Neoplasia (III)

Molecular Basis of Cancer
Overview

- Clonal expansion
- Oncogenes
- Tumor suppressor genes
- Molecular based diagnosis and therapy
Cancer Progression is Multi-stepped: Clonal Expansion & Selection

Clonal expansion:
Cancer is a big mess, but they all come from a single cell.

Proof of clonal expansion:
At end of tumor progression, when you resect the tumor, cells are genetically heterogeneous, but maintain earliest genetic alteration (indicating they come from a single cell).

Hits and alterations:
- Primary, secondary and tertiary genetic hit, but most cancers need 5-6 genetic "hits" or genetic alterations to cause cancerous proliferation.

BIG IDEA
1. Cancer undergoes clonal expansion - all derived from a single cell.
2. Tumors usually have 5-6 genetic alterations/"hits" to cause cancerous proliferation.
ONAL EXPANSION AND HETEROGENEITY

#1. What we know:
Normal cell: Mature, adult cells **CANNOT** renewably proliferate
What is new (research): Cancer stem cells may be a precursor and the "true origin of cancer" - cause still controversial and debated

Oncogenic transformation:
- Each round gains a genetic mutation
- Each color is a different round

1. Tumor cell can gain mutation to allow metastasis
2. Tumor cells can grow so big and initiate angiogenesis to continue growth

#2. Why so many mutations/genetic alterations?
- Tumor cells are "outlaws" from the immune system and must escape the body!
- Lucky cells can escape the body & immunosurveillance,

Examples of selection for mutation:
- see next slide for more info....

Repeated concept: Begin w/ NORMAL cell: 1st mutation is the same in ALL tumor cells, but there are other mutations that cause heterogeneity
#3. Tumors are genetically heterogeneous, but share same origin:
Ex. GBM (Glioblastoma) Grade 4 histology
- Different cellular morphology in different areas of the tumor but all malignant
- Malignant cells have many characteristics which are different from normal cells i.e. immune system evasion, angiogenesis, apoptosis and tumor necrosis
- **Tumors are complex:**
  - variety of genetic alterations within same tumor, but all stem from **same original cell (clonal expansion)**

#4. Treatment:
1. Can be 100s of genetic changes, but they **share signal transduction pathways**
   - Target limited # of alterations in signal transduction pathways
2. Can target first genetic variation to kill tumor cells

**EX. CML** is a specific leukemia subtype that has BCR-ABL translocation fusion protein

**Gleevec**: Gene therapy can target variation > complete remission of disease
- **Relapse of cancer due to additional mutations in BCR-ABL**
Cancer is a Genetic Disease

At each stage, specific genetic mutations occur. Ex. 1st mutation is APC/β-catenin pathway. "Gatekeeper genes".

Tumor suppressor gene: adenomatous polyposis coli

Dysplastic > "pre-cancerous cell" "caretaker genes" repair DNA mutations

beta catenin (translocates to nucleus to activate cell proliferation) binds E cadherin, cell surface protein that mediates IC interactions

APC/β-catenin FAP

RAS/BRAF 18q PIK3CA

Normal Epithelium Dysplastic ACF Early Adenoma Intermediate Adenoma Late Adenoma Carcinoma Metastasis

Chromosomal or Microsatellite Instability (HNPCC)

Tumor suppressor

Oncogene is like a stuck accelerator! (will continue to go even if you lift your foot off the pedal)

Tumor suppressor is like the brakes. Problems with the brakes, forces you to go, can't stop!

"Caretaker genes" are the mechanic. Can't take care of the DNA repairs if they are mutated
What Gene Functions can be Altered in Cancer?

- **Oncogene activation**: self-sufficiency in growth signal
- **Inactivation of tumor suppressor genes**: insensitivity to growth-inhibitory signals
- **Evasion of programmed cell death**
- **Defect in DNA repair**: can’t maintain genomic integrity
- **Expression of telomerase**: limitless replicative potential
- **Sustained angiogenesis**
- **Ability to invade and metastasize**

Cancer metabolism - use different metabolic pathways

- anti-apoptotic
  - Ex. Hypoxia, stress, radiation causes DNA damage
  - Problem must be fixed or else will signal apoptosis in the normal cell

- causes signal for cell death
Genetic Mechanisms to activate oncogenes or inactivate tumor suppressors

**Mutations.** Small-scale mutations include point mutations, deletions, and insertions. Disruption of a single gene may also result from integration of genomic material from a DNA virus or retrovirus, and such an event may also result in the expression of viral oncogenes in the affected cells.

**Genomic amplification** occurs when a cell gains many copies of a small chromosomal locus, usually containing one or more oncogenes and adjacent genetic material.

**Genomic Deletion** of tumor suppressor genes.

**Translocation** occurs when two separate chromosomal regions become abnormally fused, often at a characteristic location.
Protooncogenes are normal cellular genes which are involved in growth regulation; Oncogenes result from activation protooncogenes and lead to unrestricted growth control.
Rous sarcoma virus was discovered in 1911 by Peyton Rousis. It is a retrovirus and the first oncovirus to have been described: it causes sarcoma in chickens.

1st oncogene identified is SARC
- Oncovirus & oncogene
- Chicken virus causes sarcoma

Experiment: What can cause cancer?
1. Isolated tumor cells
2. Extract liquid portion + inject chickens
Result: Sarcoma Virus in liquid portion causing cancer
The src gene was taken up by RSV and incorporated into its genome conferring it with the advantage of being able to stimulate uncontrolled mitosis of host cells. v-src lacks the C-terminal inhibitory site, and is therefore constitutively active as opposed to c-src which is only activated under certain circumstances. v-src is an instructive example of an oncogene whereas c-src is a proto-oncogene. The c-SRC non-receptor tyrosine kinase is overexpressed and activated in a large number of human malignancies.
For example: the most common oncogenes found in human cancers are Ras oncogene. About 10-15% of all cancers carry Ras mutations. 95% of pancreatic carcinomas carry Ras mutations.

Ras genes encode proteins with GTP guanosine-nucleotide–binding activity and intrinsic guanosine triphosphatase activity.

When mutated in codon 12, 13, or 61, the RAS genes encode a protein that remains in the active state and continuously transduces signals by linking tyrosine kinases to downstream serine and threonine kinases.

1. RAS = common oncogene
2. Frequently mutated in cancers, especially pancreatic carcinomas
3. Encodes protein that bind GTP and can degrade GTP > GDP

"Hot spot" mutations in these codons can cause activation of RAS gene
Ras Activation

**Oncogene Concept:**
Mutually exclusive oncogenes - only need one mutation to activate oncogene (Ras or raf-1, but not both)

RAS activation
1. Growth signal activates receptor
2. Ras bound to GDP then activates by binding to GTP
3. Raf-1 activated and initiates MAP kinase pathway
4. Transcribes MYC proteins for cell-cycle progression

whole pathway is filled with oncogene: RAS, Raf-1, MAP Kinase, MYC protein
Oncogene Activation-Amplification

Members of four different oncogene families are often amplified: **MYC**, cyclin D1 (or **CCND1**), **EGFR**, and **RAS**.

**MYC** is amplified in small-cell lung cancer, breast cancer, esophageal cancer, cervical cancer, ovarian cancer, and head and neck cancer.

**CCND1** amplification also occurs in breast, esophageal, hepatocellular, and head and neck cancer.

**EGFR (ERBB1)** is amplified in glioblastoma and head and neck cancer. Amplification of **ERBB2** (also called **HER2/neu**) in breast cancer.
HER2/neu amplification in breast cancer cells

- Use FISH (fluorescence in situ hybridization) to determine Her2/neu amplification
  - fluorescent probe to hybridize with protein of interest

Amplification of \textit{ERBB2} in breast cancer correlates with a poor prognosis.

A monoclonal antibody against the product of this oncogene (trastuzumab) is effective in breast cancers that overexpress HER2/neu.
Oncogene Activation
-chromosomal translocation

Ex. Burkitt's lymphoma w/ chromosome 8 & 14
- MYC oncogene fused with Ig gene (very active) & induces overexpression

CML chr 9,22 translocation
- chr 22/Philadelphia chromosome: ABL-BCR fusion
- Drug targets BCR kinase (that activates expression)

chr 9 longer
chr 22 shorter
(philadelphia chr)
Tumor Suppressor Genes

Normal physiologic function of the wild type gene is to slow cell growth, cause cell differentiation, activate apoptosis or repair DNA. Require mutations (or silencing) of both alleles (Knudson’s two hit hypothesis).
Knudson’s Two Hits Theory and retinoblastoma

Inactivation of both copies of Rb tumor suppressor gene is required for tumorogenesis

*In hereditary cases:*
First hit is inherited from an affected parent and present in all cells
Second hit occurs in one of the many of retinal cells, which already carry the first hit.
Carriers of a mutant Rb gene have a 10,000-fold increased risk, children develop retinoblastoma at much younger age than sporadic cases, usually bilateral.

*In sporadic cases:*
Both hits occur somatically within a single retinal cells, whose progeny then form the tumor.
**Big ideas:**
1. Cell cycle important for tumor growth/cancer
2. Rb controls G1-S phase
   - HYPOphosphorylated = active Rb
   - NO progression of cell cycle
   - HYPERphosphorylated = inactive Rb
   - means NOT attached to E2F, progression through cell cycle
TP53 tumor suppressor gene

TP53 is mutated in more than half of human tumors.
One of its normal functions is to prevent replication of DNA which has been damaged, guardian of genome.
p53 guardian of genome

1. Fxn: Regulates cell damage
   - If damage cannot be fixed, activates apoptosis
   Normal p53 = TUMOR SUPPRESSOR
2. Mutated alleles = no regulation, cell cycle goes nuts!
B

Normal cell (p53 normal)

DNA damage

p53 activated and binds to DNA

mir-94 transcribed and processed

- Inhibits translation of growth-promoting genes (i.e., MYC, CDK4)
- Inhibits translation of anti-apoptosis genes (BCL-2)

Quiescence/senescence

Apoptosis
Gate Keeper Genes

- Affects ~1 in 7,000 individuals
- Inherited in an autosomal dominant manner
- Multiple benign tumors (adenomas) of the colon and rectum
- *APC* germline mutation

---

Familial Adenomatous Polyposis

- All polyps!
  - Some of them benign, but some gain additional mutations!

### Normal

- Affects ~1 in 7,000 individuals
- Inherited in an autosomal dominant manner
- Multiple benign tumors (adenomas) of the colon and rectum
- *APC* germline mutation
The APC/β-Catenin Pathway in Colorectal Epithelial Cells

Transcription of Growth Promoting Genes

Tcf-4  β-catenin  Gene

Normal APC = prevents transcription
Normal Colorectal Epithelial Cells

Transcription of Growth Promoting Genes

\( \beta \)-catenin

Tcf-4

Gene

APC
~80% of Colorectal Tumors

Transcription of Growth Promoting Genes

Tcf-4 β-catenin

When mutated, "releases the brakes" and transcription continues without stopping!
~5% of Colorectal Tumors

Transcription of Growth Promoting Genes

**Beta catenin** = oncogene - if mutated, you can have permanent activation that can over-ride APC
Caretaker genes

Maintain genomic stability by DNA repair or whole chromosome stability.

Caretaker genes are not oncogenic, but their loss of function increases the risk of mutations in other genes, including oncogenes and tumor suppressor genes.
Hereditary Nonpolyposis Colon Cancer (HNPCC)

- Do not arise in polyps
- Colon cancer occurs at young age
- Multiple direct family relatives
- Microsatellite instability
- DNA mismatch repair genes mutations
Replicate & polymerase bind

Mismatch mutation occur during replication

Mismatch repair proteins will bind

Normally have ability to change back/correct

Bear with me, this was all taken via screen shot.
Mismatch Repair

However if any of the DNA repair proteins are mutated (MLH1, PMS2, MSH2, MSH6) -- will not fix mismatch!
Normal Colon

Polyp

Cancer

familial adenomatous polyposis

hereditary nonpolyposis colon cancer

APC

Tumor Initiation

RAS, p53,…

Tumor Progression

FAP

Accelerated

Normal

HNPPCC

Normal

Accelerated
Genetic Landscape of Cancers

Table 1. Summary of somatic sequence mutations in five tumor types

<table>
<thead>
<tr>
<th></th>
<th>Medulloblastoma*</th>
<th>Pancreas#</th>
<th>Glioblastoma†</th>
<th>Colorectal‡</th>
<th>Breast‡</th>
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</thead>
<tbody>
<tr>
<td>Number of samples analyzed</td>
<td>22</td>
<td>24</td>
<td>21</td>
<td>11</td>
<td>11</td>
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<tr>
<td>Number of mutated genes</td>
<td>218</td>
<td>1007</td>
<td>685</td>
<td>769</td>
<td>1026</td>
</tr>
<tr>
<td>Average number of non-silent mutations per sample</td>
<td>8</td>
<td>48</td>
<td>36</td>
<td>77</td>
<td>101</td>
</tr>
</tbody>
</table>

Why such a low number?  
A: You only need to mutate the master regulators (gene transcription, protein expression, etc)

Landscape of a typical colorectal cancer. The large peaks indicate the gene mountains, small peaks indicate the hills.  
Image Courtesy of Bert Vogelstein/HHMI at Johns Hopkins

Intro: Because genome sequence is finished, we know a lot about it!  
- Past 5 years, whole genomes of cancers have been sequenced

most common childhood brain tumor

most common adult brain tumor

fund many small mounds, not as many high peaks  
Meaning: lots of different tumors, not all the same

high frequency of gene mutation are the higher peaks
Oncogenic pathways in GBMs

- Most drugs involved with these pathways

90% of cancer have genome aneuploidy - multiple chromosomes
Warburg's hypothesis

“The prime cause of cancer is the replacement of the respiration of oxygen in normal body cells by aerobic glycolysis.”-Warburg

Many of oncogenes and tumor suppressor genes are modulating the metabolic functioning of cancer cells.
<table>
<thead>
<tr>
<th>Challenges</th>
<th>Diagnosis</th>
<th>Prognosis</th>
<th>Treatment</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>-Histology</td>
<td>-Karnofsky score/age</td>
<td>-Surgery</td>
</tr>
<tr>
<td>Molecular solutions</td>
<td>-Molecular diagnosis</td>
<td>-Molecular prognosis</td>
<td>-Radiation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-Chemotherapy</td>
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<tr>
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</table>
# Glioma Molecular Diagnosis at Duke

## Immunohistochemistry

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>% of Tumor Cells Exhibiting Staining</th>
<th>Score (0–3+)</th>
<th>Date of IHC Analysis dd/mm/yyyy</th>
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<tbody>
<tr>
<td>MGMT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGFR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGFR vIII</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTEN</td>
<td></td>
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<td>pS6</td>
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<td>pMAPK</td>
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<td>Ki67 *</td>
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<tr>
<td>CD45 (LCAg)</td>
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<td>CA IX</td>
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</tr>
<tr>
<td>PDGF α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDGF β</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEGF</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>VEGF R2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIF2 α</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Interpretation of Other Analysis

- Neg: Negative
- Pos: Positive

### Types of Analysis

1. PCR
2. Protein Assay
3. Sequencing
4. IHC
5. Other

### Interpretation of FISH

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>% of Tumor Cells Exhibiting Abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR</td>
<td></td>
</tr>
<tr>
<td>7 cap</td>
<td></td>
</tr>
<tr>
<td>C-Met</td>
<td></td>
</tr>
<tr>
<td>PTEN</td>
<td></td>
</tr>
<tr>
<td>10 cap</td>
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<tr>
<td>1p21</td>
<td></td>
</tr>
<tr>
<td>9 cap</td>
<td></td>
</tr>
<tr>
<td>C-KI</td>
<td></td>
</tr>
<tr>
<td>4 cap</td>
<td></td>
</tr>
<tr>
<td>1p36</td>
<td></td>
</tr>
<tr>
<td>1p32</td>
<td></td>
</tr>
<tr>
<td>19 q13</td>
<td></td>
</tr>
</tbody>
</table>

**EGFR amplification in a GBM sample**

FISH and sequencing of cancer genomes does occur at Duke
Oncogenes as Therapeutic Targets

Table 1. Cancer Therapies That Target Oncogenic Proteins.*

<table>
<thead>
<tr>
<th>Anticancer Drug</th>
<th>Target</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoclonal antibodies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trastuzumab (Herceptin, Genentech)</td>
<td>ERBB2</td>
<td>Breast cancer</td>
</tr>
<tr>
<td>Cetuximab (Erbitux, ImClone)</td>
<td>EGFR</td>
<td>Colorectal cancer</td>
</tr>
<tr>
<td>Bevacizumab (Avastin, Genentech)</td>
<td>VEGF</td>
<td>Colorectal cancer, non–small-cell lung cancer</td>
</tr>
<tr>
<td>Small molecules</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imatinib (Gleevec, Novartis)</td>
<td>ABL, PDGFR, KIT</td>
<td>Chronic myelogenous leukemia, gastrointestinal stromal tumors, chordoma</td>
</tr>
<tr>
<td>Gefitinib (Iressa, AstraZeneca)</td>
<td>EGFR</td>
<td>Non–small-cell lung cancer</td>
</tr>
<tr>
<td>Erlotinib (Tarceva, Genentech)</td>
<td>EGFR</td>
<td>Non–small-cell lung cancer</td>
</tr>
<tr>
<td>Sorafenib (Nexavar, Bayer/Onyx)</td>
<td>VEGF, PDGFR, FLT3</td>
<td>Renal-cell carcinoma</td>
</tr>
<tr>
<td>Sunitinib (Sutent, Pfizer)</td>
<td>VEGF, PDGFR, FLT3</td>
<td>Gastrointestinal stromal tumors, renal-cell carcinoma</td>
</tr>
</tbody>
</table>

* EGFR denotes epidermal growth factor receptor, FLT3 FMS-like tyrosine kinase 3, PDGFR platelet-derived growth factor receptor, and VEGF vascular endothelial growth factor.
Potential targets and mechanisms of action of mutation-targeted drugs

Magic bullets

- Glivec inhibits ATP binding
Session-specific objectives

Explain the ways in which oncogenes develop from normal genes involved in growth regulation.

Explain how cancer develops from modifications of tumor suppressor genes.