CHAPTER

# 4 The Classification of Tumors

Malignancy is considered multiple diseases, with a total of 130 different types of cancer affecting humans. The number escalates to 1170 if subtypes or variants of cancer are included. For this reason, classification of tumors into distinctive clinicopathologic groups is essentially needed in order to guide an effective therapy and predict prognosis. The observed diversity of human tumors is explained by recent concepts of oncogenesis which consider cancer to arise from stem cells, not from adult cells, hence, a preliminary discussion of the origin, types and biology of stem cells is necessary.

## CANCER STEM CELLS

The zygote, formed by the union of sperm and ovum, is the original source of stem cells. It is totipotent since it can give rise to all embryonic and extraembryonic cell types. First, it gives rise to the three types of stem cell: trophoblast, germ cells and embryonic stem cells (Fig 4-1). Germ cells migrate to the gonads, in association with endoderm, where it provide the gametes that will import totipotency to the zygote of the next generation. Embryonic stem cells (pluripotent), located in the inner cell mass of blastocyst, will give rise to all cell types of the embryo proper. They ultimately differentiate to adult stem cells (multipotent) of the three embryonic cell layers that can give rise to a subset of cell lineages. Adult stem cells differentiate to progenitor cells which lack self renewal and have restricted capacity of differentiation (unipotent), contributing only to one mature cell type which undergoes terminal differentiation and ultimately dies by apoptosis (Fig 4-2).

Cancer stem cell (CSC) arises through mutation of an adult stem cell. Cancer stem cells have various biologic potentials which can explain the observed phenotypic diversity of tumors. Thus CSC can change from one cell type to another, either native to the part or foreign (plasticity). These phenomena are observed in tumor metaplasia. Multilineage differentiation of CSC will give rise to biphasic or mixed tumors. Cancer stem cells are also capable of changing from a well-differentiated state to a poorly-differentiated anaplastic phenotype (dedifferentiation), thus

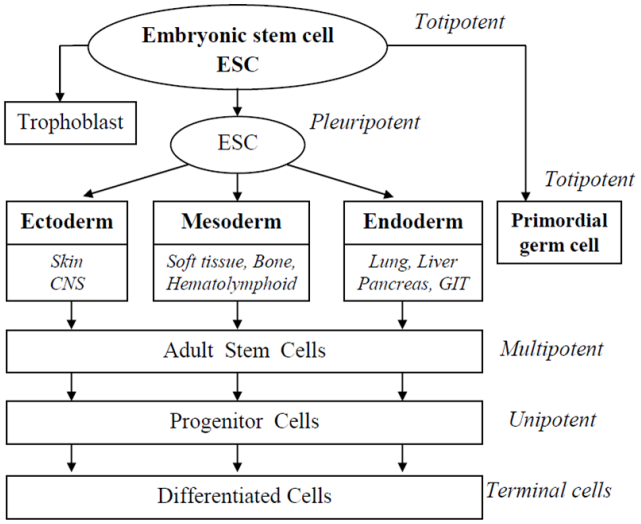


***Fig 4-1*** *The origin and differentiation potentials of stem cells. Embryonic stem cells give rise to extra -embryonic trophoblast, pleuripotent somatic stem cells and germ cells that migrate to the gonads.*

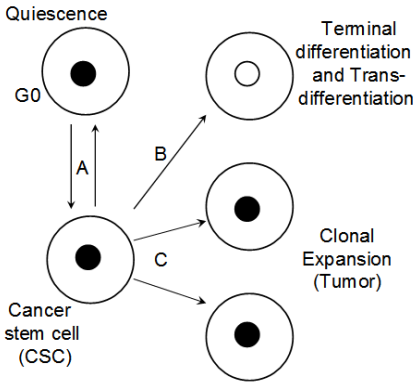


***Fig 4-2*** *Fates of cancer stem cells. (A) It enters a dormant nondividing state, (B) undergoes terminal differentiation or (C) symmetrical division resulting in clonal expansion and active growth of tumor.*

explaining the phenomenon of cancer progression with time. Moreover, CSC can radically change their growth kinetics from the usual terminal differentiation pathway (Fig 4-2). Thus, they can undergo active proliferation through symmetrical cell division resulting in clonal expansion of cancer. Conversely, they may undergo quiescence (enter Go) as observed in occult or dormant metastases, as well as, spontaneous regression of cancer. Immunohistochemical markers can identify cancer stem cells in some human cancers. Examples are: CD34 in leukemias, CD133 in brain and colon, CD44 in brain and breast cancers. Dental stem cells are CD105 positive.

## BASIS OF CLASSIFICATION

The modern classification of tumors is based on two parameters, namely: tumor cell differentiation (cell type) and its predicted biologic behavior. Cell type refers to the resemblance of tumors cells to adult or embryonic cells, and reflects lineage differentiation of stem cells, whereas, the behavior of tumor or clinical outcome is related to the proliferative and migratory potentials of cancer stem cells.

Morphology still remains the keystone in tumor diagnosis. However, in problematic cases, special ancillary techniques (electron microscopy and histochemistry) are resorted to. Electron microscopy can reveal special ultrastructural features in tumor cells which will confirm cancer phenotype (Table 4-1). Also histochemical methods can identify structural proteins, secretory products or enzymes which are diagnostic of some tumors (Table 4-2).

In recent years, immunohistochemistry has replaced histochemistry and electron microscopy as an ancillary diagnostic method (Fig 4-3 and 4-4, Table 4-2). In the following section, general aspects of immunohistochemistry is presented, but, details of their use in the diagnosis of cancer of different organs will be discussed in the special chapters.

**Table 4-1 Some Diagnostic Electron Microscopic (EM) Features of Tumors**

| EM Features | Tumor |
|---|---|
| Desmosomes, tonofilaments | Squamous cell carcinoma |
| Short microvilli | Adenocarcinoma |
| Very long microvilli | Mesothelioma |
| Small secretory granules (<100 nm) | Neuroendocrine tumors |
| Intermediate secretory granules (100-400 nm) | Endocrine pancreas |
| Large secretory granules (600-900 nm) | Acinic cell carcinoma |
| Premelanosomes and melanosomes | Melanoma |
| Thin filaments with focal densities | Myomatous tumors |
| Crystalline material | Myeloma, alveolar soft part sarcoma |
| Numerous mitochondria | Oncocytoma |
| Complex curved cell membrane | Meningioma and schwannoma |
| Birbeck granules | Langerhans cell histiocytosis |

**Table 4-2 Some Diagnostic Histochemical Methods of Malignant Tumors**

| Special Stain | Tumor |
|---|---|
| **Cytoplasmic Inclusions** | |
| Periodic acid-Shiff (PAS), neutral mucins | Gastric adenocarcinoma, erythroleukemia |
| Alcian blue (AB), PH2.5, acidic mucins | Colonic adenocarcinoma |
| PAS/AB, PH 2.5 | Intestinal metaplasia (Barrett) |
| PAS after diastase, glycogen | Ewing/PNET, rhabdomyosarcoma and renal cell carcinoma |
| Mayer mucicarmine (all mucins) | Adenocarcinoma |
| Hale's colloidal iron (acid mucins) | Chromophobe renal cell carcinoma |
| Oil red O (lipid) | Liposarcoma |
| Sudan black | Liposarcoma, myeloblastic AML |
| Toluidine blue | Mastocytoma |
| Masson argentaffin reaction | Melanoma, pheochromocytoma |
| Chromaffin reaction (zenker fixation) | Pheochromocytoma |
| Methyl green-pyronin | Plasma cell myeloma |
| **Enzymes** | |
| Myeloperoxidase | Myeloid leukemia (AML) |
| Leder chloroacetate esterase | Myeloid leukemia, mastocytoma |
| Nonspecific esterase (NSE) | Monocytic AML |
| Leukocyte alkaline phosphatase | Chronic myeloid leukemia (CML) |
| Lysozyme (muramidase) | CML and histiocytic tumors |
| Acid phosphatase | Prostatic carcinoma, hairy cell leukemia |
| Alkaline phosphatase | Osteosarcoma, seminoma |
| **Stromal structures** | |
| Masson trichrome | Fibrosarcoma, myosarcoma |
| Reticulin silver | Myelofibrosis |
| Verhoeff-van Gieson (elastic fibers) | Elastostofibroma |
| Hexamine silver (basement membrane) | Carcinomas |
| Mallory phosphotungstic acid hematoxylin (PTAH) | Gliomas |
| Congo red (amyloid) | Medullary thyroid carcinoma |

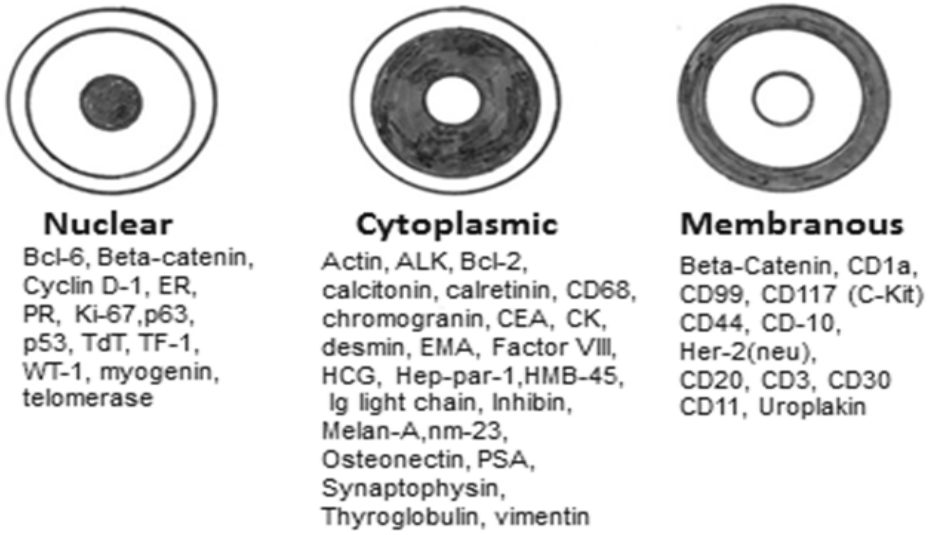


***Fig 4-3*** *The cellular* ***sites*** *of immunoreactivity of some antigens. Commonly, it is localized to a specific cellular site, but it may involve more than one site.*

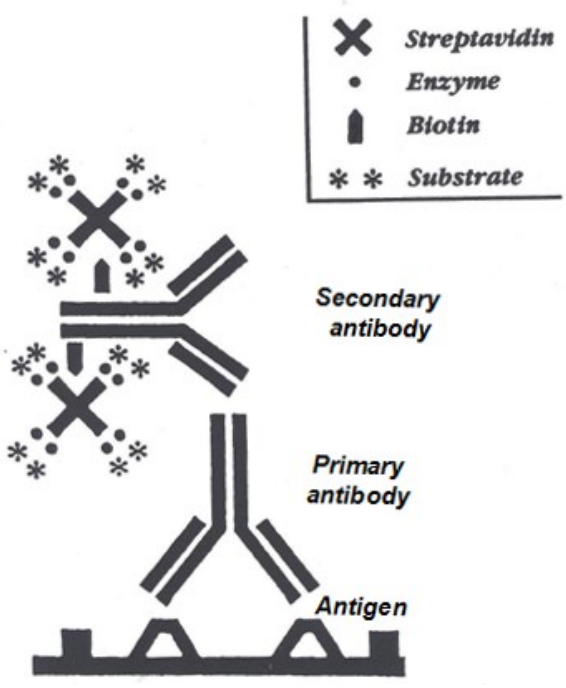


***Fig 4-4*** *The Biotin-Streptavidin (BSA) detection system takes advantage of the strong affinity of biotin to avidin to combine the chromagin to the antibody*

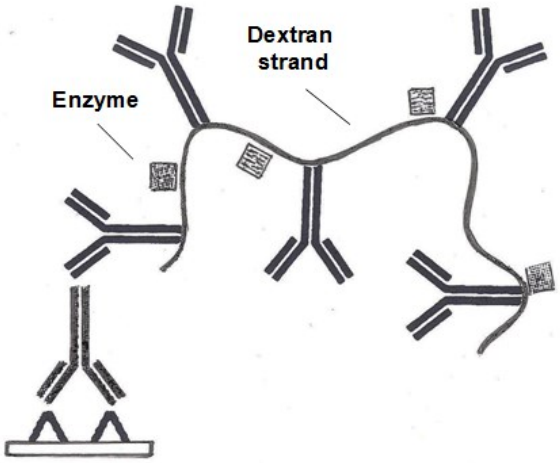


***Fig 4-5*** *The Envision detection system. It contains 10 molecules of secondary antibody and 70 molecules of enzyme on the dextran strand, hence, high sensitivity*

**Table 4-3 Classification and Immunophenotyping of Malignant Tumors**

| Tumor type | Marker |
|---|---|
| **Carcinomas** | CK, EMA |
| Squamous cell carcinoma | CK 5/6 |
| Basal cell carcinoma | CK |
| Transitional cell carcinoma | CK 7, Uroplakin |
| Adenocarcinoma | CEA |
| Choriocarcinoma | hCGT |
| **Neuroectodermal and neuroendocrine** | Chromogranin, Synaptophysin, NSE |
| MPNST | S-100 |
| Melanoma | S-100, HMB45 |
| Peripheral PNET/Ewing | CD99 |
| Gliomas | GFAP |
| **Soft tissue sarcomas** | Vimentin |
| Fibrosarcoma | Markers negativity |
| Malignant fibrous histiocytoma | CD68 |
| Leiomyosarcoma | Actin |
| Rhabdomyosarcoma | Desmin, Myogenin (Myo D1) |
| Endothelioma | CD34, CD31 |
| Mesothelioma | Calretinin |
| **Bone sarcomas** | |
| Osteosarcoma | Osteonectin, Osteocalcin |
| Chondrosarcoma | S-100 |
| Giant cell tumor | p63 |
| **Lymphomas** | LCA (CD45) |
| NHL | CD3, CD20 |
| HL Classic | CD30, CD15 |
| Myeloma | CD138 |
| **Histiocytic tumors** | |
| Langerhans cell histiocytosis | S-100, CD1a, CD207 (Langerin) |
| Non Langerhans cell histiocytosis | CD68, CD163, cathepsin B |
| Follicular dendritic sarcoma | CD21, CD23 |
| **Germ cell tumors** | PLAP |
| Yolk sac tumor | AFP |
| Sex cord tumors | Inhibin |
| **Blastemal tumors** | CK, Vimentin |
| **Tumors of vestigeal remnants** | CK, Vimentin |
| **Ectopic tumors** | Variable |
| **Mixed tumors** | CK, Vimentin |
| **Tumors of uncertain histogenesis** | Multilineage expression |
| **Undifferentiated malignant tumors** | LCA, Desmin, Chromogranin, CD99, CK, S-100, CD138, actin, C-Kit |

## IMMUNOHISTOCHEMISTRY

Immunohistochemistry makes use of cellular protein antigens to determine its differentiation (cell typing). Moreover, it can detect other antigens related to cell proliferation or aggressiveness, thus, adding a biologic perspective to conventional histopathology. Despite these merits, immunohistochemistry is not a replacement of the standard morphologic study of tumors, but rather a complementary method.

### Tumor Antigens

Antigens are present at specific sites at cellular level and are usually classified into nuclear, cytoplasmic, membranous and stromal. Nuclear antigens, include: cell cycle control proteins, DNA related enzymes (e.g. terminal deoxytransferase TdT), and viral proteins. Cytoplasmic antigens are related mainly to the structural proteins of the cytoskeleton, which are classified into: microfilaments (actin) and intermediate filaments (include: cytokeratin CK, vimentin, desmin, neurofilament NF, and glial fibrillary acid protein GFAP). Membranous or surface antigens include membrane bound cell receptors and adhesion molecules. Stromal antigens include normal proteins of extracellular matrix (e.g. collagen, laminin and fibronectin) or abnormal deposits such as amyloid.

Localization of immunoreactivity at subcellular level is diagnostic of the target antigen (Fig 4-3). When immunoreactivity is present in atypical locations, a false nonspecific positivity should be suspected. However, it is important, in this regard, to recognize two unusual immunoreactive patterns, namely: (1) paranuclear dot-like reactivity observed in cytokeratin reaction in Merkel cell tumor and small round cell tumor, desmin in desmoplastic small round cell tumor, GFAP in oligodendroglioma and CD30 and CD15 in Reed-Sternberg giant cells, and (2) Multisite reactivity, which may be combined nuclear and cytoplasmic e.g. S-100 in melanoma and CDX-2 in colonic carcinoma) or cytoplasmic and membranous (e.g. PLAP in germ cell tumors, E-cadherin and CD-15) or cytoplasmic and stromal (e.g. GFAP in astrocytomas).

### Technical Aspects

For tumor antigen integrity, formalin-fixation (10% buffered formalin) should be for a minimum of 6 hours and a maximum of 24 hours. But time could be shortened to only 20 minutes with ultrasonic treatment. Tissue sections should be prepared and undergo processing for immunostaining without delay to avoid antigen degradation. Antigen retrieval aims at the reversal of protein cross-linking by formalin fixation, hence, exposing the antigens. For most antigens, microwave heat treatment for 20 to 30 minutes at 100 °C, with either citrate buffer at pH 6.0 or Tris-HCl buffer at pH 7.0 to pH 8.0, is effective for antigen retrieval.

The demonstration of antigens in tissues by immunostaining is a two-step process. The first step is binding of the *primary antibody* to the related antigen, followed by visualization of this reaction by a *secondary or link antibody* to which are attached different enzyme systems (peroxidase or alkaline phosphatase). The primary antibody determines the specificity of the reaction, whereas, the secondary antibody with its linked enzyme causes amplification of the reaction hence increase of the sensitivity of the test. The *biotin-streptavidin* (B-SA) amplified system is a preferred method in view of its stable reagent, high sensitivity and less liability to give rise to false reaction (Fig 4-4). Recently, even more sensitive methods with fewer steps were developed namely: the *Envision method* (Dako), in which enzyme labels and secondary anti-bodies are attached to a linear dextran polymer (Fig 4-5), and the single step *Epos technique* (Dako) which also couples the primary antibody to the dextran polymer.

Two *enzyme detection systems* are available, the first uses an aqueous chromogen (AEC, red color) and needs a temporary aqueous mounting medium. The second system uses DAB chromogen (brown color) or new Fochsin (red color) and permanent mounting medium. Accurate monitoring of immunoreactivity requires that known control tissues (both positive and negative controls) be run in parallel.

The *interpretation* of immunohistochemistry requires great experience. The study should be restricted to previously selected viable parts of the tumor, avoiding the periphery of the tissue section where false positive reactions are common. Correlation with histomorphology is essential to assure that the positive reaction is in tumor cells and not normal cells. Formalin-fixed paraffin-embedded sections often display uneven intensity and patchy distribution of immunoreactivity which is probably related to irregular penetration of the fixative. Interpretation and quantitation should be limited to areas of strongest reactivity (hot spots) rather

than taking an average reading. Control tissues (both positive and negative controls) should be run in parallel.

## Clinical Applications

In clinical practice, tissue markers are valuable in three main clinical settings, namely: diagnosis of malignancy, prediction of response to therapy and prediction of prognosis.

### Diagnostic markers

1. Routine diagnostic phenotyping of tumors (Table 4-1)

2. Distinguish between malignant tumors from tumor-like reactive conditions. Examples include: (a) Follicular lymphoma (bcl-2 positive) versus reactive follicular hyperplasia (bcl-2 negative), (b) Diffuse Non-Hodgkin lymphoma which is monoclonal (expressing either kappa or lambda immunoglobulins) versus reactive paracortical hyperplasia of lymph nodes which is polyclonal expressing both kappa and lambda. (e) Normal prostatic glands show preserved basal cells (p63 positive) versus malignant glands which lack basal cells (p63 negative).

3. Distinguish between a benign and a malignant tumor, such as, dermatofibroma (CD34 negative) and dermatofibrosarcoma protuberans (CD34 positive).

4. Differential diagnosis of malignant tumors of closely similar histologic picture. Examples include: (a) Mesothelioma (Calretinin+, CEA-) from adenocarcinoma (calretinin– and CEA+), and Adrenal cortical carcinoma (chromogranin– and Vimentin+), from pheochromocytoma (chromogranin+ and vimentin-).

5. Phenotyping of problematic cases such as undifferentiated malignant tumors and metastases with unknown primary. In such cases a panel of markers is used (CK, vimentin, LCA, S-100, and chromogranin).

## Predictive Markers (Therapy Guiding)

1. Hormone receptors: ER and PR receptor studies in breast cancer; and androgen receptor in prostatic cancer, help to identify patients who would benefit from antihormone therapy.

2. HER-2 receptor overexpression in breast cancer helps to select patients for treatment with the monoclonal antibody Herceptin.

3. C-Kit (CD117) positivity in gastrointestinal stromal tumors (GIST) predicts good response to antityrosine kinase drugs (Gleevec).

4. Multidrug resistance gene (MDR-1) expression denotes resistance to anthracyclins and vinca alkaloids and indicates a shift to alternative chemotherapeutic drugs.

## Prognostic Markers

Some tumor markers are good predictors of prognosis. They are generally classified into four main groups.

*1. Proliferation markers*: such as Ki-67 (MIB -1), proliferation cell nuclear antigen (PCNA) and bromodeoxyuridine (Brd Urd). Ki-67 index may be low (<2%), intermediate (2-20%) or high (>20%). A low proliferation index is associated with benign tumors, but higher values indicate malignancy and correlates with prognosis.

*2. Invasiveness markers*: these are indicators of the local invasive potential of cancer and include: loss of cell adhesion molecules (E-Cadherin) or expression of matrix degrading enzymes (matrix metalloproteinases, such as type IV collagenase and cathepsin D).

*3. Metastatic risk markers*: these include: (a) increased new blood vessel formation (angiogenesis) as measured by microvessel density using the endothelial marker CD34 or by quantitating the expression of vascular endothelial growth factor (VEGF) by tumor cells, or (b) failure to express the metastases suppressor gene (nm-23), (c) increased expression of endothelial adhesion molecules (CD44), and (d) detection of micrometastases in lymph nodes or bone marrow using cytokeratin (CK) marker.

*4. Cancer genes alterations*: this includes expression of oncogenes (e.g. HER-2 in breast, N-myc in neuroblastoma, RET in thyroid cancer, or RAS in pancreatic cancer and melanoma). Inactivation of tumor suppressor genes (e.g. p53, Rb and PTEN) is also associated with unfavorable prognosis.

## Limitation and Problems

Interpretative difficulties with immunohistochemistry are not uncommon and may lead to two main diagnostic errors (false negative and false positive results).

*1. False negative results:* due to loss of antigen through poor fixation, or diffusion away from its specific site, the use of outdated denatured antibody, low concentration of antibody or wrong technique.

*2. False positive results* may be due to cross reactivity of the antibody with a different antigen (nonspecificity), or binding with endogenous biotin in the tissues, binding with necrotic tissue or with foreign antigens diffusing from neighboring cells. Immunoreactivity at the edge of tissue section is usually false due to dryness of the section.

# CLASSES OF CANCER

## Carcinomas

Carcinomas are malignant tumors of epithelial origin. They are the most common class of tumors, contributing about 80% of cases. They are most common in middle and old age. The growth rate is slow, hence, carcinomas are smaller in size than sarcomas. They exhibit an irregular gross margin. *Histologically,* carcinomas are composed of cohesive groups infiltrating a distinct stroma. Such cohesive pattern was proved by the electron microscopy to be due to the presence of surface attachment structures (desmosomes) as well as, interdigitating cell membranes. Lymphatic spread is the main and the early route of spread, hence common lymph node metastases. Blood spread is late, commonly affecting the liver, lungs, brain and bone in that order. Many carcinomas are highly radiosensitive.

*Squamous cell carcinoma* arises from the skin, upper areo-digestive tract, anal margin, vagina and uterus. It may also arise in areas of squamous metaplasia such as the lungs, urinary tract and gall bladder. The diagnostic features of squamous cell carcinoma is cytoplasmic keratinization, which may form nests in well-differentiated tumors.

*Transitional cell carcinoma* occurs in the urinary passages, nasal cavity and paranasal sinuses. Tumor cells have an undifferentiated cytoplasm.

*Adenocarcinomas* arises from columnar epithelium. It may arise from surface epithelium (e.g. respiratory and gastrointestinal) or from glands (e.g. liver and prostate). It may also arise on top of columnar epithelial metaplasia, such as, urinary bladder and esophagus. The diagnostic features include acinar or ductal formation, eccentric nuclei, clear cytoplasm, mucin secretion, which may be intracellular (signet-ring cells ) or extracellular.

The *choriocarcinoma,* classified under this group, refers to the fetal or gestational type which affects the uterus. The diagnostic feature is the biphasic structure with presence of both cyto and syncytial trophoblastic epithelium.

### *Immunohistochemistry*

Several antibodies are available for the diagnosis of carcinomas and are generally classified into five main groups, namely: (1) antibodies to individual cytokeratins, (2) vimentin to detect carcinomas with cytokeratin and vimentin coexpression, (3) carcinoembryonic antigen (CEA) to subclassify adenocarcinomas, (4) hormone receptors to identify tumors of endocrine target glands, and (5) organ specific markers that can point to a unique primary site.

### *Cytokeratins (CK)*

Cytokeratins are a family of more than 20 proteins, and are the most complex member of the intermediate filament family. Each of the 20 cytokeratin proteins has been given a catalog number from 1 to 20, based on its molecular weight (MW) and isoelectric point. It is also convenient to divide cytokeratins into two arbitrary biological groups, namely: high molecular weight (34βE12) and low molecular weight-CK and (LMW-CK) respectively.

A pan-cytokeratin reagent (AE1 and AE3) is a cocktail of monoclonal antibodies to high and low molecular weight keratins. This broad spectrum antibody is recommended as a primary reagent to identify epithelial lineage in general. However, different carcinomas have different cytokeratin profiles. Thus *high molecular weight cytokeratin* (HMW -CK) identifies complex epithelium and their tumors, such as: ductal, transitional and squamous carcinomas, whereas low molecular weight cytokeratin (LMW-CK) identifies carcinomas of simple epithelium, such as: hepatocellular, pancreatic and colonic carcinoma. Accordingly, carcinomas are classified into 3 main groups according to their expression to low and high molecular weight cytokeratins (Table 4-4). This scheme is helpful in the diagnostic surgical pathology of tumors. Carcinomas which are negative to both high and low cytokeratin do not exist. This classification helps to distinguish pancreatic carcinoma, from colorectal adenocarcinoma and squamous carcinoma since they belong to three different subgroups. However, it is difficult to distinguish between breast, pancreatic or lung carcinomas by this system since they lie in the same group with identical cytokeratin profile.

Some carcinomas have a unique characteristic reaction to individual cytokeratins. Thus, im

**Table 4-4 Cytokeratin Expression in Carcinomas**

| Group I<br>HMW-CK+/LMW-CK+ | Group II<br>HMW-CK-/LMW-CK+ | Group III<br>HMW-CK+/LMW-CK- |
|---|---|---|
| Breast carcinoma | Hepatocellular carcinoma | Squamous cell carcinoma |
| Pancreatic carcinoma | Renal cell carcinoma | |
| Nonsmall cell lung carcinoma | Colorectal adenocarcinoma | |
| Transitional carcinoma | Neuroendocrine carcinoma<br>Prostatic adenocarcinoma | |

HMW-CK(CK 1 to 6 and 9 to 17)
LMW-CK (CK 7, 8, 18, 19, 20)

munostaining of carcinomas for the coordinate expression of CK7 and CK20 is valuable to determine the site of adenocarcinoma of unknown primary (Table 4-5). The major utility of CK20 is to identify gastrointestinal cancer, whereas, the main value of CK7 is to identify nongastrointestinal carcinomas.

### *Vimentin Coexpression*

In the past, it was thought that cytokeratin and vimentin expression are restricted to epithelial and mesenchymal tumors respectively. However, some carcinomas are of mesenchymal origin (Table 4-6). It was subsequently shown that cytokeratin and vimentin coexpression is a feature of this subset of carcinomas, hence it is possible to identify these carcinomas (Table 4-7).

### *Carcinoembryonic Antigen (CEA=CD66)*

Expression of carcinoembryonic antigen (CEA) can be demonstrated, in variable degrees, in adenocarcinomas. It is most commonly expressed in pulmonary small cell carcinoma, colorectal and hepatocellular carcinomas (with hepatocanalicular pattern), hence it is diagnositically valuable to identify the primary site of these carcinomas. CEA expression is usually directly proportional to the degree of cellular differentiation i.e. well-differentiated adenocarcinomas tend to express CEA more than poorly-differentiated tumors. The CEA family includes true CEA, nonspecific cross-reacting antigen (NCA) and biliary glycoprotein (BGP). The latter identifies bile cancalicular structures in hepatocellular carcinoma, a sensitive and specific finding of this tumor

**Table 4-5 Expression Distribution of CK7 & CK20 in Carcinomas**

| Carcinoma Type | CK7 | CK20 |
|---|---|---|
| Transitional cell ca. | + | + |
| Pancreatic carcinoma | +/- | +/- |
| Ovarian mucinous ca. | +/- | +/- |
| Hepatocellular ca. | -- | -- |
| Renal call carcinoma | -- | -- |
| Prostatic adenocarcinoma | -- | -- |
| Squamous cell carcinoma | -- | -- |
| Lung, neuroendocrine carcinoma | +/- | -- |
| Breast duct carcinoma | + | -- |
| Non small cell lung ca. | + | -- |
| Thymoma | + | -- |
| Salivary adenocarcinoma | + | -- |
| Ovarian, serous & endometrioid | + | -- |
| Endometrial & endocervical | + | -- |
| Colorectal adenocarcinoma | -- | + |
| Merkel cell carcinoma | -- | + |

**NB** Valid results in only 80% of cases and +/-- results are unreliable

**Table 4-6 Carcinomas of Mesenchymal Origin**

| |
|---|
| Adrenal cortical carcinoma |
| Renal cell carcinoma |
| Epithelioid mesothelioma |
| Ovarian and endometrial carcinomas |
| Mullerian and mesonephric carcinoma |
| Bladder trigone carcinoma |
| Central prostate carcinoma |

**Table 4-7 Common Vimentin Coexpression in Carcinomas**

| |
|---|
| Renal cell carcinoma |
| Endometrial carcinoma |
| Salivary and sweat gland carcinomas |
| Mesothelioma |
| Thyroid carcinoma |
| Spindle cell carcinomas |

### *Hormone Receptors*

Antibodies to estrogen receptors (ER) and progesterone receptors (PR) can play an important, but limited, role in the identification of the primary site of carcinomas presenting at a metastatic site. This reaction should not be considered specific of metastatic breast carcinoma since there is a wide spectrum of tumors that have the ability to express ER and PR (e.g. ovarian, endometrial, cervical, sweat gland, thyroid and carcinoid tumors). Conversely, carcinomas which are always negative for ER and/or PR include: nonsmall cell lung cancer, colorectal and hepatocellular carcinomas. Hormone receptor studies are mainly used as predictive markers to guide hormone therapy.

### *Organ-Specific markers*

There are relatively few truly organ specific markers that can be used in the phenotyping of metastatic carcinomas (Table 4-8). In case of prostatic carcinoma, prostate specific antigen (PSA) is actually a more specific reagent than prostatic acid phosphatase (PAP) since some hind gut carcinoids can also express PAP. When used in combination, these two markers will identify more than 95% of all metastatic prostatic adenocarcinoma. A breast cancer marker is gross cystic disease fluid protein-15 (GCDFP-15) which is highly specific marker, but its sensitivity is less than 50%. Antibodies to thyroglobulin have long been considered specific and sensitive markers of both primary and metastatic carcinomas of the thyroid. Thyroid transcription factor-1 (TTF-1) is immunoreactive to thyroid carcinoma and 70% of nonsmall cell lung cancer. Uroplakins are transmembrane proteins of urothelial umbrella cells. Although displaying only moderate sensitivity, uroplakin expression is highly specific marker of transitional cell carcinoma.

Immunohistochemical markers are most valuable in the differential diagnosis of carcinomas at 4 important anatomic sites. (1) In nasopharynx to distinguish undifferentiated carcinoma (CK+) from non Hodgkin lymphoma (LCA+). (2) In the liver to distinguish primary from metastatic hepatic tumors. (3) In the brain to differentiate gliomas (GFAP+) from metastatic carcinoma (CK+). (4) In the pleura to distinguish mesothelioma from carcinoma. These problems are further discussed in the special chapters of the book.

**Table 4-8 Organ specific and Organ Associated Markers**

| **Antibodies to:** | **Identifying** |
|---|---|
| Prostatic specific antigen (PSA) | Prostate carcinoma |
| Prostatic acid phosphatase (PAP) | Prostate carcinoma |
| Gross cystic disease fluid protein-15 | Breast carcinoma |
| Thyroglobulin | Thyroid |
| Thyroid transcription factor-1(TTF-1) | Thyroid and lung carcinoma |
| Uroplakin III | Urothelial carcinomas |
| HepPar-1, GPC-3 | Hepatocellular carcinoma |
| CDX2 | Colon |
| WT1, p16 | Ovarian serous carcinoma |
| RCC | Renal cell carcinoma |

## Neuroectodermal Tumors

The neuroectoderm consists of two components, namely: the neural tube and the neural crest. The former starts as a central thickening in the ectoderm (neural plate) which becomes grooved (neural fold) and subsequently submerge in the three weeks embryo, forming the neural tube. The neural crest develops at the edges of the neural fold and also becomes submerged on each side of the neural tube. The neural tube is a stationary structure and forms the central nervous system with its supporting glial cells. Conversely, neural crest cells migrate extensively into the body and the cells become integrated into different tissues, contributing important cells such as the melanocytes, neurilemmal cells, meningeal cells, neuroendocrine cells and ganglion cells (Fig 4-6). The site distribution of paraganglia is shown in (Fig 4-7 and 4-8).

Neuroectodermal tumors are classified according to their cell differentiation into 3 main groups, namely: differentiated, neuroendocrine, and primitive (Table 4-9). Members of the differentiated group have distinctive diagnostic immunohistochemical and morphologic features (Table 4-10). Neuroendocrine tumors exhibit endocrine function, either clinically manifest or detectable by laboratory investigations. Primitive neuroectodermal tumors are highly-aggressive nonfunctioning tumors. These groups have a common feature of expressing neural markers (e.g. chromogranin and neuron specific enolase NSE) and containing neurosecretory granules demonstrated by electron microscopy. Antibodies directed against the surface glycoprotein CD99 (or MIC-2) have been widely used in the diagnosis of peripheral primitive neuroectodermal tumors (Ewing/PNET) being expressed in about 90% of cases. However, this marker has two limitation. First, it is negative in central PNET, such as: medulloblastoma, olfactory neuroblastoma and pineoblastoma. Second, it is also immunoreactive with other tumor types, such as: lymphoma, rhabdomyosarcoma, malignant fibrous histiocytoma, mesothelioma, and synovial sarcoma. However Ewing/PNET has a characteristic membrane staining pattern, whereas, other sarcomas have a diffuse cytoplasmic pattern.

### *Neuroendocrine Tumors (NET)*

The diffuse neuroendocrine system is represented by cells, spread through the body, that share neural and endocrine functions. Thus, they express neural markers as well as, may synthesize a variety of vasoactive amines and peptides that produce clinical manifestations.

Historically, one decade ago, Obendorfer (1907) introduced the term *carcinoid* to describe NETs in view of their indolent course which in less aggressive than carcinomas. Pearse (1969) introduced the term APUDomas since NETs contain the enzyme decarboxylase, hence, they can synthesize amines and peptides from aminoacids. In other words, they are capable of amine precursor uptake and decarboxyation (APUD). A unified concept of NETs was generally adopted, with the following common features: (1) Positivity to neural markers (chromogranin A, synaptophysin, neuron-specific enolase and somatostatin), (2) Capability to synthesize vasoactive amines and peptides, (3) Histologically nested pattern, fibrovascular stroma, pseudorosette formation (Homer -Wright type), uniform nuclei with finely dispersed chromatin, and (4) Electron-microscopic features of dense core, membrane-bound neurosecretory granules and synaptic vesicles.

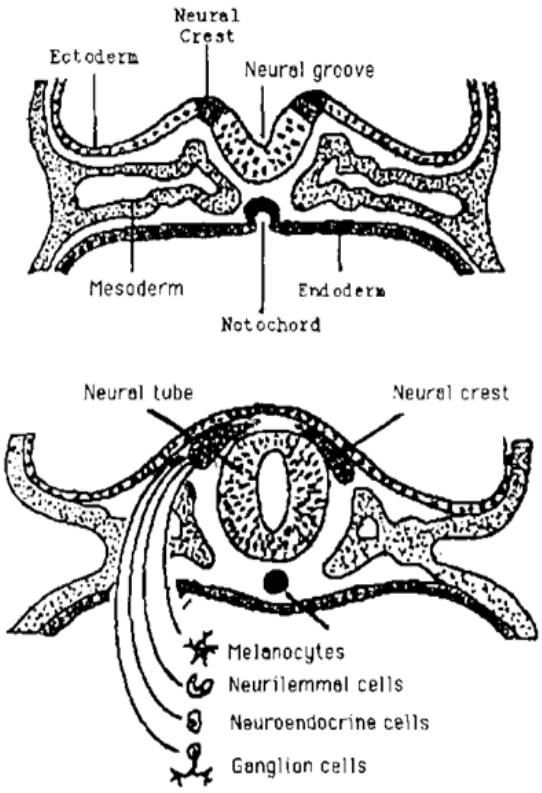


***Fig 4-6*** *Development of neuroectoderm in third week embryo. The stationary neural tube gives rise to central nervous system, whereas, the migratory neural crest contributes to a variety of peripherally-located neural structures.*

**Table 4-9 Classification of Neuroectodermal Tumors (NET)**

**I. Differentiated**
- Gliomas
- Meningiomas
- Melanocytic tumors
- Nerve sheath tumors
- Ganglioneuroma
- Granular cell tumors
- Pigmented neuroectodermal tumor of infancy

**II. Neuroendocrine**

**Epithelial**
- Carcinoid
- Small cell carcinoma
- Medullary thyroid carcinoma

**Neural**
- Neuroblastoma
- Paraganglioma

**III. Undifferentiated (PNET)**

**Central**
- Medulloblastoma
- Cerebral neuroblastoma
- Retinoblastoma
- Pineoblastoma
- Olfactory neuroblastoma

**Peripheral**
- Ewing sarcoma
- Askin tumor
- Merkel carcinoma
- Neuroepithelioma

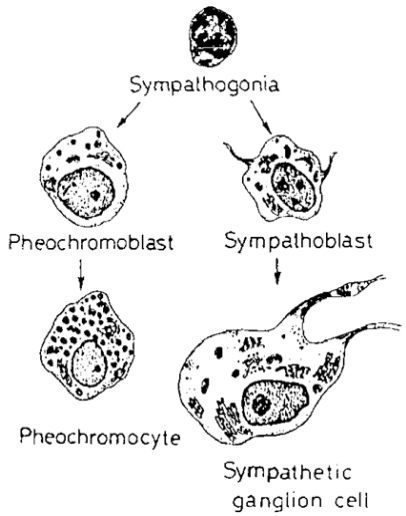


***Fig 4-7*** *Histogenesis of tumors of adrenal medulla. A primitive stem cell of neural crest origin may differentiate into neuroendocrine cell (pheochromocytoma) or neural cell (neuroblastoma).*

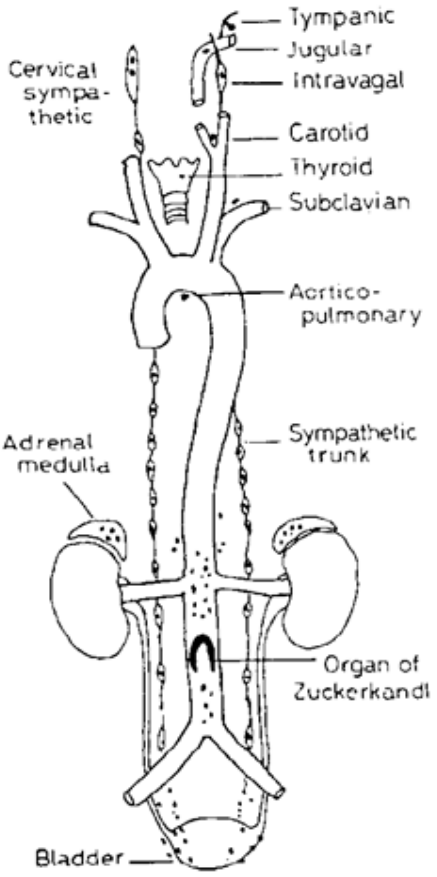


***Fig 4-8*** *The site distribution of paraganglia. Cervical and aortic ganglia are associated with parasympathetic system, whereas, thoraco-abdominal ganglia are associated with sympathetic system.*

**Table 4-10 Special Markers of Differentiated Neuroectodermal Tumors**

| Tumor | Marker |
|---|---|
| Melanoma | HMB-45 |
| Astrocytoma | GFAP |
| Ependymoma | GFAP |
| Neural tumors | Neurofilament (NF) |
| Pineocytoma | Neurofilament (NF) |
| Meningioma | EMA, Cytokeratin, Vimentin |

Abbreviations: GFAP glial fibrillary acidic protein, EMA epithelial membrane antigen

It was originally believed that all neuroendocrine tumors share a common embryologic origin from neuroectoderm (neural crest). However, it is evident at present that endoderm contributes to neuroendocrine cells of lung and gastrointestinal tract. Moreover, the anterior pituitary and pineal body are ectodermal in origin (Table 4-11). An embryologic classification of that group is presented in (Table 4-12), that reveals a striking biologic variation of neuroendocrine tumors in relation to their site of origin. The site distribution is gastrointestinal 67%, lung 25% and other locations 8%.

It is now evident that neuroendocrine tumors are indeed different entities, and their diversity is greater than their similarity. Thus, histologically, NETS of neuroectodermal origin show a neural pattern and may show pseudorosettes, whereas, NETs of endodermal origin show a carcinoma pattern, which may also be positive for cytokeratin, and lack any pseudorosettes. The precursor aminoacids uptaken by the tumors are also different (e.g. tyrosine in neuroblastoma, versus, tryptophan and histidine in carcinoids). About 10% of NETs are functioning (secrete hormones) and may be a part of multiple endocrine neoplasia (e.g. pancreatic NET and MEN-1), but, the majority are nonfunctioning. The frequency of malignancy varies considerably among these tumors, but is related mainly to the type of hormone produced (Table 4-13). Unfortunately, in most cases, malignant behavior is difficult to predict from the histopathology of tumors. A classification of NETs predictive of prognosis is difficult, but badly-needed.

A simple grading system of three categories was introduced for the gastroenteropancreatic neuroendocrine tumors (Modlin, 1997) depending on mitotic activity as determined by Ki-67 index. The WHO also introduced a new nomenclature and classification (WHO 2000, revised 2010) based on tumor cell differentiation, tumor size, mitotic activity and necrosis (Table 4-14). Most of cases fall in the well-differentiated Grade 1 tumors. Moreover, this classification is not applicable on biopsy material or NET at other sites as thyroid, adrenal or lung. In the latter location, the terms typical carcinoid, atypical carcinoid, small and large cell neuroendocrine are adopted. The practical value of these classifications awaits correlation with prognosis.

**Table 4-11 Classification and Embryologic Derivation of Neuroendocrine Tumors (NET)**

| | |
|---|---|
| I. | **Neuroectoderm** |
| | Medullary thyroid carcinoma |
| | Paragangliomas |
| | Peripheral Neuroblastoma |
| II. | **Ectoderm** |
| | Anterior pituitary tumors |
| | Pineal tumors |
| III. | **Endoderm** |
| | Bronchopulmonary tumors (BP-NET) |
| | Gastroenteropancreatic (GEP-NET) |

### *Laboratory Evaluation*

*Immunohistochemistry:* chromogranin A is present in neurosecretory granules and synaptophyin is contained in synaptic vesicles. Both are the markers of choice for this class of tumors. CD56/NCAM, which belongs to the immunoglobulin superfamily of transmembrane adhesion molecules, is also useful for NE tumors. In contrast,

**Table 4-12 Embryologic Classification of Endodermally Derived Neuroendocrine Tumors (Williams and Sanders, 1963)**

| Feature | Foregut | Midgut | Hindgut |
|---|---|---|---|
| Site | Lung, Stomach, Duodenum | Intestine, Appendix | Colon and Rectum |
| Argentaffin reaction | - | + | - |
| Hormonal product | 5-HTP histamine | 5HT (serotonin) | None |
| Carcinoid syndrome | Atypical | Typical | None |
| Product in urine | 5.HTP | 5-HIAA | None |
| Bone metastases | + | - | + |

Abbreviations: 5HTP 5-hydroxy tryptophan, 5HIAA 5-hydroxy–indole acetic acid

**Table 4-13 The Frequency of Malignancy in Functioning Neuroendocrine tumors (NET)**

| Malignancy (%) | NET |
|---|---|
| Low (10%) | Insulinoma, adrenal pheochromocytoma |
| Intermediate (20-50%) | Carcinoid, extraadrenal paraganglioma, Vipoma |
| High (80%) | Gastrinoma, Glucagonoma |
| All (100%) | Poorly-differentiated NE carcinoma, Medullary thyroid carcinoma |

N.B. The only examples of benign NET are tumorlets of lung (<5 mm) and insulinoma (<2 cm)

neuron-specific enolase (NSE) is of little help due to lack of specificity and TTF-1 due to its low expression in carcinoids (only 30% of cases). Nearly 80% of carcinoids are positive for pancytokeratin (they express CK8, 18, and 19), but lower positivity rate (about 30%) in high-grade NE tumors. The proliferation antigen Ki-67 is used to assess mitotic activity and predict prognosis.

*Laboratory investigations*: for neuroblastoma, urinary levels of catecholomines, homovanillic acid (HVA) and vanillylmandelic acid (VMA) are analysed. For pheochromocytoma, serum metanephrine, and 24 hour urinary catecholamines (VMA) and metanephrine are analysed. Patients with amine-secreting tumors should undergo 24-hour urinary 5-HIAA (for classic carcinoid syndrome) or 5-HTP (for atypical carcinoid). For patients with pancreatic NETs, a battery of 8 peptides should be analysed. The peptide profile includes the measurement of: insulin, gastrin, C-peptide, VlP, somatostatin, glucagon, ghrelin and pancreatic polypeptide.

*Nuclear imaging:* Since most NETs express somatostatin cell receptors, radioisotope imaging may be used to detect or study the extent of NETs. Moreover, somatostatin has an antigrowth and inhibitory effect on hormone secretion by endocrine glands, hence, it is used to control hypersecretory syndromes, as well as, targeted therapy of these tumors

## Sarcomas

Sarcomas are malignant tumors of mesenchymal origin which affect mainly soft tissues and bones. The relative frequency of sarcomas is much lower than carcinomas, contributing about 8% of all malignant tumors. They affect all ages, including children and are characterized by rapid rate of growth, hence, they present as bulky, circumscribed tumors, with pseudocapsule of compressed normal tissue. *Histologically,* sarcoma cells are scattered in distribution and indistinct from the stroma. This dissociation pattern is confirmed by electron microscopic studies showing absence

**Table 4-14 The Revised WHO Classification (2010) of Gastroenteropancreatic Neuroendocrine Tumors (GEP-NET)**

| WHO Terminology | Grade | Size /cm | Mitosis/10 hpf Ki-67 + % | Necrosis or Metastases | Previous Terminology |
|---|---|---|---|---|---|
| Well-differentiated tumor NE Tumor | 1 | >2 | <2 | - | Typical Carcinoid Islet cell tumor |
| Well-differentiated NE carcinoma | 2 | >2 | 2-20 | + | Atypical carcinoid |
| Poorly-differentiated NE carcinoma | 3 | >2 | >20 | + | Small or large cell Neuroendocrine carcinoma |

N.B. Hyperplastic preneoplastic lesions of NET and mixed NET are classified separately

of the desmosomes and cell membrane attachments. Sarcomas spread mainly by blood, with the lung being the most common site of metastases. Most of sarcomas are radio-resistant (lymphomas are exceptions).

Mesenchymal tumors arise from undifferentiated mesenchymal cells (stem cells) rather than adult cells. These stem cells are normally found as reserve cells in the soft tissue and bone. The mesenchymal stem cell may have a single or multiple differentiation potential (Fig 4-9). Thus many fibroblasts in tumors are in fact myofibroblasts since they also express smooth muscle protein. Moreover, the interconversion of fibroblasts and histiocytes is a well-established phenomenon and explains the histogenesis of malignant fibrous histiocytoma. Also, in case of osteosarcoma, multiple cell differentiation may occur, thus explaining the diversity of tumor subtypes. The site specificity of bone tumors is explained by the *field theory,* which postulates that the most active cells in a certain area in bone give rise to tumors which are characteristic of this area. Malignant mesenchymoma is an unusual soft tissue sarcoma, composed of at least three different cell types.

### *Immunohistochemistry*

#### *General Markers*

Vimentin is generally considered as a general marker for the families of soft tissue and bone sarcomas. However, it is rather nonspecific, because it is also expressed in some carcinomas, malignant melanoma, PNET, neuroendocrine tumors, gonadal germinomas and Langerhans cell tumors.

#### *Special Markers*

Four vascular markers are available, including: CD31, Factor VIII related antigen, CD34 and Ulex lectin. CD31 (endothelial cell adhesion molecule) is immunoreactive in 78% of angiosarcomas and 100% in Kaposi's sarcomas and appears to be a highly sensitive marker. Factor VIII related antigen is less sensitive but reactive with various benign and malignant endothelial cells. CD34 is positive in 70% of an angiosarcomas, 90% of Kaposi's sarcomas and 100% of hemangioendotheliomas. However, CD34 is nonspecific, being also immunoreactive with gastrointestinal stromal tumors and dermatofibrosarcoma protuberans. Ulex lectin is also another nonspecific endothelial marker.

Desmin is a sensitive marker of myosarcomas both smooth and skeletal muscle origin. More than 90% of rhabdomyosarcomas are positive, but the reactivity of leiomyosarcomas is variable, being more common in uterine than extrauterine tumors. Myoglobin and myogenin immunoreactivity appears specific for the skeletal muscle phenotype,

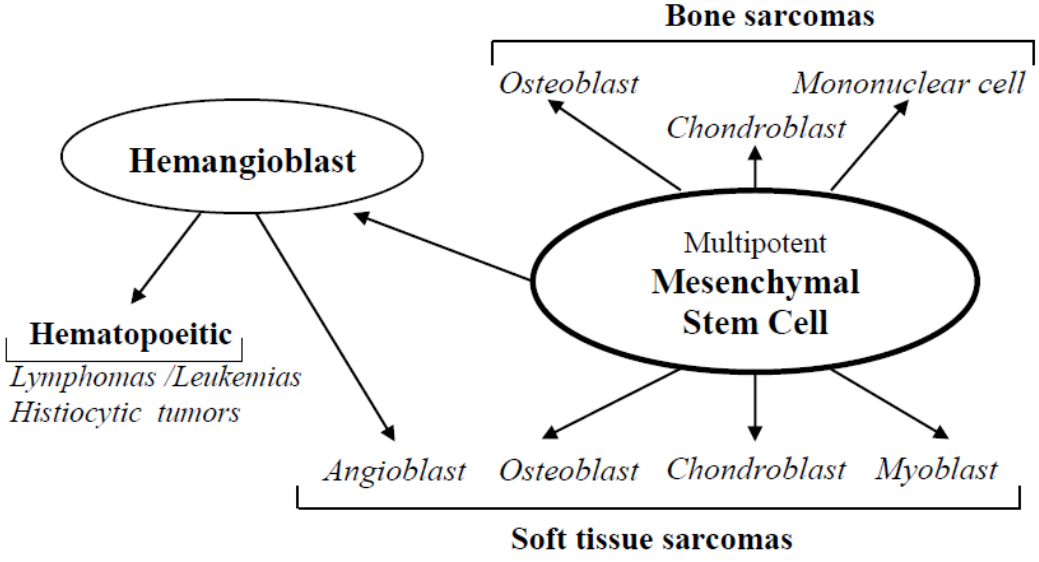


***Fig 4-9*** *Derivatives of mesenchymal cancer stem cell. It gives rise to bone, soft tissue, hematopoietic and histiocytic malignancies. Note that a hemangioblast stem cell gives rise to both hematopoeitic and vascular endothelial cells due to their close functional association.*

but for confirmation of the diagnosis of leiomyosarcoma, smooth muscle actin and smooth muscle myosin immunostains are recommended since these markers are expressed exclusively in smooth muscle tumors.

CD68 (a lysosomal glycoprotein) is a histiocytic marker, positive in 50 to 90% of cases of malignant fibrous histiocytoma. Special care is needed to assure that the reaction is in malignant cells and not reactive histiocytes. The special markers for osteosarcoma are osteonectin and bone GLA protein.

### *Cytokeratin Coexpression*

A group of soft tissue sarcomas are characterized by expression of cytokeratin in addition to vimentin. This property is made use of in the pathologic diagnosis of these sarcomas. Examples of this group are: synovial sarcoma, mesothelioma, chordoma and epithelioid sarcoma.

## Hemolymphoid Malignancies

Lymphoid tissues are classified as primary or secondary lymphoid tissue. The *primary lymphoid tissue* (central) includes the bone marrow and the thymus, which contain precursor cells responsible for cell production. *Secondary lymphoid tissues* (peripheral) include: lymph nodes, spleen, and the collection of mucosa-associated lymphoid tissue (MALT). These are the sites of more mature peripheral lymphocytes, where antigen dependent secondary differentiation occurs. Lymphocytes arises from a common precursor stem cell in bone marrow which initially differentiates into pre-B cells and pre-T cells. The latter differentiate in the thymus into helper (CD4) and suppressor (CD8) cells. Conversely, B-cells differentiate in the germinal centers of lymph nodes and the white pulp of the spleen.

Lymphomas are at present classified according to the WHO classification (2007). Hodgkin disease and myeloma are considered lymphomas in view of their origin from lymphocytes. Lymphocytic leukemias and non-Hodgkin lymphomas (NHL) are included together because of their common association. NHL is subclassified according to their cell differentiation (cell maturity) into precursor cell lymphomas and peripheral or mature cell lymphomas; and each group is further subclassified according to phenotype into B and T all types (Fig 4-10). The term Hodgkin Lymphoma (HL) is at present preferable than Hodgkin disease (HD) because of its confirmed B-cell origin. However, Hodgkin lymphoma is kept separate from NHL in view of its distinctive clinicopathologic features (Table 4-15). Two distinct phenotypes of HL are recognized by WHO, namely: HL lymphocyte predominance (CD20+) and HL classic type (CD30+, CD15+).

Histiocytic tumors (histiocytosis) are non-lymphoid in origin, but, arise from myeloid or mesenchymal stem cells. They are generally classified according to their cell differentiation and immunoexpression into two main classes, namely:

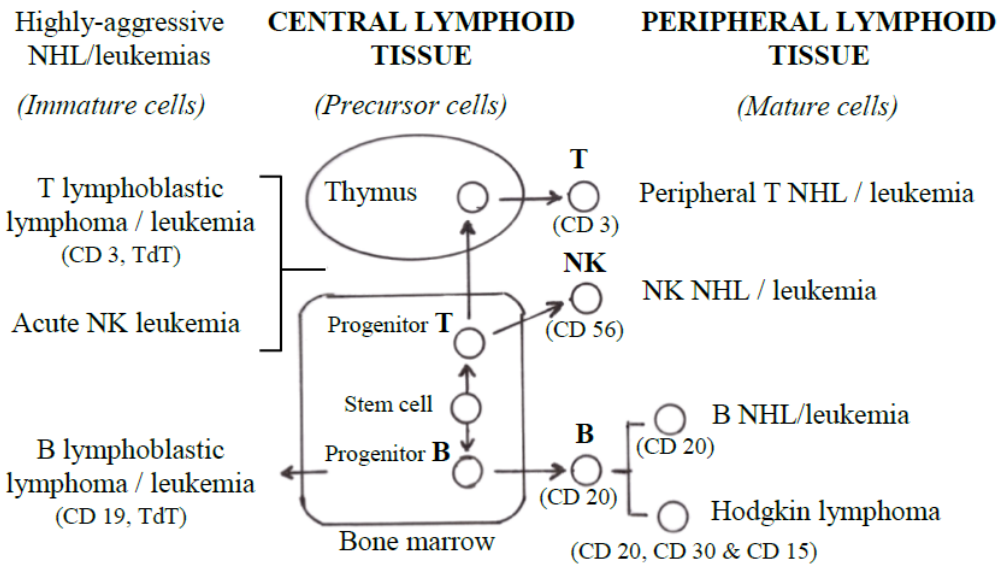


***Fig 4-10*** *The histogenesis of lymphoma/leukemia. A mother stem cell gives rise to T, Natural killer NK and B-cells. Hodgkin lymphoma and myeloma are of B-cell origin.*

**Table 4-15 Hodgkin and Non-Hodgkin Lymphomas (NHL) Compared**

| Feature | Hodgkin | NHL |
|---|---|---|
| Entity | Single | Multiple |
| Histogenesis | B (crippled) | B or T |
| Malignant cells | Reed-Sternberg | All cells |
| Spread | Contiguous LN | Noncontiguous |
| Nodal pattern | Central | Peripheral |
| Extranodal | None | May occur |
| Leukemia | None | May occur |
| Treatment | Standard | Variable |
| Prognosis | Favorable | Less favorable |

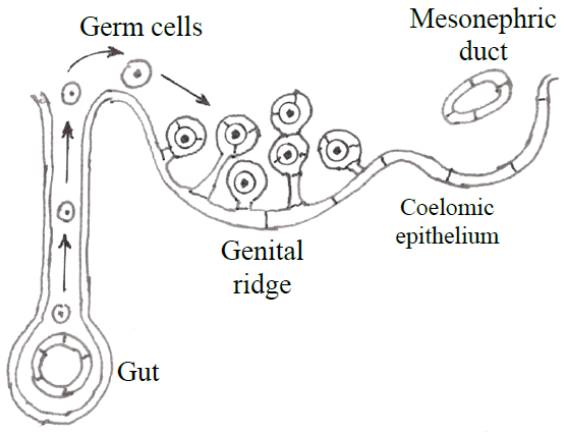


***Fig 4-11*** *The migration of germ cells. They arise from embryonic stem cells, migrate in root of mesentery to the genital ridge (the precursor of gonad), then descend to pelvis*

Langerhans cell type (CD207, S-100, and CD1a positive), and non Langerhans cell type. The latter includes tumor of phagocytic macrophages (CD163, CD68 positive) and tumors of follicular dendritic cells (CD21 and CD23 positive).

## Germ Cell Tumors

The primitive germ cells originate from embryonic stem cells during the fifth week of development and migrate in the root of mesentery from the hind gut wall first to the urogenital ridge and later to the definitive gonads (Fig 4-11). Germ cell tumors arise characteristically in abnormal or dysgenetic gonads. They arise from a diploid germ cell prior to its reduction division.

Germ cells are totipotent, with a broad spectrum of differentiation potential including both embryonic tissue (representatives of the 3 germ cell layers) and extraembryonic tissue (trophoblast and yolk sac). Hence, different tumor types are produced according to the different maturation pathways: (1) reproduction of germ cells without differentiation (seminoma and dysgerminoma), (2) differentiation to primitive embryonic epithelium (embryonal carcinoma), (3) differentiation to reproduce embryonic structures representative of the three germ cell layers but arranged in haphazard fashion with varying degrees of maturation (mature and immature teratoma), and (4) differentiation to extraembryonic structures (choriocarcinoma and yolk sac tumor).

The germ cell origin of teratomas, first proposed by Friedman and Moore in 1946, is supported by the following facts: (a) common incidence of teratomas in the gonads, (b) adequate explanation of the incidence of midline extragonadal teratomas which results from arrest of germ cells during their migratory pathway (e.g. mediastinal and retroperitoneal teratomas), (c) pineal and sacrococcygeal teratomas are explained by ectopic migration of germ cells, and d) the sex chromatin pattern of ovarian teratoma is always a female type.

## Tumors of Blastemal Origin

These are also called embryonic tumors of infancy. Unlike teratomas which contain a wide spectrum of tissue differentiation, this group of tumors consists only of cellular elements present in the particular location. Histogenetically, a clone of stem cells fail to mature during development and retains its undifferentiated embryonic nature (blastemal cell rests). A tumor, usually highly malignant, may arise from these rests. Some of these tumors are present at birth, but most develop within the first five years of life, a time when the tissues are continuing its development. These tumors may be composed of one cell type (monophasic) such as retinoblastoma, or may be composed of multiple cell types such as: hepatoblastoma, pancreaticoblastoma and pulmonary blastoma.

## Tumors of Vestigial Remnants

During embryonic development, certain structures are temporarily produced which normally undergo atrophy. However, if parts of them remain, tumors may develop from these remnants

Table 4-16):

*1. Chordoma* is a tumor of notochordal origin. This structure develops during the third week of embryologic development. It arises from ectoderm as migrating cells between the ectoderm and endoderm (Fig 4-6). This tubular structure soon breaks down and is replaced by a solid structure. With development of the bony spine the notochord disappears by the third month of embryonal life. However, notochordal residues may remain particularly at sphenoid bone or sacrococcygeal region from which chordomas may arise (Fig 4-12).

*2. Ameloblastoma* (adamantinoma) of jaw bones is an ectodermally-derived polymorphic epithelial odontogenic tumor which exhibits histologic features comparable to those of the enamel organ of the developing tooth. It arises from the dental lamina, a cell layer adjacent to the oral alveolar mucosa in embryonic life which later gives rise to the teeth. Two other histologically and histogenetically related tumors are craniopharyngeoma and ameloblastoma (adamantinoma) of the tibia. The former arises fom remnants of Rathke's pouch, and the latter arises from ectopic ectodermal cells in the bone. Some ameloblastomas of long bones, which lack definite epithelial differentiation probably represent vascular tumors (angioblastomas).

**Table 4-16 Tumors of Vestigial Remnants**

| |
|---|
| Chordoma |
| Odontogenic tumors |
| Branchial carcinoma |
| Urachal carcinoma |
| Mesonephroma |
| Mullerian carcinosarcoma |

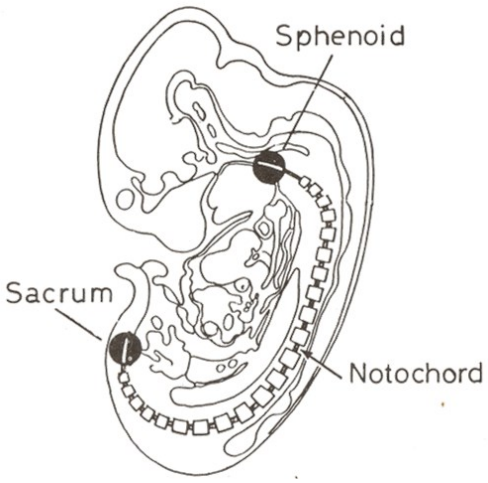


***Fig 4-12*** *Remnants of notochord, they are most common at the beginning and end of vertebral column, hence, these are common sites of chordoma.*

*3. Branchiogenic carcinoma.* The branchial apparatus in the 5 weeks embryo consists of five transverse mesodermal bars, the branchial arches, separated by grooves or clefts (Fig 4-13). Each cleft is in contact with an outpouching of the pharynx, the pharyngeal pouch, the two are separated by a thin membrane. The majority of branchial cysts arise from the second branchial cleft, cervical sinus or branchial pouch and is located in the upper neck below the angle of the mandible. The cyst is lined by squamous epithelium, hence, the evolving carcinoma is squamous in type. Before considering the diagnosis of branchial carcinoma, it is wise to rule out the possibility of neck metastases from primary squamous carcinomas somewhere in the head and neck region.

*4. Urachal carcinoma* develops from urachus an involutionary remnant of allantoic duct, which during early embryonal life connects the dome of the urinary bladder to the allantois via the umbilicus. Along the entire urachal cord (bladder, median umbilical ligament and umbilicus), glandular structure of the enteric type, may be encountered that may lead to adenocarcinomas. In the urinary bladder, adenocarcinomas of urachal origin are found in the dome or anterior wall and are intramural at onset covered by intact bladder epithelium.

*5. Mesonephroma* is a clear cell adenocarcinoma arising from mesonephric remnant (Fig 4-14). Common sites of origin are: the ovary, urinary bladder, cervix and vagina.

*6. Mullerian carcinosarcoma* (mixed mesodermal tumor) consists of a mixture of carcinoma and sarcoma. This tumor is the neoplastic expression of tissues derived from Mullerian duct remnants, which normally differentiate along both epithelial and mesenchymal lines. The paired Mullerian (paramesonephric) ducts are mesodermal in origin and are formed by an invagination of celomic epithelium adjacent to the developing gonads and closely related to the mesonephric ducts (Fig 4-14). The Mullerian ducts fuse caudally forming the uterovaginal primordium. Under hormonal influence, the Mullerian ducts undergo atrophy in males, but in females they form the uterine tubes, uterus and vagina. Mixed Mullerian tumors may

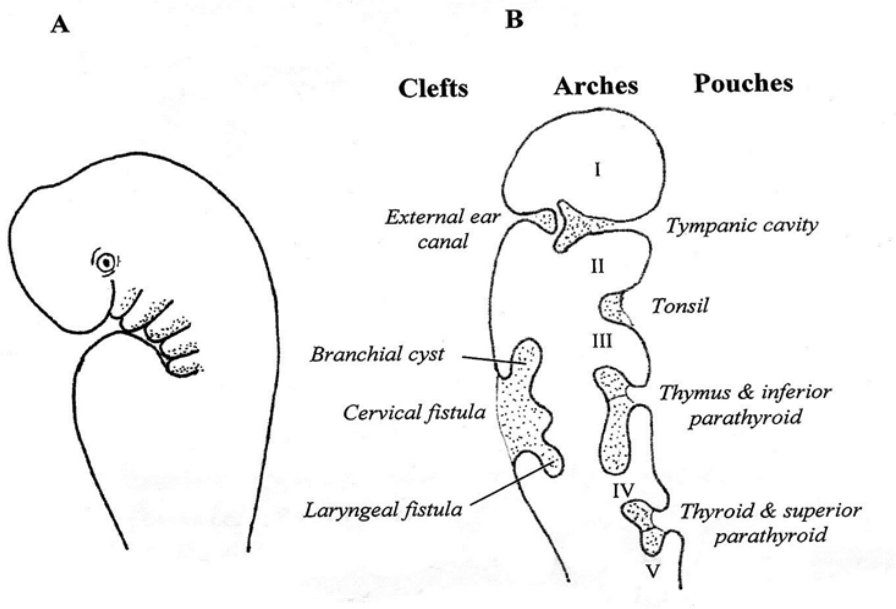


***Fig 4-13*** *Derivatives of the pharyngeal arches. (A) External view of 5-weeks embryo (B) Derivatives of pharyngeal clefts and pouches.*

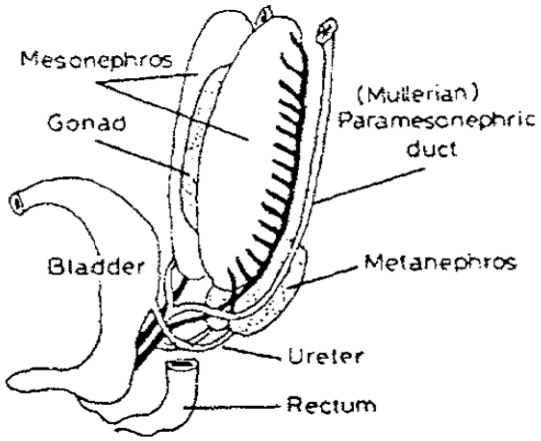


***Fig 4-14*** *Embryology of mesonephros and Mullerian duets. The latter gives rise to the internal female genitalia and are intimately related to the developing ovary and*

occur at any site of the genital tract, but are most common in the uterus of elderly patients. The tumors may be homologous and contain only tissues native to the genital canal, or heterologous if they contain foreign tissues such as striated muscle or cartilage.

## Ectopic Tumors

Ectopic or heterotopia is an abnormality in which an organ or tissue is located outside its anatomic site. Most ectopias are congenital in origin, with the malposition resulting from abnormal descent or migration of embryonic tissue (e.g. germ cells, neural crest cells and endocrine glands). However, other ectopias are acquired (e.g. mucosa associated lymphoid tissue (MALT) resulting from antigenic stimulation at ectopic sites). When ectopic tissue produce a mass lesion, it is referred to as choristoma. Tumors may arise from these ectopic tissues, and because of their unusual places may cause diagnostic problems

Ectopic tumors are classified into six main groups according to the type of ectopic tissue (Table 4-17). By far, ectopic germ cell tumors are the most common. Thus, in a pediatric series, ectopic germ cell tumors are more common (64%) than gonadal germ cell tumors (36%). The most common sites in descending order are: sacrococcygeal (45%), Gonadal (36%), mediastinal (7%), head and neck (5%), retroperitoneal (3%) and central nervous system (2%).

**Table 4-17 Ectopic Tumors and Their Unusual Sites**

| Tumor type | Ectopic sites |
|---|---|
| **1. Germ cell tumors** | |
| Teratomas | Sacrocoxygeal , mediastinal, head and neck<br>Retroperitoneum and intracranial |
| Seminomas | Retroperitoneum, mediastinal |
| **2. Neuroectodermal tumors** | |
| Gliomas (Astrocytes) | Nasal cavity |
| Ependymomas | Subcutaneous, mediastinum, lung |
| Neuroblastoma | Kidney, skin, thymus, lung |
| PNET | Pancreas, kidney, bladder, adrenal, uterus |
| Paraganglioma | Kidney, urinary bladder, pharynx, thyroid, uterus |
| Meningioma | Scalp, orbit, neck, nasal, pharynx, lung, retroperitoneal |
| Melanoma | Oral, nasal, lacrimal, gall bladder, adrenal, cervix and urinary bladder |
| Pigmented NET | Maxilla, soft tissue |
| **3. Endocrine tumors** | |
| Thyroid | Tongue, thyroglossal cyst, mediastinum |
| Parathyroid | Retroesophageal, intrathyroid, intrathymic, mediastinal |
| Pituitary | Nasopharyngeal, paranasal and parapharyngeal |
| Adrenal | Retroperitoneal, testis, ovary |
| **4. Extranodal lymphoid tissue** | |
| MALT lymphomas | Orbit, salivary, thyroid, breast, jejunum |
| Other lymphomas | Skin, brain, pleura, genitourinary, bone |
| **5. Extraskeletal Bone sarcomas** | Soft tissue |
| **6. Ectopic Epithelium (carcinomas)** | |
| Ameloblastic epithelium | Mandible, tibia |
| Salivary tumors | Mandible, lymph nodes, neck |
| Thymic epithelium (thymoma) | Thyroid, neck, pericardium, lung hilum |
| Pancreatic epithelium | Stomach, duodenum, jejunum and Meckel diverticulum |
| Liver (hepatocellular carcinoma) | Lung, spleen, pancreas, adrenal |
| Chorionic epithelium (choriocarcinoma) | Uterine tube, ovary |
| Endometriosis (endometrial carcinoma) | Ovary |

## Mixed Tumors

A mixed tumors (also known as multilineage or multimorphic) is defined as a tumor composed of more than one cell phenotype, regardless of the biologic behavior of its cellular components. It may be native or foreign to the anatomic site. Mixed tumors may arise through one of 5 mechanisms, namely: (1) multilineage differentiation of cancer stem cell, (2) transdifferentiation (metaplasia), dedifferentiation or plasticity of cancer stem cell, (3) multihit carcinogenesis of 2 cell types, (4) somatic malignancy in a teratoma and (5) a cancer metastatic into another. Mixed tumors are classified into 4 main groups, and each group is subclassified into benign and malignant (Table 4-18).

## Tumors of Uncertain Origin

These are tumors which are composed of peculiar cells which do not correspond to any of the known embryonal or adult cells, hence, they

**Table 4-18 Classification of Mixed Tumors**

| Cell types | Benign | Malignant |
|---|---|---|
| **Epithelial** | Pleomorphic adenoma<br>Hidradenoma,<br>Sebaceous adenoma<br>Trophoblastic mole | Choriocarcinoma,<br>Mucoepidermoid,<br>Sebaceous carcinoma.<br>Cholangiohepatoma,<br>Thymoma (type AB)<br>Metaplastic carcinoma<br>Ameloblastoma |
| **Neuro-ectodermal** | Ganglioneuroma<br>Pigmented neuroectodermal tumor of infancy<br>Melanotic neurofibroma | Triton tumor (ectomesenchymoma)<br>Adenocarcinoid<br>Melanotic medulloblastoma |
| **Mesenchymal** | Osteochondroma<br>Angiomyolipoma<br>Spindle cell lipoma | Dedifferentiated sarcoma<br>Mixed gliomas<br>Composite lymphoma<br>Mesenchymoma |
| **Epithelial/ Mesenchymal** | Fibroadenoma<br>Adenofibroma<br>Bronchial hamartoma<br>Benign teratoma<br>Mullerian adenomyoma<br>Mesoblastic nephroma | Malignant teratoma<br>Mullerian carcinosarcoma<br>Mesothelioma<br>Synovial sarcoma<br>Hepatoblastoma<br>Phyllodes tumor<br>Mullenian adenosarcoma<br>Nephroblastoma |

are named by cytomorphologic terms (Table 4-19). They often exhibit a complex multilineage immunohistochemical reactions, including epithelial, mesenchymal and/or neuroectodermal differentiation. All members of this group belong to soft tissue sarcomas. Their detailed clinical and immunophenotypic features are discussed in chapter 21.

**Table 4-19 Tumors of Uncertain Origin**

| |
|---|
| Synovial sarcoma |
| Alveolar soft part sarcoma |
| Epithelioid sarcoma |
| Desmoplastic small round cell tumor |
| Myxoma |
| Inflammatory myxohyaline tumor |
| Malignant rhabdoid tumor |
| Giant cell tumor |
| Parachordoma |
| Pecoma |
| Angiomatoid fibrous histiocytoma |

### Undifferentiated Tumors

In this group the tumor cells are so anaplastic that it is difficult to classify them under any of the mentioned cell types. They are usually classified as *small* cell, *spindle* cell and *pleomorphic* cell types. The tumor may be undifferentiated from the start (de novo) or may represent a focal progression in a previously differentiated tumor. In such case, the neoplasm is named *dedifferentiated tumor*. Undifferentiated spindle cell carcinoma, also known as *sarcomatoid carcinoma* because of their resemblance to sarcomas, may be very difficult to differentiate from sarcomas, even by immunohistochemical studies because the very primitive epithelial cells express both cytokerin and vimentin. Differentiation is only possible by two features: (a) the presence of islands of well-differentiated epithelium in the tumor, favors a carcinoma, and (b) the presence of other sarcoma markers as desmin, favors a sarcoma. Precise typing of these tumors is possible if cytomorphology, age of patient and immunophenotype profile are taken into consideration in the diagnosis (Table 4-20).

## BEHAVIOR CATEGORIES

Behavior categories refer to the classification of tumors according to their predicted natural history or outcome after treatment, based upon pathologic criteria. The clinical behavior of tumors varies considerably. In the majority of cases, it is easily possible to distinguish benign from malignant tumors, but in rare occasions (about 3% of cases) tumor are encountered whose behavior is either impossible or difficult to predict from their histologic picture. Practically, tumors are classified into the following 5 main behavior categories:

### I- Benign Tumors

Such tumors are composed of well-differentiated, mature-looking cells, without mitotic activity. They grow by expansion, hence, a well-circumscribed encapsulated tumor margin. Benign tumors are incapable of local invasion or producing metastases, thus, complete local excision is curative. Examples include: adenoma, papilloma, fibroadenoma, lipoma, angioma, neurofibroma and myoma.

**Table 4-20 Classification of Undifferentiated Malignant Tumors**

| Cell Morphology | Pediatric | Adults |
|---|---|---|
| **Round Cell** | Neuroblastoma (CD56)<br>Lymphoma (LCA)<br>Rhabdomyosarcoma (Desmin)<br>Ewing /PNET (CD99)<br>Synovial sarcoma (CK, CD99) | Lymphoma (LCA)<br>SCLC (chromogranin )<br>Carcinoma (CK)<br>Melanoma (S-100)<br>Synovial sarcoma (CK, CD99) |
| **Spindle cell** | Rhabdomyosarcoma (Desmin )<br>Synovial sarcoma (CK, CD99)<br>MPNST (S-100)<br>Leiomyosarcoma (Actin )<br>Fibrosarcoma (all negative ) | Sarcomatoid carcinoma (CK)<br>Melanoma (S-100)<br>Synovial sarcoma (CK, CD99)<br>MPNST (S-100)<br>Leiomyosarcoma (Actin)<br>GIST (C-kit)<br>Fibrosarcoma (all negative ) |

## II- Locally-Aggressive Tumors

These are well-differentiated, but locally invasive to surrounding normal tissues. They have a high risk of local recurrence with inadequate excision, but no distant metastases. Examples are listed in (Table 4-21).

## III- Tumors of low Malignant Potential (LMP) (Borderline) (Rarely metastasizing)

The tumors are moderately differentiated, may recur, and are associated with a low risk of lymph node or distant metastases (<5% of cases). Examples are presented in (Table 4-22).

## IV- Malignant Tumors

There are moderately to poorly-differentiated, show hypercellularity, active mitosis and abnormal mitosis. They are capable of permeative destructive local invasion, and have a high-risk of both local recurrence, as well as, distant metastases (>5%). The classification of malignant tumors is previously presented in (Table 4-3). Patients with malignant disease present in one of 8 clinical settings (Table 4-23).

## V- Indeterminate Tumors

This class is also called *uncertain* in WHO terminology. Tumors in this group are impossible to predict their biologic behavior from their histology picture. A tumor may appear well-differentiated and produce metastases, and another looks pleomorphic and clinically behave in a benign way. For this reason, we have to resort to other parameters (risk or prognostic factors) for help to suggest a possible clinical behavior. Examples of this category are presented in (Table 4-24).

A correct diagnosis of the behavior category of a tumor is of vital importance, since the policy of treatment is based upon this diagnosis. A misdiagnosis can result in a harmful therapy or inadequate treatment. To minimize this error, it is advisable to follow the steps of diagnostic scheme shown in (Fig 4-15), with consideration of differential diagnosis of both tumor-like lesions, as well as, closely similar tumors. The final diagnosis should be based upon the integration of pathologic, clinical, radiologic and immunohistochemical information.

**Table 4-21 Locally-Aggressive Tumors**

| |
|---|
| **Epithelial** |
| Verrucous squamous carcinoma |
| Ameloblastoma |
| Inverted papilloma |
| Villous adenoma |
| **Mesenchymal** |
| Fibromatosis |
| Dermatofibrosarcoma |
| Giant cell tumor |
| Myxoma |
| Hemangioendothelioma |
| Myopericytoma |

**Table 4-22 Tumors of Low Malignant Potential (Rarely Metastasizing) ***

| |
|---|
| **Epithelial** |
| Ovarian borderline tumors |
| Basal cell carcinoma |
| **Neuroectodermal:** |
| Pigmented NET of infancy |
| **Mesenchymal** |
| Infantile fibrosarcoma |
| Inflammatory myofibroblastic tumor |
| Solitary fibrous tumor |
| Plexiform MFH |
| Angiomatoid MFH |
| Kaposi sarcoma |
| Phyllodes tumor |

* Metastasis occur in < 5% of cases

**Table 4-23 The Eight Clinical Presentation Categories of Malignant Tumors**

| |
|---|
| Malignant, in primary site |
| Multiple tumors, in same organ |
| Multiple tumors, in different organs |
| Recurrent tumor |
| Secondary malignancy by local invasion |
| Metastatic malignancy with known primary |
| Metastatic malignancy with unknown primary |
| Metastatic, uncertain whether it is primary or metastatic |

**Table 4-24 Tumors of Indeterminate Biologic Behavior (Uncertain)**

| |
|---|
| **Epithelial** |
| Adrenal cortical tumors |
| **Neuroendocrine** |
| Paragangliomas |
| Typical pulmonary carcinoid |
| Well-differentiated GEP-NET |
| **Other tumors** |
| Gastrointestinal stromal tumor (GIST) |
| Sex cord tumors |
| Perivascular epithelioid cell tumor (PEComa) |

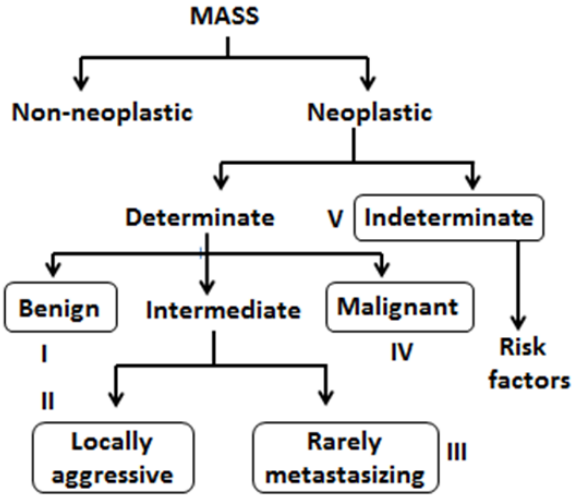


***Fig. 4-15*** *Flow diagram of diagnosis of behavior categories of tumors. It is important to recognize at first tumor-like lesions and tumors of indeterminate behavior.*

## PROGNOSTIC FACTORS

Prognostic or risk factors, are parameters that affect prognosis of a given tumor. Identification of these risk factors is important for two reasons. First, it can help to predict the biologic behavior of tumors in the indeterminate category. Second, it can help in refinement of patient stratification and tailor treatment to individual patient according to tumor biologic characteristics (risk-adapted therapy).

Prognostic factors are generally classified into three main groups, namely: *(1) Patient factors:* such as age and general condition or performance status, *(2) Tumor factors:* such as site, size and stage, and *(3) biomarkers:* such as mitotic activity (Ki-67 index), tumor burden (LDH level), ploidy, chromosomal abnormalities, Oncogene expression, drug resistance or cancer spread markers.

*A prognostic index* combines multiple prognostic factors together. Thus, each prognostic factor is given a score, and the addition of scores creates an index (e.g. Nottingham prognostic index in breast cancer and the international prognostic index (IPI) in non-Hodgkin lymphoma).

*Survival analysis* is the method of choice to evaluate the effectiveness of a given treatment, or to compare different treatments (clinical trials), or to evaluate the importance of different prognostic factors of a given cancer. Survival curves are usually calculated by Kaplan-Meir method which displays the cumulative probability of patient survival during a specified period (5 or 10 years). The starting point is taken as the date of start of treatment or date of diagnosis. Patients are followed periodically, and their status recorded (living free of disease, living with residual or recurrent disease, or dead). It is possible to calculate *disease-free survival (DFS)* by selecting cancer-free patients only, or *overall survival (OS)* by including all patients. In highly-aggressive types of cancers with very short clinical course, the *median survival* in months may be calculated and used for comparison.

When multiple risk factors of a given cancer are evaluated to determine their significance, the statistical analysis is done in two steps. First, a *univariate analysis* of survival is done for each risk factor using the log-rank test. The second step, is a *multivariate analysis* using Cox proportional hazard model, to be applied only in the variable which proved to be significant by the log-rank test of the first step. Multivariate analysis will detect *independent variables* (risk factors) which are truly significant and allow comparison of their relative importance *(hazard ratio).*

### Grading Of Cancer

Grading is defined as the histologic estimate of the degree of malignancy. Originally, Broder graded malignant tumors into four grades with increasing malignancy, but nowadays, it is customary to use three grades only. To simplify statistical analysis, tumors may even be classified into only two groups namely: low grade tumors and high grade tumors. Grading is based on

microscopic estimation of cellular differentiation, anaplasia and mitotic activity. A poorly-differentiated tumor, with marked anaplasia and mitosis, is a grade III tumor. Also, an undifferentiated or embryonic tumor is usually automatically graded as grade III.

There are four limitations for tumor grading: a) it is based on *subjective* estimate, hence there is marked interobserver variation in grading the same tumor by different pathologists, b) *variability in place,* thus tumor grade may vary in different areas of the tumor, c) *variability in time,* which means an increase of grade with time because of tumor cell progression with time, since, high-grade cells tend to overgrow the low-grade ones, and d) *irrelevance*, since in some neoplasms, the tumor type or location is more predictive of the natural history than tumor grade.

In case of carcinomas, grading is only useful in certain sites as: invasive duct carcinoma of the breast and transitional carcinoma of the urinary bladder, but, in many carcinomas, the histologic type of the tumor is more important parameter than the grade. For example, mucinous carcinoma of the colon is an unfavorable tumor in spite of its rather bland histology. Also, thyroid tumor types give more information on the natural history of tumor and its spread pattern than tumor grade.

The grading of soft tissue sarcomas has been very complex, and generally tumor types are considered more important than grades. The National Cancer Institute, Bethesda (NCI) system developed by Costa (1984) considered tumor necrosis as of key importance in predicting prognosis. Certain histologic types, without necrosis and showing minimal mitosis, usually respond well to therapy and are grouped as grade I (e.g. myxoid and well-differentiated liposarcoma, dermatofibrosarcoma, leiomyosarcoma, hemangiopericytoma and malignant Schwannoma). Sarcomas showing up to 15% necrosis are graded as grade II and cases with more necrosis as grade III. Recently, soft tissue sarcomas are simply graded by a French system into low and high grades only (WHO, 2000). This system has a definite prognostic value.

In brain tumors, it is customary to grade the neoplasm into 4 grades, however, within the astrocytoma group, the anatomic location and age of patient appears to be more important. Thus, cystic astrocytomas of the cerebellum in children behaves as a benign tumor as compared to cerebral astrocytomas in adults.Other favourable astrocytoma types include: optic nerve glioma and juvenile pilocytic astrocytoma.

## SCORING OF CANCER

In prostatic carcinoma, a scoring system was developed by Gleason, taking into consideration the variability of grade in different areas of the tumor. Five grades are recognized based upon the glandular pattern rather than anaplasia. The primary grade (most common) and the secondary grade (second most common) are combined together to obtain the *Gleason's score* (values between 2 and 10). Patients with more than 6 Gleason's score are associated with unfavorable prognosis.

## STAGING OF CANCER

Staging is defined as the anatomic extent of malignant disease as evaluated by clinical or pathological examination. Staging is more important than grading in the selection of treatment modality and evaluation of patient survival.

The staging of cancer is based on the size of primary tumor, its local invasion, spread to regional lymph nodes, and the presence or absence of blood-borne metastases. The main staging system in current use is the TNM system developed by the International Union Against Cancer. This system is based on the assessment of three components, namely: (T) the extent of the primary tumor, (N) the absence or presence and extent of regional lymph node metastasis and (M) the absence or presence of distant metastasis. The addition of numbers to these three components indicates the extent of the malignant disease. So the system is a "shorthand notation" for describing a particular malignant tumor. In the recent classification, both the clinical and pathological stages for a given site generally coincide.

An alternative staging classification is the SEER system in which malignant tumors are simply classified into 3 stages: localized, regional and distant. In case of carcinomas, a fourth stage of carcinoma in situ is added (Fig 4-16).

### GTM Stage

Soft tissue sarcomas and bone sarcomas are the only malignant tumors in which the grade (G) is taken into consideration in staging of tumors *(Enneking System).* The tumors are graded into high and low grades, according to three parameters, namely: tumor differentiation, mitotic rate and

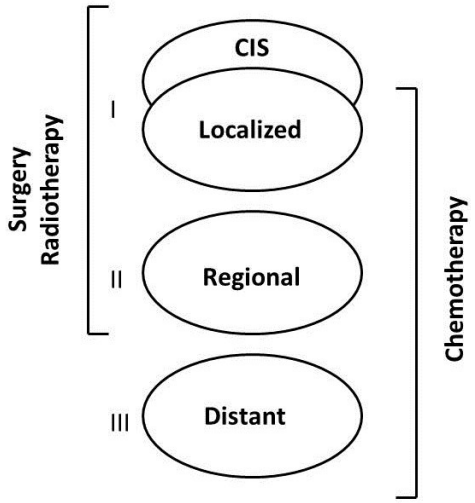


***Fig 4-16*** *The SEER staging system and scope of therapy. Carcinomas are classified into 4 stages, whereas, sarcomas into 3 stages due to absence of carcinoma in situ (CIS).*

amount of tumor necrosis. The extent of tumor is categorized into: intracompartemental: (Tl), extracompartemental (T2) or metastatic (M), whether nodal or distant. Based upon these parameter, three stages are recognized (Table 4-25)

In case of thyroid carcinoma and in view of important effect of histologic type and age prognosis, staging is essentially based on these two parameters (chapter 19).

**Table 4-25 Enneking Staging System for Sarcomas of Soft Tissue and Bone**

| | | | |
|---|---|---|---|
| Stage I | Low grade | without | metastasis |
| Stage II | High grade | without | metastasis |
| Stage III | Sarcomas | with | metastasis |

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