MOLECULAR BASIS OF ONCOGENESIS

INTERUNIVERSITY CERTIFICATE IN HUMAN GENETICS

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Molecular basis of oncogenesis

I - Cancer Biology

II - 4 lines of evidence for the genetic basis of oncogenesis
1. Tumorigenic retrovirus
2. Transfection of tumoral DNA
3. Cytogenetics
4. Inherited cancers

III - Cancer = a genetic disease

IV - New insights from NGS studies
Somatic cell fates

Self-renewal

Differentiation

Apoptosis

Cancer = disruption of the cellular homeostasis

I - CANCER BIOLOGY

Definition
Multi-step process

Normal tissue → in situ tumor → locally invasive cancer → metastases
Common Hallmarks

Emerging Hallmarks:
- Sustaining proliferative signaling
- Evading growth suppressors

Enabling Characteristics:
- Deregulating cellular energetics
- Resisting cell death
- Inducing angiogenesis
- Activating invasion and metastasis
- Avoiding immune destruction
- Enabling replicative immortality
- Tumor-promoting Inflammation

Interaction with tumor microenvironment

Adapted from Hanahan and Weinberg, 2011
• The Rous Sarcoma Virus (1909) \(\rightarrow\) chicken sarcoma

**II - 4 lines of evidence for the genetic basis of oncogenesis**

1. **Tumorigenic retrovirus**

   - A viral **oncogene** is responsible for transformation
   - Viral oncogenes = mutated versions of normal cellular **proto-oncogenes** (1970)
     - \(v\text{-src}\) (avian sarcoma) \(\rightarrow\) c-src
     - \(v\text{-abl}\) (murine leukemia) \(\rightarrow\) c-abl
     - \(v\text{-myc}\) (avian lymphoma) \(\rightarrow\) c-myc
     - ...
Viral Oncogenesis: rare in human tumors

- HBV
- Hepatocarcinoma
- HCV Hepatocarcinoma
- EBV Burkitt lymphoma, Hodgkin lymphoma
  Lymphoma of immunosuppressed patients
  Nasopharyngal carcinoma, Gastric carcinoma
- HHV8 Kaposi sarcoma
  Plasmablastic lymphoma
- HPV Cervical cancer
- HTLV1 Adult T-cell leukemia / lymphoma
II - 4 lines of evidence for the genetic basis of oncogenesis

2. DNA Transfection

Transfer of the tumor phenotype to normal cells

- Isolation & sequencing of tumor DNA
- Identification of mutated genes = oncogenes:
  - Ex: RAS family
II - 4 lines of evidence for the genetic basis of oncogenesis

2. DNA Transfection

Ras proteins: **HRAS, KRAS, NRAS**

- Key components of signal transduction pathways
- Hotspot activating mutations
- ~15-20% of human cancers

http://www.nature.com/bjc/journal/v102/n4/fig_tab/6605534f1.html
II - 4 lines of evidence for the genetic basis of oncogenesis

3. Cytogenetics

- 1890: nuclear and mitotic alterations in cancer cells (Von Henseman)
- 1914: assumption that clonal chromosomal anomalies induce cellular malignant transformation (Boveri)
- 1956: human chromosome number (Tijo & Levan)
- 1970: chromosome bands (Caspersson)
- 1970-...: discovery of multiple recurrent cytogenetic alterations, correlations between karyotypic aberrations, diagnosis and prognosis
- 1975-...: expansion of molecular biology, cloning of involved genes, functional studies (genes, proteins)
- 1990-...: therapeutic applications
- 2001: human genome cartography
Main consequences of chromosomal aberrations

- Translocations
  - Chimeric gene
  - Transcriptional deregulation

- Gain / amplification

- Deletion
Chromosomal translocations:

2 types of functional impact

1/ Translocation leading to a chimeric gene
   → expression of a new chimeric protein

2/ Translocation leading to upregulation by promoter swap
   → increased / ectopic expression of a normal protein
4 lines of evidence for the genetic basis of oncogenesis

3. Cytogenetics

Translocation leading to a chimeric gene t(9;22)(q34;q11) in chronic myelogenous leukemia
4 lines of evidence for the genetic basis of oncogenesis

3. Cytogenetics

Translocation leading to a chimeric gene  
t(9;22)(q34;q11) in CML

Constitutive activation of a chimeric tyrosine kinase
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3. Cytogenetics

Translocation leading to upregulation t(8;14)(q24;q32) in Burkitt lymphoma

Constitutive activation of a normal MYC protein driven by immunoglobulin enhancers
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3. Cytogenetics

Genomic amplification
HER2 in breast cancer

Normal breast cell

Her2+ breast cancer cell

~20,000 receptors

Up to 1-2 million receptors
Deletion
1p/19q in oligodendroglioma
Most of the cancers are sporadic

1/10,000 à 1/100,000 are inherited
  - ~5% of adult cancers
  - Autosomal dominant transmission of the inherited predisposition

Linkage and positional cloning studies (80’s-90’s) :
  - identification of new cancer genes
  - Usually, recessive, loss of function mutations -> 2 main types of genes :
    - “Gate-keeper” = Cell control (TP53, RB) = tumor suppressor genes
    - “Care-taker” = Genome stability / DNA repair (ATM, MMR complex)

Genes involved in inherited predisposition are also mutated in somatic cancers
  Ex: TP53 mutated in > 50% cancers
II - 4 lines of evidence for the genetic basis of oncogenesis

4. Inherited Cancers

2 categories of loss of function genes
“Gatekeeper” = cellular proliferation & cell cycle control

Ex:
- RB controls the G1-S checkpoint → Retinoblastoma
- TP53 controls G1 progression → Li-Fraumeni syndrome
- APC controls proliferation → Familial adenomatous polyposis
II - 4 lines of evidence for the genetic basis of oncogenesis

4. Inherited Cancers

2 categories of loss of function genes
“Caretaker” = Genome stability / DNA repair

Ex: BRCA1/2
MMR DNA repair complex
ATM

→ Breast/ovarian cancers
→ Lynch syndrome
→ Ataxia-telangiectasia

Double-strand DNA breakage

DNA mismatch

Ashok R. et al, NEJM 2003
III - Cancer = a genetic disease

Alteration of master genes for cellular homeostasis

- Growth factors (GF)
  & cytokines
  ex: IL3
- GF receptors
  ex: HER-2
- (Sub-)membran proteins
  ex: RAS
- Cytosolic / sub-membran kinase proteins
  ex: ABL
- Nuclear transcription factors
  ex: MYC
- Cellular cycle regulator proteins
  ex: TP53
- Apoptosis regulator proteins
  ex: BCL2

Hanahan and Weinberg, 2011
Activating = gain of function

- Alteration of one allele « dominant »
- « oncogene »
  
  *Ex : RAS, MYC, ABL,…*

Inactivating = loss of function

- Alteration of both alleles « recessive »
  (1 allele : « negative dominant »)
- « Gatekeeper” = Cell control (tumor suppressor genes)
  *Ex : RB, TP53, APC,…*
- “Care-taker” = Genome stability
  *Ex : MMR complex, ATM, BRCA1/2…*

Quantitative gain / Overexpression :
- genomic amplification
- translocation « promotor swap »

Qualitative gain / Coding sequence change :
- activating mutation
- translocation « fusion gene »
- viral integration

Quantitative loss / loss of expression :
- deletion
- monosomy
- methylation

Qualitative loss / Coding sequence change :
- inactivating mutation
- indel
III - Cancer = a genetic disease

**Clonality & multistep process**

- **Clonal proliferation**: cells originating from a single common ancestral cell

**Initiation**  
- Genetic alteration  
- Environmental factor

**Promotion**  
- Genetic alteration  
- Selective advantage  
- Variable number of mutational events

**Tumoral progression**  
- Genetic alteration

- Normal tissue  
- Pre-malignant lesion  
- Primary tumor  
- Metastasis

○ : normal cell, ○ : pre-malignant cell, ○ : malignant cell without metastatic ability, ○ : malignant cell with metastatic ability

- Accumulation of synergistic and ordered genetic damages through mutation in master genes
  - Selective advantage
  - Variable number of mutational events
The prevalence of somatic mutations vary across human cancer types:
- cell type
- age
- mutagenic exposures
- ...

Ludmil B. Alexandrov et al, Nature 2013
It is crucial to distinguish driver from passenger mutations

- **Passenger mutations** → large majority
  - neutral: do not confer growth advantage
  - mutational exposures, genome instability,…

- **Driver mutations** → in key genes for the cell type
  - confer growth advantage
  - role in oncogenesis
  - clinical utility
  - good candidate for targeted treatments
  - recurrent

  **Caution !!!**

  + genes known to be involved in cancer
## IV – New insights from NGS studies

### Specific mutational signatures

<table>
<thead>
<tr>
<th>Signature</th>
<th>Prevalence in cancer samples</th>
<th>Probable association</th>
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<tbody>
<tr>
<td>Signature 1A</td>
<td>11.7%</td>
<td>Age</td>
</tr>
<tr>
<td>Signature 1B</td>
<td>60.7%</td>
<td>Age</td>
</tr>
<tr>
<td>Signature 2</td>
<td>14.4%</td>
<td>APOBEC (Cytidin deaminases: C → U)</td>
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<tr>
<td>Signature 3</td>
<td>9.5%</td>
<td>BRO1/2 mutations (Large del)</td>
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<tr>
<td>Signature 4</td>
<td>12.1%</td>
<td>Smoking</td>
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<tr>
<td>Signature 5</td>
<td>14.4%</td>
<td>DNA MMR deficiency</td>
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<tr>
<td>Signature 6</td>
<td>2.6%</td>
<td>Ultraviolet light</td>
</tr>
<tr>
<td>Signature 7</td>
<td>5.0%</td>
<td>2.0%</td>
</tr>
<tr>
<td>Signature 8</td>
<td>0.6%</td>
<td>Immunoglobulin gene hypermutation</td>
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<tr>
<td>Signature 9</td>
<td>0.5%</td>
<td>Pol μ mutations</td>
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<tr>
<td>Signature 10</td>
<td>0.6%</td>
<td>Terazosinamide</td>
</tr>
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<td>1.4%</td>
<td>2.2%</td>
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<tr>
<td>Signature 12</td>
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<td>APOBEC</td>
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<td>1.8%</td>
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</tr>
<tr>
<td>Signature 21</td>
<td>12.5%</td>
<td>12.5%</td>
</tr>
</tbody>
</table>

**MMR, mismatch repair**

Several sub-clones with common and unique mutations

- Different models of tumor progression
IV – New insights from NGS studies

Clonal evolution

Reconstitution from single cell exome + copy number data

Ex: 2 breast cancers

Wang Y et al, Nature 2014
- Minor existing clones may emerge during treatment
- Secondary mutations

Kleppe M et al  Nat Medicine 2014
• **First generation of targeted therapies:**
  → Search for the primary genetic event = major clone
  Targeting this clone → target all the malignant cells
  ex: Tyrosin kinase inhibitor (Imatinib) and *BCR-ABL* in CML
  Emergence of resistances: selection of existing minor clones
  novel mutations

• **NGS area and intra-tumoral clonal heterogeneity:**
  *Frequent cancers* → *very large number of rare genomic diseases*
  → Search for:
  - key drivers mutations
  - minor existing clones which may emerge during treatment
    High coverage NGS studies
    Combined targeted therapies → personalized treatment
    (unknown toxicities ? Increased costs ?)