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Review Article

Round Cell Tumors: Classification and Immunohistochemistry

Abstract

Round cell tumors as the name suggest are comprised round cells with increased nuclear-cytoplasmic ratio. This group of tumor includes entities such as peripheral neuroectodermal tumor, rhabdomyosarcoma, synovial sarcoma, non-Hodgkin's lymphoma, neuroblastoma, hepatoblastoma, Wilms' tumor, and desmoplastic small round cell tumor. These round cells tumors are characterized by typical histological pattern, immunohistochemical, and electron microscopic features that can help in differential diagnosis. The present article describes the classification and explains the histopathology and immunohistochemistry of some important round cell tumors.

Keywords: *Ewing sarcoma, lymphoma, round cell tumor*

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Introduction

The term round cell tumor describes a group of highly aggressive malignant tumors composed of relatively small and monotonous undifferentiated cells with increased nuclear-cytoplasmic ratio.[1] Malignant small round cell tumors (MSRCT) is a term used for tumors composed of malignant round cells that are slightly larger or double the size of red blood cells in air-dried smears.[2]

This group of neoplasms is characterized by small, round, relatively undifferentiated cells. Differential diagnosis of small round cell tumors is particularly difficult due to their undifferentiated or primitive character. Tumors that show good differentiation are generally easy to diagnose, but identification of the diagnostic, morphological features is difficult when a tumor is poorly differentiated, therefore, no definitive diagnosis may be possible.[3] Fine-needle aspiration cytology (FNAC) plays an important role in the diagnosis of these tumors.[2,4,5]

Classification

On the basis of round cell pattern

A. Diffuse round cell pattern
   1. Ewing's sarcoma
   2. Primitive neuroectodermal tumor (PNET)
   3. Merkel cell carcinoma
   4. Embryonal rhabdomyosarcoma (ERMS)
   5. Small cell carcinoma
   6. Lymphoma
   7. Leukemic infiltrate.

B. Septate or lobulated round cell pattern
   1. Small round cells are divided by fibrous/fibrovascular septate
   2. Ewing's sarcoma
   3. Alveolar rhabdomyosarcoma (ARMS).

C. Alveolar/pseudoalveolar round cell pattern
   This pattern includes focal, poor cohesion of the round cell population resulting in pseudo alveolar appearance
   1. ARMS
   2. PNET.

D. Round cell pattern with rosettes
   A rosette' is like a flower, with the cells being arranged radially around a central area
   1. Flexner's (also called Flexner - Winterstein, true rosettes) - contain clearly delineated empty central lumen
      e.g., neuroblastoma, PNET
   2. Homer Wright rosette-center has no lumen, but abundant fibrillary material
      e.g., neuroblastoma.

E. Round cell pattern with hemangiopericytomatous vascular pattern
   e.g., poorly differentiated synovial sarcoma, Mesenchymal chondrosarcoma.

F. Round cell pattern with other components

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1. Pseudo glands - poorly differentiated synovial sarcoma
2. Cartilage - mesenchymal chondrosarcoma.

According to size of round cell

1. Small round cell - Squamous cell carcinoma, PNET, Ewing's sarcoma, melanoma, rhabdomyosarcoma (RMS), Langerhans cell disease, lymphoma, adenocarcinoma, neuroendocrine carcinoma, Merkel cell carcinoma, olfactory neuroblastoma
2. Large round cell - Squamous cell carcinoma, adenocarcinoma, melanoma, RMS, lymphoid tumors, paraganglioma.

On the basis of origin

I. Neurogenic origin: Ewing's sarcoma/PNET, neuroblastoma, retinoblastoma, medulloblastoma, Merkel cell tumor, paragangliomas, small cell tumor of lung
II. Mesenchymal origin
   1. Myogenic differentiation
      a. ERMS
      b. ARMS.
   2. Osteoid differentiation
      a. Small cell osteosarcoma.
   3. Chondroid differentiation
      a. Mesenchymal chondrosarcoma.
   4. Adipose tissue like differentiation
      a. Myxoid/round cell liposarcoma.

Hematolymphoid origin

a. Lymphoma/"reticulum cell sarcoma."

Malignant soft tissue tumors of uncertain type

a. Desmoplastic small round cell tumor (DSRCT)
b. Poorly differentiated synovial sarcoma.

Ewing's Sarcoma and Primitive Neuroectodermal Tumor

Ewing's sarcoma is a sarcoma of bone classically described under small round cell tumors. There is considerable clinical and histologic overlap between this tumor and the PNET. Ewing's sarcoma arises within the bone, but can also occur within the soft tissue (extraosseous Ewing's sarcoma) and PNET arises within soft tissues.[6] This neoplasm mainly affects the pelvis and the femur region and predominates in the second decade of life [Table 1].

PNET is a small round cell malignancy of primitive, neuroectodermal tissue or pluripotential, migratory neural crest cells that arises from the soft tissue or bone, commonly affecting older children and adults.[7] The term, "PNET" includes MSRCTs of the thoracopulmonary region (Askin's tumor), extraskeletal Ewing's sarcoma, peripheral neuroblastoma, and peripheral neuroepithelioma.[2,8]

FNAC reveals the presence of tumor cells that are arranged in relatively small, tight clusters with the presence of round or irregular nuclei lacking nucleoli. These small blue cells have a high nucleocytoplasmic ratio. Two population of cells have been described large chief cells and smaller dark cells.[2]

The cytoplasm of these cells is pale blue and contains variable numbers of punched-out vacuoles which correspond to glycogen deposits, can be well demonstrated by periodic acid–Schiff (PAS) staining. However, the presence of large amounts of intracellular glycogen is not a specific finding as while up to 35% of all Ewing's sarcoma cases do not contain detectable glycogen, many other childhood tumors do contain detectable glycogen.[2]

The tumor shows variable numbers of pseudorosettes; fibrillary matrix and Homer Wright rosettes are seen at times, and mitotic figures are rarely detected.

Radiographic features

Onionskin or sunburst appearance.

Special stains

PAS with diastase (glycogen present in 75% of cases), immunohistochemistry.

Table 1: Histological characteristics of Ewing Sarcoma and PNET

Histological types	Characterization
General	Sheets and large nests of uniform, small, polygonal cells with scanty cytoplasm and indistinct cell borders are present
	Dispersed chromatin with hyperchromasia and variable mitotic figures
	Rosettes are absent
Typical	Round cells with varying proportions of large clear cells and smaller hyperchromatic cells are present
	Cytoplasm is ill-defined, scanty, pale staining and vacuolated as the result of intracellular deposits of glycogen
	Hemorrhage with vascular lakes or sinuses are seen
	Filigree pattern (association of distinct vascular structures with degenerated or necrotic ghost cells) larger tumor cells
	Metaplastic bone or cartilage
Atypical	Cells have increased nuclear size or cellular atypism
	Moderate amount of glycogen
	Lobular architecture, increased extracellular matrix, or alveolar pattern with no evidence of myoblastic differentiation
	Increased mitoses (>2/HPF) and cellular pleomorphism
	Spindle cells, usually at the tumor margin, but not diffuse

HPF – High-power field

Neuroblastoma

It is the third most common malignant extracranial solid tumor of childhood. Neuroblastoma and its related variants are derived from primitive neural crest cells that migrate from the mantle layer of the developing spinal cord and populate the primordial of the sympathetic ganglia and adrenal medulla [Table 2].[9]

Rhabdomyosarcoma

RMS is the most commonly found soft tissue sarcoma in children wherein the cancer cells are thought to arise from skeletal muscle progenitors. These tumors are currently classified into ERMS, ARMS, and pleomorphic RMS (PRMS) subtypes.[9]

Subtypes

I. ERMS: Mostly affects the children younger than 10 years of age. Features include:
   a. Varying degree of cellularity with alternating densely packed hypercellular areas and loosely textured myxoid areas
   b. A mixture of poorly oriented small undifferentiated hyperchromatic round- or spindle-shaped cells and varying number of differentiated cells with eosinophilic cytoplasm characteristic of rhabdomyoblasts[9,10]
   c. A matrix containing little collagen and varying amount of myxoid material.

II. ARMS: Composed of large aggregates of poorly differentiated round or oval tumor cells that show central loss of cellular cohesion and formation of irregular alveolar spaces. The individual cellular aggregates are separated and surrounded by dense hyalinized fibrous septa
   Cells at the periphery of alveolar spaces adhere in a single layer to the fibrous septa while the cells at the center of the alveolar spaces are loosely arranged or free floating.[11]

III. PRMS: Composed of spindle-shaped cells arranged in a fascicular pattern with eosinophilic stringy cytoplasm.

Special stains

1. PAS with or without diastase: For intracellular glycogen
2. Colloidal iron and alcian blue: For extracellular mucinous material
3. Masson's trichrome, phosphotungstic acid hematoxylin.

Desmoplastic Small Round Cell Tumor[12]

DSRCT is a rare neoplasm that was first described by Gerald and Rosai in 1989.[12,13] It is a high-grade tumor that mostly affects abdominal cavity and visceral organs. It differs from other childhood tumors due to its clinical features, morphology, and its immunohistochemistry staining pattern.[2,14] Tumors are composed of sharply demarcated nests of varying size with small round or oval cells embedded in the hypervascular desmoplastic stroma. Large tumor cell nests have central necrosis [Table 3].[15]

Table 2: Histological characteristics of Neuroblastoma and variants

Features	Classic Ewing's sarcoma	Atypical Ewing's sarcoma	PNET
Cell shape	Uniform, round	Irregular	Irregular
Chromatin	Fine	Coarse	Coarse
Nucleoli	Pinpoint	More prominent	Prominent
Glycogen	Abundant	Moderate	Scanty
Rosettes	Absent	Absent	Present

PNET – Primitive neuroectodermal tumor

Table 3: Immunohistochemical Data on Desmoplastic Round Cell Tumor

Histological types	Characterization
General	Neuroblasts with varying stages of differentiation
Neuroblastoma	Homer-wright rosettes
	Undifferentiated type - no ganglionic differentiation
	Poorly differentiated type - <5% differentiating cells
	Differentiating type - >5% differentiating cells
Ganglioneuroblastoma, nodular	Has primitive neuroblasts along with maturing ganglion cells
	Contains gross nodules of neuroblastoma abutting large expanses of ganglioneuroma. Also known as composite neuroblastoma
Ganglioneuroblastoma, intermixed	Consist of nests of neuroblasts situated in the ganglioneuromatous stroma
Ganglioneuromna	Mature and fully differentiated tumor characterized by a mixture of Schwann cells and ganglion cells
Special stains/immunoperoxidase	**Characterization**
Immunoperoxidase	NSE, neurofilament protein, S100, desmin

NSE – Neuron-specific enolase

The arrangement of the cells
1. Large nests with central necrosis
2. Tubular like structures
3. Trabeculae separated by fibrovascular septa reminiscent of a "Zellballen" pattern.

Other features include
1. Tumor cells have cleared out cytoplasm or a signet ring appearance
2. Rhabdoid like foci in which tumor cells have paranuclear intracytoplasmic hyaline inclusions composed of aggregates of intermediate filaments
3. Wright like rosettes.

Immunohistochemical Data on Desmoplastic Round Cell Tumors[15]

Marker	Number of positive cases (%)
Desmin (dot like pattern)	39/39 (100)
Cytokeratin (CAM 5.2 and AE1/AE3)	37/39 (95)
EMA	24/25 (96)
Vimentin	22/27 (81)
CD57 (Leu7)	10/15 (67)
NSE	18/25 (72)
Synaptophysin[15]	3/19 (16)

EMA – Epithelial membrane antigen; NSE – Neuron-specific enolase

Willm's Tumor

Willm's tumor (WT) or nephroblastoma is the cancer of kidneys that typically occurs in children rarely in adults [Table 4].

Markers for Wilm's tumor

Cytokeratin, Desmin, WT-1 protein expression, NB84, CCN-3 protein.[17]

Immunohistochemistry of Round Cell Tumors

Markers:[9]

1. CD 99
   - CD 99 is a transmembrane glycoprotein of 30–32 KDa
   - It plays a role in cellular adhesion and regulation of cellular proliferation
   - Normal tissue that commonly displays strong expression of CD99 include:
     1. Cortical thymocytes
     2. Sertoli cells
     3. Endothelium
     4. Pancreatic islets
     5. Ependyma
     6. Epithelium (urothelium, squamous epithelium, columnar epithelium)
   - It is specific for:
     1. Ewing's sarcoma - 90%
     2. Lymphoblastic lymphoma - 90%
     3. Synovial sarcoma - >75%
     4. Mesenchymal chondrosarcoma - 50%
     5. Osteosarcoma and desmoplastic round cell tumor - Rare
     6. Neuroblastoma - Never reported (-).
2. NB 84
   - It is sensitive marker for neuroblastoma (75%), Ewing's sarcoma (16%–25%)
   - Also positive for RMS, WT, osteosarcoma, desmoplastic round cell tumor.
3. S-100
   - It is a marker for benign and malignant nerve sheath tumors
   - Composed of two subunits α and β that combine to form 3 isotypes: α-α isotype found in the myocardium, skeletal muscle and neurons, α-β isotype found in chondrocytes, glia and skin adnexae, β-β isotype found in Langerhans and Schwann cells.
4. Desmin
   - It is the intermediate filament protein associated with both smooth and skeletal muscle differentiation
   - Rarely expressed by myofibroblasts and their corresponding tumors
   - In skeletal muscles desmin is localized to Z-zone between myofibrils when it serves as binding material for contractile apparatus. In smooth muscles, it is associated with cytoplasmic dense bodies
   - Desmin can also be expressed by nonmuscle cells including fibroblastic reticulum cells of lymph nodes, submesothelial fibroblast, and endometrial stromal cells
   - Expressed in PNET, desmoplastic round cell tumors, neuroblastoma, mesothelial cells and tumors, WT.
5. Cytokeratins
   - Used for distinguishing epithelial from nonepithelial tumors (lymphomas, sarcomas, melanomas)
   - Expressed in: Carcinoma, epithelial sarcoma, leiomyosarcoma, mesothelioma
   - Also expressed by round cell tumors such as

Table 4: Histological variants of Willm's tumor

Histological types	Characterization
General-favorable histology	Mixture of cell types differentiating into blastema, epithelium, and stroma
	This triphasic pattern is the most common but mono- and bi-phasic tumors are also identified
Blastemal	Resembles condensed mesenchyme of the embryonic kidney
	Small closely packed and mitotically active cells with minimal differentiation
Diffuse blastemal	Large sheets of blastema
	May extend beyond kidney and diffusely infiltrative
Serpentine blastemal	Frequent pattern with undulating cords of blastemal cells in a loose, myxoid stroma[16]
Nodular blastemal	Blastemal islands are rounded
Basaloid blastemal	Serpentine or nodular patterns are outlined in a distinctive epithelial layer
Epithelial	Recapitulates various stages of normal nephrogenesis resembling collecting ducts or nephrons and glomeruli
	Heterologous elements of mucin, squamous, and ciliated epithelium may occur
Stromal	Myxoid and spindle cells resembling embryonic mesenchyme are present
	Skeletal muscle most common element
	Various elements including cartilage, adipose tissue, bone, mature ganglion cells, and neural tissue

Table 5: Screening for undifferentiated round cell tumors

Antibody to	Small cell carcinoma	Melanoma	Lymphoma	PNET	Rhabdomyosarcoma	Poorly differentiated synovial sarcoma	Desmoplastic round cell tumor	Neuroblastoma
Pan cytokeratin	+	-	-	Variable	-	+	+	-
S-100 protein	-	+	-	-	Variable	Variable	-	Rare
CD45	-	-	+	-	-	-	-	-
Desmin	-	-	-	Rare	+	Variable	+	-
CD99	-	-	Variable	+	Variable	+	Rare	-
Myogenin/$MyoD_1$	-	-	-	+	+	+	-	-
NB-84	-	-	-	Rare	Rare	-	Rare	+
PAX5			+		+			+

+: Reactive, -: Non reactive, PNET: Primitive neuroectodermal tumor

Ewing's sarcoma/PNET, RMS, WT, desmoplastic round cell tumor.

6. Myogenic transcription factor
   - Help in the differentiation of mesenchymal progenitor cells to myogenic lineage and subsequent maintenance of the skeletal muscle phenotype.
7. PAX 5
   - PAX 5 is a member of the paired box transcription factors involved in the development and is expressed in hematopoietic malignancies of B-cell lineage
   - Expressed in cases of neuroendocrine carcinomas, urothelial tumors, Merkel cell carcinoma, glioblastoma, and neuroblastoma
   - Also positive for B-cell lymphoblastic lymphomas, WT and ARMS [Table 5].[9,18]

Conclusion

The ubiquitous distribution and diverse histology of different round cell tumors pose a challenge in their diagnosis. The diagnostic aids like the use of special stain, immunocytochemistry, flow cytometric immunophenotyping, and reverse-transcriptase polymerase chain reaction help to differentiate and diagnose these group of tumors. The early diagnosis of these tumors implicates the appropriate therapeutic modalities, including neo-adjuvant chemotherapy in advanced malignancy.

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Conflicts of interest

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