Characteristics of Cancer

- Not a single disease
  - vary in age of onset
  - rate of growth
  - different metastatic potential
  - different responses to treatment
  - may represent small genetic alterations common among cancers

Characteristics of Cancer

- Cancer is leading cause of death in western world
- cancer is the 2nd leading cause of death in US after cardiovascular disease
- Highest mortality rates are from lung, colorectal, breast and prostate cancers

Characteristics of Cancer

- Considered disease of aging
  - diagnosis of most cancer occurs at 67 y
  - rare in children but is leading cause of death between ages 1-14 (leukemia is leading cause with others being osteosarcoma, lymphoma, Wilms' tumor [kidney])

Characteristics of Cancer

- ⌘ 8 million Americans have had cancer
  - ⊠ 50% considered cured
  - ⊠ 1 in 3 will eventually get cancer
- ⌘ Steady rise in death rates from cancer over last 50 years
  - ⊠ in 1930 there were 143 deaths/100,000
  - ⊠ in 1950 there were 157/100,000
  - ⊠ in 1990 there were 174/100,000
  - ⊠ major increase due to lung cancer deaths
- ⌘ Why? Smoking, environment, diet, life style changes

Characteristics of Cancer

- ⌘ More people now being cured of cancer
  - ⊠ in 1940's 1 in 4 people diagnosed with cancer survived 5 yrs
  - ⊠ in 1990's 40% live > 5 yrs
  - ⊠ due to better diagnostic techniques as well as treatment techniques

Characteristics of Cancer: malignant disease

- ⌘ Most common types of malignant neoplasms arise from epithelial cells
  - ⊠ cells have supportive stroma of blood vessels and connective tissue
    - ⊠ neoplasm
    - ⊠ tumor
    - ⊠ benign
    - ⊠ malignant
  - ⊠ must differentiate benign from malignant cells in order to determine treatment and prognosis

Characteristics of Cancer: malignant disease

⌘ Malignant Tumors
- invade and destroy adjacent tissue
- metastisize thru blood and lymph
- anaplastic (less well differentiated)
- grow rapidly
- continue to grow even with host starvation (cachexia-wasting)

⌘ Benign Tumors
- encapsulated and do not invade
- remain localized
- resemble normal tissue
- grow more slowly than malignant

Characteristics of Cancer: malignant disease

⌘ Suffix --oma indicates benign tumor when attached onto tissue type (except for lymphoma, melanoma and thymoma).
- Malignant tumors are indicated by the terms carcinoma (epithelial in origin) or sarcoma (mesenchymal in origin) preceded by the histologic type (I.e., adenocarcinoma of breast).

⌘ Most human malignancies arise from epithelial tissue
- those arising from stratified squamous epithelium called squamous cell carcinomas
- those arising from glandular tissue are adenocarcinomas

Classification of Human Tumors

⌘ When a malignant tumor no longer resembles the tissue of origin it is *anaplastic* or *undifferentiated*.

⌘ Some tumors arise from *pluripotent* stem cells and may contain several tissue elements (I.e, teratomas of the uterus (contain hair, nails, bone, glandular epithelium, etc...)

⌘ Neoplasms of hemopoeitic system have no benign counterparts

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Macroscopic Features

⌘ Pathologist-
- looks at overall appearance of tumor
  - encapsulation/invasiveness/site of growth
  - site of growth can give suggestions as to clinical course of growth--
    - likelihood & route of metastasis
    - effects of tumor on body functions
    - type of treatment that can be used
    - whether it is the primary site of growth (difficult)
  - accessibility of tumor-- surgical removal (resection), drug accessibility (brain, etc...)

Macroscopic Features

⌘ Pathology-
- Some tissue sites are more easily prone to metastasis than others (liver, lung, breast, prostate)
  - 5 yr survival rate of breast cancer patients is 85% if no sign of metastasis, but only 30% when more than four axillary nodes are involved.
- Determination of potential organ dysfunction (small tumors may cause a lot of dysfunction while Lg may cause none)

Cytologic Features of Tumors

❖ Morphology of cancer cells different than that of normal cells:
- more variable in size and shape
- nucleus larger with greater nuclear/ cytoplasmic ratio and nucleus containing large nucleoli
- # of cells undergoing mitosis higher (20/1000 cells with mitotic figures)
- presence of "giant cells"

Histological Classification

⌘ Histological classification of neoplasms determines the growth patterns, likelihood of metastasis, type of treatment, and prognosis: Example--

☒ Lung carcinomas fall into 3 categories
- epidermoid or squamous cell
- undifferentiated (small or large cell)
- adenocarcinomas

Types of Lung Carcinomas

☒ Epidermoid- grow more slowly and remain localized. Grow in larger bronchi and may cause obstruction of airway (bring to physician earlier than other types)

☒ treatment is surgery. 5 yr survival 50%

☒ undifferentiated small cell- occur in smaller bronchioles and have high growth rate, are invasive and 70% will have distant metastases by time of diagnosis.

☒ Treatment is chemotherapy and life expectancy less than 1 year

☒ have abnormal levels of hormones in blood

☒ change in histologic cell type after chemo- from small cell carcinoma to squamous cell carcinoma

Types of Lung Carcinomas

⌘ Bronchogenic carcinoma- arise in periphery of lung and do not produce signs of pulmonary effects until late in disease. This is not associated with smoking as are other two types.

☒ Treatment by surgery, but prognosis poor

⌘ Leukemias--arise from hematopoietic stem cells of bone marrow; faulty differentiation and growth regulation. Abnormal cells out number normal cells and are not functional

☒ 4 types-- acute myelocytic leukemia (AML), chronic myelocytic leukemia (CML), acute lymphocytic leukemia (ALL) and chronic lymphocytic leukemia (CLL)

Grade and Stage of Neoplasms

⌘ Grading- based on degree of <u>differentiation</u> and on estimate of growth rate (<u>mitotic index</u>)-- thought that less differentiated tumors more aggressive (too simple).
  - Grade I-- 75% to 100% differentiation
  - Grade II-- 50%-75% differentiation
  - Grade III-- 25%-50% differentiation
  - Grade IV-- 0%-25% differentiation

⌘ Also based on amount of infiltration and amount of stromal tissue in and around tumor

⌘ Imp't to grade for prognosis and for measuring effectiveness of treatment

Tumor Staging

⌘ Staging-- to provide a common language to describe the extent of disease (2 agencies-- Union Internationale Contre le Cancer (UICC) and American Joint committee for Cancer Staging and End Results Reporting (AJCCS)
  - to aid in planning treatment
  - to provide categories for estimating prognosis and evaluating results of tx
  - to facilitate exchange of information

⌘ Both utilize the T (tumor), N (involvement of LN) and M (+/- metastasis)-- TNM system

Tumor Staging

⌘ Four methods involved in staging
  - Clinical-- estimation of disease based on physical exam, clinical lab tests, x-ray films and endoscopic examination
  - Radiographic staging-- evaluation of progression based on radiography
  - Surgical staging-- direct exploration of extent of disease by surgery
  - Pathologic staging-- use of biopsy to determine degree of spread, depth of invasion, and involvement of LN

Tumor Staging

⌘ Stage I-- $(T_1N_0M_0)$ Primary tumor limited to the organ of origin. No evidence of nodal or vascular spread. Tumor usually can be removed by surgery. LTS (long term survival) is from 70-90%

⌘ Stage II--$(T_2N_1M_0)$ primary tumor spread into surrounding tissue and LN immediately draining area. Tumor may be operable but not completely resectable. LTS 45-55%

⌘ Stage III--$(T_3N_2M_0)$ primary tumor is large with invasion into deeper tissues. Not resectable. LTS 15--25%

Tumor Staging

⌘ Stage IV-- $(T_4N_3M_+)$ Large primary tumor (>10 cm), invading adjacent tissues. Extensive LN involvement and distant metastases. LTS < 5%

⌘ Lymph node involvement--
- determined by size,
- firmness,
- amount of invasion,
- mobility,
- # of nodes involved

Cancer treatment

⌘ Early diagnosis essential
- surgery
- radiation
- chemotherapy

⌘ used together these treatments more effective (*adjuvant therapy*= utilizing anticancer drugs with surgery or radiation)

⌘ aggressiveness of malignant disease dictates how radical a treatment should be

⌘ Cancers generally not diagnosed in time to be treated effectively

Diagnostic Procedures

- ⌘ <u>Electron microscopy</u> (EM)-- tissue must be prepared, fixed.... From biopsy
- ⌘ <u>Immunohistochemistry</u>-- uses Ab's diredted against TAA's (tumor associated antigens) (Table 1-5 page 15) [BCG, PSA, CEA, AFP, CA-125]
- ⌘ <u>Cytogenetics</u>-- examines chromosomes from a population of cells & ID abnormalities (I.e., breaks, exchanges, translocations, deletions
- ⌘ Abnormal gene expression-- southern (DNA) & northern (RNA) blotting, PCR
- ⌘ <u>Tumor markers</u>-- by ELISA and RIA and Western blot (oncogenes over-expressed)

Epidemiology of Cancer

- ⌘ Trends--
  - ☒ 20-30 yr latency
  - ☒ incidence of cancer goes up with age (about 67)
    - ☒ early diagnosis & diagnostic techniques ???
  - ☒ with decrease in death due to other sources incidence of cancer increases
  - ☒ 25% of males and 20% of females who were born in 1985 will die of cancer (up from 18% and 16% from 1975 figures)

Epidemiology of Cancer

- ⌘ If you exclude respiratory tract and skin (melanoma) cancer, the incidence in cancer in males and females has stayed flat since the 1950's
- ⌘ overall mortality rates have not changed much since the 1950's
- ⌘ cure rates for childhood leukemia, Burkitt's lymphoma, testicular cancers and Hodgkin's disease have increased (does not change overall cure % because these are relatively rare)
- ⌘ 50% of all cancer deaths due to lung, breast and colorectal cancers
- ⌘ SEE TABLEs & CHARTs

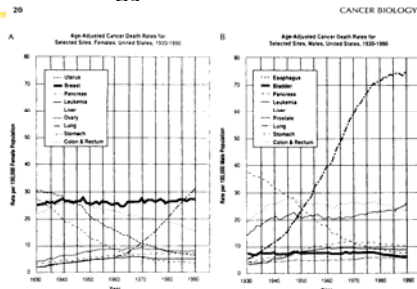## Epidemiology of Cancer

CANCER BIOLOGY

Figure 2-1 Age-adjusted cancer death rates for selected sites, United States, 1930 to 1990. (A) Females. (B) Males. Age-adjusted to the 1970 U.S. standard population. (From Boring et al.[2])

Epidemiology of Cancer

THE EPIDEMIOLOGY OF HUMAN CANCER

Table 2-1 Summary of Changes in Cancer Incidence and Mortality, 1950–89 and 5-Year Relative Survival Rates, 1950–88 (by Primary Cancer Site, Males and Females)

Epidemiology of Cancer

⌘ *Decrease in Mortality Rates (1973-1989)*
- breast (-4.6%)
- brain (-8.1%)
- colorectal (-18.3%)
- ovarian (-26%)
- stomach (-28.5%)
- bladder (-29.6%)
- cervical (-40.8%)
- testicular (-64.2%)
- Hodgkin's (-55.3%)
- leukemia (-16.6%)

⌘ *Increase in mortality Rates (1973-1989)*
- lung cancer
- brain cancer
- melanoma
- leukemia (over 65)
- pancreas (over 65)
- multiple myeloma

Epidemiology of Cancer

⌘ 5 yr survival rates for certain cancers has increased:
- ⊠ melanoma
- ⊠ prostate
- ⊠ testicular
- ⊠ bladder

⌘ 5 yr survival rates for other cancers have stayed the same since 1950's (harder to get early diagnosis
- ⊠ stomach   pancreas   lung
- ⊠ liver   esophagus

Epidemiology of Cancer

⌘ Cancer incidence rates are highest for:
- ⊠ black males > white males > black females > white females

⌘ These differences may reflect differences in
- ⊠ environmental exposures to carcinogens
- ⊠ diet
- ⊠ smoking habits
- ⊠ life style

Epidemiology of Cancer

⌘ Blacks have higher incidence and mortality in:
- ⊠ esophagus
- ⊠ uterine cervix
- ⊠ stomach
- ⊠ liver
- ⊠ prostate
- ⊠ larynx

⌘ Whites have higher incidence and mortality in:
- ⊠ melanoma
- ⊠ other skin cancers
- ⊠ breast
- ⊠ uterine corpus
- ⊠ ovary
- ⊠ testis
- ⊠ bladder
- ⊠ brain

Epidemiology of Cancer

⌘ Lung Cancer
- 172,000 case new in 1994
  - incidence rate for men 80/100,00 in 1990, down from 87/100,000 in 1984
  - incidence rate for women increasing, up to 41/100,000 in 1990
  - 153,000 deaths occurred in 1994 due to lung cancer
- adenocarcinomas account for 35% of lung malignancies
- large cell undifferentiated carcinomas account for 15%

Lung Carcinomas

- adenocarcinomas account for 35% of lung malignancies. Treatable by surgery if localized, but may metastisize to brain
- large cell undifferenciated carcinomas account for 15%. Contain large anaplastic cells and metastisize early. Poor prognosis.
- Small cell lung cancers (SCLC)- 10% of lung neoplasms. Grow rapidly, metastasize early, poor prognosis. Produce a variety of neuroendocrine substances (ACTH, ADH, calcitonin) that cause symptoms of hormone imbalance

Colorectal Cancer

⌘ New cases of colonic cancer expected to reach 107,000 in 1994 and cause over 49,000 deaths (for rectal cancer 42,000 incidence and 7,000 deaths)
- incidence increases with age
- 1/3 of colorectal cancer patients diagnosed while cancer is still localized and 5 yr survival rates 90% when detected while still localized. Survival rates for patients with distant metastases < 7%
- Predisposing factors-- familial adenomatous polyposis, chronic ulceration colitis high fat diet, low fiber diet

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Colorectal Cancer

- Familial polyposis--
  - autosomal dominant trait that leads to multiple adenomatous polyps in colon by age 30, and undergo malignancy
  - mutation in specific gene identified-- called APF gene (thought to be a tumor suppressor gene) Mutations in p53 and *ras* oncogene occur later in tumor progression
- Treatment by surgery, radiation, chemotherapy or combination

Pancreatic Cancer

- 28,000 new cases per year
  - 25,000 deaths occurred in 1994
  - accounts for 2.5% of all annual diagnoses of cancer, but responsible for 5% of deaths
  - LTS is 3%
  - silent until well advanced
- Risk factors
  - age (65-79)
  - smoking
  - sex (30% more common in men, 60% > in blacks)
  - diets high in fat

Breast Cancer

- 182,000 new cases in 1994. Incidence rate is about 108/100,000.
  - There was an apparent increase in the 1980's, but may be due to diagnosis (mammography screenings, etc...). Found more smaller tumors.
  - Overall mortality rate has not been affected much with earlier diagnosis (why?).
  - LTS 92% for localized tumors, 18% when spread
- Risk factors-- age >50, early menarche, late menopause, first childbirth after 30, nulliparity, family history, obesity, high fat diet

Breast Cancer

- ⌘ Two genes involved
  - ☑ BRCA1 and BRCA2
  - ☑ treatment includes surgery (mastectomy), radiation, chemotherapy (tamoxifen [anti-estrogen] useful in combination with chemotherapy for postmenopausal women for tumors that are ER+ or PR+)and hormonal modifications

Ovarian Cancer

- ⌘ Accounts for 4% of all cancers in women
- ⌘ annual rate of 24,000 in 1994 and causes an estimated death rate of 13,600 in 1994
  - ☑ no symptoms until late in disease
  - ☑ use of serum marker (glycoprotein CA-125) is elevated in 50% of women with ovarian cancer
- ⌘ Risk factors--
  - ☑ age over 60
  - ☑ multiparity
  - ☑ history of breast cancer
  - ☑ life style and dietary factors (higher in industrial nations (not Japan) and higher in Caucasian women of European descent.

Ovarian Cancer

- ⌘ Treatment--
  - ☑ surgery
    - ☒ both ovaries and uterus and fallopian tubes
    - ☒ Drug treatment with taxol (from bark of Pacific yew tree
    - ☒ LTS 41%
      - • 88% for women diagnosed with localized
      - • 17% for patients with metastases
      - • Only 23% diagnosed prior to metastasis

Uterine Cancer

- 70,000 new cases in 1994
  - 15,000 invasive cervical carcinomas
  - 55,000 carcinomas *in situ*
  - uterine endothelial cancer accounted for 31,000 new cases
- Death rate from uterine cancer has decreased 70% over past 40 years (early diagnosis)
- Risk Factors:
  - early age of 1st sexual intercourse
  - multiplicity of sexual partners
  - smoking
  - papillomavirus
  - prolonged estrogen therapy/obesity/failure to ovulate

Uterine Cancer

- 75% of endometrial uterine cancer occurs after age 50 and only 4% before age 40
- Peak diagnosis of endometrial cancer age 58-60 while peak diagnosis of cervical cancer is 48-50
- treat by hysterectomy with/wo radiation. Chemo used for metastatic disease and most effective with poorly differentiated tumors that are not hormone dependent
- 5 yr survival for in situ is 100% & for endometrial cancer is 93%

Bladder Cancer

- Bladder cancer is the most common malignant neoplasm of the urinary tract
- 51,200 new cases in 1994: 38,000 in men and 13,200 in women. 4th most common cancer in men and 9th most common in women
- mortality rates decreasing (early diagnosis)
- most common in 50-70 age group
- Risk Factors:
  - smoking
  - parasitic infection (schistosomal)
  - certain chemicals (aniline dyes)
- LTS-- superficial CA-90%: regional 45%: metastatic 9%

Prostate Cancer

- 200,000 in 1994 with 38,000 mortalities
  - disease of aging/ rare in men under 55 and 1000/100,000 in men over 75 (Black males have highest incidence: reasons unknown)
    - Digital exam
    - PSA
    - ultrasonography
  - Treatments:
    - Surgical
    - cryosurgery
    - hormonal
- LTS has improved from 50%-78% over past 30 years

Leukemia

- Involve blood forming lineage of cells that are blocked in differentiation and immature cells found in blood. Depending upon cell lineage and state of differentiation disease is classified as acute or chronic myelogenous leukemia (AML or CML) OR acute or chronic lymphocytic leukemia (ALL and CLL)
- 28,600 new cases each year. Leukemia strikes more adults than children, and ALL accounts for 80% of the leukemias of children

Leukemia

- Peak age for ALL is 3-4 years and siblings of affected children have a 4X greater risk of getting disease suggesting a genetic factor
- Viral infection associated with adult T cell lymphocytic leukemia but not in children
- translocations of certain chromosomes associated with specific leukemias (*bcr-abl* chimeric gene and activation of *myc* and *bcl-2*
- In 1950's childhood ALL was 100% fatal, but now 73% of all patients survive. LTS for AML and CML still low (20%-25%)

Skin Cancer

- Non-Melanoma most common in US. >700,000 new cases/yr
  - most are slow growing, locally invasive, do not readily metastasize, treated easily
  - 2 most common types
    - basal cell carcinoma (BCC) accounts for most cases-- due to sun damaged skin caused by UV rays. Rarely affects dark skinned people. Depletion of ozone layer causing increase in incidence
    - Squamous cell carcinoma (SCC) is cancer of keratinizing cells of epidermis. Occurs later in life than BCC and occurs more commonly in women. UV radiation
    - treatment by surgery, cryo-, laser-
    - overall cure rates > 90%

Skin Cancer

- Malignant Melanoma
  - aggressive, invasive, metastatic
  - increasing 4% per year in US
  - more common in fair skinned people
  - due to exposure to sunlight (UV)
  - treatment by surgery and chemotherapy. In metastatic disease response rate is only 15%-20%

Variation in Cancer Incidence

- Varies greatly in different parts of the world
  - environmental exposures (viruses, mold from *Aspergillus flavus* {aflatoxin})
  - diet (fat content, PCB intake, etc...)
  - social customs (chewing betel nut, carrying hot coals, etc...)
  - exposure to parasitic infections (schistosomal parasites)
  - average life expectancy
- *Cancer clusters*- primarily environmental, life style (delayed childbearing)

Factors in Development of Cancer

⌘ (Table 2-5, page 36) Categories for exposure to carcinogenic agents
- occupational
- medical
- social
  - 2-6% human cancers due to occupational exposure
    - tobacco= 25-40%, alcohol=2-4%, diet=10-70%, reproductive patterns & behavior=1-13%, drugs and medical procedures=0.5-3%, geophysical factors (UV)= 2-10%, infection=1-10%, pollution=1-5%

Causes of Cancer

⌘ Cigarette smoking- (also passive)
- lung, mouth, pharynx, larynx, esophagus, urinary bladder, pancreas, kidney
- mutagens & carcinogens in smoke
  - benzo[a]pyrene, dibenza[a]anthracene, nickel, cadmium, urethane, formaldehyde, nitrogen oxides

⌘ Alcohol-
- interacts with smoking-- liver, mouth, larynx, etc

⌘ Diet-- preservatives, smoking, salt, fat (saturated), fiber, nitrates, BBQ, saccharine (promotion vs initiation)
- stomach, colon, pancreas, breast, ovary, uterine endometrium, prostate

Causes of Cancer

⌘ Diet:
- Different in various parts of the world--
  - Stomach-- Japan (smoked or pickled fish due to nitrates and salt content)
  - colon, pancreas, breast, ovary, endometrium, prostate-- US (dietary fat: in US 40-45% of calories come from saturated fat compared to Japan 15-20%). High fiber content lowers risk (may be due to decreased time of exposure to toxins)
  - agents in food for taste and preservatives-- cooking style (broiled vs fried vs BBQ)
    - saccharin (promotor)
    - BHT (promotor)
    - nitrites

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Causes of Cancer

⌘ Sexual development/patterns/behavior
  ⊠ duration of hormonal exposure- longer duration leads to breast cancer
    ⊠ early age of menarche
    ⊠ delayed 1st pregnancy
    ⊠ delayed menopause
  ⊠ cancer of cervix associated with early and frequent sexual contact with many partners
    ⊠ also human papillomavirus
⌘ Occupational- 2-4% of all deaths due to occupation
  ⊠ (Table 2-5) asbestos (lung), benzene (leukemia), naphthylamine (urinary cancer), coal soot (skin, due to PAH's and association with DNA repair mechanisms)

Causes of Cancer

⌘ Herbicides-
  ⊠ phenoxy compounds, dioxin, furan, agent orange
  ⊠ stored in fat and last a long time in body
⌘ Air and water pollution-
  ⊠ nitrosamines: potent carcinogen (normal in low concentrations, but higher around industry
  ⊠ > 50 chlorinated hydrocarbons found in sewage treatment effluents
  ⊠ in one study 325 organic chemicals ID in drinking water of 80 cites and only 10% of these have been tested for carcinogenicity (AMES test)
    ⊠ benzene, ether, carbon tetrachloride, chloroform, PCB's, vinyl chloride, etc...

Causes of Cancer

⌘ Radiation
  ⊠ Ultraviolet- low energy, does not penetrate deeply (people with *xeroderma pigmentosa* have > risk to skin cancer, people closer to the equator, light skinned people, people at higher elevation)
    ⊠ basal cell carcinoma- invades locally but never metastatic
    ⊠ squamous cell carcinoma- invades locally and may metastasize
    ⊠ melanoma- highly malignant and rapidly metastatic

Causes of Cancer

⌘ Radiation
  - Ionizing radiation-
    - x-ray and radium- type of cancer depends upon dose, age at time of exposure and sex of individual
    - w/i 25-30 yrs after whole body exposure there is increased incidence of leukemia and cancers of the breast, thyroid, lung, stomach, salivary gland
  - Radon-
    - radioactive gas (due to decay of radium 236) is ubiquitous. Causes bronchogenic carcinoma
    - closed environments
    - lifetime exposure to 4 pCi/L causes 1% increase in lung cancer (most households have < 2 pCi/L)

Causes of Cancer

⌘ Drugs:
  - anticancer drugs give risk for cancer- cyclophosphamide... are alkylating agents known to interfere with DNA
  - 2nd cancers arise later in life from previous treatments (20 years later)
  - immunosuppressive drug treatments (organ transplant)

⌘ Hormones:
  - Estrogen, progesterone (rate of cell proliferation higher in nulliparous women [lower hormone levels?]). Progesterone reg mitotic div in breast epithelium

Causes of Cancer

⌘ Hormones-
  - inc risk with higher body weight thought to be due to inc conversion in levels of adrenal androgens to estrogen and lower levels of sex-hormone-binding globulin in obese women.
  - Use of "unopposed" estrogen therapy w/o progesterone inc endometrial cancer
  - oral contraceptives do not seem to increase risk of breast cancer much.
  - Use of diethylstilbestrol (DES) increases risk 1.5 X for breast cancer (vaginal adenocarcinomas in women from mothers treated with DES

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Causes of Cancer

- Infection-
  - infection with certain viruses
    - Epstein-Barr- Burkitt's lymphoma
    - hepatitis B- liver cancer
    - human T cell lymphocytic virus- leukemia
    - human papillomavirus- cervical cancer
    - HIV- kaposi's sarcoma & non-Hodgkins disease
  - Parasites
    - schistosomal infection- bladder cancer
    - chonorchiasis- cholangiosarcoma of liver

Causes of Cancer

- Genetic Factors
  - inherited cancers
    - 1-2% of total cancers
      - retinoblastomas- dominant gene. If have gene you have a 95% probability and will develop 3-4 tumors (oncogenesis at cellular level is rare (gene alone is not sufficient- 2 event hypothesis- one dominant gene and one mutation [spontaneous])
      - 20-40% of Wilms' tomors
      - familial multiple polyposis
  - 50 forms of hereditary cancers
    - retinoblastoma- long arm of chromosome 13 has deletion in band 14 (del 13q14) results in loss of RB1 allele in cells with already mutated RB1 (tumor suppressor gene=RB1)

Causes of Cancer

- Wilms' tumor
  - renal cancer
  - deletion of short arm of chromosome 11 in band 13 (del 11p13)
  - suggest tumor suppressor gene-
- Li-Fraumeni syndrome
  - sarcomas, breast cancer, leukemia
  - associated with loss of part of chromosome 17 which has the tumor suppressor gene p53

Causes of Cancer

- Predisposition to cancer
  - involves interaction of genetic and environmental factors
    - xeroderma pigmentosum (extreme sensitivity to light and incidence of skin cancer of 100%)
    - autosomal recessive trait (homozygous recessive)
    - defect in DNA repair
  - defects in ability to metabolize foreign chemicals (xenobiotics) especially those that are carcinogenic
  - PAH's and AHH arylhydrocarbon hydroxylase (Ah locus-->p450 enzyme induction)

Causes of Cancer

- Avoidability of cancer
  - life style accounts for 80% a cancers and therefore can be avoided
  - ideal life style
    - do not smoke or drink
    - should eat a diet low in fat, rich in fiber and yellow vegetables
    - should protect from hazardous chemicals in work and home and avoid unneeded x-rays, avoid excessive exposure to sunlight
    - woman should do above and have at least one child early in reproductive life and avoid multiple sex partners
  - cannot avoid pollution or infections

Oncogenes & Protooncogenes

TABLE 8-4. ONCOGENES AND PROTO-ONCOGENES

Oncogene	Virus	Protein Encoded by Oncogene	Protein Encoded by Proto-oncogene
Peripheral Membrane Proteins with Tyrosine-specific Protein Kinase Activity			
v-src	Rous sarcoma virus, B77 ASV	pp60^{v-src}	pp60^{c-src}
v-abl	Abelson murine leukemia virus	p160$^{v-gag-abl}$	pp145^{c-abl}
v-fes/fps	Snyder-Theilen FeSV; class II ASVs including Fujinami sarcoma virus	pp85$^{v-gag-fes}$ pp130$^{v-gag-fps}$	pp92$^{c-fes/fps}$
v-yes/fgr	Y73 Esh sarcoma virus Gardner-Rasheed FeSV	pp90$^{v-gag-yes}$ pp70$^{v-gag-fgr}$	p55$^{c-yes/fgr}$
v-kit	Hardy-Zuckerman FeSV	pp80$^{v-gag-kit}$	?
Transmembrane Receptors with Tyrosine-specific Protein Kinase Activity			
v-erbB	Avian erythroblastosis virus (AEV)	P45^{v-erbB}	EGF receptor: 150K
v-fms	McDonough FeSV	P140$^{v-gag-fms}$	Macrophage colony stimulating factor (CSF1) receptor: 170K
v-ros	UR2 ASV	P68$^{v-gag-ros}$	P68^{c-ros}
v-erbA	AEV	P75$^{v-gag-erbA}$	Thyroid hormone receptor: 70K
Cytoplasmic Serine/Threonine-specific Protein Kinases			
v-mos	Moloney murine sarcoma virus (Mo-MSV)	P37^{v-mos}	P41^{c-mos}
v-mil-raf	MH2 ASV; Moloney murine sarcoma virus strain	P100$^{v-gag-mil}$ P75$^{v-gag-raf}$ and P90$^{v-gag-raf}$	P72$^{c-mil/raf}$
Plasma Membrane-associated GTP-Binding Proteins with GTPase Activity			
v-ras	Harvey and Kirsten murine sarcoma virus (Ha- and Ki-MSV)	P21^{v-ras}	P21^{c-ras}
Growth Factor			
v-sis	Simian sarcoma virus (SiSV)	P28^{v-sis}	P32^{c-sis}; PDGF
Nuclear DNA-binding Proteins			
v-myc	Avian myelocytomatosis virus (MC29)	P110$^{v-gag-myc}$	P62^{c-myc}
v-myb	Avian myeloblastosis virus (AMV)	P45^{v-myb}	P75^{c-myb}
v-fos	FBJ murine osteosarcoma virus	P55^{v-fos}	P55^{c-fos}
v-ets	E26 avian acute leukemia virus	P135$^{v-gag-myb-ets}$	P54^{c-ets}, P75^{c-myb}
v-ski	SKV770		

Genetic alterations in cancer cells

- In 1914 Boveri thought that only one abnormal chromosome could cause the malignancy of a cancer cell
  - Philadelphia chromosome (Ph[1]) in patients with chronic myelocytic leukemia (CML). Due to translocation of a piece of chromosome 22 to chromosome 9. > 90% of patients with CML have Ph[1] chromosome in leukemic cells
- Chromosome banding imp't for ID of genes
  - each band contains 5×10^6 nucleotide pairs and deletions or duplications of 2×10^6 nucleotide pairs is difficult to detect

Genetic alterations

- Average protein with a MW of 50,000 is about 1200 nucleotide pairs (how many AA's is this translated into??).
  - Approximately 1000 genes could be duplicated or deleted without being detected (5×10^6 div by 1200).
  - The entire gene does not have to be deleted of duplicated for its function to be altered
  - THUS- many mutations detected by banding underestimate the number of cancers that have altered gene function

Genetic alterations

- All cancer cells have some alteration of gene expression
  - chromosomal translocations
  - inversions
  - deletions
  - amplifications
  - point mutations
  - aneuploidy (duplications or deletions of whole chromosomes)
- most studies come from leukemias and lymphomas (easier to obtain single cell suspensions than from solid tumors)

Genetic alterations

✿ Translocations & inversions
- ☒ > 100 have been observed and are associated with specific cancer types
- ☒ Ph1 chromosome arose through reciprical translocation and fusion of a small piece of chromosome 9 to chromosome 22 and a piece of 22 to 9. The Ph1 chromosome represents the fusion of *abl* (from 9) to the *bcr* (22) and the resulting fusion-chimeric protein
  - ☒ reciprocal translocation between long arm (q) of chromosome 9, band 34, and band 11 of the q arm of chromosome 22 {t(9:22)(q34;q11) } This involved a breakpoint near the Abelson (*abl*) protooncogene
- ☒ Burkitt's lymphoma has a t(8;14) translocation of the *myc* from 8 to 14 and is near the Ig heavy chain Cu, and results in activation of *myc* when it should be quiescent.

Photographs (left) and diagrams (right) of human chromosomes 9 and 22 and the products of the translocation that creates the Philadelphia chromosome (Ph1, lower right) commonly found in human leukemic cells and its reciprocal. The translocation juxtaposes the genes bcr and abl normally found on separate chromosomes. Arrows denote breakpoints.
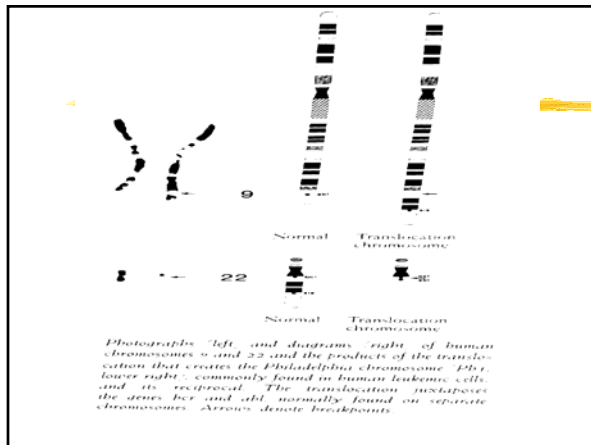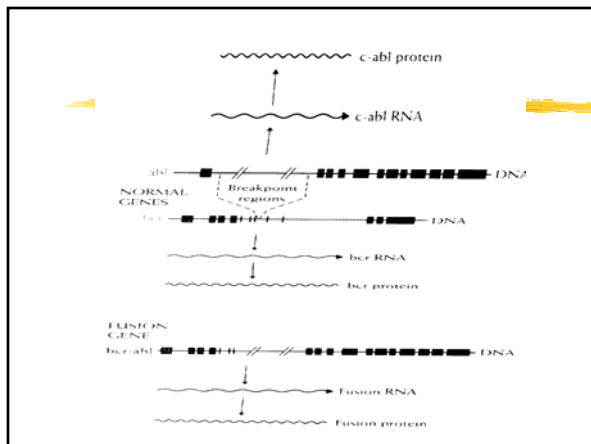
Genetic alterations

⌘ Abl oncogene
   ⊠ breakpoints for translocation occur at variable sites, but always in intron regions
   ⊠ in translocation to chromosome 22 it is inserted into the midpoint of the *bcr* gene which encodes a GTP*ase* activating protein (GAP)
   ⊠ a chimeric *bcr-abl* protein is made which has high tyrosine activity
⌘ Conundrum-
   ⊠ similar translocations occur in other cancer types with different aggressiveness, may be due to different breakpoints and different sized protein products
☞ **similar patterns of altered gene expression can lead to different endpoints in different cell types**
☞ **Different patterns of gene alteration can produce common phenotypic changes in cells**

Genetic alterations

⌘ Chromosomal Deletions
   ⊠ most common defects observed in solid tumors are deletions in specific gene sequences
      ⊠ seen as a loss of a part of a banding region or the loss of heterozygosity of a specific allele
      ⊠ gene amplification
      ⊠ single base substitutions or point mutations
   ⊠ these changes may result in activation of an oncogene or inactivation of a tumor suppressor gene

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Genetic alterations

⌘ Chromosome deletions that are most common in certain tumor types:
   ⊠ del (13)(q14q14) in retinoblastoma (loss of Rb tumor suppressor gene)
   ⊠ 11p13 deletion in Wilms' tumor
   ⊠ deletion of DCC (deleted in colon cancer) gene
   ⊠ deletions in long arm of chromosome 5 (del 5q) of cancers such as acute non-lymphocytic leukemia [region is 5q21-31 and contains genes that code for growth factors and growth factor receptors involved in myeloid differentiation

Genetic alterations

⌘ Chromosome deletions
- 3p13-23 small cell carcinoma & adenocarcinoma of lung, renal cells carcinoma & ovarian adenocarcinoma
- 1p32-36 region in neuroblastoma and glioma
- 1p11-22 deletions in melanoma, breast adenocarcinoma, and others
- 1q21-23 in uterine and bladder adenocarcinomas
- 6q11-27 in melanoma, glioma & ovarian carcinoma
- 7q21-34 lost in uterine leiomyoma, prostate, glioma
- p53 tumor suppressor lost from chromosome 17p in many cancers

Genetic alterations

⌘ Induction of cancer involves at least 2 hits: 1st may be through genetically inherited gene while 2nd occurs after birth
- normal allele may be enough to give normal growth characteristics, but if it is also mutated then cancer occurs

⌘ Gene amplification:
- increase in copy number (small or 10-100 fold)
  - n-myc gene in stage III and IV neuroblastoma
  - EGF (her-2/neu genes) in breast and ovarian cancers
  - Trisomy (8 in ALL, 9 in myeloproliferative cancers, 12 in lymphomas, also 3 and 7 in other cancers

Genetic Alterations in Cancer

⌘ Aneuploidy
- localized tumors have lower % of aneuploidy than tumors that have metastasized (> in undifferentiated tumors)

⌘ Point Mutations
- lead to single base changes in DNA sequence. During repair bases are put in incorrectly

⌘ Loss of heterozygosity-
- one allele is damaged, translocated, deleted- increased risk of cancer due to potential for other allele to be mutated with no backup

Table 3-3 Deletion and Loss of Heterozygosity in Solid Tumors

Mapping Human Genome

⌘ **3000 megabases (3X10^{15}) containing 50,000-100,000 genes (only 2500 mapped so far). ID of specific alterations in genes**
  - techniques used to map genes
    - use of hybrid cells (chromosomes of one cell type are lost and the other retained, banding appearances different on mouse and human, and by process of elimination the assignment of enzyme or marker to a specific chromosome can be made based on chromosomes still present)
    - *in situ* hybridization-uses cDNA that is radiolabeled to a specific site on metaphase chromosomes
    - southern blot- use of restriction enzymes and examination of fragments for specific genes and linkages

Mapping Human Genome

⌘ **Inappropriate production by cancer cells of proteins and other cellular products = ectopic production**
  - due to alteration of gene expression :
    - gene amplification
    - rearrangement
    - translocation
    - point mutations
  - produces symptoms of non-cancerous disease, produced by cancer cells (p 75)
    - increased transcription (mutation in regulatory gene?)
    - increased mRNA levels (gene amplification, decreased mRNA degradation)
    - clonal expansion of normal cells that produce protein in high amount
    - aging increases "leakiness" of gene expression

Table 3-5 Ectopic Hormones Produced by Various Human Cancers

Hormone	Tumors Producing Hormone Ectopically	Associated Clinical Syndrome
ACTH	Carcinomas of lung, colon, pancreas, thyroid, prostate, ovary, cervix, thymoma, pheochromocytoma, carcinoid tumors	Cushing's syndrome
ADH	Carcinomas of lung, duodenum, pancreas, ureter, prostate, thymoma, lymphoma, Ewing's sarcoma	Inappropriate antidiuresis, hyponatremia
Calcitonin	Carcinomas of lung, breast, prostate, bladder, pancreas, liver, esophagus, stomach, colon, larynx, testis, carcinoid tumors, insulinoma, pheochromocytoma, melanoma	No apparent syndrome
Erythropoietin	Hemangioblastoma, uterine myofibroma, pheochromocytoma, carcinoma of liver, ovary	Polycythemia (erythrocytosis)
Gastrin	Carcinoma of pancreas	Zollinger-Ellison syndrome (gastric hypersecretion with intractable peptic ulceration)
Glucagon	Carcinoma of kidney	Hyperglycemia, malabsorption, gastrointestinal stasis
Growth hormone	Carcinomas of lung, stomach, ovary, breast	Hypertrophic pulmonary osteoarthropathy, acromegaly
HCG	Carcinomas of breast, stomach, small intestine, pancreas, biliary tract, liver, colon, rectum, lung, ovary, testis, melanoma	Gynecomastia, precocious puberty
Prolactin	Carcinomas of lung, kidney	Galactorrhea, gynecomastia
PTH	Carcinomas of kidney, lung, liver, adrenal, pancreas, parotid, ovary, testis, spleen, breast	Hypercalcemia
TSH	Carcinomas of lung, breast	Hyperthyroidism

Specific cancer types with gene alterations

⌘ Retinoblastoma:
- 2 genetic hits: 1st is inherited, 2nd is somatic mutation
- due to deletion of 13(13q14) of RB1 gene (1st tumor suppressor gene identified)
- occurs early on since proliferation of retinal cells tapers off with age. Need proliferation to "fix" mutations, and chance of 2nd mutation occurring is higher in proliferating cells

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Specific cancer types with gene alterations

⌘ Wilms' Tumor
- pediatric kidney cancer (nephroblastoma)- arises in primitive cells of developing kidney
- In some rare Wilms' tumors there is a deletion of a gene within the band p13 of chromosome 13
  - codes for a zinc finger DNA-binding protein that is expressed in blast cells of developing kidney

⌘ Li-Fraumeni Syndrome-
- Breast cancers and sarcomas appear earlier than they should (15-44 years) Risk decreases with age
- autosomal dominant gene
- due to p53 on chromosome 17p (point mutation). Tumor suppressor

Specific cancer types with gene alterations

- ⌘ Leukemias-
  - arise from stem cells in BM (myeloid=granulocytic) or lymphoid=lymphocytic cell lineages)
  - may be rapid (acute) or slower (chronic) onset
    - in acute leukemias the chromosomal translocations result in activation of transcription factors that affect differentiation
    - in chronic leukemias translocation events affect genes that induce cell proliferation and survival
  - reciprocal translocation of long arms of chromosomes 9 and 22 consistent finding in CML and results in chimeric *Bcr-Abl* protein
    - chromo 9 is of paternal origin and 22 maternal
    - 9q34 is hypermethylated (inactive) and 22q11 hypomethylated (active); activation of "downstream" genes with hypomethylation

Specific cancer types with gene alterations

- ⌘ 11q23 translocations found in acute leukemias and lymphomas
  - (4;11) (6;11) (9;11) and (11;19) most common
  - gene located at breakpoint is the myeloid-lymphoid leukemia gene= MLL
  - mRNA transcripts are altered from normal cells
  - depending upon where the breakpoint is and which chromosome receives the translocated genes, different types of transformed cell lineages can result

Specific cancer types with gene alterations

- ⌘ **Lymphoma**-
  - translocation between chromosomes 8 and 14 in Burkitt's lymphoma
  - 80% of patients with BL have a t(8;14)(q24;q32) translocation [other translocations have chrom 8 with chromo 22 and 2]
  - all of these have Ig genes [14q32 has Ig Heavy chain genes: 22q11 has lambda light chains: 2p11 has kappa light chains
  - THUS, Ig genes involved in some key way in BL
  - All of these translocations involve translocation of *c-myc* proto-oncogene from chromosome 8 to other chromosomes
  - translocation may cause activation of *c-myc* when Ig genes are activated

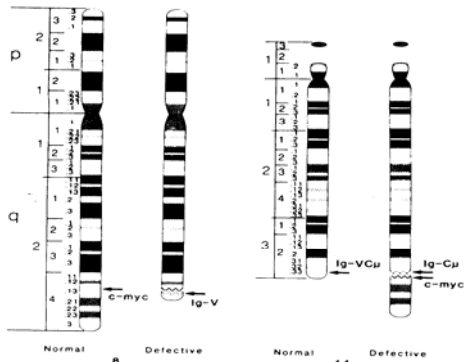## Burkitt's Lymphoma Translocations

Specific cancer types with gene alterations

⌘ Colorectal Cancer

- easy to study from surgical resections. Tumors are clonal and have good evidence that there are hereditary factors, environmental factors, oncogene activation and tumor suppressor inactivation
- Model of tumor progression shown in Fig 3-6 (p 83) Due to total accumulation of changes rather than specific sequence of alterations
- >90% if colon cancers have 2 or more genetic alterations and many have 4 or 5 alterations [later tumors have more changes]
- Autosomal dominant Familial Adenomatous Polyposis syndrome (FAP) gene has been cloned and is called APC. Mutations are point, frameshift or deletions and inactivate the APC gene (tumor suppressor). Located on 5q21
- 2nd gene locus in 5q21 is MCC (mutated in colorectal cancer)
- Loss or inactivation of APC and MCC inc proliferation and increases chances for subsequent genetic alterations to occur

Specific cancer types with gene alterations

⌘ Colorectal Cancer

- Another early change is loss of methyl groups from DNA. Hypo-methylation associated with activation of silent gene
- activation of ras protooncogenes occurs in many colorectal cancers, and mutations occur in codons 12 or 13 to cause irregular activity (cannot be turned off)- GTPase activity
- Additional losses or inactivation of Tumor suppressor genes occurs sequentially as tumor progresses:
- 18q (may contain DCC gene which produces cell adhesion protein)
- loss of 17p which contains p53 tumor suppressor gene
- may also lose DNA repair genes

Colorectal Tumor Cell Progression
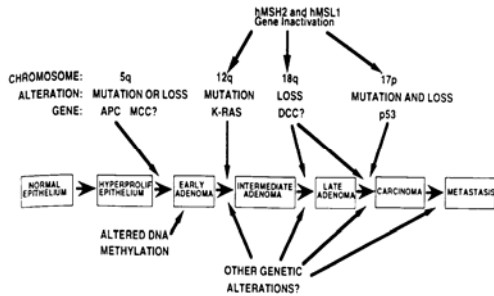
Specific cancer types with gene alterations

⌘ Breast Cancer

- show LOH at a number of different gene loci and p53 is affected in both hereditary and sporadic breast cancer
- one heritable trait is located on 17q21=BRCA1(zinc-finger DNA binding), is linked to 45% of affected families with breast cancer only and 100% of breast cancer families that had at lest one case of ovarian cancer
- BRCA2 located on 13q (high risk for breast but not ovarian cancer)

Conclusions

⌘ Multiple (multiplicity of) genetic alterations associated with individual cancers and show up consistently in cancer of different tissue types

- in one tissue type p53 may be important in an early step of stem cell proliferation and differentiation, whereas in other tissues it may be more important in later stages

⌘ Oncogene activation/mutation also contributes to unregulated growth

⌘ Early changes associated with causes of cancer and those that occur later associated with progression

⌘ Loss of ability to repair DNA

Phenotypic Characteristics of Cancer Cells

- Study of cells *in vitro* is used commonly but is not best
  - change of karyotype
  - selection of aberrant cells types that are different from original cancer
- Study of changes *in vivo* even more difficult
  - hard to mimic "real life" factors
    - nutritional state
    - hormones
    - infections
    - influence of promotors

Phenotypic Characteristics

- In 1940's Earle *et al.* cultured fibroblasts with methylcholanthrene (1ug/L) and found that within 6 days they became transformed, and when injected into mice produced sarcomas (8% of "normal" cells also caused tumors: (WHY?)

- Other chemicals later shown to do same transformations

- To cause tumors in animals, must put in susceptible animal (i.e., nude mice, specific strain, etc....)

Phenotypic alterations

- Cytologic alterations:
  - **increased cytoplasmic basophilia**
  - **increased number and size of nuclei**
  - **increased nuclear/cytoplasmic ratio**
  - **formation of clusters and cords of cells**
- Alteration in growth characteristics:
  - **immortality**
  - **decreased density dependence (loss of contact inhibition)**
  - **Decreased serum requirements**
  - **loss of anchorage dependence**
  - **loss of cell cycle control (do not stop in G1 or at G1/S)**
  - **resistance to apoptosis**

Phenotypic alterations

⌘ Changes in cell membrane structure and function
- agglutability by plant lectins
- changes in cell surface glycoproteins
- appearance of TAA's
- increased uptake of AA's, hexoses and nucleosides

⌘ Loss of cell-to-cell and cell-extracellular matrix interactions that determine differentiation processes

Phenotypic alterations

⌘ Loss of response to differentiation agents
- through loss or change of receptors
- loss of signal transduction processes
- loss of genes

⌘ Altered signal transduction pathways:
- Ca^{++}
- PKC
- TK
- Ca^{++}- dependent kinases and enzymes
- GF receptors/phosphorylation & dephosphorylation mechanisms

Phenotypic alterations

⌘ Increased expression of oncogene products
- due to:
  - chromosomal translocation
  - amplification
  - mutation

⌘ Loss of tumor suppressor gene function
- due to:
  - deletion
  - mutation

Phenotypic alterations

⌘ Overproduction of growth promoting factors
- TGF-alpha
- PDGF
- hematopoietic growth factors (CSF's, IL's)

⌘ Genetic instability:
- progressive loss of regulation

⌘ Alterations in enzyme patterns:
- inc levels of enzymes involved in proliferation
- inc invasiveness
- inc proteases/collagenases/glycosidases

Phenotypic alterations

⌘ Increased production of fetal proteins (may be ectopic)
- CEA
- AFP
- CG (chorionic gonadotropin)

⌘ Ability to produce tumors in experimental animals
- Nude (athymic), SCID, transgenic mice, knockout mice, retroviral infection, microinjection,

⌘ Ability to avoid hosts immune system response

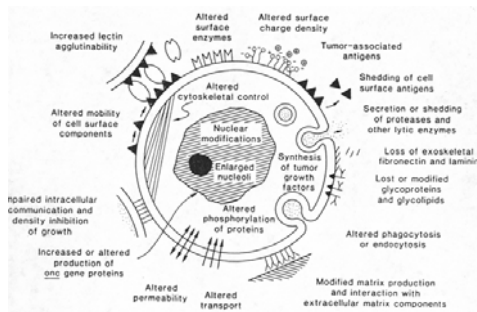## *Figure of Cellular Alterations*

Figure 4-1 Some cellular alterations observed after neoplastic transformation. (Modified from Nicholson.)

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Clonal derivation of cancer

⌘ Tumors arise from a single cell and therefore are clones of the original mutated cell
- ☒ in primary cancers, all cells have the same abnormal chromosome arrangement (i.e., Ph1)
- ☒ all cells of a given multiple myeloma produce same type of Ig
- ☒ tumors arising in women whose cells are heterozygous for the X chromosome-linked iso-enzymes of glucose-6-phosphate dehydrogenase express only one of the two possible iso-enzyme types (only one X expressed in cells/randomly determined, and if all cells express same isotype then tumor comes from one cell)

Single cell clonal theory

⌘ Phenotypic alterations can occur from a single cell that has cloned into cancer mass:
- ☒ neoplastic cells unstable and go through evolutionary changes as tumor progresses
  - ☒ tumor initiation occurs from interaction of carcinogen with susceptible cell (initiation & promotion)
  - ☒ induced change provides selective growth advantage over normal cells
  - ☒ other cells may be induced but never grow or are killed
  - ☒ proliferation "fixes" genetic alteration and after a lag period and genetic instability, there appear variants
  - ☒ many variants may be killed (metabolic disadvantage/immune killing) but those with selective advantage continue to grow
  - ☒ Increased metastatic potential and characteristics appear
  - ☒ microenvironment plays imp't role in tumor characteristics

Alterations of Cellular Differentiation

⌘ Cancer develops from cells that are capable of dividing
- ☒ all tissues retain some capacity for cell division
- ☒ when dividing cannot differentiate
  - ☒ differentiation is the sum of all the processes by which cells in a developing organism achieve their specific set of structural and functional characteristics
    - requires progressive restriction of genomic expression
      - due to internal programming
      - environmental influences (neighboring cell interactions, growth factors, hormones, position to other cells and circulation, etc...)

Alterations of Cellular Differentiation

⌘ Difference between **totipotent** (can develop into any cell type) vs **pluripotent** (develops one of cell types peculiar to their tissue of origin)

⌘ Mechanisms of cell differentiation:
- ◩ 1) asymmetric cell divisions (from zygote-->adult)- gastrula, cytoplasm differences
- ◩ 2) selective changes in gene structure and transcription
- ◩ 3) selective translation of mRNA's
- ◩ 4) differential response to microenvironment

Function of Interphase Chromosomes

⌘ When cells are preparing for cell division and enter the mitotic (M) phase of cell cycle, chromosomes condense. During metaphase they line up alone the equatorial plate (replicated pairs) and get ready for division

⌘ Most of cell cycle is in Interphase, where cell makes proteins specific for differentiation

⌘ Some single genes range in size from 30,000 to 1 million bases.

Chromosomes

⌘ Noncoding DNA (silent) makes up 90% of mammalian genome [includes satellite DNA; long interspersed repeated elements and smaller nontranscribed DNA] Plays important role in determining which genes are silent and which are transcribed in a given tissue

⌘ Condensed regions not as available to RNA transcription enzymes as uncoiled DNA

⌘ Packaging of chromatin plays large role in damage by carcinogens (non-condensed more available for damage)

Gene Activation

⌘ H1 histone present in lower concentrations in active chromatin and displacement of histones may play a role in uncoiling of DNA to make it more able to complex with RNA polymerase

⌘ methylation of DNA is decreased in active genes

⌘ There are differences in composition of nuclear matrix proteins between normal and cancer cells (these are non-histone proteins and may contain proteins such as Lamins A and C). There are *cis* and *trans* regulatory proteins

Promoters & Enhancers

⌘ Presence of *Promoter* sequences present in both prokaryotic and eukaryotic cells, but regulation more complex in eukaryotic cells

  ☒ includes packaging, methylation, binding of nuclear proteins

  ☒ *cis* regulatory elements- act on gene sequences on the same DNA strand (but my be several hundred base pairs away) vs *trans* regulatory elements which control genes on other chromosome strand

    ☒ two types of *cis*: sequences near transcription initiation site (*promoters*) and *enhancers* (activator genes)that are more remote from gene (facilitates access of RNA polymerase to initiation sites). Upstream or downstream- 10,000 bases away

    ☒ both promoters and enhancers contain **TATA** boxes

Promoters and Enhancers

⌘ Viruses contain similar elements to enhancers

  ☒ enhancer sequences in viruses located within long terminal repeat regions (LTR), and these sequences can activate cellular genes (i.e., protooncogenes) which can lead to cell transformation

⌘ Enhancer-Promoter cross-talk

  ☒ necessary for coordination of cell processes and gene activation control

    ☒ 1) Enhancer and Promoter elements may be brought together by binding of distant DNA sequences and allosteric change in DNA

    ☒ 2) Protein-Protein interactions to "bridge" regions

    ☒ 3) "tracking mechanism" to convey signal from one domain to the other

Promoters & Enhancers

⌘Control mechanisms:

☑different transcription factors can bind to promoter and enhancer regions

☒may need both binding in order to activate gene

☒activity may be only 1/2 maximal if only one binds

☒may be "strong" and "weak" promoters and enhancers

Transcriptional Factors

⌘Transcriptional Factors--

☒multidomain regions that have DNA-binding, activation, nuclear-localization and ligand binding domains

☒DNA binding domains characterized by ::
- helix-turn-helix
- zinc fingers

☒many TF's form dimers through leucine-rich "zipper" domains (i.e., *jun-fos* interactions)

Repressor Substances

⌘Some have DNA binding domains but lack activation domains and compete with TF's for binding sites

⌘Some can form heterodimers with activators and block ability of TF's to bind to DNA

⌘Some may bind to TF's that are already bound to DNA and prevent activation by blocking interaction with TATA boxes

Repressor Factors

⌘ Transcriptional activators and repressors can be encoded for by the same gene (by alternate mRNA splicing)

⌘ Activators and repressors coexpressed in cells (balance of two is important)

⌘ function of activators and repressors is often regulated by postranscriptional modifications such as phosphorylation (kinase enzymes)

Promotor/Enhancer-Specific Transcription Factors

⌘ AP-1/*Fos*/*Jun*: trancriptional activator

◰ AP-1 binding sites bind to *fos/jun* oncogene proteins as well as the tumor promoter phorbol ester (TPA) binding factor and glucocorticoid receptor

⊠ AP-1 binding sites also known as TPA responsive-element (TRE) and glucocorticoid receptor element (GRE)

⊠ for factors to be active they must dimerize (i.e., *fos* must form a luecine zipper with *jun*) to form a **heterodimer** (homodimers of *jun* are weakly active and homodimers of *fos* do not occur)

⌘ ATF/CREB: ATF/CRE enhancer sequence (TGAACGTCA) is the activating transcription factor (ATF) binding site also known as the cyclic AMP response element (CRE) that binds the cAMP response element binding protein (CREB)

Hormone Receptors

⌘ Receptors for Steroid Hormones:

◰ glucocorticoids, estrogen, progesterone)

⊠ estrogen and progesterone receptors are cytosolic- after binding to ligand they migrate through nuclear membrane and bind to specific promoter/enhancer sequences of DNA (hormone response elements (HRE's) to activate or repress gene activity

⊠ Thyroid hormone receptor may function as a repressor in absence of ligand and as a gene activator in presence of ligand

Post-transcriptional Regulation

⌘1. Splicing of pre-mRNA into mRNA
⌘2. Capping (5'-methly cap) & polyadenylation
⌘3. Nuclear-cytoplasmic transport
⌘4. Initiation of translation
⌘5. Alternate translation from overlapping genes
⌘6. Turnover of mRNA
⌘7. Protein folding and processing
⌘8. Post-translational modifications of protein
⌘9. Intracellular translocation of mature protein

Role of Microenvironment in Differentiation

⌘Positional effects- ex.=embryonic cells
⌘extracellular matrix (ECM)-cell communication
  - Laminin- glycoprotein: helps in binding cells to ECM, migraton of cells, growth, and differentiation (active sites on molecule such as IKVAV sequence that induces *c-fos* and *c-jun* which stimulate proliferation, and SIKVAV-containing peptide which stimulates angiogenesis and tumor growth
  - Fibronectin- glycoprotein: anchor cells. Involved in cell spreading, cell movement and proliferation (need both ECM and fibronectin *in vitro* for growth)

Role of Microenvironment in Differentiation

⌘Proteoglycans- composed of core protein and multiple glycosaminoglycan (GAG) chains (repeating disaccharide sugars).
  - In glycoproteins 30-40% is carbohydrate; in proteoglycans about 90% is carbohydrate
  - part of membrane surface receptors and there are different types with varied functions:
    - cell-to-cell adhesion
    - binding to growth factors/ protease inhibitors/cell-ECM interactions

Role of Microenvironment in Differentiation

⌘ ECM-- normal cells require tissue specific ECM but tumor cells will grow well on ECM from a number of tissues
- ECM has direct effect on gene transcription via triggering intracellular signaling system
- exogenous factors (hormones & GFs) act as cofactors with ECM to induce gene transcription
- Integrity and function of ECM is involved in determining the metastatic potential of malignant cells (induction of proteases, glycosidases, heparinases, collagenases

Role of Microenvironment in Differentiation

⌘ Growth Factors-- (Table 5-4) Paracrine, autocrine, endocrine production

⌘ Ionic and pH effects-- growth stimulatory effects of mitogens show transient increase in intracellular pH and cation influx (CA^{++}) into cells

Reversability of Differentiation

⌘ Malignant neoplastic transformation occurs only in cells that are capable of dividing
- terminally differentiated cells will not develop into tumors, but any step in process towards terminal differentiation is reversible
- stem cells more susceptible to oncogenic agents (stem cells in liver?)

⌘ Difference between cancer and normal cells is the lack of normal differentiation. Carcinogens act on some step in differentiation such that they block the normal progression of gene activation and leave some of the differentiation genes off and proliferation genes on. May occur at one or more stages and can explain why there is a great diversity in chemically induced tumor cells in differentiation grade

Summary/Discussion Question

⌘ **Be able to discuss how any changes or alterations in each of these parameters that affect differentiation will give rise to a cancer cell**

☒ i.e., **list all of the parameters, giving examples of each, and discuss how gene activation is affected, how normal gene progression might be affected, and how changes in receptor and signal transduction processes are involved in cancer development**

Causes of Cancer

⌘ What causes the cellular alterations that produce cancer

☐ strongly related to environmental factors and life style

☐ cancers have common characteristics (have talked about)

☐ long latent period (> 20 years?) before <u>detectable</u> tumor found

☒ growth of tumor 1st limited by host defenses or lack of blood supply

☒ genetic instability leads to more aggressive growing tumor (more embryonic) and biochemical divergence occurs even though tumor was clonally derived.

Causes of Cancer

⌘ History

☐ 1761-- John Hill observed that nasal cancer appeared in people who used snuff excessively

☐ 1775-- Percival Pott observed that scrotal skin cancer appeared in chimney sweeps

☐ 1875-- Volkman and Bell observed that skin that was in contact with tar and paraffin oils (polycyclic aromatic hydrocarbons (PAHs) had increased cancer

☐ 1895-- Rehn found the development of bladder cancer in people who worked with analine dyes

☐ 1915 1st observation in animals that repeated coal tar application to ears of rabbits produced cancer (PAHs in coal tar)

Causes of Cancer

⌘ Since 1940s other carcinogens found:
  - 2′,3′-dimethyl-4-aminoazobenzene (liver)
  - 2-acetylamino-fluorene
  - halogenated hydrocarbons
  - urethane
  - beryllium salts
  - nitrosamines
  - intercalating agents
  - asbestos
  - vinyl chloride
  - diethylstilbesterol
  - aflatoxins (naturally occurring)

Causes of Cancer: Activation of onco/protooncogenes

⌘ Carcinogens activate cellular protooncogenes and oncogenes by a number of mechanisms:
  - base substitution (point mutation)
  - chromosomal translocations
  - gene amplification

⌘ *Ras* protooncogene mutations (liver, skin, lung and mammary tumors)
  - G-->A base transition , or G-->T or A-->T with different mutagens and tumor site
  - introduction of apurinic sites in DNA cause mutations in proto-oncogenes and in *ras* induce a CAA to CTA mutation in codon 61 (single AA change causes *ras* to remain active and can't be turned off).

Causes of Cancer

⌘ Most interactions of carcinogens result from covalent bond formation between carcinogen and the nucleophilic sites in proteins (i.e., sulfur, oxygen, and nitrogen atoms in cysteine, tyrosine, histidine) and nucleic acids (purine or pyrimidine ring nitrogen and oxygen)

⌘ Parent compound may have to be incubated with liver homogenate 1st in order for active metabolite to be formed

⌘ Ames Test for mutagenicity *NOT* carcinogenicity (not all mutagens are carcinogens) [use of *Salmonella typhimurium*]

Causes of Cancer (PAHs)
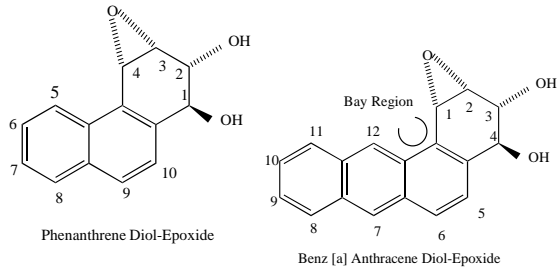
⌘ Mediated through epoxides

Phenanthrene Diol-Epoxide

Benz [a] Anthracene Diol-Epoxide

Causes of Cancer (PAHs)

⌘ When injected into new born mice the diol epoxide form has 20 X greater activity for cancer than the diol form.
- ◹ Proof of necessity for epoxide formation and carcinogenicity comes from studies of antagonists of metabolic formation and with inducers of the inactivation of the diol expoxide compounds.

⌘ Ingestion of drugs, smoking, exposure to halogenated hydrocarbons and diet influence the metabolism (and carcinogenicity) of PAHs (through activation of p450 enzymes)

⌘ PAH metabolites are DNA adducts (bind to DNA to cause dysfunction)

Causes of Cancer (Adduct Formation)

⌘ Biological consequences of adduct formation:
- ◹ stabilize the distortion of the helix by inserting between stacked bases, and lead to a frameshift mutation during replication of DNA (alkylated bases cause misrepair with wrong base during during DNA replication)
- ◹ induction of instability of purine glycosidic bonds between purine and deoxyribose. Results in loss of base (apurinic site- "open site) which is generally filled by "A" resulting in base substitution.
- ◹ Specificity of binding at 300 bp sequence prior to RNA polymerase binding site leading to dysregulation of gene transcription.

Cause of Cancer: Tumor Initiation, Promotion&Progression

⌘ Cancer is a multistage process
- Rous- found that papillomas in rabbits regressed after a period of time, but could be made to reappear if skin was stressed by punching holes in it or by applying irritants like turpentine or cholorform
- tumors could exist is latent (dormant) form and tumor induction vs tumor growth involved different mechanisms (i.e., **initiation** and **promotion**)

⌘ 1941- Berenblum found that mice that had a single skin painting of carcinogen (methylcholanthrene) only a few animals got papillomas, but if same area painted repeatedly with croton oil (not carcinogenic) almost all animals developed skin cancers

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Cause of Cancer: Tumor Initiation, Promotion&Progression

⌘ Complete carcinogen- one that has both initiation and promoting activity (vs incomplete)

⌘ Tumor promoting- is not carcinogenic but augments formation of cancers after initiation
- If promoting agent given before initiating agent then no carcinomas develop

⌘ Progression Stage- cell proliferation caused by promoting agents allows cellular damage done by initiator to be "fixed" and cells clonally expanded. Genetic instability is present and as cells grow there are more genetic alterations
(leads to increased growth, invasiveness & metastasis)

Cause of Cancer: Tumor Initiation, Promotion&Progression

⌘ Initiation can occur after a single, brief exposure to a potent initiating agent.
- Leads to transformation into a dormant tumor cell that appear within one mitotic cycle (1 day)
- initiation is irreversible
- change is fixed in genes and passed onto daughter cells

⌘ Promoting agent can be given up to a year later with a high % of tumors obtained
- promotion is a slow, prolonged phase that lasts throughout the latent period, and may be reversible or arrested by anti-carcinogenic agents

Cause of Cancer: Tumor Initiation, Promotion&Progression

⌘ Tumor promotion is a cell proliferation phase that propagates the initiated damage and leads to altered clone of cells. Most promoting agents are mitogens.

⌘ During clonal expansion the tumor losses growth control and escapes from host defenses

⌘ Tumor progression is thought to be irreversible because of the large amount of genetic changes that occur. Takes years, and development of cell heterogeneity occurs (invasive & metastatic potential and Ag specificity, resp to hormones/drugs, immune sys and state of differentiation)

Mechanisms of Tumor Initiation

⌘ Initiation can come about by either direct genotoxic (mutational) events or by epigenetic events that modulate gene expression without directly reacting with the base sequence of DNA

- Mutational Theory depends on 3 kinds of evidence:
  - **1-- Agents that damage DNA are frequently carcinogenic**
  - **2-- Most carcinogenic agents are mutagens (tested for in microorganisms- AMES test). 90% of all carcinogens are mutagenic (not all mutagens are carcinogenic)**
  - **3-- Incidence of cancer in patients with DNA repair deficiencies is increased**
    - **xeroderma pigmentosa/ ataxis telangiectasia/ Fanconi's anemia/ Bloom's syndrome**

Mechanisms of Tumor Initiation

⌘ Source of carcinogens:

- **environmental**
  - **PAHs, industrial pollution, pesticides, smoking, alcohol, etc....**
- **Spontaneous mutations- from inherent error rate in the fidelity of DNA replications and/or repair**
  - background mutation rate is ~1.2-1.4 X 10^{-10} mutations/base pair/cell division (one mistake in 10 billion base pairs per each cell division).
  - There are ~10^{14} cells in human, with a genome of ~2 X 10^9 base pairs, that undergo 10^{16} division cycles throughout life. 2.8 X 10^{15} single base (point) mutations arise in lifetime. If this occurs the 2.8 X 10^{15} spontaneously arising tumors could arise (too many).

Spontaneous Mutations & Cancer

⌘ **Why most mutations are not productive**

⌘ Most base changes are not in coding sequence

⌘ not all mutations produce a cancer cell (do not occur in oncogene or suppressor gene

⌘ more than one mutation necessary to produce a cancer cell (25-50% of human colonic cancers contained 9 or more mutations!)

Spontaneous Mutations & Cancer

⌘ At least 2 mutations necessary: therefore required mutation rate would be the square of 1.4 X 10^{-10} (i.e., this would give about 300,000 spontaneous cancer cells in a lifetime
  - ☑ Most of these die or are eliminated by immune system
  - ☑ If more than two mutations necessary then # of cells decreases more

⌘ Mechanisms of spontaneous mutations
  - ☑ depurination (wrong base added in by DNA poly)
  - ☑ deamination
  - ☑ oxygen radical damage
  - ☑ errors in DNA replication

Mechanisms of Tumor Promotion

⌘ Promotors appear to act by cell membrane interactions but also may act by direct DNA interaction (rare)
  - ☑ Phorbol esters (TPA= [1-O-tetradecanoyl-13-acetate]) most examined of Tumor Promotors
    - ☒ found in diet, cigarette smoke, and environment
    - ☒ produce a wide variety of biochemical changes in cell

    - ☒ Effects of TPA due to ability to activate a calcium-dependent protein kinase (protein kinase C, PKC)
      - is calcium dependent and phospholipid-dependent
      - cytosolic form of PKC inactive-->translocated to membrane and binds to receptor (DAG or TPA). TPA has long life and provides strong signal for proliferation
      - 9 different isozymes of PKC
      - serine-threonine kinase and stimulates AP-1 formation

Validity of Tests for Carcinogenicity

⌘ 60-90% of human cancers attributable to environmental causes and life style factors.
- Smoking, diet, UV exposure, sexual practices, parasitic and viral infections, industrial pollution, pesticides
- Therefore most cancers are preventable

⌘ Methods for detection of carcinogens
- rodents exposed to "maximum tolerated dose" (MTD) for life span of animal
  - expensive and time consuming (3-4 yrs)
  - doses are orders of magnitude higher than what humans would ever encounter (i.e., saccharine studies: must consume 25kg/day to reach levels of carcinogenicity)

Validity of Tests for Carcinogenicity

⌘ What is appropriate dose and time to use?
⌘ Is there a threshold below which is OK to use?

⌘ Ames Test for mutagenesis in bacteria *Salmonella* is widely used short term test:
- does not detect certain kinds of chemical carcinogens (i.e., those that do not bind to DNA)
- over predicts carcinogenicity for other

⌘ Agent can increase the incidence of cancer by:
- genotoxic mechanisms- damaging cells DNA
- nongenotixic- increase spontaneous genetic error to occur during proliferation

Validity of Tests for Carcinogenicity

⌘ In Ames test:
- 30-50% of both natural and synthetic chemicals are carcinogens, mutagens, teratogens and clastogens (DNA damaging agents)
- Irradiation Carcinogenesis:
- x-rays and UV (causes T--T dimers, and T--C dimers) radiation produce damage to DNA
- leads to repair processes which are error prone
- cell proliferation is required to "fix" initial damage into a heritable change and to then allow for clonal expansion to occur (fixation occurs after 1st division)

⌘ Repair- incision by endonucleases, excision of ss DNA strand containing pyrimidine dimers, replication of excised region (DNA polymerase) and ligation

Irradiation Carcinogenesis

⌘ When low doses of chemical carcinogens and x-rays are used together, these two types of agents act synergistically to produce malignant transformation

⌘ Heavy exposure to sunlight is directly related to skin cancer. UV irradiation is a **complete** carcinogen, but promotion by other factors may help

⌘ In **RADIATION** carcinogenesis, the damage to DNA (I.e, mutagenic and carcinogenic effect) is due to the generation of **FREE RADICALS**

Radiation-- Oxygen Radicals

⌘ X-rays and gamma rays have a low rate of energy transfer and penetrate deeply into tissue (in contrast, protons and α particles have a high energy transfer and generate more radical ions locally, and have low penetrations through tissue

⌘ Damage to DNA includes

  ▱ single and double stranded breaks

  ▱ point mutations- due to misrepair deletions

  ▱ chromosomal translocations

Free Radical Formation

⌘ H_2O ------> H_2O^+ + e^-

⌘ H_2O^+ + H_2O ------> H_3O^+ + OH^- (hydroxyl radical)

⌘ e^-_{ag} + H_2O -----> OH^- + H^+ (hydrogen radical)

⌘   *AGING AND CANCER*

⌘ O_2 ----> O^-_2 ----> H_2O_2 ----> $^*OH$ ---> H_2O

SOD

| Superoxide Anion | Hydrogen Peroxide | catalase | Hydroxide Radical |
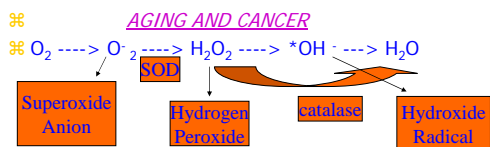
Genetic Events that follow DNA Damage

⌘ 1. Induction of early response genes (*c-jun*)

⌘ 2. Induction of later-response genes (TNF, PDGF)

⌘ 3. Activation of interleukin-1 and PKC

⌘ 4. Activation of oncogenes (*c-myc and K-ras*)

⌘ These may lead to events of carcinogenesis, but latent period may last as long as 7-10 years for leukemia, 10-15 years for bone, 27 years for brain, 20 for thyroid, 22 for breast, 25 for lung, 26 intestinal cancer, and 24 for skin cancer

DNA Repair mechanisms

⌘ 1. Abnormal precursor degradation- to prevent incorporation into DNA

⌘ 2. Visible light-activated photoreactivation repair- removal of UV induced pyrimidine dimers

⌘ 3. Strand break repair- DNA ligase, exonuclease, and polymerase activities

⌘ 4. Base excision repair- recognizes simple base alterations and corrects alteration

⌘ 5. Nucleotide excision repair- recognizes bulky DNA base adducts: (most used mechanism)

☒ pyrimidine dimers, and base cross-links

DNA repair

⌘ DNA damage recognition and repair complex contains proteins that function in concert with transcription factors and RNA polymerase

⌘ Actively transcribed genes are repaired more rapidly and completely than the overall genome repair of same cells

☒ actively transcribed protooncogenes and suppressor genes (subject to more damage) are repaired quickly

☒ Mutations may accumulate on non-transcribed strand and protooncogenes that are not active may not become obvious until they are transcribed (cancer latency??)

Viral Carcinogenesis

⌘ One way to determine whether virus caused cancer was to innoculate susceptible animal with filtered extracts (Rous in 1911 transmitted chicken leukemia from filtered tumor extracts)

⌘ Gross showed that mouse leukemia passed from parent to offspring. Had to use mice that were less than 48 hours old to successfully do this. Could not pass it to adult mice. (why??)

Viral Carcinogenesis

⌘ RNA oncoviruses classified by morphologic criteria:

▱ Type A= non-infectious, bud into intracellular membranes rather than through the plasma membrane, and stay within the cell. They have an active reverse transcriptase and exist as a **proviral** form in chromosomal DNA.

▱ Type B= have spikes on outer envelope, but from cells and are identified primarily in murine species to cause mammary tumors (MMTV)

▱ Type C= distributed among birds and mammals, induce leukemias, sarcomas

▱ Type D= RNA oncoviruses- retroviruses

Viral Carcinogenesis

⌘ Oncoviruses can be transmitted through germ line cells (vertical transmission= parent --> offspring) (horizontal transmission --> person to person)

⌘ Later in life exposure to chemicals, hormones, irradiation, chronic irritation (or other agents) trigger expression of oncogenes. Carrier animal may remain disease free during life, but transmit virus to offspring

Viral Carcinogenesis-Human

- Proof:
  - 1) epidemiologic data showing correlation between living in an area of endemic viral infection and type of cancer
  - 2) serological evidence of antibody titers to viral antigens in patients with specific cancer
  - 3) evidence for insertion of viral DNA into a cancer host's genome
  - 4) evidence for a specific chromosomal translocation
  - 5) data showing viral infection of cell in culture that gives transformation and ability of cells to produce tumors in nude mice
  - 6) development of cancers in transgenic mice produced by embryonic gene transfer of viral genes

Viral Carcinogenesis-Human

- Types of Virally induce Himan cancers:
  - Epstein-Barr (*c-myc* translocation chromo 8--> 14)
    - Burkitt's lymphoma
    - nasopharyngeal carcinoma
    - B-cell lymphomas
    - Hodgkin's lymphoma
  - Hepatitis (HBV)
    - hepatocellular carcinoma
      - point mutations on chromosome # 17 of p53 gene
  - Papillomavirus (HPV) [60 serotypes]
    - cervical cancer (HVP 16 and 18)
  - HTLV- retrovirus, RT, (exogenous DNA, not integrated)
    - human T cell leukemia
    - HIV: Kaposi's sarcoma (maybe herpes associated also??)

Oncogenes

- Provirus- viral genetic component part of host chromosome and passed onto daughter cells
  - must be a DNA virus or RNA virus must have RT
- Protovirus- genome of oncogenic viruses arose from normal cellular DNA that had been altered by exogeneous carcinogen or by altered viral excision processes
- Viral oncogenes are cellular protooncogenes (that are altered in at least one base such that they lose ability to be regulated)

Oncogenes

⌘ Many of the oncogenic viruses that arose from genetic recombination events, are replication-defective, and do not form complete viruses unless the cells are co-infected with a "helper" virus

⌘ The transforming oncogenic sequences are not necessary for virus replication, but provide selective advantage for host cell proliferation

Oncogenes Associated with Retroviruses

⌘ **Class I** - Growth Factors (autocrine or paracrine)
  ◻ _sis_ , gp28, PDGF-like GF, secreted, growth factor
⌘ **Class II** - Receptor & non-receptor Tyrosine Kinase
  ◻ _src_, pp60, tyrosine kinase, plasma membrane
⌘ **Class III** - Membrane-associated G proteins
  ◻ _H-ras & K-ras_, p21, mutant GTP binding protein, plasma membrane (constitutively active in GTP binding state)
⌘ **Class IV** - Protein-serine/threonine kinases
  ◻ _raf_, p90, cytoplasm, signaling serine PK
  ◻ _mos_, p37, cytoplasm, cytoplasmic factor PK

Oncogenes Associated with Retroviruses

⌘ **Class V** - Cytoplasmic Regulators
  ◻ _crk_, p47, cytoplasm, SH2/SH3 (sarc homology regions), domain signaling protein
⌘ **Class VI** - Nuclear transcription factors (DNA binding)
  ◻ _myc_, p47, nucleus, sequence-specific transactivator (basic region/helix-loop-helix)
  ◻ _fos_, p55, nucleus, sequence transactivator with c-jun (AP-1), leucine zipper
  ◻ _jun_- p65, nucleus, sequence transactivator with c-fos (AP-1), leucine zipper

Characteristics of Oncogenic DNA and RNA Tumor Viruses

- ⌘ Productive infection with cell lysis
- ⌘ Abortive infection leads to cell transformation with little or no virus production
- ⌘ Transformation is inefficient (10^6-10^7 virus particles/transformation)
- ⌘ Some induce cellular DNA synthesis

- ⌘ Productive infection leads to transformation, no cell lysis
- ⌘ Abortive infection leads to transformation (no virus)
- ⌘ Transformation efficient (10^2-10^4 virus particles/transformation)
- ⌘ Induce cellular DNA synthesis

Activation of Oncogenes

- ⌘ By mechanisms previously described:
  - ⊠ point mutations
  - ⊠ gene rearrangement
  - ⊠ gene amplification
  - ⊠ increased transcription due to alterations in packaging
  - ⊠ insertion of retrovirus enhancer regions (LTRs) next to c-onc genes

Specific Oncogene Functions

- ⌘ *Ras*- members are *Hi-ras*, *Ki-ras* and *N-ras*
  - ⊠ tumorigenic *ras* genes differ from normal cellular counterparts by having a single point mutation at codon 12, 13, 61 or 117, or by alternate mRNA splicing
  - ⊠ different mutagens cause different point mutations and this gives distinct events in initiation phases, and in the promotion-progression phases of skin cancer
  - ⊠ Normal function of p21 *ras* is to interact with tyrosine kinase receptors to activate a signal transduction pathway. To do this p21 has to bind GTP (**on** signal) and then hydrolyze it (**off** signal)

Specific Oncogene Functions: Ras

- *Ras* p21 mutated at codons 12 or 61 has a structure that prevents the ability to bind to the GTPase activating protein (GAP), thereby keeping p21 in the GTP bound or activated mode.
- Evidence suggests that the action of a single oncogene in **INSUFFICIENT** to cause neoplastic growth of normal diploid cells. The action of at least two oncogenes is required
- *Ras* acts cooperatively with *myc*

Specific Oncogene Functions: Ras

- *Ras* is part of the signal transduction pathway for tyrosine kinase receptors: i.e., EGF, PDGF, FGF
- Ultimately, **MAP kinase** (mitogen activated protein kinase) is phosphorylated and activated and its substrates include the *jun/fos* AP-1 complex.
- This pathway is always turned "on" when *ras* is mutated, and leads to constitutive gene activation through AP-1

Specific Oncogene Functions: *Myc*

- *Myc* plays role in cell proliferation and differentiation.
- Expression of *c-myc* is higher in proliferating cells and falls as terminal differentiation occurs. Continued expression of *myc* blocks differentiation
- Rearrangement of *myc* by chromosomal translocation is of 1º importance (occurs between chromosomes 8-->14, 8-->2 and 8-->22: all involving Ig genes). Under control of Ig genes

Specific Oncogene Functions: Myc

⌘ c-Myc is a DNA binding, nucleophosphoprotein that is a transcription factor.

⌘ It has a <u>transcriptional activation domain, a DNA binding domain, a nuclear localization signal, a site for phosphorylation by a nuclear protein kinase, a helix-loop-helix motif, and a leucine-zipper motif</u> (may have to form a dimer to become active

⌘ *Max* dimerizes with *myc* and binds to concensus sequence CACGTC (*max* homodimers bind to this as well and competes with *max-myc* binding)

Specific Oncogene Functions: Myc

⌘ c-myc induced transformation results from high expression a a normal coding sequence rather than activation by a point mutation

⌘ myc induces ornithine decarboxylase, cyclin A, and cyclin E (all involved in cell proliferation)

⌘ Increased *c-myc* in some cell types (B lymphocytes) is associated with programmed cell death (apoptosis)

Specific Oncogene Functions: Src

⌘ Normal protein compared to transforming viral protein shows :

◻ a number of scattered single amino acid difference between residues 1-->514, but especially at Thr 338-> Ile: also truncations at carboxyl terminus regulate activity

◻ is a tyroxine kinase and activity enhanced by phosphatases (enzymes that de-phosphorylate)

◻ phosphorylation of tyrosine 527 in C-terminus of *Src* is inhibitory and dephosphorylation of this residue stimulates Scr kinase activity. In mutants, Tyr-527 is either missing or underphosphorylated (i.e., active)

Specific Oncogene Functions: bcl-2

- *bcl-2* activated by a chromosomal translocation in non-Hodgkin's B cell lymphomas *(called bcl because it was found in B-cell lymphomas)*
  - puts bcl-2 gene from chromosome 18q21 with the Ig heavy chain locus on chromosome 14q32 resulting in increased expression of bcl-2 when Ig gene is active (under influence of IGH enhancer gene [IGH= immunoglobulin heavy chain])
  - enhances lymphocyte survival by inhibiting apoptosis in some cells, but not all
    - produces two proteins by alternative splicing of mRNA
      - Bcl2-α and Bcl2-β

DNA Tumor Viruses

- 3 main groups:
  - papovaviruses- human wart virus, SV40, polyoma
  - adenoviruses- tumorigenic in new born animals
  - herpesviruses- Epstein-Barr, Leukemogenic virus (Marek's disease in chickens)
- SV40- found in resus monkey kidney cells. No human disease, no cancer
  - DNA enters nucleus and is transcribed into "early" and "late" mRNAs

DNA Tumor Viruses : SV40

- Transcription of early genes is required for synthesis of viral proteins that are involved in replication of SV40 DNA.
  - Also contain information for transformation and code for the intra-nuclear T antigen.
  - Late mRNA transcribed after DNA replication and codes for the viral structural proteins.
  - Viral DNA integrated into host cell genome
  - Transformed cells do not make "late" mRNA nor do they produce viral proteins, but they do make T antigen.

DNA Tumor Viruses : SV40

- ⌘ T antigen required for initiation of viral DNA synthesis, for induction of host cell DNA synthesis, and for establishment and maintenance of transformed state.
- ⌘ Large and small T antigens. Coded for by same gene (A gene) and have same 5′ and 3′ ends. Differential splicing accounts for two products with small T missing a portion of A gene transcript
- ⌘ Large T necessary for transformation, inducing a number of host genes: thymidine kinase, rRNA genes

DNA Tumor Viruses : Papilloma

- ⌘ Induce benign epithelial tumors in some animal species, including humans: warts (skin, anal and genital, and subtype is associated with cervical cancer).
  - ▣ Subtypes 16, 18, 31 and 33 considered high risk
  - ▣ produce oncoproteins E6 and E7: can immortalize cells after numerous passages
  - ▣ viral genomes integrated into host cell DNA
  - ▣ low % of women infected with HPV develop cervical cancer, but high % of cervical cancer patients are + for HPV.

DNA Tumor Viruses : Papilloma

- ⌘ Series of progressive events involving genetic instability of cells transformed by high risk HPVs
  - ▣ association of E6 and E7 with the tumor suppressor protein p53
  - ▣ high risk HPV-16 and -18 E6 proteins bind to p53 and cause its degradation by a ubiquitin-mediated process
  - ▣ expression of both E6 and E7 necessary for efficient progression to cancer. E7 binds to and inactivates another tumor suppressor protein RB

DNA Tumor Viruses : Adenovirus

- Adenovirus Type 12 produces tumors in new-born hamsters (31 serotypes and -12, -18 and -31 highly oncogenic in new born rodents)
  - at least part of adenovirus genome becomes integrated into host DNA and production of virus induce nuclear T antigen required for transformation
  - Adenovirus E1 gene codes for E1A and E1B proteins responsible for oncogenic potential of virus
    - Expression of E1A alone can immortalize rodent cell cultures, but coexpression of E1B is necessary for complete transformation
    - E1A binds to and inactivates RB, and also stimulates DNA synthesis, and production of epithelial cell growth factor
    - E1B complexes with and disrupts action of p53

DNA Tumor Viruses : Herpes

- 5 types of Herpes viruses infect humans:
  - HSV-1 (Herpes simplex), HSV-2, HZV (herpes zoster), cytomegalovirus (CMV), and Epstein-Barr virus(EBV)
  - EBV can immortalize B lymphocytes in culture and express a variety of nuclear antigens (EBNA 1-6)
    - EBNA-2 involved in immortalization of B cells: is a transcription factor to enhance the expression of several viral and host genes (c-myc enhanced)
    - EBNA-2 blocks the anti-proliferative effect of α-IFN on B cells (IFNs may act as tumor suppressor factors for B cells)
  - Presence of viral genome in cell may not be necessary to maintain transformed state "hit and run" hypothesis