MUTATIONS AND CANCER
1. Relationship between mutagens and carcinogens

- Mutagenicity and carcinogenicity are clearly correlated. One study showed that 157 of 175 known carcinogens (approximately 90 percent) are also mutagens. The somatic mutation theory of cancer holds that these agents cause cancer by inducing the mutation of somatic cells. Thus, understanding mutagenesis is of great relevance to our society.

- Understanding the specificity of mutagens in bacteria has led to the direct implication of certain environmental mutagens in the causation of human cancers. Ultraviolet light and aflatoxin B$_1$ have long been suspected of causing skin cancer and liver cancer, respectively.

- Now, DNA sequence analysis of mutations in a human cancer gene has provided direct evidence of their involvement. The gene in question is termed $p53$ and is one of a number of tumor-suppressor genes; these encode proteins that suppress tumor formation. A sizable proportion of human cancers have mutated tumor-suppressor genes.
2. AFB₁, UV light, and cancer

- **Liver cancer** is prevalent in southern Africa and East Asia, and a high exposure to AFB₁ in these regions has been correlated with the high incidence of liver cancer.
  - When *p53* mutations in cancer patients were analyzed, G→T transversions, the signature of AFB₁-induced mutations, were found in liver cancer patients from South Africa and East Asia but not in patients from these regions with lung, colon, or breast cancer.
  - On the other hand, *p53* mutations in liver cancer patients from areas of low AFB₁ exposure did not result from G→T transversions.

- Sequencing *p53* mutations has also strengthened the link between UV and human skin cancers.
  - The majority of invasive human squamous cell carcinomas analyzed so far have *p53* mutations, all of them mutations at dipyrimidine sites, most of which are C→T substitutions when the C is the 3′ pyrimidine of a TC dimer.
  - This is the profile of UV-induced mutations. In addition, several tumors have *p53* mutations resulting from a CC→TT double base change, which is found most frequently among UV-induced mutations.
3. The p53 tumor-suppressor gene

- Mutations in $p53$ are associated with many types of tumors, and estimates are that **50% of human tumors lack a functional $p53$ gene**.

- The **active p53 protein is a transcriptional regulator** that is activated in response to DNA damage. Activated wild-type p53 serves double duty:
  - it prevents progression of the cell cycle until the DNA damage is repaired
  - under some circumstances, it induces **apoptosis** (programmed cell death).

- In the absence of a functional $p53$ gene, the p53 apoptosis pathway does not become activated, and the cell cycle progresses even in the absence of DNA repair.

- This progression elevates the overall frequency of mutations, chromosomal rearrangements, and aneuploidy and thus increases the chances that other mutations promoting cell proliferation or blocking apoptosis will arise.
4. How p53 arrests the cell cycle in G1

When DNA is damaged, the p53 protein becomes activated. Activated p53 stimulates the transcription of the gene that encodes the Cdk inhibitor protein p21. The p21 protein binds to S phase cyclin-Cdk complexes and inactivates them, so that the cell cycle arrests in G1. It is not known how DNA damage activates p53.
The cell cycle is under a positive and direct control of the cyclin-dependent kinase family (CDK) and their regulatory subunits. CDKs control the cell cycle in part by hyperphosphorylation and inactivation of negative regulators of the cell cycle, such as the RB protein responsible for susceptibility to retinoblastoma, and associated proteins p107 and p130. It has been proposed that after genotoxic stress, the accumulation of p53 protein induces a cell cycle arrest at the G1 phase; this arrest allows the repair of DNA damage before its replication in the S phase.

Accumulation of active p53 induces the expression of different proteins that regulate the cell cycle. p21 (encoded by the Waf1 gene, called also Cip1, Sdi1 or Pic1) inactivates the Cdk-Cyclin complex by forming a Cdk2/A or E Cyclin/Proliferating Cell Nuclear Antigen/Waf1 complex. Formation of this complex leads to the accumulation of hypophosphorylated pRb, causing the release of E2F, which is necessary for the induction of DNA synthesis.
6. p53 and apoptosis

- p53 can induce apoptosis (programmed cell death) by two independent mechanisms, as shown below. One pathway depends on the function of p53 as a transactivator of transcription by upregulating the expression of Bax, IGF-BP3 and Fas proteins, and by downregulating the expression of Bcl2, IGF-1R and IGFII. The up- and down-regulation of these proteins, respectively, has been correlated with the induction of programmed cell death processes.

  ![Diagram of p53 and apoptosis pathways]

  The second pathway is independent of the p53 transcriptional function but is dependent on p53 protein-protein interactions: p53 protein can bind to cellular proteins involved in DNA synthesis such as replicating protein antigen (RPA), and in DNA repair such as TFIIH, including xeroderma pigmentosum group B (XPB) and D (XPD) DNA helicases, p62 and topoisomerase I.
7. Mutations in tumor suppressor genes are recessive at the cellular level

- P53 inactive alleles are recessive, in the sense that both copies of the wild type p53 alleles must be inactivated in order to produce the mutant phenotype.

- Recessivity **at the cellular level** is a major characteristic of tumor suppressor genes.

- Other recessive tumor suppressor genes are also implicated in the repair of DNA damage. Genes that, when inactivated, produce the phenotype of elevated mutation rates are important contributors to the progression of tumors in humans.

- Such tumor-suppressor mutations that interfere with DNA repair promote tumor growth indirectly, because their elevated mutation rates make it much more likely that a series of oncogene and tumor-suppressor gene mutations will arise, corrupting the normal regulation of the cell cycle and programmed cell death.
8. Oncogenes and tumor-suppressor genes

- Tumors do not arise as a result of single genetic events but rather are the result of multiple-hit processes, in which several mutations must arise within a single cell for it to become cancerous.

- Two general kinds of mutations are associated with cancer: oncogene mutations and mutations in tumor-suppressor genes.
  - Oncogenes are mutated in such a way that the proteins that they encode are activated in tumor cells carrying the dominant mutant allele. A tumor cell will typically be heterozygous for an oncogene mutation and its normal allelic counterpart.

- Roughly 100 different oncogenes have been identified. How do their normal counterparts, proto-oncogenes, function?
  - Proto-oncogenes generally encode a class of proteins that are selectively active only when the proper regulatory signals allow them to be activated. Many proto-oncogene products are elements of cell cycle positive control pathways, including growth-factor receptors, signal transduction proteins, and transcriptional regulators. Other proto-oncogene products function to negatively regulate the apoptotic pathway.
9. The oncogene Ras

- Ras is one such oncogene product. In normal cells, it helps to relay signals by acting as a switch. When receptors on the cell surface are stimulated (by a hormone, for example), Ras is switched on and transduces signals that tell the cell to grow. If the cell-surface receptor is not stimulated, Ras is not activated and so the pathway that results in cell growth is not initiated.

GTP-Ras is short-lived because a protein stimulates conversion to the inactive GDP-Ras, and therefore, continued stimulation with growth factors is necessary for continued cell growth. An exception is those cells that harbor particular mutated forms of Ras protein, the oncogenic forms, for instance mutations where the protein does not get converted efficiently to GDP-Ras.

In about 30% of human cancers, Ras is mutated so that it is permanently switched on, telling the cell to grow regardless of whether receptors on the cell surface are activated or not.
10. Oncogenic Ras mutations

- Oncogenic Ras proteins have been found to be of two types:
  - Most transforming mutations in the Ras protein affect the conformation of the guanine nucleotide binding pocket such that either the Ras protein is unable to hydrolyze bound GTP, or such that GAP (the GTPase Activating Protein) cannot increase the GTP hydrolytic activity of Ras. These mutations are the ones most frequently found in human tumors and occur in residues 12, 13, 59 or 61.
  - In the second type of mutants, the strength of guanine nucleotide binding of the mutated protein is affected, leading to a high intrinsic rate of release. When the protein rebinds nucleotide it is most likely to be GTP, due to the higher intracellular concentration of GTP relative to GDP. These mutations (residues 116, 119) are not as oncogenic as the ones affecting stimulated GTPase.

- Activating Ras mutations are present in greater than 50% of colorectal adenomas and carcinomas, and the vast majority occur at codon 12. Ras abnormalities are one of the earliest events in the stepwise progression of colorectal neoplasms, being detectable even in histologically unremarkable epithelium and aberrant crypt foci adjacent to cancers. Amongst other gastrointestinal malignancies, K-ras mutations are one of the most common genetic abnormalities in pancreatic and bile duct carcinomas, detectable in greater than 75% of tumors.
11. Li-Fraumeni Syndrome

- The Li-Fraumeni syndrome was originally described in 1969 and characterized by individuals with an increased risk of breast cancer, sarcomas, leukemia, and CNS tumors. In the 1990s a mutation in the p53 tumor suppressor gene located on chromosome 17 was found to be associated with this syndrome, with over 70% of Li-Fraumeni families having a mutation in p53. A Li-Fraumeni like syndrome in which individuals do not carry a mutant p53 but in whom a similar spectrum of multiple tumors is seen has also been described.

- The risk of developing a second malignancy in patients with Li-Fraumeni syndrome is 50% at 30 years. It has been suggested that the predisposition for cancer in these individuals is exacerbated by an increased susceptibility to DNA-damaging agents and ionizing radiation received as treatment of a first cancer.
Malignancies typical of Li-Fraumeni syndrome include:

- bilateral breast cancer diagnosed at age 40 (I-2)
- a brain tumor at age 35 (II-1)
- soft tissue sarcoma at age 19 and breast cancer at age 33 (II-3)
- breast cancer at age 32 (II-5)
- osteosarcoma at age 8 (III-3)
- leukemia at age 2 (III-4)
- soft tissue sarcoma at age 3 (III-5)
13. Sporadic vs hereditary tumors

The two-hit theory of hereditary cancer was proposed by Knudson in 1971 in relation to retinoblastoma, a rare childhood tumor of the eye. 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. A second somatic mutation was hypothesized to be necessary for promoting tumor formation. Given the high likelihood of a somatic mutation occurring in at least one retinal cell during development, the dominant inheritance pattern of retinoblastoma in some families could be explained. In the nonhereditary form of retinoblastoma, both mutations were proposed to arise somatically within the same cell.
14. Inherited syndromes of cancer due to germline mutations in tumor suppressor genes

<table>
<thead>
<tr>
<th>Cancer/Cancer Syndrome</th>
<th>Gene</th>
<th>Chromosomal Location</th>
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<tbody>
<tr>
<td>Breast and ovarian cancers</td>
<td>BRCA1</td>
<td>17q21</td>
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<tr>
<td>Breast cancer</td>
<td>BRCA2</td>
<td>13q12-13</td>
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<tr>
<td>SBLA/Li-Fraumeni syndrome</td>
<td>p53</td>
<td>17p13</td>
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<tr>
<td>Retinoblastoma</td>
<td>RB1</td>
<td>13q14</td>
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<tr>
<td>HNPCC</td>
<td>MSH2</td>
<td>2p</td>
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<tr>
<td></td>
<td>MLH1</td>
<td>3p21.3-23</td>
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<tr>
<td></td>
<td>PMS1</td>
<td>2q31-33</td>
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<tr>
<td></td>
<td>PMS2</td>
<td>7p22</td>
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<tr>
<td>Familial adenomatous polyposis</td>
<td>APC</td>
<td>distal to 5'</td>
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<td>Melanoma</td>
<td>MLM</td>
<td>9p21</td>
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<tr>
<td>Neurofibromatosis</td>
<td>NF1</td>
<td>17q11.2</td>
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<tr>
<td>von Hippel-Lindau disease</td>
<td>VHLS</td>
<td>3p25</td>
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<tr>
<td>MEN 2A, MEN 2B, FMTC</td>
<td>RET</td>
<td>10q11.2</td>
</tr>
<tr>
<td>Wilms' tumor</td>
<td>WT1</td>
<td>11p13</td>
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</tbody>
</table>

HNPCC = hereditary nonpolyposis colorectal cancer
SBLA = sarcoma, breast and brain tumor, leukemia, laryngeal and lung cancer, and adrenal cortical carcinoma
MEN = multiple endocrine neoplasia syndromes
FMTC = familial non-MEN medullary thyroid carcinoma

Mutations in the BRCA1/2 and in the HNPCC genes are especially important from an epidemiological point of view.