Oncogenes & Tumor Suppressor genes

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Lecture overview

• What are oncogenes
• How do oncogenes function in cancer cells?
• How are oncogenes “turned on”?
• What are tumor suppressor genes?
• How do TSG function in cancer cells?
• How is TSG function lost?
Oncogenes and Tumor Suppressor genes

**Oncogenes**
- Genes that promote cell growth and/or motility which, when upregulated or deregulated, imbue cells with the cancerous properties of unregulated growth and motility

**Tumor suppressor genes**
- Genes which normally suppress cell growth and/or motility which are frequently downregulated in tumor cells, allowing for unchecked growth/motility
Gain of oncogene(s) + Loss of TSG = CANCER

*Cancer is a genetic disease
Oncogenes

• The term “oncogene” was coined in 1969 by R. Huebner & G. Todaro
• Genes that have the potential to cause cancer (proto-oncogenes)
• Transform healthy cells – cause them to gain “hallmarks of cancer”
• First discovered in viruses, later in cells
Src – the first oncogene

- The first oncogene was discovered before we understood what oncogenes really are!

Peyton Rous, 1911 – cell-free extract from chicken tumors can cause new tumors when injected into healthy chickens

Rous Sarcoma – a form of cancer which infects chickens

Rous Sarcoma Virus (RSV) – a retrovirus which generates a cDNA that inserts into the chickens’ DNA

v-Src – the gene which gets expressed by RSV

v-Src = oncogenic retrovirus
Other viral oncogenes

- Rous Sarcoma      src
- Abelson leukemia  abl
- Avian erythroblastosis  erbB
- McDonough feline sarcoma  fms
- H-Z feline       kit
- Murine sarcoma 3611  raf
- Simian sarcoma   sis
- Harvey sarcoma   H-ras
• Many oncogenes identified in viruses

• But... **Most human cancers are not viral in origin**

• Cellular versions of oncogenes = **proto-oncogenes**
Src in human tumors

Src expression/activity upregulated in:

• Breast ca
• Pancreatic ca
• Ovarian ca
• Head & Neck ca
• Lung ca
• Gastric ca
• Colon ca – C-terminal truncation identified
c-Src

• A kinase
• Cell motility, growth, morphology, proliferation
• The first proto-oncogene
• Activity is tightly regulated
  – po-Y527
v-Src is a CA version of c-Src

- c-Src – interaction between po-Y527 and SH2 domain hold c-Src in a kinase inactive state
- Dephosphorylation of Y527 releases the interaction
- v-Src lacks regulatory Y527  →  constitutively active
Src – the first (proto)oncogene

- 1976 – v-Src is the viral version of **c-Src**
- c-Src is a **proto-oncogene**
Activation of proto-oncogenes

Proto-oncogenes are tightly regulated in healthy cells

- **Mutation**
  - H-ras, K-ras, N-ras
  - EGFR
- **Gene amplification**
  - Myc
  - ErbB2/HER2
- **Chromosomal translocations**
  - Myc
  - Bcr/Abl
Activation of Ras by mutation

Oncogenic H-Ras mutation in bladder ca:

Glycine $\rightarrow$ Valine (G12V)

Common point mutations found in oncogenic Ras:
- aa 12
- aa 13
- aa 61
The effect of oncogenic point mutations on Ras signaling

- Ras is a GTPase – binds to and hydrolyzes GTP
- In the GTP-bound form, Ras is active
- Hydrolysis of GTP kills Ras activity
- G12V mutant Ras loses GTPase activity, remains active
Table 4.2 A list of point-mutated ras oncogenes carried by a variety of human tumor cells

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Proportion (%) of tumors carrying a point-mutated ras gene&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas</td>
<td>90 (K)</td>
</tr>
<tr>
<td>Thyroid (papillary)</td>
<td>60 (H, K, N)</td>
</tr>
<tr>
<td>Thyroid (follicular)</td>
<td>55 (H, K, N)</td>
</tr>
<tr>
<td>Colorectal</td>
<td>45 (K)</td>
</tr>
<tr>
<td>Seminoma</td>
<td>45 (K, N)</td>
</tr>
<tr>
<td>Myelodysplasia</td>
<td>40 (N, K)</td>
</tr>
<tr>
<td>Lung (non-small-cell)</td>
<td>35 (K)</td>
</tr>
<tr>
<td>Acute myelogenous leukemia</td>
<td>30 (N)</td>
</tr>
<tr>
<td>Liver</td>
<td>30 (N)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>15 (N)</td>
</tr>
<tr>
<td>Bladder</td>
<td>10 (H, K)</td>
</tr>
<tr>
<td>Kidney</td>
<td>10 (H)</td>
</tr>
</tbody>
</table>

<sup>a</sup>H, K, and N refer to the human H-RAS, K-RAS, and N-RAS genes, respectively.

Oncogene activation by mutation

WT EGF Receptor (EGFR) – ligand binding stimulates activation

Mutant EGFR – Constitutive activation
Oncogene activation by gene amplification

- Multiple copies of Myc, ErbB2 → greater expression
- Pro-growth advantage of tumor cells with greater expression

Breast ca survival based on Her2/neu expression (Slamon et al, 1987)
Table 4.3 Some frequently amplified chromosomal regions and the genes they are known to carry

<table>
<thead>
<tr>
<th>Name of oncogene⁵</th>
<th>Human chromosomal location</th>
<th>Human cancers</th>
<th>Nature of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDM4/MDMX</td>
<td>1q32</td>
<td>breast, colon, lung, pre-B leukemias</td>
<td>p53 inhibitor</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>3q26.3</td>
<td>lung SCC, ovarian, breast</td>
<td>PI kinase</td>
</tr>
<tr>
<td>erbB1/EGFR</td>
<td>7q12-13</td>
<td>glioblastomas (50%); squamous cell carcinomas (10-20%)</td>
<td>RTK</td>
</tr>
<tr>
<td>cab1-erbB2-grb7</td>
<td>17q12</td>
<td>gastric, ovarian, breast carcinomas (10-25%)</td>
<td>RTK, adaptor protein</td>
</tr>
<tr>
<td>k-sam</td>
<td>7q26</td>
<td>gastric, breast carcinomas (10-20%)</td>
<td>RTK</td>
</tr>
<tr>
<td>FGF-R1</td>
<td>8p12</td>
<td>breast carcinomas (10%)</td>
<td>RTK</td>
</tr>
<tr>
<td>met</td>
<td>7q31</td>
<td>gastric carcinomas (20%)</td>
<td>RTK</td>
</tr>
<tr>
<td>K-ras</td>
<td>12p12.1</td>
<td>lung, ovarian, colorectal, bladder carcinomas (5-20%)</td>
<td>small G protein</td>
</tr>
<tr>
<td>N-ras</td>
<td>1p13</td>
<td>head and neck cancers (30%)</td>
<td>small G protein</td>
</tr>
<tr>
<td>H-ras</td>
<td>11p15</td>
<td>colorectal carcinomas (30%)</td>
<td>small G protein</td>
</tr>
<tr>
<td>c-myc</td>
<td>8q24</td>
<td>various leukemias, carcinomas (10-50%)</td>
<td>TF</td>
</tr>
<tr>
<td>L-myc</td>
<td>1p32</td>
<td>lung carcinomas (10%)</td>
<td>TF</td>
</tr>
<tr>
<td>N-myc-DDX1</td>
<td>2p24-25</td>
<td>neuroblastomas, lung carcinomas (30%)</td>
<td>TF</td>
</tr>
<tr>
<td>akt-1</td>
<td>14q32-33</td>
<td>gastric cancers (20%)</td>
<td>ser/thr kinase</td>
</tr>
<tr>
<td>akt-2</td>
<td>19q13</td>
<td>ovarian carcinomas</td>
<td>ser/thr kinase</td>
</tr>
<tr>
<td>cyclin D1-exp1-hst1-ems1</td>
<td>(11q13)</td>
<td>breast and squamous cell carcinomas (25-50%)</td>
<td>G1 cyclin</td>
</tr>
<tr>
<td>cdk4-mdm2-sas-gli</td>
<td>12q13</td>
<td>sarcomas (10-30%), HNSCC (40%), B-cell lymphomas (25%)</td>
<td>CDK, p53 antagonist</td>
</tr>
<tr>
<td>cyclin E</td>
<td>19q12</td>
<td>gastric cancers (15%)</td>
<td>cyclin</td>
</tr>
<tr>
<td>akt2</td>
<td>(19q13)</td>
<td>pancreatic, ovarian cancers (30%)</td>
<td>ser/thr kinase</td>
</tr>
<tr>
<td>AIB1, BTAK</td>
<td>(20q12-13)</td>
<td>breast cancers (15%)</td>
<td>receptor co-activator</td>
</tr>
<tr>
<td>cdk6</td>
<td>(19q21-22)</td>
<td>gliomas (5%)</td>
<td>CDK</td>
</tr>
<tr>
<td>myb</td>
<td>6q23-24</td>
<td>colon carcinoma (5-20%), leukemias</td>
<td>TF</td>
</tr>
<tr>
<td>ets-1</td>
<td>11q23</td>
<td>lymphoma</td>
<td>TF</td>
</tr>
<tr>
<td>gli</td>
<td>12q13</td>
<td>glioblastomas</td>
<td>TF</td>
</tr>
</tbody>
</table>

⁵The listing of several genes indicates the frequent co-amplification of a number of closely linked genes; only the products of the most frequently amplified genes are described in the right column.

⁶Abbreviations: TF, transcription factor; RTK, receptor tyrosine kinase; CDK, cyclin-dependent kinase; G protein, guanine nucleotide-binding protein; HNSCC, head-and-neck squamous cell carcinomas.


Table 4.3 The Biology of Cancer (© Garland Science 2014)
Chromosomal rearrangement

• Genetic instability
  ➔ Place strong promoter in front of an oncogene
  ➔ formation of novel hybrid proteins
t(8;14)

- Burkitt’s Lymphoma
- Fusion of chromosomes 2, 14, or 22 to chromosome 8
- Place Myc under the Ig promoter
Bcr-Abl

- Chronic myelogenous leukemia (CML)
- Abl – oncogene (TK) located on cr 9
- Breakpoint cluster region – cr 22
- t(9;22)
- Result: CA version of Abl
Summary so far

• Oncogenes were first discovered in the DNA of tumor-causing viruses
• Activation of proto-oncogenes drive tumorigenesis
• Oncogenes are activated by gene amplification, activating mutations, and chromosomal rearrangements
Oncogenes → cancer, end of story, right?!  WRONG

I'M SORRY
I CAN'T HEAR YOU OVER THE SOUND OF HOW WRONG YOU ARE
• Scientists expected oncogenes to be genetically dominant

• BUT – fusion of cancer and normal cells create non-tumor forming hybrid

NO TUMOR!
Results of fusion studies

- Tumor phenotype is recessive to normal phenotype
- Normal cells have properties which suppress tumorigenesis
- **Tumor suppressor genes**
Additional support for TS theory: Retinoblastoma

• Familial
  – Parent previously had the disease (carry one disease allele)
  – More likely to get sporadic “second hit”
  – Presents in both eyes

• Sporadic
  – Requires two hits per cell (one per allele)
  – Less frequent
  – Presents in one eye
Tumor Suppressor genes

• Tumor suppressor genes function as growth suppressors in healthy cells
• The loss of tumor suppressor genes causes cancer
• Cancer = gain of oncogenes + loss of TSG
How to TSG get lost?

• Direct inactivating mutations
  – Rare \((10^{-6} \text{ per cell generation})\)
  – 2 alleles \(\rightarrow\) even more rare \((10^{-12} \text{ per cell generation})\)

• Mutations during mitosis
  – Not all that rare
  – Loss of heterozygosity (LOH)
Loss of Heterozygosity

Normal cell

Cell with 1 mutant allele

(S phase)

Abnormal mitosis

Normal mitosis
Loss of Heterozygosity

2 separate mutations (rare)

1 mutation + LOH (more likely)
Mechanisms of TSG inactivation

- Gene deletion
- Direct mutation
- Loss of Heterozygosity
- Epigenetic silencing (promoter methylation)
Promoter methylation

- Promoters rich in the sequence cytosine-guanosine (CpG)
- Cytosine gets methylated
- HDAC protein complexes recognize methyl-CpG
- HDAC removes histone acetylations
- Histones instigate “closed” DNA conformation
- → turn off transcription
NF1 as a tumor suppressor

NF1

• Lost in neurofibromatosis
• A GTPase Activating Protein
  – Induces hydrolysis of GTP
  \[\rightarrow\text{inactive Ras}\]
• Loss of NF1 functionally mimics hyperactivation of Ras
Genetic changes → Cancer

**Oncogenes**
- Gene amplification
- Insertion of powerful (viral) promoters
- Activating mutations

**Tumor suppressors**
- Gene deletion
- Silencing mutations
- Loss of heterozygosity
- Promoter hypermethylation