPCC).

• HNPCC: autosomal, dominantly-inherited

• Natural history of HNPCC:
  – Carriers do not have an obvious pre-malignant condition (e.g., the histology of their colon is normal).
  – Carriers are predisposed to develop colorectal carcinoma.

• HNPCC results from inherited lesions in genes encoding components of the DNA mismatch repair system:
  – hMSH2, hMLH1, hPMS1, or hPMS2

Mismatch repair defects in HNPCC

• mismatch repair $\supseteq$ incorrectly paired nucleotides

• malignant cells of HNPCC tumors exhibit genetic instability at the nucleotide level

• this underlying genetic instability accelerates malignant progression.

• HNPCC accounts for 2-4% of colorectal carcinomas

• In addition, ~13% of sporadic colorectal carcinomas display mismatch repair deficiency; these all harbor somatic mutations in one of the mismatch repair genes implicated in HNPCC
Tumor Suppression

• tumor suppressors inhibit tumor formation by a variety of different mechanisms:

• negative control of cell growth...
  – by regulating cell cycle progression (e.g., Rb, p16)
  – by regulating signal transduction pathways (APC)

• maintenance of genomic integrity...
  – by regulating the cellular response to DNA damage (p53, ATM, Chk2)
  – by functioning as effectors of DNA repair (hMSH2, hMLH1)
Methods to isolate tumor suppressor genes

- **Linkage analysis of family pedigrees**: identify polymorphic markers that co-segregate with tumor susceptibility
- **Loss of heterozygosity (LOH) in tumor cells**: identify polymorphic markers that exhibit tumor-specific LOH
- **Positional cloning**: search for transcription units (candidate genes) and analyze each for:
  - germline mutations that co-segregate with tumor susceptibility (in familial tumors)
  - somatic mutations that arise uniquely in the malignant cells (in sporadic tumors).
Chromosome 13 maternal & paternal homologues

the second Rb mutation (in somatic cells)

Rb\textsuperscript{m1} \times \textcolor{green}{\text{Rb}\textsuperscript{m2}}

\textit{de novo} mutation

\textcolor{red}{\text{LOH}}

chromosome loss

\textcolor{red}{\text{LOH}}

chromosome loss & reduplication

\textcolor{red}{\text{LOH}}

gene conversion

Chromosome 13 maternal & paternal homologues