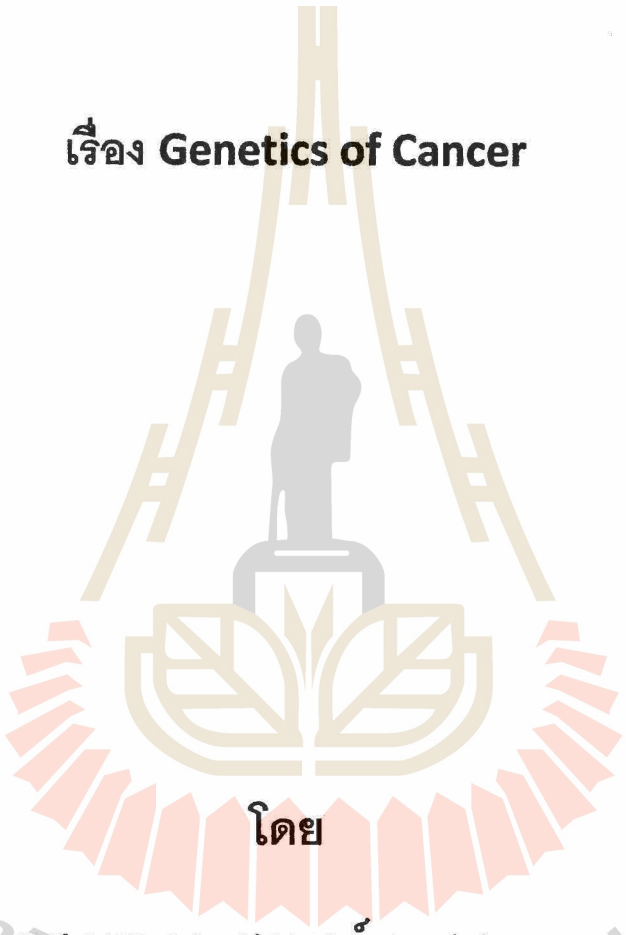
เอกสารวิชาการ

เรื่อง Genetics of Cancer



โดย

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มหาวิทยาลัยเทคโนโลยีสุรนารี

OTOP 58

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Genetics of Cancer

A. The molecular basis of cancer

The fundamental principles of malignant transformation include;

1. Nonlethal genetic damages lie at the heart of carcinogenesis.
2. A tumor is formed by clonal expansion of a single precursor cell that has incurred genetic damage. This concept is supported by the study of X-linked isozyme cell marker as illustrated in 1. The picture shows that the tumor cells of women are composed of cells, which produces only one type of X-linked isoenzyme;Glucose-6-phosphate dehydrogenase. Normally the cells of female would consist of two populations of cells with different X-linked isozymes. This finding supports the notion that tumor cells originate from only one initiated cell.

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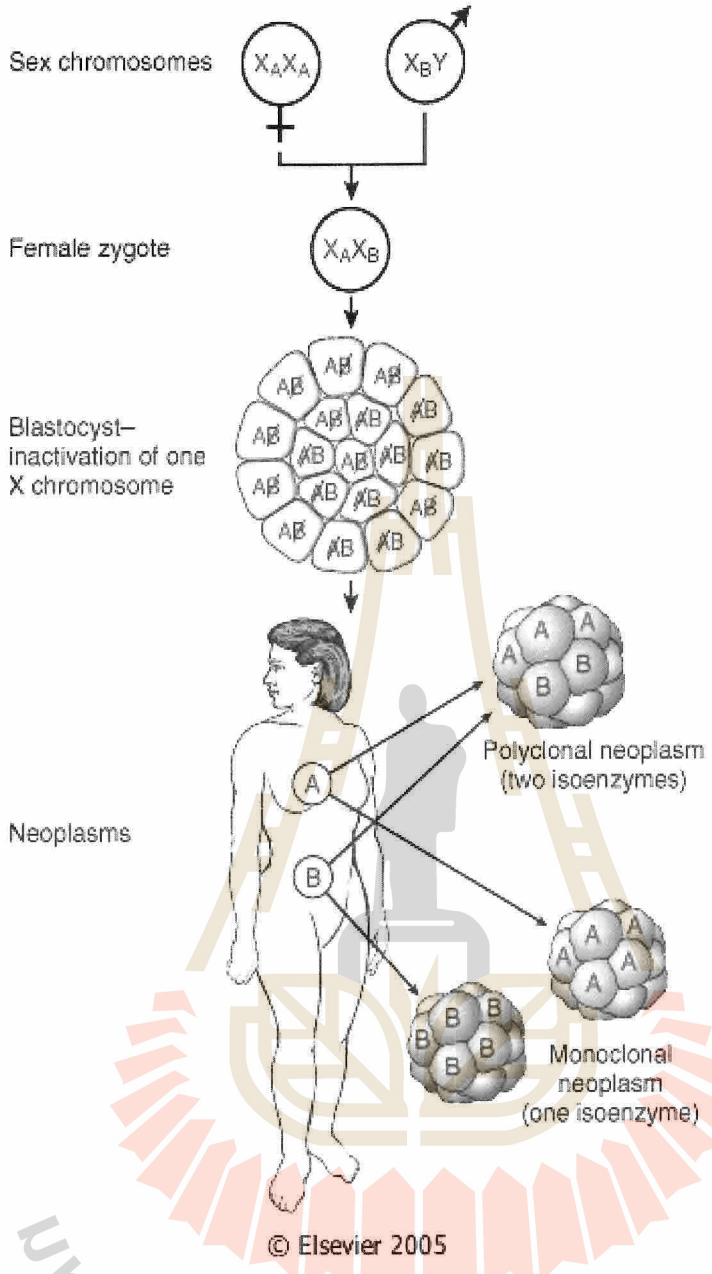

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Figure 1.1 The X-linked isozyme cell marker as evidence of the monoclonality of the cancer cells. (Source: *Pathologic Basis of the Disease 8th ed, Kumar V, Abbas AK, Fausto N. 2010*)

3. Four classes of normal regulatory genes are the targets for genetic damage
   - Growth promoting proto-oncogenes
   - Growth inhibiting tumor suppressor gene
   - Gene that regulates programmed cell death (apoptosis)
   - Genes involved in DNA repair
4. DNA repair genes affect cell proliferation and survival indirectly by influencing the organism to repair nonlethal genetic damage
   - Defect in DNA repair genes predispose to mutations and neoplastic transformation
   - Both alleles of DNA repair genes must be inactivated to induce the genomic instability

5. Carcinogenesis is the multistep at both genetic and phenotypic level
   Malignant phenotypes are acquired by a stepwise fashion called
   "tumor progression"
   (Malignant phenotypes: Excessive growth, local invasion, metastasis)
   - These changes result from the accumulation of genetic lesions

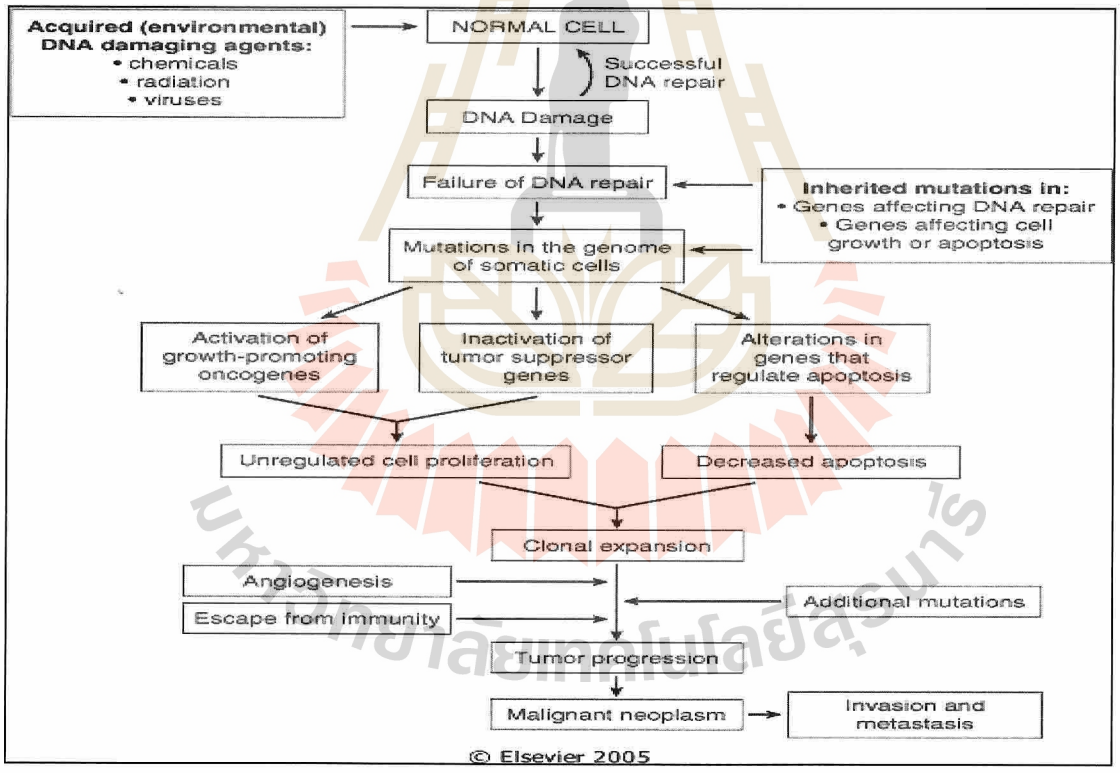

Figure 1.2 Simplified scheme of the molecular basis of cancer (Source; *Pathologic Basis of the Disease 8th ed, Kumar V, Abbas AK, Fausto N. 2010)*

Physiological alterations of malignant cells

Fundamental changes in cell physiology of cancer cells are;

1. Self sufficient in growth signal
   - Ability to proliferate without external stimuli
   - Results from oncogene activation
2. Insensitivity to growth inhibitory signal
   - Tumor cells not response to normal inhibitor of cell proliferation: TGF-β and CDK inhibitor
3. Evasion of apoptosis
   - From inactivation of *p53* or other genes
4. Defect in DNA repair
   - Tumor cells fail to repair DNA damage cause by mutagen
5. Limitless replicative potential
   - Associated with maintenance of telomer length and function
6. Sustained angiogenesis
   - Induce by VEGF
7. Ability to invade and metastasis
   - From intrinsic genetic changes or external factors

Normal cell cycle (review)

1. Cell cycle is controlled by cyclin and CDKs (cyclin-dependent kinase)
2. CDKs are present in inactive form throughout the cell cycle
3. Cyclins are synthesized at a specific time during cell cycle-> activate the CD
4. Phosphorylaton of Cyclin-CDK complex directly drives the cell cycle progression

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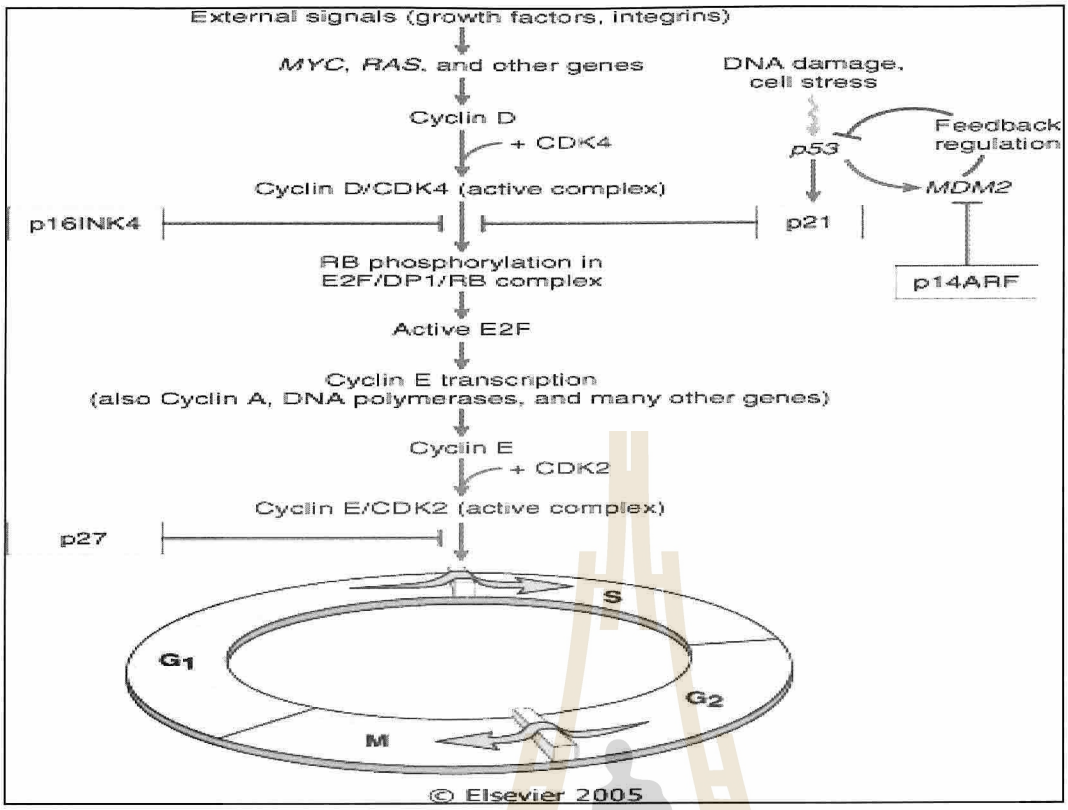

Figure 1.3 Normal Cell Cycle. (Source: *Pathologic Basis of the Disease 8th ed, Kumar V, Abbas AK, Fausto N. 2010*)

<u>Molecular basis of multistage carcinogenesis</u>

The tumor development and progression can be divided into 3 stages.

Initiation. The key alterations include;

1. Irreversible with memory
2. Required fixative by cell division
3. Genotoxic agent: Ionizing radiation
4. Depend upon xenobiotic metabolizing capacity of the cell

Promotion. The key alterations include;

1. Provide growth advantage for the initiated cell
2. Reversible
3. Epigenetic alteration
4. Modulated by variety of environmental factors: age , diet, hormonal status, etc.
5. Promoted lesions are seen microscopically and/or grossly

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Progression. The key alterations include;

1. Irreversible
2. Growth of altered cells responsive to environmetal factor
3. Discernable alterations in the cell genome
4. Evolving chromosomal abnormalities
5. Benign or malignant tumors seen

Critical genetic alterations in multistage carcinogenesis

1. Activation of cellular proto-oncogene
2. Inactivation of tumor suppressor genes
3. Inactivation of putative metastatic tumor suppressor genes
4. Increasing the genomic instability; aneuploidy, DNA methylation etc.

Major genes involved in carcinogenesis

1 Oncogenes have the following characteristics;

- Genes involved in promoting cell proliferation
- Behave as dominant fashion (One allele can induce transformed phenotype)
- Normal version called proto-oncogene

2. Five broad classes of oncogenes

1. Secreted growth factor
2. Cell surface receptor
3. Component of intracellular signal transduction system
4. DNA-binding nuclear proteins; transcription factors
5. Component of the network of cyclin, cyclin-depenent kinases and kinase inhibitors

3. Mechanisms for oncogene activation

1. Gene amplification
2. Point mutation
3. Chromosome translocation
4. Transposition to an active chromatin domain

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6.2 Tumor suppressor gene

Functions

- Inhibit malignant transformation
- Both alleles have to be inactivated before changing in the behavior of the cells

"Recessive mode of action"

- Major classes; gene involved in

: Cell cycle check-point components

: Induction of apoptosis

: Maintain genome stability

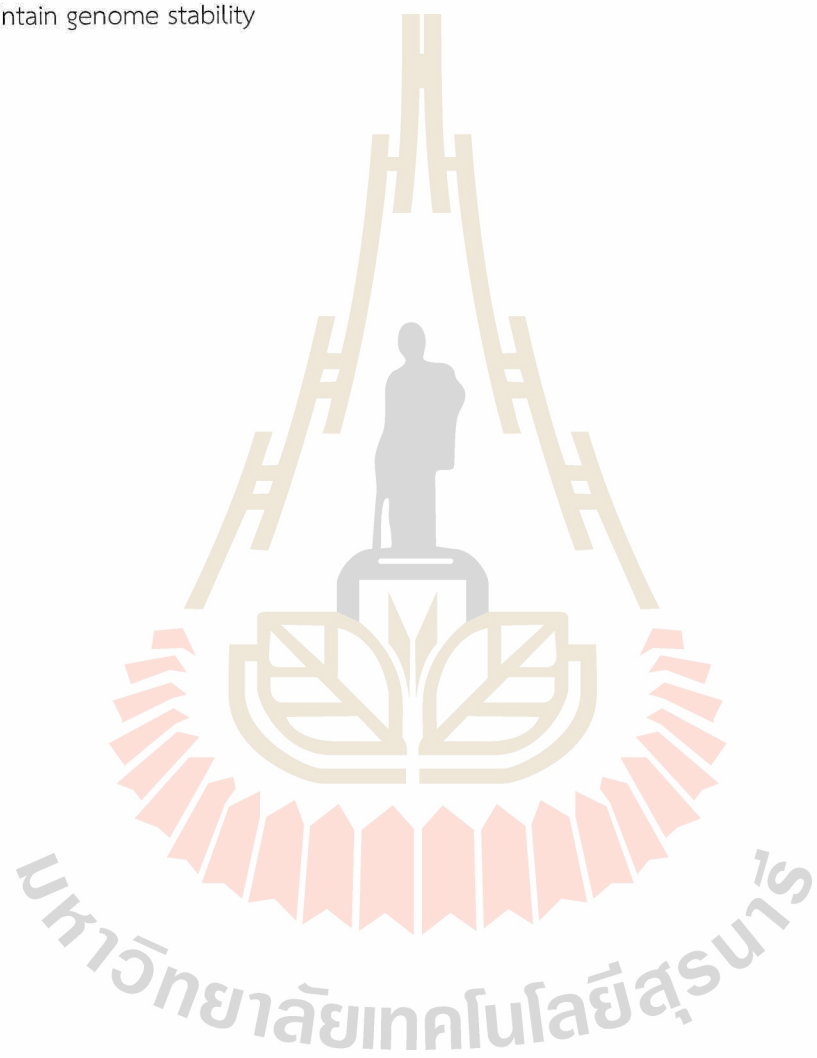

Table 1.1 Selected tumor suppressor genes in human cancer (Source: *Dechsukhum C.*)

Selected tumor suppressor genes in human cancer

Subcellular location	Gene	Function	Associated tumor
Cell surface	TGF-β receptor	Growth inhibition	Colon
	E-cadherin	Cell adhesion	Stomach, Breast
Cytosol	APC	Inhibiyion of Signal transduction	Stomuch Colon, Pancreas
Nucleus	*Rb*	Cell cycle control	Retinoblastoma Osteosarcoma Breast, Colon
	p-53	Cell cycle control, Apoptosis regulation	Most cancers
	WT_1	Nuclear trancription factor	Wilms' tumor
	BRCA-1	DNA repair	Breast, Ovary
	BRCA-2	DNA repair	Male and female Breast

Biology of Tumor Growth

There are 4 phases of tumor progression

- Transformation
- Growth of the transformed cells
- Local invasion
- Distant metastasis

The factors influence the tumor cell growth

- Kinetic of tumor cell growth
- Tumor angiogenesis
- Tumor progression and heterogeneity

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Factors that determine the tumor cell proliferation

- Doubling time of the tumor cells
- Growth fraction of the tumor cells
- Rate of cells which are lost

Doubling time of the tumor cells

- Tumor have equal of longer doubling time than normal cell
- Tumor cells are easily trigged to enter the cell cycle than normal cells as the cell cycle check point proteins are deranged

Growth Fraction

- In early stage -> most tumor cells are in the proliferative pool
- Later stage -> tumor cell exit the proliferative pool by
  Shedding
  Lack of nutrient
  Differentiation
  Reversion to Go
- Late stage -> Most tumor cells are in Go stage
- In the clinical detectable tumor-> Most tumor cells are in the non-proliferative pool
- In rapidly growing tumor-> Growth fraction =20%

Determination of Rate of Tumor Cell Growth

Rate of Tumor Cell Growth is determined by the excess of cell production over cell loss

Concepts in tumor cell kinetic

- The rate of growth depends on the growth fraction and degree of imbalance between cell proliferation and cell losses
- Growth fraction of the tumor cells has a potential effect on their susceptibility to chemotherapy

Tumor Angiogenesis

- Neovascularization is required for tumor growth beyond 1-2 mm. Distance
- Effect of neovascularization on tumor growth
  - Supply nutrient and oxygen
  - Endothelium produces mitotic growth factors that stimulate tumor cell proliferation; IGF, PDGF, GM-CSF, IL-1
- The tumor induces neovascularization by production of 2 major angiogenic growth factors
  - VEGF

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- bFGF

- Angiogenesis if controlled by antiangiogenetic agents secreted by tumor cells
  - Angiostatin
  - Endostatin
  - Vasculostatin
- P53 controls the angiogenesis by stimulation the production of antiangiogenic factor Thrombospondin

Tumor Progression and Heterogeneity

8.1 General Features

- Tumor progression is the multistages process in which tumor increase their malignant properties including
  - Accelerated growth
  - Invasiveness
- Ability to form distant metastasis is the result of tumor heterogeneity which occurs during tumor progression
- Multiple accumulated mutations are the underlying molecular changes of this process
- Defects in caretaker genes or DNA repair genes are the major causes of genetic instability in tumor cells which make the cell prone to multiple mutational events
- The aggressive tumor clones are selected by immunogenic or non-immunogenic pressure
- The tumor cells are less antigenic and require less growth factors to grow

8.2 Mechanism of Invasion and Metastasis

- Metastatic property of tumor cells require multiple characteristics of the tumor cells
- There are two phases of metastasis
  - Invasion of the extracellular matrix
  - Vascular dissemination and homing of the tumor cells

Invasion of the extracellular matrix

Major steps are required for tumor movement to the vascular compartments

- Detachment of the tumor cells from each other
- Attachment to the matrix component
- Degradation of extracellular matrix
- Migration of the tumor cells

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Detachment of tumor cells from each others

- Down-regulation of E-cadherin or mutations in catenin
- Attachment of matrix components, Tumor cells express receptors for (esp. Integrins)
- Laminin
- Fibronectin

Degradation of extracellular matrix

Certained types of proteases are secreted by tumor cells to degrade the extracellular matrix

- Serine protease
- Cysteine (Cathepsin D) protease
- Matrix metalloproteases (MMP9,MMP2); type IV collagenase

Tumor cell migration

- There are two groups of proteins which drive tumor cell migration
- Tumor cell derived motility factors
- Cleavage products of matrix components; collagen or laminin

Vascular dissemination and homing of the tumor cells

- Tumor cells aggregate with themselves or with platelets in the circulation
- Receptor on the tumor cells partially indicates the site of metastasis
- Chemoattractants recruit the tumor cells to the target sites (IGF-1, 2)
- Inhibitors of the proteases secreted by the target organs make them unresponsive to the tumor growth

8.3 Molecular genetic of metastasis

- Alteration in multiple genes is required for tumor cells to have the metastatic property
- One or few genes are enough to suppress the tumor metastasis
- Metastatic-suppressor genes are:

  NM23 in mouse tumor model

  KAI-1 in prostate cancer

  Kiss gene in melanoma

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9. Clinical features of tumors

9.1 Scope of study

- Effect of a tumor on the host
- The grading and clinical staging of tumor
- The laboratory diagnosis of neoplasms

9.2 Effect of tumor on host

The clinical effects on the host are the result of

- Location and impingement on adjacent structure
- Functional activity such as hormone synthesis
- Bleeding and secondary infections when they ulcerate through the adjacent natural surfaces
- Rupture or infarction of tumor

9.2.1 Local and hormonal effect

- Endocrinopathy induced by the endocrine or nonendocrine tumor
  Islet cell tumor -> hypoglycemia
- Primary or secondary tumor to the endocrine organs -> endocrinopathy
- Metastatic tumor to the pituitary gland -> severe hormonal insufficiency
- Ulcer , bleeding or infarction from rapid growing of tumor
- Melena or hematemesis in tumor of intestine

9.2.2 Cancer cachexia

- Is the wasting syndrome characterized by Loss of body fat and lean body mass
- Profound weakness from anorexia and anemia
- Caused by the soluable cytokines from tumor or host cells in response to the tumor
- Increased metabolic rate and decreased food intake
- Increased metabolism of both muscle and fat tissue
- Is the effect of some cytokines produced by the tumor cells or macrophages; TNF-α, IL-1, IFN-γ

9.2.3 Paraneoplastic syndrome

- Is the symptom complex of cancer bearing patients
- Characteristic of paraneoplastic syndrome
- Is not explained by local or distance spread of tumor

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- Is not related to the elaboration of hormone indigenous to the tissue from which the tumor arises
- May cause significant clinical problem or mortality of the patients
- May mimic metastatic disease which confounds the treatment

The clinical significance of the paraneoplastic syndrome

- May represent the earliest manifestation of an occult cancer

The common paraneoplastic syndromes

Endocrinopathy

- Due to ectopic hormone production
- Lung carcinoma induces > 50% of Cushing's syndrome by ACTH production
- Hypercalcemia
- Caused by the production of parathyroid-related hormone (PTHRH)
- Caused by cytokines; IL-1, TGF-α, TNF or dihydroxy vitamin D
- Commonly found in carcinoma of breast, lung, kidney and ovary

Neuromyopathy paraneoplastic syndrome

- Due to the immune response to the normal neuronal cell as a result of tumor antigen stimulation
- Various clinical spectrum
- polymyopathy, polyneuropathy, cortical cerebellar degeneration and a myastenic syndrome

Acanthosis nigricans

- Gray-black patch of verrucous hyperkeratosis of skin
- Is associated with some forms of malignancy in 50% of patients over 40 year-old

Hypertrophic osteopathy

- Found in 10% of bronchogenic carcinoma
- Characterized by

  Periosteal new bone formation at the distal end of metacarpal or metatarsal bones and proximal phalanges

  Arthritis of adjacent joint

  Clubbing of fingers

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Vascular and hematologic manifestation

- Disseminated intravascular coagulation (DIC): commonly found in acute promyelocytic leukemia and prostatic adenocarcinoma
- Non-bacterial endocarditis: found in advanced mucin producing adenocarcinoma

Grading and staging of cancer

- Is the parameter of cancer used to compare between different treatment of cancer
- Used to stratify the tumor into different comparable groups according to
- Degree of differentiation -> grade
- Extend of spread of cancer within a patients -> stage

Grading of tumor

- Is based on the degree of differentiation and mitosis of the tumor cells
- Presumed to be c rrelated with the patient aggressiveness
- Indicates the degr e of differentiation of the tumor cell -> how closed the tumor resemble the norr al counterpart
- Vary from I-IV

Staging of cancer is depended on

- The size of primary tumor
- The extend of spread to the regional lymph node
- The presence or absence of blood-borne metastasis

There are two systems currently used

- TMN system
- AJC system

TNM system

T = Tumor size vary from T0-T4 (T0 -> in situ cancer)

N = Lymph node metastasis N1-N3 denotes involvement of the increasing number of lymph node and the range of nodes

M = Metastasis

M0 = No metastasis,

M1 and M2 = Presence of metastasis

AJC proposed by the American Joint Committee

Incorporate within each stage the size of primary tumor, lymph node

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involvement and the distance metastasis vary from I-IV

Laboratory diagnosis of cancer

Histologic and cytologic method

- Both histologic examination and clinical data are important for the accurate diagnosis of cancer
- Radiation effect can mimic the cancerous process etc.
- Is limited by the tissue sampling which has to be adequate, representative and well-preserved

Several sampling methods are currently used

- Excisional biopsy
- Needle aspiration
- Cytologic smear
- Frozen study is the quick and (intraoperative diagnosis of cancer -> highly accurate)
- Inadequate sampling and other pathologic change may cause inaccurate diagnosis

Fine-needle aspiration

- The specimens are obtained by aspirating the cells and attendant fluid with a small bore needle followed by cytologic examination of smear slides
- Is widely used for the assessment of the palpable lesions such as breast, thyroid, lymph node and prostate or other deep-seated lesions in the assistance of imaging technique
- Less invasive and easily performed
- Extremely reliable and useful technique

Cytologic examination or pap smear

- Widely used in the detection of the early cancer of cervix
- Can be used to screen the other carcinoma such as endometrium, bronchogenic, uninary bladder, prostate and gastric carcinoma
- Detection of tumor cells in body fluid: abdominal, pleural, joint and cerebrospinal fluid (CSF)
- Diagnosis is based on the morphology of individual tumor cell or a group of cells

Immunohistochemical study

- To demonstrate the tumor cell products or surface markers
- Some application include
    - Categorization of undifferntiated malignant tumor by using antibody to the

intermediate filaments

Keratin -> epithelial cell origin

Actin -> muscle cell origin

- Categorization of leukemia and lymphoma

  To detect the T or B cell origin of tumor cells

- Some application includes

  - Determination of origin of metastatic tumor By detection of the tumor specific protein

    PSA = prostate cancer origin

    Thyroglobulin = thyroid gland origin

  - Hormone receptor in breast cancer: Estrogen and progesterone

  - c-erbB2 expression in breast cancer -> poor prognosis

Molecular diagnosis

Have several clinical applications in cancer management

- To diagnose the malignant neoplasms such as hematopoietic malignancy
- Detection of tumor specific translocat on by PCR of cytogenetic methods:

  t(9;22) in chronic myeloid leukemia

  t(11;22) in Ewing's sarcoma
- For using as a prognostic indicator of cancer

  N-myc amplification in neuropblastoma -> poor-prognosis

Detection of minimal residual disease

- *RT-PCR to detect the chimeric transcript in the patients*
- bcr-c-ble transcript in chronic myeloid leukemia
- k-ras mutation in colon cancer

Diagnosis of hereditary predisposition to the cancer

Detect the germ-line mutations of the tumor suppressor genes in the unaffcted individual of the family for the risk assessment of the cancer risk

- BRCA-1 and BCRA-2 in breast cancer family
- RET gene mutations in endocrine tumor syndrome

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Flow cytometry

- Used to quantitatively measure the surface antigen and DNA content in the tumor cells for the diagnosis and prognosis indication
- Surface antigen in leukemia and lymphoma cell -> used to classify the tumor types
- Chromosomal DNA content -> indicates the prognosis of the patients
- Aneuploidy -> poor prognosis

Tumor markers

- Are biological indicator of the presence of tumor
- Cell surface antigen, cytoplasmic protein, enzyme and hormone
- Can be detected in plasma of other body fluid
- Mainly used to support the diagnosis of the tumor in conjunction with other standard methods or for the prognosis indication

Major tumor markers

Carcinoembryonic antigen (CEA)

- Normally produced by the cell of gut, liver and pancreas of the fetus
- Can be detected in

  60-90% of colon cancer

  50-80% of pancreatic cancer

  25-50% of gastric cancer

- Can be found in benign conditions: alcoholic cirrhosis, hepatitis, Crohn disease and ulcerative colitis
- Lacks sensitivity and specificity to detect the early cancer
- Clinical value is to detect the recurrence of cancer
- CEA detected 6 wks after tumor resection of colon cancer indicates the recurrence

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Alphafetoprotein (AFP)

- A glycoprotein normally synthesized in the yolk sac, liver and gastrointestinal tract of the fetus
- Usually detectable in the liver and germ line tumor the testis
- Can also be found in other benign conditions: cirrhosis, toxic liver disease, hepatitis and pregnancy
- High level of AFP is useful in detection of hepatocellular cancinoma and germ cell tumor of the testis
- Used to detect the recurrence of the liver and germ cell tumor of the testis

Cancer Epidemiology

- Provides the valuable data to the cause of human cancer
- Indicate the environmental and racial and cultural influences on the cancer risk

Cancer incidence

- Indicate the individual likelihood of cancer development
- American have 1/5 chance to die from cancer as reflex by the overall 23% mortality rate of cancer
- Change of incidence over the period of time indicate the change in cancer causing factor
- Age-adjusted incidence rate has changed over the years
- In the last 50 year -> mortality rate increase in men and slightly decrease in women
- The change in the incidence and mortality rate likely attributed to the change in environmental factor, life style and the cancer treatment modality

Geographic and environmental factor

- Variation in cancer incidence between races reflects the role of both environmental and genetic factor in cancer development
- Japanese have 7-8 time higher mortality rate of gastric cancer than do American but 2 times less in death rate due to lung cancer
- Immigration study indicates the more influence of environmental factor than genetic factor
- Japanese immigrants in America have the closed cancer incidence to the American

Major environmental factor for cancer

- UV, dietary carcinogen
- Overweight
- Alcohol abuse
- Increase risk of oropharynx, larynx and esophagus and liver from induction of cirrhosis
- Smoking is associated with
- 77% of lung cancer among men and 43% among women
- Oral, pharynx, larynx, esophagus and pancreas and urinary bladder cancer
- HPV infection is associated with cervical carcinoma

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- Age

  Is the important factor in cancer development

  Higher rate of cancer incidence in

  older age group > 55-74 years

  young age group -> 10% of overall mortality

- 60% are caused by leukemia and tumor of nervous system

Hereditary

- Has important role in cancer development
- Hereditary forms of cancer can be divided into 3 catagories

  a. Inherited cancer syndrome

  b. Familial cancer

  c. Autosomal recessive syndrome of defective DNA repair

a. Inherited cancer syndrome

- Caused by mutation of a single gene
- Affected individual increases risk of development of specific type of tumor
- Autosomal dominant trait of inheritance
- *Retinoblastoma due to the Rb gene mutation (10,000 higher risk in cancer development*
- *Familial polyposis coli from APC gene mutation -> 100% will develop colon cancer at the age of 50*
- Predisposition to the specific type of cancer
  - *MEN syndrome 2 -> increase risk for thyroid, adrenal, parathyroid carcinoma*
- Is associated with marker phenotype
  - *Colon cancer is associated with the benign tumor of the large intestine (adenomatous polyp)*
- Can show the complete or incomplete penetrance

b. Familial cancer

- Charaterized by young-age onset cancer, found in 2 or more cancer cases in the relatives of the index cases
- Not related to the inherited gene mutation
- Multiple of bilateral cancer
- Dominant of multifactoral mode of expression

a. Autosomal recessive syndrome of defective DNA repair

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- Characterize by chromosome or DNA instability
- Xeroderma pigmentosum for skin cancer

Other mode of genetic predisposition

Caused by the genetic polymorphism of the metabolizing enzymes: P450 in carcinogen detoxification

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