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Cancer as a Genetic Disease

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Section A

Introduction

About Cancer

- Cancer refers to at least 100 forms of disease
- Cancer is an infectious disease
- Cancer is a disease of aging
- Cancer is an environmental disease

About Cancer

- Cancer is a genetic disease
  - Not monogenic like MD or CF, but multigenic
- Cancer is caused by mutations in somatic cells
- Cancer can be caused by mutagens, chemicals that damage DNA, or viruses
- Cancer is caused by an accumulation of mutations in different genes in a single cell
- Cancer is caused by altered expression of genes or by accumulation of mutations in a single cell

- Mutations, or damage to the genome, can be caused by mistakes during normal DNA replication
  - 1×10^{-10} mutations/bp/cell division
  - 2×10^{9} bp, so about one mistake (mutation) in every 10 cell divisions of a stem cell)
- There is an intrinsic chemical instability of certain DNA bases that might give rise to mistakes or mutations
- The DNA can be attacked by free radicals, ionizing radiation, UV radiation, and chemical carcinogens—producing mutations

- Finally, viruses, such as human papilloma virus (HPV), contain proteins that bind to and inhibit p53 and RB leading to an increase of genomic instability and an increase in mutations
- "Random mutations in the genes which control proliferation or apoptosis are responsible for Cancer" (Bertram, J.S. 2001. Molecular aspects of medicine. 21: 167-223.)

Five Major Pathways: Cancer Cells

- There are five major pathways that must be activated or inactivated in a cell for the cell to become a cancer cell
  - Growth stimulatory signals
  - Growth inhibitory signals
  - Apoptosis resistance
  - Infinite proliferative capacity
  - Angiogenic potential

How Is Cancer Studied in the Laboratory?

- Cancer in "cell culture"—transformed cells
- Normal cells and transformed cells differ in:
  - Morphology
  - Contact inhibition
  - Serum (growth factor) dependence
  - Anchorage dependence
  - Tumorigenicity

Tumor Cells and Viruses

- Tumor cells contained a “cancer” gene
- Tumor viruses contained “cancer” genes (oncogenes), which had cellular counterparts
  - Over 25 distinct cellular genes, now referred to as proto-oncogenes, have been identified
- Oncogenes are involved in signaling pathways that increase proliferation

The Proto-Oncogene

- Often the presence of the proto-oncogene is not sufficient to cause cancer
- However, increased expression of the proto-oncogene may cause cancer
- Genomic instability or the integration of retroviruses may give rise to cancer by increasing the expression of proto-oncogenes
- Tumors have chromosomal abnormalities
  - Gain and loss of chromosomes, translocation, and amplifications

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Section B

Oncogenes

- Many B cell tumors carry chromosomal translocations that join the **c-myc** proto-oncogene to one of the immuno-globulin genes

Oncogenes and Tumor Viruses

- Oncogenes were isolated from tumor viruses, and they were also isolated from tumor tissue
- The oncogenes from viruses and tumor tissue were not always precisely the same as the proto-oncogene

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Section C

Tumor Suppressor Genes

Rapid Progress in the Field of Oncogenes

- One of the reasons that progress was rapid in the field of oncogenes was that the addition of oncogenes to cells was genetically dominant
- However, in some experiments using cancer cells in culture, the tumorigenicity phenotype was recessive to normal growth

Growth-Suppressing Genes

- These experiments led to the idea that cells had growth-suppressing genes
  - If these genes were inactivated by mutation or deletion, then cancer would result
- There are numerous tumor-suppressor genes and anti-oncogenes
- These tumor suppressor genes code for proteins that normally act as checkpoints to cell proliferations or cell death

Retinoblastoma (RB) Susceptibility Gene

- One of these tumor suppressor genes is the retinoblastoma (RB) susceptibility gene
- This gene was isolated by positional cloning
- It was known that retinoblastoma, tumors of the eye in children, were associated with deletion of 13q14.11
- Probes to this region of chromosome 13 were isolated
- Regions of chromosome 13 near this region were tested for expression as mRNA, presence of closely related sequences in other species (mouse, rat, etc.), and deleted, rearranged, or mutated DNA of retinoblastoma cells

Continued

Retinoblastoma (RB) Susceptibility Gene

- Another tumor suppressor gene, p53, was first isolated because a mutated form of p53 was a dominantly acting oncogene
- Mutation in p53 are now known to be associated with increased susceptibility to cancer

Some Presumptive Suppressor Genes

- RB
  - Chromosome 13q
- Wilms tumor gene WT1
  - Chromosome 11p
- Deleted colon carcinoma, DCC
  - Chromosome 18q
- Neurofibromatosis gene type 1 NF1
  - Chromosome 17q
- P53
  - Chromosome 17q
- Gene involved in familial adenomatous polyposis coli, APC
  - Chromosome 5q

Li-Fraumeni Syndrome

- A mutant p53 gene is responsible for inherited cancers in the Li-Fraumeni syndrome

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Section D

Cancer Is a Multigenic Disease

- Cancer is caused by mutations in many genes

Some Questions

- What do cancer genes code for? what processes are altered that give rise to cancer?
  - Signal transduction
  - Cell cycle checkpoints
  - DNA repair
  - Balance between cell division and apoptosis
  - Angiogenesis

Molecular Diagnosis

- My vision for the future is that we will no longer be diagnosed with breast cancer or prostate cancer or brain cancer—but rather with a cancer having the following genes malfunctioning
- This approach to diagnosis—having a molecular diagnosis—will then allow us to tailor the treatment of the individual

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Section E

Therapies

Potential and Actual Therapies

- Surgery
- Conventional chemotherapy
- Radiation therapy
- Hormone therapy
- Monoclonal antibodies
- Antibody toxin conjugates
- Antibody radionucleotide therapy
- Patient-specific vaccines
- Immunotherapy
- Specific protein kinase inhibitors
- Gene therapy
- Virotherapy (October 2003 *Scientific American*)
- Antisense therapy
- Anti-angiogenesis therapy

Gleevec

- Remember we talked about the fusion between BCR gene and the ABL gene
- The result was the p210 BCR-ABL protein—that is a new protein kinase
- Drug STI-571 (Gleevec) was developed as an inhibitor of the BCR-ABL protein
- The drug was effective in early-stage disease, but resistance developed in blast-stage disease
- The mechanism of resistance was a single mutation in p210 or an increase in the BCR-ABL gene number

Reference: *Nature,* 2001. 412 pp. 281–282.

Retinoblastoma (Rb) Protein

- Cell-cycle master switch
  - Implicated in DNA replication, differentiation, DNA repair, cell-cycle checkpoints, and apoptosis
- Interacts with many (over 100?) different cellular proteins—for example:
  - Cyclin-dependent kinases (phosphorylate Rb to turn on cell proliferation)
  - E2F, a transcription factor, to sequester it

Protein Kinases ATM and ATR

- Part of the DNA damage response pathway (DRP)
- DRP controls DNA repair, telomerase composition and length, transcriptional programs, and apoptosis
- DRP proteins sense damage, transduce the damage signal, and affect a response
- DRP includes:
  - p53, ATM, BRCAI, Rad 55, ChKl, and ChK2.

Deranged Pathways

Spaghetti Junction	
The pathways that, when deranged, lead to cancer are complex, interconnected, and many. This table lists some of the better-understood ones.	
Additional Pathways Perturbed in Cancer	**Representative Cancer Types**
WNT/Beta-catenin/APC	Colorectal cancer, melanoma
Transforming Growth Factor-b (TGF-b)	Hereditary nonpolyposis colon cancer, gastrointestinal, prostate, ovarian cancer
Ras	Hematopoietic, breast, ovarian, pancreatic, bladder cancer
c-Myc	Breast, bladder, B cell leukemia, melanoma, and many more
Notch	Hematopoietic cancer
Jak/Stat	Hematopoietic cancer
Nfkb	Hematopoietic cancer
BCR-ABL molecular defect	Chronic myelogenous leukemia
Sonic hedgehog/Patched	Basal cell carcinoma
Epidermal Growth Factor Receptor (EGFR)	Breast cancer, lung cancer, ameloblastoma, many other cancers of epithelial origin
Telomerase Expression	Multiple human cancers

Schultz, L.B., September 22, 2003. Aberrant signaling, *The Scientist*, 10–12.

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Section F

Cancer and Genomics

- Recent strategies for studying cancer that involve analysis of multiple samples
  - Genomics: high-throughput technologies for analyzing genes
  - Transcriptomics: high-throughput technologies for analyzing messenger RNAs
  - Proteomics: high-throughput technologies for analyzing proteins

Reference: Olli Kallioniemi, March 25, 2004. Profile of a tumor. *Nature;* 428, pp. 379–382.

An Example of Genomics

- An example of genomics would be:
  - An experiment analyzing the expression of 70 informative genes in breast tumors to associate the messenger RNA expression of certain of these genes to good or poor prognosis

References: van't Veer, L.J., et al. 2002. *Nature* 514, 530–536.
Van de Vijver, M.J., et al. 2002. *N. Engl. J. Med.* 347, 1999–2009.

- The outcome of this genomic study would be to help clinicians distinguish between:
  - Those women having breast tumors who have a poor prognosis and might need adjuvant therapy or chemotherapy
  - Those women who have a good prognosis—whereby some of those treatments may not be necessary

- Proteomics
  - An analysis of blood serum using mass spectrometry to identify up to 15,000 peaks, which represent fragments of protein that are defined by their mass-to-charge ratios
- Purpose of this technology
  - To determine if there are tumor markers for certain cancers that show up in the blood

Continued

- Proteomics
  - Analysis of levels of specific phosphorylated proteins in tumor samples
- Purpose of this technology
  - This analysis is to determine correlations of certain signaling pathways in tumors that might help target inhibitor studies

Mutations Leading to Cancer

- It is known that mutations in about 1% of human genes (a total of about 290 genes) are reported to contribute to cancer
- It is estimated that there are around 500 new anticancer drugs currently in clinical trials

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Section G

Multidrug Resistance and Cancer

Multidrug Resistance and Cancer

- Multidrug resistance (MDR) in human malignancies is likely to be a major cause of failure in cancer chemotherapy
- Characterization of independently derived MDR cell lines clearly illustrates that MDR can be mediated by the overproduction of a 170 kDa glycosylated plasma membrane protein called P-glycoprotein
  - "P" in this case stands for permeability

How Can a Cell Become Drug Resistant?

- Decreased uptake
- Increased degradation
- Increased efflux of the drug
- All of these mechanisms can be imagined for a particular drug, but the unique thing about MDR is that it is characterized by **simultaneous acquisition of resistance to drugs that are structurally and functionally unrelated**
  - The cells become resistant to a number of unrelated drugs by a selection protocol to a single cytotoxic drug

Characteristics of the Chemotherapy Drugs

- Characteristics of the chemotherapy drugs
  - Predominantly alkaloids
  - Antibiotics of plant and fungal origin
- Resistance to these compounds is thought to be achieved by lowered intracellular drug concentration: increased efflux

Overproduction of the P-glycoprotein

- Overproduction of the P-glycoprotein is caused by a gene amplification event: 100–200 copies of gene

- Amplification of CHP1 homologous sequences correlated with MDR
  - Areas of up to 500 kb are amplified up to 100–200 times
- Highly resistant cells have karyotypic changes:
  - HSR (homogeneously staining regions)
    - Staining with Giemsa
  - Double minute chromosomes
  - Chromosome translocations

Continued

- This 600 bp sequence with no internal Eco R1 sites detected multiple bands
  - One indication that P-glycoprotein is encoded by a multigene family
- Hamster probe could hybridize to mouse and human
    - Conserved nucleotide sequences

P-glycoprotein

- P-glycoprotein is also found widespread in human cancers
  - Including leukemias, lymphomas, sarcomas, and carcinomas
- MDR cell lines can be isolated from drug-sensitive tumor cells

Resistance of Human Tumors to Chemotherapy

- Resistance of human tumors to chemotherapy follows a similar pattern in two situations:
  - For many tumors (adenocarcinomas of the colon and kidney), malignant cells are primarily unresponsive to multiple chemotherapeutic agents (intrinsic drug resistance)
  - In other cases (childhood leukemias and neuroblastomas), there is a good response initially to chemotherapy but tumors become refractory to therapy (acquired drug resistance)
    - Drugs of proven chemotherapeutic value include vinblastine, daunorubicin, and actinomycin D

Potential Clinical Implications

- If MDR is found (using cDNA probes for mRNA levels of MDR1 gene), then non-toxic drugs able to reverse the multidrug resistance phenotype might be useful in converting drug-resistant tumors to drug-responsive tumors
- Antibody against P-glycoprotein linked to toxin will specifically kill multidrug resistant cell lines

"An Ideal World" versus "The Real Cellular World"

"In an ideal world, cell division, survival, and death are in sync, promoting homeostasis with neither unregulated growth nor inappropriate cell loss. However, the real cellular world is laden with oncogenes and tumor suppressor genes whose products interact in overlapping pathways that, when dysfunctional, can lead to cancer."

Source: *The Scientist,* September 22, 2003, pp. 10–12.