CHAPTER 16: CANCER

- genetic disease, but is not an inherited disease in most cases
- genetic alterations in the DNA of a somatic cell
  - because of these, cancer cells become freed from many restraints to which normal cells are subjected
    -> normal cells: do not divide unless stimulated by body’s homeostatic machinery, do not survive if damage incurred is irreparable, do not wander away from a tissue to start new colonies elsewhere in body
- cancer cells experience breakdown in regulatory influences that protect body
- cancer cells proliferate uncontrollably
  - produce malignant tumors: invade surrounding healthy tissue
  - malignant tumors tend to metastasize: spawn renegade cells that break away from the parent mass, enter the lymphatic/vascular circulation and spread to distant sites in the body → secondary tumors (metastases)
    - secondary tumors: no longer amenable to surgery
- can be treated as long as the tumor remains localized
  - by surgical removal
- three major types of cancer
  - breast cancer
  - prostate cancer
  - colon cancer
- most current treatments
  - chemotherapy & radiation
  - however, they lack the specificity needed to kill cancer cells without damaging normal cells also
  - result: patients cannot usually be subjected to high enough doses of chemicals/radiation to kill all tumor cells

I. Basic Properties of a Cancer Cell

- to study cancer cells: Obtain cells by removing malignant tumor, dissociating the tissue into its separate cells, and culturing in vitro.
  - behavior of cancer cells most easily studied when cells are growing in culture.
- normal cells can be converted to cancer cells
  - by treating them with carcinogenic chemicals, radiation, or tumor viruses
  - cells transferred in vitro by chemicals or viruses can generally cause tumors to suitable host animals
- most important characteristic: loss of growth control

<table>
<thead>
<tr>
<th>NORMAL CELLS</th>
<th>MALIGNANT CELLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal cells</td>
<td>Cancer cells</td>
</tr>
<tr>
<td>Normal cells grow in monolayer</td>
<td>Cancer cells grow in clumps (foci)</td>
</tr>
<tr>
<td>- grow in culture dish until they cover the surface as a monolayer</td>
<td>- grow in multilayered clumps (a.k.a. foci)</td>
</tr>
<tr>
<td>- respond to inhibitory influences from environment</td>
<td>- not responsive to signals that cause normal cells to cease growth and division</td>
</tr>
<tr>
<td>- need stimulatory growth signals to grow</td>
<td>- ignore inhibitory growth signals</td>
</tr>
<tr>
<td>- depend on growth factors usually added to the growth medium: epidermal growth factor &amp; insulin, which are present in serum (blood)</td>
<td>- continue to grow in absence of stimulatory growth signals</td>
</tr>
<tr>
<td>- limited capacity for cell division: undergo aging process rendering them unfit to continue to grow</td>
<td>- can proliferate in the absence of serum</td>
</tr>
<tr>
<td>- cell cycle does not depend on interaction between growth factors and their receptors (located at cell surface)</td>
<td>- cell cycle does not depend on interaction between growth factors and their receptors (located at cell surface)</td>
</tr>
<tr>
<td>- immortal: continue to divide indefinitely</td>
<td>- immortal: continue to divide indefinitely</td>
</tr>
</tbody>
</table>
and divide after a number of mitotic divisions
- telomerase: absent
  - belief: one of the body's major defenses that protects against tumor growth
- apoptosis (self-destruction) when chromosome content becomes disturbed
- telomerase: present
  - enzyme that maintains the telomeres at the ends of the chromosomes; allows cells to continue to divide
- aneuploidy: unstable; have highly aberrant chromosome complements
  - result of: defects in mitotic checkpoint; or presence of abnormal number of centrosomes
- often depend on glycolysis (anaerobic metabolic pathway)
  - high metabolic requirements
  - inadequate blood supply within tumor
- if reduced oxygen (hypoxia), cells activate transcription factor HIF: induces the formation of new blood vessels and promotes the migratory properties of the cells
  - leads to spread of tumor
- although, even if plenty oxygen, glycolysis=ATP (aerobic glycolysis)
- increased uptake of glucose compared to normal cells
  - can be used to locate metastatic tumors by using PET scans

## II. The Causes of Cancer
-1775: Percivall Pott (British surgeon)
- environmental agent->cancer
  - chronic exposure to soot from chimney sweeps leads to:
    - → cancer of the nasal cavity
    - → cancer of the skin of the scrotum
  - soot has carcinogenic chemicals that alter the genome
  - carcinogenic chemicals: directly mutagenic or converted to mutagenic compounds by cellular enzymes
- Tumor viruses
  - 2 groups (depending on nucleic acid found within mature virus particle):
    - DNA tumor viruses (ex. polyoma virus, simian virus 40 (SV40), adenovirus, herpes-like viruses)
    - RNA tumor viruses (retroviruses; structure similar to HIV)
- transform mammalian cells into cancer cells
- carry genes whose products interfere with the cell's normal growth-regulating activities
  - associated with only a small % of human cancers
- Other viruses: linked to as many as 20% of cancers worldwide
  - increase risk of developing the cancer, rather than being sole determinant responsible for the disease
- HPV (Human Papilloma Virus): viral infection->cancer
  - transmitted via sexual activity
  - although present in about 90% of cervical cancers, most women infected with the virus will never develop this malignancy
  - causes cancers of mouth and tongue in both men and women
- hepatitis B virus: associated with liver cancer
- Epstein-Barr virus: associated with Burkitt's lymphoma in areas where malaria is common
- HHV-8: herpes virus associated with Kaposi's sarcoma
- *Helicobacter pylori*: stomach-dwelling bacterium
  - causes ulcers, gastric lymphomas
- chronic inflammation triggered by pathogen: causes many cancers linked to viral and bacterial infections
- IBD (Inflammatory Bowel Disease): increases risk of colon cancer
- causes of certain cancers are obvious (ex. smoking->lung cancer, exposure to UV radiation->skin cancer, inhaling asbestos fibers->mesothelioma)
- however, causes of most types of human cancer unidentified
- Environmental factors important: ex. individuals who moved from Asia to United States/Europe no longer exhibit high rate of gastric cancer (as occurs in Asia) but instead have elevated risk of colon & breast cancer (characteristic of Western countries)
- Diet also important: ex. cancer rates are higher among obese individuals (elevated levels of insulin & insulin-like growth factor (IGF-1)); calorie-restricted diet protects against cancer; animal fat and alcohol can increase risk; isoflavones in soy, sulforaphanes in broccoli, and EGCG in tea reduce risk
- Drugs interfering with action of estrogen (ex. tamoxifen, raloxifene) or metabolism of testosterone (ex. finasteride): can reduce breast/prostate cancer incidence
- Nonsteroidal anti-inflammatory drugs (NSAIDs) (ex. aspirin, indomethacin): can reduce risk of colon cancer; inhibit cyclooxygenase-2, an enzyme that catalyzes synthesis of hormone-like prostaglandins which promote growth of intestinal polyps
- Antidiabetes drug metformin: reduce risk of cancer; lowers circulating levels of insulin and IGF-1

III. The Genetics of Cancer
- Cancer results from the uncontrolled proliferation of a single wayward cell. (monoclonal)
- Any of the trillions of cells in our body may have the potential to change in genetic composition and grow into a malignant tumor
- Malignant transformation requires more than a single genetic alteration
  - Those we inherit from our parents (germ-line mutations)
    - Not a major factor
  - Those that occur during our own lifetime (somatic mutations)
    - Greatest impact
- Development of a malignant tumor (tumorigenesis)
  - Progression of permanent genetic alterations in a single line of cells (may take decades to complete)
  - Cells in the line become increasingly less responsive to body’s normal regulatory machinery and better able to invade normal tissues
    - Tumorigenesis: requires that the cell responsible for initiating the cancer be capable of a large number of cell divisions
- Most common solid tumors
  - Breast, colon, prostate, and lung
  - Arise in epithelial tissues (high level of cell division)
    - Example: leukemia - develop in rapidly dividing blood-forming tissues
  - Cells of most tissues
    1. Stem cells
      - Possess unlimited proliferation potential
      - Have capacity to produce more of themselves
      - Can give rise to all of the cells of the tissue
      - Have the opportunity to accumulate the mutations required for malignant transformation
  2. Progenitor cells
    - Derived from stem cells
    - Possess a limited ability to proliferate
  3. The differentiated end products of the tissue
    - Lack the capability to divide
- General scenarios: origin of tumors
  1. Tissue stem cells - from within the relatively small population of stem cells that inhabit each adult tissue
  2. Progenitor cells - can give rise to malignant tumors by acquiring certain properties, (i.e. unlimited proliferation) as part of the process of tumor progression
only those tumors with cells that maintain the length of their telomeres will be capable of unlimited growth.
- cells with no telomerase enzyme will soon die off, and then all the cells in the tumor will contain telomerase.
  - activation of telomerase expression: epigenetic change, one that results from the activation of a gene that is normally repressed.
  - once activated, it is a permanent, inheritable alteration.
- even after cells become malignant, cancer cells continue to accumulate mutations and epigenetic changes that make them increasingly abnormal = genetic instability
  - makes disease difficult to treat with chemotherapy because cells often arise within the tumor mass that are resistant to the drug
- tumor progression - accompanied by histological changes (changes in cell’s appearance)
  - often produce “precancerous” cells - cells that gained some cancer cell properties
  - Pap smear: test to detect precancerous cells in the epithelial lining of the cervix
- benign tumors
  - contain cells that have proliferated to form a mass that poses little threat of becoming malignant
  - all the moles we have in our body are examples
  - pigment cells - compose a mole
    - undergone genetic changes that would lead them to being malignant tumors, causing them to enter senescence (permanent state of growth arrest) → “forced senescence” to restrict the development of cancers
- A. Tumor-Suppressor Genes and Oncogenes: Brakes and Accelerators
  - Tumor-suppressor genes (1960s)
    - act as cell’s brakes
    - encode proteins that restrain cell growth
    - prevent cells from becoming malignant
    - suppresses the formation of the tumor
    - to be inactivated, mutation or deletion
  - Oncogenes
    - encode proteins that promote the loss of growth control
    - also proteins that promote the conversion of a cell to a malignant state
    - most act as accelerators of cell proliferation
*proto-oncogenes
- have potential to subvert the cell’s own activities and push the cell toward the malignant state

1. mutation in gene - alters structure and function of encoded protein
2. gene amplification - overexpression of gene
3. rearrangement of DNA - brings new DNA segment, altering either its expression or the structure of the encoded protein

- for a cell to be malignant, should first lose its tumor-suppressor function, accompanied by the conversion of a proto-oncogene to an oncogene
- the functions of the products:
  - a. tumor-suppressor genes
    - loss of function = transformation of normal cell to cancer cell
    - encode proteins that act as negative regulators of cell proliferation, which is why their elimination promotes uncontrolled cell growth
    - products also help maintain genetic stability, which may be a primary reason that tumors contain such an aberrant karyotype
    - examples: genes that encode:
      - transcription factors (TP53, WT1)
      - cell cycle regulators (RB, INK4a)
      - components that regulate G proteins (NF1)
      - phosphoinositide phosphate (PTEN)
      - protein that regulates protein degradation (VHL)
    - retinoblastoma
      - found first tumor-suppressor gene to be studied and cloned
      - rare childhood cancer of the retina of the eye
      - gene: RB
      - 1. occurs at high frequency and at young age in members of certain families → cancer can be inherited
      - 2. occurs sporadically (noninherited) at an older age among members of the population at large
- deletion of interior portion of one pair of homologous chromosome in the cells of the retinal cancer
  - indicates that the chromosomal aberration had been inherited from one of the parents
- inherited as a dominant genetic trait
- children inherit a strong disposition toward developing retinoblastoma, rather than the disorder itself
- 10% of individuals who inherit a chromosome with an RB deletion never develop the retinal cancer
- development of retinoblastoma requires that both copies of RB gene be eliminated/mutated
  - the cancer arises as the result of two independent “hits” in a single cell
- sporadic retinoblastoma
  - tumor develops from retinal cell
  - both copies of RB gene underwent successive spontaneous mutation
  - may have normal cells that lack the RB mutation and tumor cells; both alleles of the gene were mutated
- germ-line retinoblastoma
  - person inherits chromosome with RB deletion
  - halfway along the path to becoming malignant
- mutation or deletion of the remaining RB allele in any of the cells of the retina produces a cell that lacks a normal RB gene, thus cannot produce a functional RB gene product
  - explains why people who inherit abnormal RB gene are so highly predisposed to developing the cancer
- the second “hit” fails to occur in approx. 10% of individuals who do not develop the disease
- mutations in RB alleles are also common in sporadic breast, prostate, and lung cancers among individuals who have inherited two normal RB alleles
  - to suppress cancerous phenotype, cells from tumors are cultured in vitro, and a wild-type RB gene back into the cells are reintroduced
a. sporadic retinoblastoma
- begins with 2 normal copies of RB gene
- retinoblastoma occurs wherein retinal cell accumulates independent mutations in both alleles of the gene

b. inherited retinoblastoma
- begins with one abnormal allele of RB gene (usually deletion)
- all cells have at least one of their two RB genes nonfunctional
- retinal tumor occurs if the other RB allele in a retinal cell becomes inactivated, usually a result of a point mutation

- The role of pRB in Regulating the Cell Cycle
  - pRB: protein encoded by RB gene
  - helps regulate the passage of cells from the G1 stage to S phase
  - key targets of pRB: E2F family of transcription factors
    - E2F proteins bind to pRB, which prevents a number of genes encoding proteins required for S-phase activities.
    - E2F-pRB: act as a gene repressor
    - pRB releases its bound to E2F once it is phosphorylated, then the transcription factor E2F will activate its gene expression = cell’s irreversible commitment to enter S phase
  - a cell that loses pRB = RB mutation
    - cell also loses its ability to inactivate E2F
    - pRB contains at least 16 diff serine and threonine residues
    - pRB - negative regulator of the cell cycle
      - importance: DNA tumor viruses encode a protein that binds to pRB, blocking its ability to bind to E2F
        - by using these pRB-blocking proteins, these viruses accomplish the same result as when the RB gene is deleted, leading to the development of human tumors

- The Role of p53: Guardian of the Genome
  - TP53 gene: tumor-suppressor gene
    - if absent, responsible for Li-Fraumeni syndrome, a rare inherited disorder
    - victims of disease: high incidence of breast, brain cancer, and leukemia
(like retinoblastoma) affected individuals inherit one normal & one abnormal(or deleted) allele of the TP53 = highly susceptible to cancers that result from random mutations in the normal allele

- TP53 - most commonly mutated gene in human cancers

- p53 is important in preventing a cell from becoming malignant
  - serves as a transcription factor that acts as a crucial player in a cell’s response to stress
  - Protein p21: initiates cell cycle arrest
  - when a cell experiences DNA damage, p53 activates a gene that encodes a protein called p21: inhibits the cyclin-dependent kinase that normally drives a cell through the G1 checkpoint = progression through the cell cycle is arrested
    - gives the cell time to repair the genetic damage before it initiates DNA replication
    - failure to repair DNA damage → production of abnormal cells, which have the potential to become malignant
  - other than cell cycle arrest, p53 also prevents development of cancer by directing a genetically damaged cell to apoptosis (ridding the malignant potential)
    - BAX gene: encodes a protein that initiates apoptosis
  - levels of p53 in healthy G1 is very low (keeps lethal action under control), however, if cell sustains genetic damage (UV light, chemical carcinogens, etc), the concentration of p53 rises rapidly
  - MDM2 - protein that degrades p53
    - binds to p53 and guides it out of the nucleus, into the cytosol
    - in cytosol: MDM2 adds ubiquitin molecules to p53 → destruction
    - mice that lack a gene encoding MDM2 die at an early stage of development, because their cells undergo p53-dependent apoptosis
    - mice lacking genes that encode both MDM2 and p53 survive to adulthood, but are highly prone to cancer
  - ATM - protein that phosphorylates p53
    - once phosphorylated, p53 is no longer able to interact with MDM2

- Functioning TP53 gene:
  - cancer cells with DNA damage, most likely to become apoptotic
  - if cancer cells lose p53 function, they become resistant to treatment
    - colon, prostate, pancreatic cancer: lack TP53 gene

- The Role of p53: Promoting Senescence

- senescent cells: remain alive and metabolically active, but do not divide permanently (i.e. moles), and may be ingested by phagocytic immune cells
- pathways leading to senescence:
  - INK4a gene - tumor-suppressor gene
    - often disabled in human cancers
    - encodes protein p16 - inhibits cyclin-dependent kinases required for progression through the cell cycle
    - encodes protein ARF - stabilizes p53
      - inhibits MDM2
      - inactivation of the TP53 gene (within senescent cells) causes the cells to resume their progress towards full malignancy
- Other Tumor-Suppressor Genes
  - FAP: Familial adenomatous polyposis coli
    - inherited disorder; development of adenomas (thousands of premalignant polyps) from epithelial cells
    - if not removed → malignant tumors
due to APC deletion (similar to RB deletion)
  - APC - tumor-suppressor gene at chromosome 5
- Breast Cancer
  - approx. 1 in 8 women in the US, Canada, and Europe
  - 5-10%: inherited gene
gen: BRCA1 and BRCA2
  - may also cause ovarian cancer (has a high mortality rate)

b. Oncogenes
- RAS - oncogene mutated most frequently in human tumors
  - encodes a GTP-binding protein: on-off switch for a number of key signaling pathways controlling cell proliferation and metabolism
  - FTPase activity cannot be stimulated = continuous proliferation signals along the pathway
  - no approved drug to block RAS function yet
- I. Oncogenes That Encode Growth Factors or Their Receptors
  - 1983 Brain Tumors (gliomas)
    - cancer-causing simian sarcoma virus: contained oncogene (sis) from the cellular gene for growth-factor PDGF (protein in human blood)
    - large amounts of PDGF = uncontrolled proliferation of cells
  - Avian erythroblastosis virus
    - carries oncogene erbB - encodes EGF receptor
      - missing: part of protein that binds to growth factor
      - malignant cells have more receptors that normal cells, thus, are stimulated to divide under conditions that would not affect normal cells
- II. Oncogenes That Encode Cytoplasmic Protein Kinases
  - Raf - serine-threonine protein kinase
    - heads the MAP kinase cascade: primary growth-controlling signaling pathway in cells
    - mutations turn Raf into an enzyme that remains in the “on” position = most likely to convert the proto-oncogene into an oncogene = cell’s loss of growth control
    - most closely linked to melanoma
    - due to BRAF mutations
  - SRC - 1st oncogene to be discovered, also a protein kinase
    - SRC mutations appear only rarely among the repertoire of genetic changes in human tumor cells
- III. Oncogenes That Encode Transcription Factors
  - MYC - regulates expression of proteins and noncoding RNAs involved in cell growth and proliferation
  - most commonly altered in human cancers
  - Burkitt’s lymphoma
    - common type of cancer in Africa
    - results from the translocation of a MYC gene to a position adjacent to an antibody gene
    - disease occurs primarily in persons who have also been infected with Epstein-Barr virus (causes only minor infections) in people living in Western countries (not associated with tumorigenesis)
- IV. Oncogenes That Encode Proteins That Affect the Epigenetic State of Chromatin
  - most important factors in determining the epigenetic state of chromatin:
    - 1. DNA methylation
      - silences genes
    - 2. histone modifications
      - either activate or repress gene transcription
  - mutations in oncogenes that encode proteins that affect one of the two can promote tumorigenesis by increasing/decreasing transcription factors of genes involved
    - example: Acute Myeloid Leukemia
      - mutations in DNMT3A - gene that maintains DNA methylation patterns during DNA replication
reduction in level of DNA methylation = genetic instability & increased transcription of certain proto-oncogenes

V. Oncogenes That Encode Metabolic Enzymes
- tumor cells depend much more on glycolysis than do normal cells
  - glioblastoma or Brain Cancer
  - mutations in the TCA cycle enzyme isocitrate dehydrogenase (IDH1 and IDH2)
  - these mutations cause the enzyme to lose its normal activity
  - disruption of these epigenetic processes would likely result in the aberrant regulation of gene expression within tumor cells

VI. Oncogenes That Encode Products That Affect Apoptosis
- BCL-2 - oncogene most closely linked to apoptosis
  - becomes oncogenic when gene is translocated to an abnormal site on the chromosome
    - follicular B-cell lymphomas - human lymphoid cancer
      - translocation of the BCL-2 gene next to a gene that codes for the heavy chain of antibody molecules
      - overexpression of the BCL-2 gene → suppression of apoptosis in lymphoid tissues (abnormal cells → lymphoid tumors)
  - may also reduce the effectiveness of chemotherapy (keeps tumor cells alive and proliferating despite damage by the drug treatment)

b. The Cancer Genome
- more than 350 genes are “cancer genes”
- each type of cancer has its own characteristic complement of frequently-mutated genes
- adenoma - benign growths (polyps) that have the potential to develop into malignant tumors if not taken away during a colonoscopy
- colorectal cancer
  - “mountains” - genes that are mutated at a high frequency, in a large proportion of tumors
  - “hills” - genes that are mutated at a lower frequency
- drivers - mutations that cause or contribute to the malignant phenotype
- passengers - genes that constitute a “hill”, genes that tend to become mutated for some reason but have no effect on the phenotype of the cancer cell
- cancer can be thought of not simply as a disease of aberrant genes but as one of aberrant cellular pathways
- cancer as a “pathway” disease rather than a “genetic” disease - suggests that disrupting/restoring any one of the key steps in a single essential pathway may be sufficient to derail the malignant cells and lead to tumor regression
- cancer is a gradual multistep progression of individual point mutations, not universally shared

b. The Cancer Genome
- more than 350 genes are “cancer genes”
- each type of cancer has its own characteristic complement of frequently-mutated genes
- adenoma - benign growths (polyps) that have the potential to develop into malignant tumors if not taken away during a colonoscopy
- colorectal cancer
  - “mountains” - genes that are mutated at a high frequency, in a large proportion of tumors
  - “hills” - genes that are mutated at a lower frequency
- drivers - mutations that cause or contribute to the malignant phenotype
- passengers - genes that constitute a “hill”, genes that tend to become mutated for some reason but have no effect on the phenotype of the cancer cell
- cancer can be thought of not simply as a disease of aberrant genes but as one of aberrant cellular pathways
- cancer as a “pathway” disease rather than a “genetic” disease - suggests that disrupting/restoring any one of the key steps in a single essential pathway may be sufficient to derail the malignant cells and lead to tumor regression
- cancer is a gradual multistep progression of individual point mutations, not universally shared

DNA microarrays or DNA chips - used to analyze gene expression
- have shown:
  - 1. progression of tumor is correlated with a change in expression of particular genes
  - 2. certain cancers can be divided into subtypes with diff clinical features (depending on gene-expression profiles)
  - 3. the gene-expression profile of tumor can reveal how aggressive (lethal) the cancer is
IV. New Strategies for Combating Cancer

- surgery, chemotherapy, radiation: “brute-force strategies”->kill both cancer cells and normal cells
- Targeted therapies: attack only cancer cells, inactivate particular protein that doesn’t allow cancer cell to grow/survive, or target cancer cells based on patient’s unique pattern of somatic mutations
- anticancer strategies can be divided into 3 groups:
  1. those that depend on antibodies or immune cells to attack tumor cells
  2. those that inhibit the activity of cancer-promoting proteins
  3. those that prevent the growth of blood vessels that nourish the tumor

- Immunotherapy:
  -(1800s) William Coley, New York physician: spontaneous remissions
  -found patient with inoperable neck tumor who had gone into remission after contracting streptococcal infection beneath skin to be free of cancer
  -developed bacterial extract (toxin) that stimulates immune system to attack malignancy when injected under skin/into tumor
  -can cure uncommon soft-tissue sarcomas
  ->finding: the body is capable of destroying tumors
  lissa is awesome

- 2 treatment strategies: (1) Passive immunotherapy (2) Active immunotherapy

  (1) Passive immunotherapy: antibodies are administered as therapeutic agents
  -antibodies recognize and bind to specific proteins on targeted tumor cells’ surface, killing cells directly or via other elements of the immune system
  -monoclonal antibodies: first developed in mid-1970s
  -attempts to use these proteins as therapeutic agents foiled in next 20 years for a number of reasons
  -these antibodies were produced by mouse cells and encoded by mouse genes->recognized as foreign and cleared from bloodstream before they had a chance to work
  -humanized antibodies: largely human proteins except for small part that recognizes antigen, which remains “mousey”;
    researchers able to produce antibodies with a completely human amino acid sequence
  -Herceptin: humanized antibody directed against a cell-surface receptor (HER2) that binds a growth factor that simulates proliferation of breast cancer cells; inhibits activation of receptor by the growth factor and stimulates receptor internalization
  -approx. 25% of breast cancers are composed of cells that overexpress the HER2 gene, causing cells to be sensitive to growth factor stimulation
- prognosis of patients whose tumors overexpressed HER2 improved because of Herceptin (ex. reduced chance of recurrence of disease by about 50% in a 4-yr. period in a study of 3000 women with early-stage breast cancer)
- combining Herceptin with another monoclonal antibody (Omnitarg) that interferes with HER2 dimerization can increase survival
- Rituxan: most effective humanized antibody to date; (1997) treatment of non-Hodgkin’s B-cell lymphoma; binds to a cell-surface protein (CD20) present on malignant B cells in approx. 95% of cases of the disease
- binding of antibody to CD20 protein inhibits cell growth and induces cells to undergo apoptosis
- Vectibix: directed against EGF receptor; approved as single-agent treatment of EGFR-expressing metastatic colon cancer; remains in circulation long enough to be administered once every other week since it is a fully human protein
- Arzerra: human monoclonal antibody for treatment of lymphocytic leukemia; binds to CD20 on surface of B cells
- Yervoy: human monoclonal antibody for treatment of melanoma; blocks CTLA-4, a protein that normally inhibits body’s T cells from carrying out an immune response
- a number of antibodies are being developed that contain a radioactive atom or a toxic compound conjugated to the antibody molecule
- antibody targets the complex to the cancer cell, then the associated atom or compound kills targeted cell
- Zevalin and Bexxar: radioactively labeled anti-CD20 antibodies; treats non-Hodgkin’s B-cell lymphoma
- Adcetris: anti-CD30 antibody linked to a toxic compound; treats Hodgkin’s lymphoma

(2) Active (or adoptive) Immunotherapy: involves person’s own immune system more against malignant cells
- the immune system has evolved to recognize and destroy foreign materials, but cancers are derived from a person’s own cells.
- although many tumor cells contain proteins not expressed by their normal counterparts (tumor-associated antigens) or mutated proteins (ex. \(BRAF^{V600E}\)) different from those in normal cells, they are still host proteins in host cells.
- as a result, the immune system fails to recognize these proteins as inappropriate
- strategies to artificially stimulate immune system
  - patient is inoculated with a protein known to be present in their cancer cells (ex. HER2 in person with breast cancer, CEA (carcinoembryonic antigen) in person with pancreatic cancer)
  - if the body can be stimulated to mount an immune response against the protein, that response has the potential to attack the cancer cells with that protein on their surface
  - patient’s immune cells are isolated, modified/stimulated in vitro, allowed to proliferate in culture, then reintroduced to patient: shown to be effective in shrinking size/extent of tumor and significantly increasing patient’s expected time of survival
- 2 examples of this general type of cell-based immunotherapy:
  - both need to be personalized to the patient:→ extremely expensive
    - DCVax
      - currently in phase II trials for glioblastoma (highly lethal brain tumor)
      - dendritic cells taken from patient’s blood, stimulated in vitro with antigens from patient’s own tumor, then injected back into patient
      - involves exposure of dendritic cells to specific proteins to generate immune response:→ "cancer vaccine"
      - "manufacturing process" takes about 10 days and provides enough cells for several years of treatment
      - vaccine may extend median survival of patients to more than 3 yrs
      - as of July 2010: 33% of treated patients had survived at least 4 yrs and 27% had survived at least 6 yrs
      - fewer than 5% of patients given conventional treatments survive 5 yrs
    - Aug 2011: phase I trial on 3 patients with advanced chronic lymphocytic leukemia (CLL)
    - Phase I trials: normally carried out to determine:
      (1) whether a drug is safe
      (2) its proper dosage
    - in this case, the treatment resulted in the complete remission of disease in 2 of the patients and partial remission in the 3rd
to carry out the treatment: Carl June & his colleagues at the Univ. of Pennsylvania
isolated T cells from blood of patients, then genetically engineered these cells using a disabled
HIV1 viral vector to carry a specific, high-affinity TCR that would allow the T cells to react with
& kill host cells with CD19 protein on their surface
- T cells carry a T cell receptor or TCR, a multisubunit protein, on their surface which
determines the specific molecules (antigens) a given T cell will react with and which
target cells the T cell will attack/kill
- for the most part, T cells do not possess TCRs that allow them to react with other
cells, protecting the body from self-attack
- B cells: only cells that normally carry CD19->both the normal versions and those part
of the CLL cancer
- once T cells were genetically modified and numbers expanded in culture, T cells were infused
back into patient
- within 1-2 weeks, patients were stricken with a sickness characterized by very high fever,
lowered blood pressure, kidney distress->result of interaction between infused T cells (and
their progeny formed in their body after infusion) and the huge number of cancerous cells that
these patients carried
- it was estimated that each infused T cell killed more than 1000 CLL cells, leaving patients with
a small number of genetically engineered T memory cells derived from the infusion, a virtual
absence of normal B cells due to their destruction by T cells, and the absence of of malignant
cells or a greatly reduced tumor
- memory T cells: prevent reemergence of CLL and suppression of formation of normal B cells,
which might make patients more susceptible to infection
- use of genetically modified T cells: risk->T cells can attack any cell that happens to carry the
targeted antigen
- cell-based immunotherapies may cause severe autoimmune side effects

-Inhibiting the Activity of Cancer-Promoting Proteins

cancer cells: contain proteins present at abnormal concentration or display abnormal activity
- "oncogene addiction": dependency of growth and/or survival or tumor cells on continued activity of these
proteins
- selectively blocking the activity of one of these proteins can kill all malignant cells
- researchers->synthesis of small-molecular-weight compounds that inhibit activity of cancer-promoting proteins
- some drugs were custom-designed to inhibit particular protein of known structure, others were identified by
randomly screening many compounds synthesized by pharmaceutical companies
- protein-inhibiting compound: once identified, is tested for effectiveness against approx. 60 different types of
cultured cells isolated from different human cancers, allowing researchers to reconstitute the genetic
heterogeneity and diverse drug sensitivities found among human cancers
- success against cultured cells leads to tests of the agent in immunocompromised mice carrying human tumor
transplants (xenografts)
Chronic Myelogenous Leukemia (CML): caused by a translocation that brings a proto-oncogene (ABL) in contact with another gene (BCR) to form a chimeric gene (BCR-ABL). Blood-forming cells that carry this translocation express a high level of ABL tyrosine kinase activity, causing cells to proliferate uncontrollably and initiate tumorigenesis.

Gleevec: compound that binds to the inactive form of the protein and prevents its phosphorylation by another kinase required for ABL activation, selectively inhibiting ABL kinase. Confirmed that elimination of a single required oncogene product could stop the growth of a human cancer.

Most cases of resistance result from mutations in the ABL portion of the fusion gene, prompting the development of a 2nd generation of targeted inhibitors that remain active against most of the mutated forms of the ABL kinase. These drugs can treat Gleevec-resistant cases of CML and suggest that the ideal drug regimen may consist of many different inhibitors that target different parts of the same protein, ensuring that drug-resistant mutants will not emerge.

Zelboraf (PLX4032): drug that treats metastatic melanoma driven by a mutated version of BRAF, one in which the normal valine residue at position 600 is replaced by a glutamic acid (BRAFVal600Glu), changing the shape of the active site; specifically blocks activity of the mutant enzyme without affecting the normal protein.

- The mutation occurs in approx. 50% of melanoma patients.
- Patients also develop resistance to Zelboraf like Gleevec but resistance appears much earlier (typically after 7 mos.) and in most cases results from mutations in other genes rather than secondary mutations in BRAF.

Resistance to Zelboraf: due to MAPL pathway becoming constitutively active, bypassing the cells’ need for BRAF kinase activity, which the drug inhibits.

Studies are underway to combine Zelboraf with inhibitors of downstream components of the pathway (ex. MAPK or ERK).

### Table 16.3 Examples of Small-Molecule Targeted Therapies That have Either Been FDA Approved or Are Being Tested

<table>
<thead>
<tr>
<th>Drug</th>
<th>Target</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gleevec, Tasigna</td>
<td>BCR-ABL, KIT, PDGFR</td>
<td>Tyrosine kinase inhibitor</td>
</tr>
<tr>
<td>Sprycel</td>
<td>KIT/ABL1, SRC family</td>
<td>Tyrosine kinase inhibitor</td>
</tr>
<tr>
<td>Iressa, Tarceva</td>
<td>EGFR</td>
<td>Tyrosine kinase inhibitor</td>
</tr>
<tr>
<td>Zactima</td>
<td>VEGFR, EGFR</td>
<td>Tyrosine kinase inhibitor</td>
</tr>
<tr>
<td>Sutent, Votrient</td>
<td>VEGFR, PDGFR, KIT</td>
<td>Tyrosine kinase inhibitor</td>
</tr>
<tr>
<td>Tykerb</td>
<td>EGFR, HER2</td>
<td>Tyrosine kinase inhibitor</td>
</tr>
<tr>
<td>Nexavar</td>
<td>BRAF, KIT, VEGFR</td>
<td>Kinase inhibitor</td>
</tr>
<tr>
<td>GDC-0973</td>
<td>MEK</td>
<td>Inhibits MAPK cascade</td>
</tr>
<tr>
<td>Velcade, Carfilzomib</td>
<td>proteasome</td>
<td>Inhibits protein degradation</td>
</tr>
<tr>
<td>Zolinza, Istodax</td>
<td>HDACs</td>
<td>Inhibits histone acetylation (epigenetic effect?)</td>
</tr>
<tr>
<td>Envedge</td>
<td>Smoothened of His pathway</td>
<td>Blocks cell growth and survival pathway</td>
</tr>
<tr>
<td>Torisel, Affinitor</td>
<td>mTOR</td>
<td>Blocks cell-survival pathway</td>
</tr>
<tr>
<td>BKM120, PX866</td>
<td>PI3K</td>
<td>Blocks cell-survival pathway</td>
</tr>
<tr>
<td>BEZ235, BGT226</td>
<td>PI3K, mTOR</td>
<td>Blocks cell-survival pathway</td>
</tr>
<tr>
<td>Perifosine, MK2206</td>
<td>PKB/AKT</td>
<td>Blocks cell-survival pathway</td>
</tr>
<tr>
<td>Trisenox (arsenic trioxide)</td>
<td>NF-κB</td>
<td>Blocks cell-survival pathway</td>
</tr>
<tr>
<td>Suilimet</td>
<td>CDK2</td>
<td>Induces apoptosis</td>
</tr>
<tr>
<td>Tamoxifen, Raloxifene</td>
<td>Estrogen receptor</td>
<td>Blocks estrogen action</td>
</tr>
<tr>
<td>Aminodes, Aromasin</td>
<td>Aromatase</td>
<td>Inhibits estrogen synthesis</td>
</tr>
<tr>
<td>Zytiga</td>
<td>CYP17</td>
<td>Inhibits androgen synthesis</td>
</tr>
<tr>
<td>Genasense, ABT-263</td>
<td>BCL-2</td>
<td>Induces apoptosis</td>
</tr>
<tr>
<td>17-AAG</td>
<td>HSP90</td>
<td>Inhibits molecular chaperone</td>
</tr>
<tr>
<td>Nudins</td>
<td>p53</td>
<td>Inhibits p53-MDM2 interaction</td>
</tr>
<tr>
<td>PRIMA-1</td>
<td>p53</td>
<td>Restores mutant p53 activity</td>
</tr>
<tr>
<td>PX-478</td>
<td>HIF-1</td>
<td>Inhibits this transcription factor that is activated by hypoxia</td>
</tr>
<tr>
<td>Veliparib, Rucliparib</td>
<td>PARP-1</td>
<td>Blocks HR-based DNA repair</td>
</tr>
<tr>
<td>Dacogen, Vidaesa</td>
<td>DNMT</td>
<td>Inhibits DNA methylation</td>
</tr>
</tbody>
</table>

-Chronic Myelogenous Leukemia (CML): caused by a translocation that brings a proto-oncogene (ABL) in contact with another gene (BCR) to form a chimeric gene (BCR-ABL). Blood-forming cells that carry this translocation express a high level of ABL tyrosine kinase activity, causing cells to proliferate uncontrollably and initiate tumorigenesis.
It is unlikely that a tumor will harbor cells resistant to 2 drugs targeting 2 different pathways or 2 different proteins in the same pathway.

Combined drug therapy: may block emergence of resistant cells; effective in blocking resistant strains of HIV virus, which is highly mutable like cancer cells.

Unfortunately, inhibiting some key signaling proteins in cancer cells can have serious side effects as a result of their actions in normal cells.

Failure to develop drugs eradicating solid, epithelial-based tumors (carcinomas)
- Tumors are genetically complex or cells are not dependent on a single oncogene product and aberrant signaling pathway as are blood-cell cancers and melanoma.
- Iressa: inhibitor of tyrosine kinase of the EGF receptor (EGFR); originally tested on lung cancer patients because these tumors were known to exhibit high levels of EGFR
- Initial clinical trials: approx. 10% of patients in the United States & 30% of Japanese patients responded positively to the drug; remaining population unaffected

Subsequent studies on cancers with EGFR mutations indicated that targeting the mutant EGFR only works if the patient has wild type KRAS.
- Patients with mutant KRAS -> non-responders
- Lung and colon cancers are now tested for KRAS mutations before starting anti-EGFR therapy.
- Xalkori: drug effective in treating lung cancer patients with normal EGFR genes but expressed an EML4-ALK fusion protein from chromosomal translocation; inhibits ALK kinase.
- Approved along with a "companion diagnostic" test: fluorescence in situ hybridization (FISH): to identify rearrangement which occurs in about 5% of patients.
- Verify that not all cases of a particular cancer can be treated alike.
- Poly(ADP-ribose) polymerase (PARP): enzyme involved in DNA metabolism and DNA repair.
- PARP-1 inhibitors: can treat breast & ovarian cancers that exhibit deficiencies in BRCA1 or BRCA2.
- BRCA proteins: involved in DNA repair.
- Such cancer cells are more dependent on other DNA repair pathways than normal cells, including those that require PARP-1.
- When PARP-1 is inhibited in BRCA-deficient tumor cells, certain types of DNA damage cannot be repaired, causing cells to die by apoptosis.
- This type of treatment is based on "synthetic lethality": mutations or inhibition of only 1 protein (ex. BRCA or PARP) has no effect on cell viability, but mutations and/or inhibition of 2 different proteins leaves the cell unable to carry out one/more essential functions.
- Targets cancer cells that have lost function of a particular tumor suppressor protein (ex. BRCA1 or BRCA2).
- A similar strategy may target cancer cells that have lost p53 function because such cells should also be vulnerable to drugs that don't affect normal cells, which have tumor-suppressive pathways.
- It might be possible to find genes sensitive to synthetic lethality from cancer genomics studies, looking for either:
  1. 2 genes that are never mutated together in the same tumor.
  2. Genes that are never mutated in any cases of a particular cancer -> genes are presumably required for cancer cell survival and represent possible drug targets.

The Concept of a Cancer Stem Cell
- Cancer stem cells: maintain the tumor and promotes its spreading.
- Cells capable of propagating or regrowing the tumor.
- Has consequences for cancer therapy: if the drug kills off the tumor mass, but not cancer stem cells, then the possibility of the regrowth of the tumor is likely.
- Example: Gleevec - drug for CML patients, they should keep taking to keep their disease in remission.

Inhibiting the Formation of New Blood Vessels (Angiogenesis)
- New blood vessels form (angiogenesis) as a tumor grows in size.
- Blood vessels deliver nutrients and excrete waste of tumors, and also provide the conduits for cancer cells to spread.
- VEGF: growth factors secreted by cancer cells to promote angiogenesis.
- Overexpressed in most solid tumors.
- Angiogenic inhibitors: stops tumor growth.
- Tumors treated with these did not become resistant to repeated drug application.
- Target normal, genetically stable endothelial cells.
- tumor cells are genetically unstable and can evolve into resistant forms, thus they can become resistant to the usual chemotherapeutic agents
  - Avastin - antibody
    - directed against VEGF
    - blocks VEGF from binding to and activating its receptor, VEGFR
    - could prolong the lives of patients with metastatic colorectal cancer for many months
- early detection: best anticancer strategy
  - Mammography - detects breast cancer
  - Pap smears - detects cervical cancer
  - colonoscopy - detects colorectal cancer