Mechanisms of oncogenesis

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Neoplasms

- Second (to cardiovascular disease) cause of death worldwide
- In highly developed countries is, or soon will be #1!
- The most common neoplasms in Poland (new cases in 2012):
  
  **Males:**
  - lung 14 468
  - prostate 10 866
  - colon 9 452
  - urinary bladder 5 024

  **Females:**
  - breast 16 855
  - colon 7 319
  - lung 6 342
  - uterus 5 594

Cancer

Uncontrolled proliferation of cells and resistance to death promoting factors:
- No response to proapoptotic stimuli
- Escape from immune system
- Clonal growth

Neoplastic transformation

- Imbalance between pro-neoplastic (oncogenes) and anti-neoplastic proteins (oncosuppressors genes and genomic stability genes) - cancer gene mutations enhance net cell growth
- Genetic, epigenetic and environmental causes.
- Multi-step process! Usually several events need to occur in order to form tumor. This may take up to 30 years!

„Vogelgram”

Eric Fearon & Bert Vogelstein

(Cell. 1990;61(5):759-67)

Mutation

APC
β-catenin
K-ras
p53
other

Adenoma
Late adenoma
Cancer
Late cancer
Metastases

Genome stability genes, MMR

Time:
- Decades
- 2-5 years
- 2-5 years
3 gene classes that contribute to oncogenesis

- Protooncogenes
- Antioncogens (tumor suppressor genes)
- Stability genes (DNA repair genes)

Protooncogenes

- Normal (non-mutated) genes coding proteins necessary for proper function of the cell. Their function is usually positively correlated with increased pace of cell cycle: transcription factors, regulators of transcription, regulators of cell cycle, receptors and their ligands
  - Ras (the most often mutated protooncogene in cancers)
  - Src
  - Myc (c-Myc, N-Myc, L-Myc)
  - EGFR1 (breast cancer)
  - Cyclin D1

- Point mutation:
  - Hyperactive protein

- Gene amplification:
  - Additional copies of the gene – too much product

- Chromosomal translocation:
  - too much of the normal product
  - Fusion protein - too much of the product
  - Fusion protein – hyperactive product

- Only activating mutations lead to oncogenesis.
- Dominant genes – mutation of one allele leads to disease

Tumor-suppressors

- Proteines coded by these genes inhibit cell cycle and, in consequence, slow down the rate of cell divisions.
  - p53
  - Rb (retinoblastoma)
  - p16INK4a
  - NF1, NF2
  - APC

- Tumor progression is related to the loss of function of these genes (e.g. inactivating mutations).
- Recessive genes - both copies of the gene have to be altered.
- Exceptions:
  - Haploinsufficiency
  - Dominant-negative mutations
Accumulation of mutated p53

Colon cancer  Breast cancer

Stability genes

- During replication errors occur 1:1Mbp
- Environmental factors may increase the incidence of mutations (UV-, gamma-, X-radiation, alkylating agents)

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<th>Examples</th>
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<td>Cyclophosphamide, Chlorambucil</td>
<td>Bulky DNA-addicts, DNA crosslinks</td>
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<td>Aromatic hydrocarbons</td>
<td>Soot, tobacco smoke, chimney soot, smoked meat</td>
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<td>Amines and azides</td>
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<td>Nitrosamines</td>
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<td>Asbestos</td>
<td>Asbestos</td>
<td>Inflammation driven proliferation, fixing genetic mistakes</td>
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<td>UV radiation</td>
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<td>Pyrimidine dimers cause miscopling of DNA</td>
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<td>Ionizing radiation</td>
<td>X-rays, α-, β-, γ-radiation</td>
<td>DNA-breakage, base changes</td>
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Stability genes

- Repair of mutations acquired during replication is controlled by stability genes such as:
  - ATM, ATR (AT)
  - BRCA1, BRCA2 (breast ca. ovarian ca.)
  - MLH1, MSH2, MLH3, MSH6, PMS1, PMS, TGFB2 (NHPCC)
  - NBS1 (nibrin) Nijmigen syndrom
  - Rad50
- Like the oncosuppressors, they are recessive – both copies must be defective for cancerogenesis.

Summary

- Both alleles of gene are equally expressed.
- Gain of function mutations
  - Are not found in oncosuppressors or stability genes
  - Are dominant

Summary

- Loss of function mutations
  - Are not found in protooncogens
  - Are recessive
  - How do we know it? → Two hit hypothesis

AG Knudson jr.
PNAS, 1971
Two hit model of oncogenesis, cont.

Telomerase

- Elongates telomers
- Normally active only in embryonic cells or adult stem cells
- Found to be active in some cancers

Loss of heterozygosity (LOH)

- If one of the alleles of tumor-suppressor gene is not functional (e.g. mutated allele is inherited from one of parents), the other allele is sufficient to exercise the function of the gene.
- For the neoplastic transformation it is than enough to acquire mutation only in one remaining, healthy (WT) gene (Alfred Knudson’s two hit model)
- Usually the first mutation is subtle point-mutation (as the ones inherited or germline) when the second is a chromosomal mutation (such as deletion of a fragment of the chromosome). This leads to the loss of the entire allele of the gene, hence the cell has only one (mutated) copy of the gene left. The cell is not a heterozygote any more!

Hereditary cancer

- Protooncogenes:  
  - KIT, PGDFRA
- Stabilizing genes (most often mutated in hereditary form of disease)  
  - ATM, BRCA1, BRCA2
- Tumor-suppressors  
  - APC, Axin2, PTCH, p53, CDKN2a (p16ink4a i p16arf), NF1, NF2

Cancer predisposition hereditary disorders

Li-Faumeni syndrom

- Defective p53 gene
- Various types of cancer with the early onset: sarcomas (bone sarcoma), breast cancer, brain tumors (glioblastoma), lymphomas
- The onset of cancer in 50% of syndrom affected patients is before 40 years of age

Cancer predisposition hereditary disorders

Familial adenomatous polyposis

- Responsible gene: APC (Adenomatous polyposis coli - chromosom 5, germ line mutation; 30% mutation de novo (hereditary os sporadic)
- 1% of all colorectal cancers. Patients have thousands of polips in the colon (2. i 3. decade of life)
- Average age of colorectal cancer in these patients 39.
- 90% will develop cancer before the age of 45
- Other manifestations include: other GI tract cancer, benign tumors
Cancer predisposition hereditary disorders

HNPCC (Lynch syndrome)
- Germ line mutations in stability genes: MLH1, MSH2, MLH3, MSH6, PMS1, PMS2
- Up to 5% of colorectal cancer cases
- Endometrial cancer (in females)
- Colorectal cancer 44 years of age (average)

Cancer predisposition hereditary disorders

Nijmegen breakage syndrome
- Recesive disorder of gene NBS1, (nibrin, DNA repair protein that fixes dsDNA breaks)
- Firs case reported in Nijmegen (Holland) but the disease is typical for central Europe Czech Republic, Poland
- Survival up to 52years
- 1000x more likely to develop NHL i ALL
- 40% before age of 20 will develop NHL
- Heterozygotes 4x more likely to develop cancer than healthy indyviduals! (haploinsufficiencya) probably up to 2% of cancers in Polsce

Other mechanisms of the gene function loss (mutation phenocopies)
- Epigenetic, e.g.: methylation of the gene promoter shuts down function of the gene. If WT allele is methylated, only mutated allele produces altered protein
  - p16\textsuperscript{Kip1} – in over 40% of cases promoter methylation is the „second hit” (mutations 0-2%, LOH 0-10%)
- Posttranslational modification of protein coded by healthy (WT) allele
  - E.g. through inactivating phosphorylation. „Cross-talk” between signaling pathways

Other mechanisms

Epigenetic
- Onkomirs – mikroRNA associated with cancer development
  - miR 17
  - miR 19
  - miR 21
- Antyonkomirs
  - miR 143
  - miR 145

Other genetic changes

- Translocation of chromosomes –chimeric proteins of abnormal function
  - T(9;22) – translocation of a part of gene c-Abl under a control of a promoter of a gene Bcr
  - Philadelphia kariotype
  - protein Bcr/Abl – CML and ALL

Oncogenic viruses

- HTLV1/2 (T-cell leukemia)
- EBV (Burkitt lymphoma, nasopharyngeal cancer )
- HBV i HCV (hepatitis and liver cancer)
- HPV (cervical cancer; vaccine
  - HPV code for proteins E6 i E7 which inhibit p53 i Rb. Phenocopy of the mutation of these genes!
"Crosstalk" between signaling pathways

Signaling pathways dysregulated by Bcr/Abl oncogene

Gene therapy strategies

Vectors

Cathionic lipids

Retroviruses
Genome editing

“Repair” of the mutations
- ZNF-nucleases
- TALEN
- CRISPR/Cas9

Elements of CRISPR-Cas9
- PAM - Protospacer-adjacent motif
- CRISPR - Clustered regularly interspaced short palindromic repeats
- tracrRNAs - Trans-activating CRISPR RNAs
- crRNA – CRISPR RNA
- Cas9 - endonuclease

Genome editing with CRISPR/Cas9

Homework
Please read this article:

Cancer genes and the pathways they control
Bert Vogelstein & Kenneth W. Kinzler
Nature Medicine VOLUME 10 | NUMBER 8 | AUGUST 2004

- Pdf file available through ssl service of our University (full text)

The End