SOMATIC MUTATIONS AND CANCER

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TO UNDERSTAND,
TO TREAT
TO PREVENT CANCER
AT BEST FOR ALL
FORMATION OF A TUMOR RESULTS FROM SOMATIC MUTATIONS AND DARWINIAN SELECTION
SOMATIC MUTATIONS IN CANCER: SYNOPSIS

1. No cancer without somatic mutations
2. The rate of mutation is important
3. Mutations are stochastic events
Rarely, errors can take place in the course of DNA replication.
NUMEROLOGY OF SOMATIC MUTATIONS

- Estimated rate of somatic mutation in an individual gene: $2 \times 10^{-7}$/cell division
- Approximate number of cells in human body: $10^{14}$ (about $10^9$ per G)
- Approximate number of ‘deep’ cell divisions to make an adult: $10^{15}$
- Approximate number of genes in human genome: 24,000.
- Approximate number of expected somatic mutations accumulated in an adult: $5 \times 10^{12}$
SOMATIC MUTATIONS IN CANCER

• How many mutations
• Which cells are mutated
• What causes mutations
• What kind of mutations
• What genes are mutated
THE INCIDENCE OF CANCER DEPENDS STRONGLY ON AGE
SOMATIC MUTATIONS IN CANCER

• How many mutations
• Which cells are mutated
• What genes are mutated
• What kind of mutations
• What causes mutations
Self-sufficiency in growth signals

Evading apoptosis

Insensitivity to anti-growth signals

Sustained angiogenesis

Tissue invasion & metastasis

Limitless replicative potential

(From Hanahan D, Weinberg RA
Cell. 2000 Jan 7;100(1):57-70.)
ONCOGENE ADDICTION

...The apparent dependency of some cancers on one or a few genes for the maintenance of the malignant phenotype

Bernard Weinstein

Science 297:63, 2002
Cancer Res 68:3077, 2008
MODELS OF ONCOGENE ADDICTION

QuickTime™ and a decompressor are needed to see this picture.

(From Torti & Trusolino EMBO Mol Med 3:623, 2011)
BINDING TO CERTAIN SPECIFIC DNA ELEMENTS IS CRUCIAL TO THE FUNCTIONS OF p53
p53: SOMATIC MUTATIONS versus GERM-LINE MUTATIONS
Figurative depiction of the landscape of somatic mutations present in a single cancer genome.

FEATURES OF HUMAN RETINOBLASTOMA ARE REMARKABLY CONSERVED

Original tumor

Xenograft from above

(From Zhang et al., Nature, 2012)
GENOMIC PROFILE OF RETINOBLASTOMA IN TWO INDIVIDUAL PATIENTS

From Zhang et al., *Nature*, 2012
RETINOBLASTOMA HAS FEW MUTATIONS WHEN COMPARED TO OVARIAN CANCER

From Zhang et al., Nature, 2012
Circos plots for the primary tumor, metastasis, and xenograft genomes of a basal breast tumor

(From Ding et al., *Nature* **464**:999, 2010)
PATTERNS OF SOMATIC GENOMIC REARRANGEMENTS IN PANCREATIC CANCER

(From Campbell et al., Nature 467: 1109, 2010)
TENTATIVE TIMELINE OF PANCREATIC CANCER

(From E G Luebeck
CLONAL EVOLUTION IN TWO PATIENTS WITH CLL DEMONSTRATED BY 'ULTRA-DEEP' SEQUENCING

QuickTime™ and a decompressor are needed to see this picture.

(From Patel et al., NEJM 366:1079, 2012)
COMPLEXITY OF SOMATIC MUTATIONS IN ACUTE MYELOID LEUKAEMIA

(From Patel et al., NEJM 366:1079, 2012)
CLONAL EVOLUTION FROM MDS TO AML

(From Walter et al., NEJM 366:1090, 2012)
WNT AND SHH SUB-TYPES OF MEDULLOBLASTOMA ARE ANATOMICALLY DISTINCT

(From Gibson et al., Nature 468:1095, 2010)
Other major initiatives accessible on line:

WELLCOME TRUST SANGER INSTITUTE CANCER GENOME PROJECT
http://www.sanger.ac.uk/research/projects/cancergenome/

NIH-NCI CANCER GENOME ANATOMY PROJECT
http://cgap.nci.nih.gov/
SOMATIC MUTATIONS IN CANCER

• How many mutations
• Which cells are mutated
• What genes are mutated
• What kind of mutations
• What causes mutations
EPIGENETICS

.... The switching on and off of genes during development, the segregation of gene activities following somatic cell division, and the stable (somatic) inheritance of a given spectrum of gene activities in specific cells’.....
.....possibly explained by ‘changes in the pattern of DNA methylation’.

Robin Holliday 1989
DEVELOPMENT and DIFFERENTIATION: GOING DOWN DIFFERENT PATHWAYS

C D Waddington 1957
DEVELOPMENT and DIFFERENTIATION: GOING DOWN DIFFERENT PATHWAYS
DEVELOPMENT and DIFFERENTIATION: GOING DOWN DIFFERENT PATHWAYS
DEVELOPMENT and DIFFERENTIATION: GOING DOWN DIFFERENT PATHWAYS
DEVELOPMENT and DIFFERENTIATION: 
IS THE PROCESS REVERSIBLE?
A MODEL OF HOW CHROMATIN CAN REGULATE GENE EXPRESSION
Figure 2. Schematic representation of epigenetics associated with active and silenced loci

Zelent, A. et al. Mol Cancer Ther 2005;4:1810-1819
POINT MUTATION
<table>
<thead>
<tr>
<th>Substitutions at specific dinucleotides</th>
<th>COLON</th>
<th>BREAST</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>5’-TpC-3’</td>
<td>79 (11.4)</td>
<td>257 (30.7)</td>
<td>336 (21.9)</td>
</tr>
<tr>
<td>5’-CpG-3’</td>
<td>309 (44.4)</td>
<td>139 (16.6)</td>
<td>448 (29.2)</td>
</tr>
<tr>
<td>Substitutions at CG base pairs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CG to TA</td>
<td>413 (59.3)</td>
<td>289 (34.5)</td>
<td>702 (45.8)</td>
</tr>
<tr>
<td>CG to GC</td>
<td>48 (6.9)</td>
<td>239 (28.5)</td>
<td>287 (18.7)</td>
</tr>
<tr>
<td>CG to AT</td>
<td>93 (13.4)</td>
<td>148 (17.7)</td>
<td>241 (15.7)</td>
</tr>
<tr>
<td>Substitutions at TA base pairs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA to CG</td>
<td>56 (8.0)</td>
<td>72 (8.6)</td>
<td>128 (8.3)</td>
</tr>
<tr>
<td>TA to GC</td>
<td>51 (7.3)</td>
<td>35 (4.2)</td>
<td>86 (5.6)</td>
</tr>
<tr>
<td>TA to AT</td>
<td>35 (5.0)</td>
<td>55 (6.6)</td>
<td>90 (5.9)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>696</td>
<td>838</td>
<td>1534</td>
</tr>
</tbody>
</table>

SOME SPECIFIC TYPES OF SOMATIC MUTATIONS FOUND IN TUMORS
COPY LOSS
COPY GAIN
REECIPROCAL TRANSLOCATION

GENE FUSION
TYPES OF MUTATIONS IN HUMAN CANCER

(From Futreal et al., 2004)
FUSION GENES
IN EPITHELIAL (and other) CANCERS

- RET-PTC1  
  Papillary thyroid cancer
- TMPRSS2-ERG  
  Prostate cancer
- ETV6-NTRK3  
  Breast cancer
- ARID1A-MAST2  
  Breast cancer
- EML4-ALK  
  Adenocarcinoma of the lung
- MYB-NFIB  
  Adenoid cystic carcinoma of salivary gland
- KIAA1549-BRAF  
  Glioma
- EWS-FLI1  
  Ewing sarcoma
- SYT-SSX2  
  Synovial sarcoma
QuickTime™ and a decompressor are needed to see this picture.
CHROMOTHRYPSIS IN MEDULLOBLASTOMA IN LI-FRAUMENI PATIENTS

(From Rausch et al., Cell 148:59, 2012)
CORRELATION BETWEEN p53 STATUS AND CHROMOTRYPSIS IN MEDULLOBLASTOMA

Maximum amplicon count per chromosome

(From Rausch et al., Cell 148:59, 2012)

Coined term and reported occurrence in several types of tumors, including:

- Osteosarcoma (~25%)
- Neuroblastoma 10
- Medulloblastoma 4
- Prostate 1
- Multiple myeloma (~1.3%)
- Colon common
The intrinsic rate of somatic mutation of an individual may be a determinant of the risk of cancer.
Rare GPI(−) granulocytes can be found in a normal person

CD55, CD59 (GPI-linked)

CD11b (transmembrane)

(Araten et al..Proc Natl Acad Sci U S A. 96:5209,1999)
Rare GPI(−) red cells are found in a normal person and are susceptible to complement lysis

Rare GPI(−) granulocytes are present in most normal persons

(Araten et al.. Proc Natl Acad Sci U S A. 96:5209, 1999)
GPI(−) granulocytes from normal persons have PIG-A mutations

(Araten et al.. Proc Natl Acad Sci U S A. 96:5209, 1999)
The Rate of Somatic Mutation Can Be Measured in Humans

CD48, CD55, CD59-PE

HLA-DR FITC

PBL from PNH patient

Unsorted LCL from normal subject

Flow-sorted GPI(+) cells

Same after 27 days culture

The Rate of Somatic Mutation Can Be Measured in Humans

(Araten et al.. Cancer Res. 2005)
ADVANTAGES OF *PIG-A* AS A SENTINEL GENE

1. X-linked gene: therefore mutations are phenotypically expressed.
2. The *PIG-A* product is a subunit of an enzyme required for GPI synthesis: therefore mutations can be detected by testing for GPI-linked proteins.
3. GPI-linked proteins are ubiquitously expressed on the cell surface: therefore a variety of cells can be studied individually by flow cytometry.
4. In view of (2), amplification provides high sensitivity.
5. In view of (3), multiple proteins can be analyzed, thus avoiding artefacts.
Biological correlates of $\mu$

- Normal range
- Genetic determinants
- Environmental factors that affect $\mu$
- Acquired changes in $\mu$
- Risk of cancer
- Changes of $\mu$ in cancer
THE MUTATION RATE IS INCREASED IN CONDITIONS ASSOCIATED WITH INCREASED SUSCEPTIBILITY TO CANCER

(Araten et al.. Cancer Res. 2005)
Personalized Cancer Medicine

What is it?

Optimizing the care of each individual patient with cancer in terms of the tumor and in terms of the host.
PCM - II

Optimizing the care of each individual patient with cancer

*in terms of the tumor:*

it means to make a full diagnosis at the molecular level

*in terms of the host:*

it means to identify factors that may affect the course of the tumor and/or the response to therapy
PCM - III

Personalized medicine is very important:
It means to treat the patient as a whole person
Anti-angiogenici
Anti-infiammatori
Immunomodulatori

Farmaci che agiscono sul DNA e sulla mitosi
(*chemioterapici classici*)

Inibitori di un *signal transduction pathway* importante in un certo tumore
(*p.es. sunitinib*)

Interferenza con molecole mutate oncogeniche
(*p.es. imatinib, gefitinib*)

Interferenza con molecola iper-espressa in un tumore
(*p.es. trastuzumab*)
People prefer to be satisfied with a single causative factor....

[In fact], accidental factors and constitutional factors both play a role.... We refuse to posit any contrast in principle between the two sets of aetiological factors.... [that] regularly act jointly....

Sigmund Freud, 1912