Knudson’s “two-hit” hypothesis

"Knudson’s analysis of the age-specific incidence of retinoblastoma led him to propose that two “hits” or mutagenic events were necessary for retinoblastoma development. Retinoblastoma occurs sporadically in most cases, but in some families it displays autosomal dominant inheritance. In an individual with the inherited form of the disease, Knudson proposed that the first hit is present in the germline and thus in all cells of the body. However, the presence of a mutation at the susceptibility locus was argued to be insufficient for tumor formation, and a second somatic mutation was hypothesized to be necessary for promoting tumor formation."

Knudson, 1971: “two-hit” model

“An easily understood, workable falsehood is more useful than a complex, incomprehensible truth.”
Cancer is a disease of genomic instability

- It is profoundly nontrivial to take a mature cancer cell line and divine the iterative sequence of steps that led to its formation.

One:
“self-sufficiency in growth”
- The majority of cells in the adult human (60,000 billion of them) are mitotically quiescent – most in G₀, but some elsewhere (G₂)
- Some are unequivocally postmitotic (neurons, muscle cells)
- The decision to exit quiescence and begin proliferation is VERY tightly regulated

1967 (Hartwell) – screen in budding yeast for cell division control (cdc) mutants.

A forward genetic screen
A phenomenon →
a list of genes involved in the phenomenon →
an understanding of mechanism
1. Take organism
2. Mutagenise
3. Isolate mutants relevant to phenomenon under study
4. Determine what genes are affected (mutated) in each mutant
5. Determine the order of action of gene products in the process under study

Mitosis
Begin DNA replication
End DNA replication
S phase
G₁
G₂
G₀
Gene X does its “thing”
Permissive: Cells at all points in cell cycle
Restrictive: Cells all arrest at the point in the cell cycle where mutated gene exerts its function

Yeast Cdc28p and human CDK1
Burkitt’s lymphoma

The mutation in Burkitt’s lymphoma puts Myc downstream of the Ig enhancer – this overexpressed Myc and thus creates a hypermorph. This is a dominant mutation – a “hypermorph.”

Immediate early genes: myc, jun, fos

A summary

To make a transition to malignancy, a cell must be under the erroneous impression that it is receiving a signal from the body to leave quiescence and begin proliferating. This typically occurs via a dominant mutation in a “protooncogene”:
1. A growth factor receptor (ret).
3. A transcription factor downstream of #2 (myc).
The mutation creates a constitutively active form of the protein (i.e., it’s a neomorph or a hypermorph).

Two:
insensitivity to anti-growth signals and evasion of apoptosis

Mutations in protooncogenes are dominant (they yield neomorphs and hypermorphs), and thus heterozygosity is sufficient for cancer. The second major class of genes mutated in cancer – the “tumor suppressors” – are ablated via recessive mutations (but see below!), and thus both alleles must be lost (LOH) for cancer to proceed. The name “tumor suppressor” stems from the fact that introducing a wild-type allele of one of them into a cancer cell will arrest its growth.

“Malignancy of somatic cell hybrids”

The studies of Ephrussi et al. and Harris provided compelling evidence that the ability of cells to form a tumor is a recessive trait. They observed that the growth of murine tumor cells in syngeneic animals could be suppressed when the malignant cells were fused to normal cells, although reversion to tumorigenicity often occurred when the hybrids were propagated for extended periods in culture. The reappearance of malignancy was found to be associated with chromosome losses. Stanbridge and his colleagues studied hybrids made by fusing human tumor cell lines to normal, diploid human fibroblasts. Their analysis confirmed that hybrids retaining both sets of parental chromosomes were suppressed, whereas hybrids containing only one set of chromosomes lost the ability to form tumors. Moreover, it was demonstrated that the loss of specific chromosomes, and not simply chromosome loss in general, correlated with the reversion to tumorigenicity. The observation that the loss of specific chromosomes was associated with the reversion to malignancy suggested that a single chromosome (and perhaps even a single gene) might be sufficient to suppress tumorigenicity. To directly test this hypothesis, single chromosomes were transferred from normal cells to tumor cells, using the technique of microcell-mediated chromosome transfer. It was found that the transfer of a single chromosome 11 into the HeLa cervical carcinoma cell line suppressed the tumorigenic phenotype of the cells. Many studies have now demonstrated that transfer of even very small chromosome fragments will specifically suppress the tumorigenic properties of certain cancer cell lines.
Our two best friends

1. p53 is a sensor of DNA damage – its function is to arrest the cell when DNA is damaged, and either allow the damage to be repaired, or send the cell into apoptosis.

2. pRb senses cell stress and evaluates levels of growth factor signaling – its function is to prevent progression through G1 until adequate signals have been received.

Mutations in p53 are the most common genetic event in cancer, closely followed by inactivation of the pRb pathway – in general, practically all human cancers have somehow inactivated both p53 and pRb.

Tumor suppressors: a summary

- Li-Fraumeni syndrome and retinoblastoma are caused by germline loss-of-function mutations in p53 and pRb.
- From a biochemical standpoint, those mutations yield loss of function proteins – thus, these alleles should be recessive.
- Both diseases, however, exhibit autosomal DOMINANT inheritance, because heterozygosity for a mutation in pRb reveals a loss-of-heterozygosity event for the other allele, and, in the case of p53, the mutant allele is frequently an antimorph (dominant negative) because p53 binds DNA as a tetramer.
Causes and treatments

Diagnostics of sporadic cancer

"For most types of cancer [other than prostate and colorectal], there are no molecular markers available. For example, there are no molecular markers that can be used to detect lung cancer at early stages – the time when it is most amenable to therapy…"


www.epigenomics.com
www.oncomethylome.com

Note: I am not endorsing either of the two companies, but the work that they do is directly relevant to this issue. fdu

Aflatoxin (liver cancer)

Consumption of food contaminated with aflatoxins, toxic metabolites of some species of Aspergillus fungi, has been associated both with human and animal hepatocellular carcinoma. Foods susceptible to Aspergillus infection include peanuts/groundnuts, corn/maize, and oil seeds and nuts, such as cottonseed, almonds, and pistachios. Ecologic studies of humans have revealed associations of high consumption of aflatoxin in moldy peanuts, corn, and soy products with increased rates of HCC in several high-incidence areas of Asia and Africa.

Ounce of prevention

1. Do not smoke.
2. If you have female children, make sure your daughter(s) gets the new HPV vaccine.
3. Wear sunscreen.
5. Fruits and vegetables (folate and B₁₂).
6. Broccoli and other cruciferous vegetables.
Detailed characterization of the particular base substitutions in the p53 gene has revealed distinctly different spectra of p53 mutations in different types of cancer. For example, most p53 mutations in colorectal cancers appear to have arisen spontaneously as a result of deamination of methylated cytosine bases, leading to C→T transition mutations. By contrast, many of the p53 mutations seen in lung cancers are transversion mutations (e.g., G→T) that may have arisen as result of direct interactions of p53 gene sequences with carcinogens present in tobacco smoke. Furthermore, some of the most compelling data to link mutagenic and carcinogenic agents with cancer induction have come from study of the p53 mutations seen in skin (squamous cell) cancers and hepatocellular cancers. In squamous cell cancers arising in ultraviolet light-exposed skin areas, a sizeable fraction of the p53 mutations presumably arose from the generation of pyrimidine dimer premutagenic lesions. Similar studies of the p53 gene in hepatocellular cancers arising in individuals from geographic areas with very high exposures to aflatoxin have identified mutations that are similar to those generated by aflatoxin in in vitro studies.

Spontaneous meCpG deamination (colon cancer)
Should be 4% of all NN – in fact, is 0.8%.
Methylation:
C → 5mC
CpG → 5mCpG
5mCpG → TpG → TpA
deamination → MMR
CpG → UpG → CpG
(no mutation)

A Russian proverb that aptly describes most current cancer treatment modalities
«Лучшее средство от кровотечения из носа – жгут на шею».
“The best cure for a nosebleed is a torniquet on the neck.”

“Chemo” drugs
Intrastrand DNA crosslinking
Depolymerization of microtubules
Apoptosis (programmed cell death)
Cell cycle arrest

“Fused transcript of abl and bcr genes in chronic myelogenous leukaemia”

Abl Src
gleevec
“... 400 mg of oral imatinib daily “

Progression-free survival at 18 months – 89%

Kantarjian et al. (2002) NEJM 346: 645

Trastuzumab (Herceptin).

“The HER2/neu oncoprotein is a receptor tyrosine kinase that transduces growth promoting signals. Binding the HER2/neu oncoprotein with specific antibodies leads to growth inhibitory signaling and promotes apoptosis. Trastuzumab (herceptin) is a humanized monoclonal antibody developed in mice that binds the HER2 receptor. Clinical trials in patients with metastatic breast cancer whose tumors overexpressed HER2/neu demonstrated that herceptin had activity as a single agent, with objective response rates ranging from 11 to 26%.”

Tarceva OKd for new use by FDA
Lung cancer drug from Genentech to treat pancreas
SF Chronicle – 11-3-05

*Genentech Inc. and its partner OSI Pharmaceuticals Inc. said Wednesday that their lung cancer drug Tarceva received Food and Drug Administration approval for use in pancreatic cancer, the most deadly type of tumor.

Tarceva is not a cure, but in a clinical trial of patients with advanced pancreatic cancer, it boosted the number of patients who survived for at least a year from 19 percent to 24 percent. Doctors say that data, announced earlier this year, marked the first time in 10 years that a new treatment has delivered a significant survival gain.

The American Cancer Society estimates that about 32,180 people in the United States will be diagnosed with pancreatic cancer in 2005, and about 31,800 will die. The average life expectancy for a patient with metastatic pancreatic cancer is three to six months, according to the Pancreatic Cancer Action Network, a patient advocacy organization.
And you thought LOD scores were boring

Human breast cancer is usually caused by genetic alterations of somatic cells of the breast, but occasionally, susceptibility to the disease is inherited. Mapping the genes responsible for inherited breast cancer may also allow the identification of early lesions that are critical for the development of breast cancer in the general population. Chromosome 17q21 appears to be the locale of a gene for inherited susceptibility to breast cancer in families with early-onset disease. Genetic analysis yields a lod score (logarithm of the likelihood ratio for linkage) of 5.96 for linkage of breast cancer susceptibility to D17S74 in early-onset families and negative lod scores in families with late-onset disease.


A strong candidate for the 17q-linked BRCA1 gene, which influences susceptibility to breast and ovarian cancer, has been identified by positional cloning methods [within a 600 kb region of 17q]. The BRCA1 gene is expressed in numerous tissues, including breast and ovary, and encodes a predicted protein of 1863 amino acids. This protein contains a zinc finger domain in its amino-terminal region, but is otherwise unrelated to previously described proteins. Identification of BRCA1 should facilitate early diagnosis of breast and ovarian cancer susceptibility in some individuals as well as a better understanding of breast cancer biology.

Very important distinction

“Tumorigenesis in individuals with germline BRCA1/2 mutations requires somatic inactivation of the remaining wild-type allele.”

“Somatic BRCA1 mutations in sporadic breast cancer have not been described.”

BRCA1: 1,863 aa  BRCA2: 3,418 aa
Not related by sequence to each other.
“Breast and Ovarian Cancer Risks Due to Inherited Mutations in BRCA1 and BRCA2”

1. “The lifetime risk of breast cancer among female mutation carriers was 82%.”

2. “Lifetime risks of ovarian cancer were 54% for BRCA1 and 23% for BRCA2 mutation carriers.”

Prophylactic bilateral mastectomy (and/or oopherectomy) for BRCA1/2 mutation carriers

“A study of 139 women with deleterious BRCA1 or BRCA2 mutations who were followed at the Rotterdam Family Cancer Clinic. To reduce their risk of breast cancer, 76 of these women chose to undergo prophylactic bilateral mastectomy, whereas the remaining 63 were followed according to a surveillance protocol consisting of a monthly breast self-examination, a biannual breast examination by a health care professional, and annual mammography. … No breast cancers were observed in the 76 women who underwent prophylactic bilateral mastectomy, whereas eight were detected in the surveillance group. This study … supports the report by Hartmann et al. that prophylactic bilateral mastectomy has an efficacy of at least 90 percent in women classified as at high risk on the basis of a family history of breast cancer. Together [these studies] suggest that the strategies to reduce the risk of breast cancer in high-risk women, prophylactic bilateral mastectomy is the most effective.

Two decades of research have convincingly shown that most women with breast cancer can safely be treated with breast-conserving surgery instead of mastectomy. Thus, it is difficult to accept that prevention should be more extreme than the cure. In this era of molecular medicine, we strive for cancer-prevention options that are more targeted and less invasive than surgical extirpation. Chemoprevention for breast cancer that is as effective and safe as prophylactic bilateral mastectomy is a hope for the future.

Andrea Eisen and Barbara Weber (2001) NEJM 345: 208

Risks appear to be increasing with time: Breast cancer risk by age 50 among mutation carriers born before 1940 was 24%, but among those born after 1940 it was 67%.

Physical exercise and lack of obesity in adolescence were associated with significantly delayed breast cancer onset.

The biggest puzzle of them all

The likeliest function of BRCA1/2 is in abetting homology-directed DNA repair of double-strand breaks.

Why oh why then do women with mutations in BRCA1/2 develop cancer of the breast and of the ovaries? DNA repair needs to occur in all tissues!

“Hereditary Cancer Testing”

“Myriad Genetic Laboratories is a leader in cancer predisposition testing. Myriad discovered the BRCA1 and BRCA2 genes that cause hereditary breast and ovarian cancer, and now offers the most accurate clinical tests available to determine predisposition to cancer: BRACAnalysis for hereditary breast and ovarian cancer, COLARIS for hereditary colon and endometrial cancer, and MELARIS for hereditary melanoma and pancreatic cancer. These tests are unparalleled in accuracy, quality and customer service.”