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The Use of Ultrastructure in the Diagnosis of Neoplasm

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General principles

I. Electron microscopy is time-consuming and expensive. In deciding whether a neoplasm is to be examined ultrastructurally for diagnostic purpose, pathologists should first consider whether there is a reasonable chance the technique will reveal diagnostically significant structures not discernable by light microscopy. We use electron microscopy most frequently to:

1) Distinguish poorly differentiated carcinomas from sarcomas and lymphoma. Basal lamina often surrounds clusters of epithelial cells and, although poorly differentiated carcinoma cells rarely display fully developed desmosomes or junctional complexes, primitive junctions between carcinoma cells are common. When they are present, desmosomes with tonofilaments are virtually diagnostic of carcinoma. Microvilli, sometimes associated with a terminal bar, are also characteristic of epithelial neoplasms. The presence of lumen is important to indicate the epithelial nature of cells. They may be intracytoplasmic or intercellular(Table 1). Sarcomas usually lack junctions between cells and if they are present they are typically poorly formed and sparse. Fine filaments concentrated along the periphery of the cell suggest mesenchymal differentiation. Lymphoma cells characteristically lack all of the above features.

Table 1. Intracytoplasmic Lumina(lined by rather stunted microvilli and containing amorphous material).

Breast Carcinoma Cells*	Adenomatoid tumor
Renal cell carcinoma	Gastric carcinoma
Mesothelioma	Benign breast disease

* Breast carcinoma is considered to have the endocytoplasmic lumina as a more constant feature.

2) Identify cytoplasmic structures which are associated with specific types of differentiation. These include, but are not limited to, membrane bound granules, melanosomes, filaments with contractile bodies. Crystals, keratohyaline granules, Weibel-Palade bodies, and mucin droplets. These are discussed further below. How often is electron microscopy needed for diagnosis? In our experience, in about 5% of neoplasms electron microscopy is essential for diagnosis, and in a further 10-15% it makes a significant contribution to the accuracy of the diagnosis. Because electron microscopy is time-consuming and because the equipment and supplies are expensive, it will not be freely available in all laboratories. When it is not, priorities need to be established to determine which cases should have ultrastructural examination. The first step in this process is to ask whether there is a reasonable chance the technique will reveal diagnostically significant structures which cannot be discerned by light microscopy. This decision can only be made after careful examination of light microscopic sections. Once it is decided electron microscopy is capable of providing an answer to a specific question, then the need for using the technique can be divided into three levels of urgency.

Priority 1: the neoplasm under study cannot be classified by light microscopic techniques, and only ultrastructural examination will permit precise classification. The differential diagnosis by light microscopy includes neoplasms which will be treated in different ways or neoplasms which have different ways or neoplasms which have different clinical behavior.

Priority 2: a neoplasm cannot be classified by light microscopy, but all the differential diagnostic possibilities

include neoplasms which are treated in similar ways or which have similar prognosis or both.

Priority 3: the diagnosis is certain by light microscopy, but ultrastructural confirmation is of interest. When there is limited access to electron microscopy, priorities of this sort are important. Ideally, priority 1 tumors should be examined by electron microscope even if tissue must be transported for some distance and/or removed from the paraffin block.

II. It is rare to find a single ultrastructural feature which is diagnostic of a single type of neoplasm. Rather, it is almost always the sum of the ultrastructural features together with the light microscopic findings which are useful. For example, Z-bands and Z-band material are a marker for skeletal muscle differentiation, but skeletal muscle differentiation is not limited to rhabdomyosarcoma, as it can be found in Wilms' tumor, hepatoblastoma, mixed Müllerian tumor, etc. Premelanosomes are characteristic of neoplastic melanocytes, but can be found in squamous cells, macrophages, and the glandular cells in Paget's disease. Cytoplasmic microfilaments are found both in myofibroblasts as well as in smooth muscle cells. Cell to cell junctions are present in many neoplasms as are "neurosecretory" granules. Weibel-Palade bodies are probably the only exception to this rule, since they are exclusively found in endothelial cells and endothelial cell neoplasms.

Assessment of Electron Micrographs

Electron micrographs should be examined in some sort of organized sequence. The features we evaluate are as follows:

a) cell contours and cell margin differentiation
b) intercellular junctions
c) cytoplasmic granules
d) cytoplasmic filaments
e) patterns of cytoplasmic organelles
f) nuclear features
g) basal lamina
h) stroma. These features as they relate to the diagnosis of poorly differentiated tumors and tumors of uncertain differentiation are discussed below:

1) In general, cell contours are of limited diagnostic usefulness; however, elongate interdigitating cell processes are most frequently found in adenocarcinomas, epithelioid sarcomas, meningiomas, neural tumors, and parathyroid adenomas. Pinocytotic vesicles are most prominent in perineural cells, endothelial cells, and smooth muscle cells. Intracellular lumina with microvilli are indicative of adenocarcinoma. Numerous long interwoven surface microvilli are characteristic of mesothelioma.

2) Because cell junctions are characteristic of epithelial cells and some mesenchymal cells, knowledge of the ultrastructural morphology of the attachments between cells, i.e., tight junctions, intermediate junctions, desmosomes, and primitive junctions is useful(Table 2). Most carcinomas upon which EM would be performed have primitive incompletely developed cell contacts varying from thickening to poorly formed junctional complexes. We prefer to label these incomplete cell junctions as "primitive cell junctions." The outstanding exceptions are poorly differentiated squamous cell carcinoma associated with pseudosarcomatous reaction. Well formed desmosomes with tonofibrils(cytokeratin filaments) may be found in squamous tumor cells not easily recognized as demonstrating squamous differentiation by light microscopy.

Table 2. *Differential Diagnosis of Desmosomes vs. Desmosome-like Junctions*

Desmosomes	Desmosome-like junctions
Parallel dense plaque in the opposing membranes	Parallel dense plaque as subplasmalemmal densities
Bundles of tonofilaments converging upon the plaque	Absence of detectable tonofilaments converging upon the plaque
Widened intercellular space of 25nm	The intercellular gap is unchanged or narrowed
Presence of dense material called intermediate line in the intercellular space	Absence of intermediate line

Intercellular junctions may be found between sarcoma cells, but they are unusual in the most commonly encountered sarcomas. Sarcomas regularly demonstrating cell junctions are neurosarcoma, endothelial sarcoma, synovial sarcoma, leiomyosarcoma, and epithelioid sarcoma. Junctions are not a feature of fibrosarcoma, malignant fibrous histiocytoma, or osteosarcoma. Other neoplasms which develop cell junctions are mesotheliomas, thymomas, sex cord stromal tumors of the ovary, and many central nervous system tumors. Occasionally melanomas will demonstrate cell to cell junctions.

3) Cytoplasmic granules and inclusions, the result of celluar secretion, are perhaps the most useful structures for identifying cellular differentiation ultrastructurally. Some of the common granules and inclusions helpful diagnostically and the tumors in which they may be found are listed in Table 3. None of these are specific for one type of tumor, and even the "neurosecretory granules" considered the hallmark of endocrine cells may be found occasionally in other tumors, such as adenocarcinoma. Moreover, neurosecretory granules morphology is not a reliable way to identify a specific type of endocrine neoplasm or its secretion product, since tumors may secrete ectopic hormones or may secrete more than one type of hormone. However, the granules of pheochromocytoma, insulinoma, and renin-secreting tumors are usually characteristic(Tabel 4). While Weibel-Palade bodies seem to be present only in endothelial cells, they are often absent in all except the most well differentiated vascular neoplasms. Pre-melanosomes, the marker for melanoma, come in several varieties: 1) the familiar oblong membrane bound structure with a striped core composed of transversely arranged parallel filaments. This is the only form we consider diagnostic of malignant melanoma in a poorly differentiated neoplasm. 2) helical structures with central zigzag coiled filaments; 3) small membrane bound granules containing electron dense amorphous granules(Table 5).

4) Cytoplasmic filaments useful for diagnosis come in two forms(Table 6).

Table 3. Cytoplasmic Granules and Inclusions of Diagnostic Importance

Type of granules	Tumors
Mucin(must be classic)	Adenocarcinoma
	Acinic cell carcinoma
Neurosecretory	Carcinoid and atypical carcinoid
	Neuroblastoma
	Medullary carcinoma of thyroid
	Pheochromocytoma(sometime "halo" granules)
	Oat cell carcinoma
Other endocrine granules	Various
Keratohyaline	Squamous cell carcinoma
Pre-melanosomes	Malignant melanoma and some other melanocytic tumors
Langerhan's granule	Histiocytosis and other conditions
Rhomboidal and elongated crystalloides	Alveolar soft part sarcoma
Weibel-Palade bodies	Neoplasms of endothelial differentiation
Immunoglobulins	Lymphomas with plasmacytoid differentiation

Table 4. Comparative Features of Secretory Granules for the Gastrointestinal and pancreatic Endocrine System.

Cell type	Endocrine hormone	Granule morphology	Histochemical reactivity with:
Alpha	Glucagon	200-400nm with round, black core and a gray halo.	Anti-glucagon
Beta	Insulin	250-350nm, round to oblong with a paracrystalline core and a clear halo.	Anti-insulin
Delta	Somatostatin	200-450nm, round, gray-black	Anti-somatostatin
G	Gastrin	200-400nm, round with variable electron-dense core	Anti-gastrin
S	Secretin	100-200nm, round or oblong with electron-dense core and narrow clear halo	－
I	Vasoactive Intestinal Polypeptide(VIP)	140-190nm, round and variable electron density	Anti-Vip

Cell Type	Endocrine Hormone	Granule Morphology	Histochemical Reactivity with:
EC(Enterochromaffin)	Foregut-5HTP[a]	150-250nm, uniformly round, with variable density	Argyrophil or nonreactive
	Midgut Serotonin	70-500nm, pleomorphic, uniformly dense	Argentaffin antiserotonin
	Hindgut-no secretion	154-235nm, round, granular with variable density	Nonreactive
Parafollicular	Calcitonin	200-400nm, dense-core secretory granules	anti-calcitonin
Adrenal gland	Epinephrine	200-270nm, large, round or elongated, medium density with particulate structure	−
	Norepinophrine	200-270nm, small, electron-dense granules lying in a vacuole	−

[a]5-hydroxytryptophane

Table 5. Melanin Granules

Stage I	No melanin present, small vesicles developed from Golgi apparatus
Stage II	Elongated vesicles with patterned membrane structures. Folded membrane forming a helical structure. There is no melanin deposit. Double membrane bound
Stage III	Presence of specks of melanin in the previous Stage II structure, double membrane bound
Stage IV	Electron-dense granules without discernible internal structures. Unit membrane bound; 0.3-0.7um

Table 6. Cytoskeleton Components

Class	Structure	Size	Cell type	Immunocytochemistry
Microtubule	Tubules	25nm	Almost all cells	Anti-tubulin
Neurotubule	Tubules	25nm	Neural cells	Anti-tubulin
Intermediate	Keratin(tonofilaments)	9-10nm	Epithelial	Anti-keratin
filaments	Desmin	10nm	Muscle cells	Anti-desmin
	Skeletin	10nm	Muscle cells	
	Vimentin	10nm	Mesenchymal cells	Anti-vimentin
	Neurofilaments	10nm	Nerve cells	Anti-neurofilaments
	Glial filaments	10nm	Glial cells	Anti-glial fibrillary acidic protein
Thin	Actin	5nm	Striated and smooth	Anti-myosin
filaments			muscle	Anti-myoglobin
			Smooth and skeletal muscle;	Anti-actin
			brush border of epithelial cells	

a) microfilaments(which are synonymous with myofilaments), and

b) intermediate filaments. The 6.0-7.0nm microfilaments (thin filaments) are mainly actin but a few are composed of myosin, while the 15.0 nm microfilaments(thick filaments) are composed of myosin. Bundles of disorganized thick and thin filaments, remnants of sarcomeres, and Z-bands and Z-band material are all diagnostic of skeletal muscle differentiation(skeletal muscle differentiation is not limited to rhabdomyosarcoma, but may be seen in other tumors). Caution must be taken to distinguish regenerating muscle fibers from neoplastic muscle cells.

Smooth muscle cells are characterized by the following:

1) parallel arrays of numerous 6.0-7.0nm filaments containing contractile bodies

2) dense attachments plaques along the cell membrane

3) basal lamina

4) pinocytotic vesicles. The more poorly differentiated the smooth muscle cell, the fewer of these structures will be found. Our Minimal criteria for smooth muscle differentiation is numerous microfilaments averaging 10.0nm in diameter comprise glial filaments as well as several different types of cytoskeletal filaments, such as tonofilaments, desmin, keratin, and filamin. Unless these filaments are arranged specifically such as Rosenthal fibers or tonofilaments in squamous cells, they cannot be subdivided by ultrastructural examination. However, antibodies against the protein in these cytoskeletal filaments have been developed, and are beginning to be used diagnostically. Antibodies against glial fibrillary protein are now a

standard technique for identifying central nervous system tumors and polyclonal antibodies against desmin, keratin, and filamin are being evaluated.

5) The arrangement of the type of organelle present in tumor cell cytoplasm is rarely of diagnostic help. Large numbers of tubules formed of smooth ER or concentric layers of smooth ER are features of steroid-producing cells as are mitochondria with tubular cristae, but such features are usually found in steroid-producing cells which are well differentiated enough to be recognized with the light microscope. Numerous mitochondria are the hallmark of oncocytes, but they are also present in a large number of other types of cells. To be sure a cell is oncocytic, mitochondria should be packed together or the mitochondria should be pleomorphic and contain lamelliform cristae. Stacks of parallel arranged endoplasmic reticulum, often in a circular profile, is characteristic of plasma cells, acinic cell carcinoma of the salivary gland, and lymphomas with plasmacytoid features. Annulate lamellae, more common in carcinomas and Sertoli-cell tumors, may be present in any neoplasm. Lysosomes of the phagolysosome type are particularly prominent in carcinomas of the thyroid and prostate and in some large cell lymphomas. Complexes composed of two or more organelles are of little or no diagnostic aid, but are often found in hairy cell leukemia, paraganglioma, and adrenocortical neoplasms.

6) The presence of basal lamina or basement membrane material, attributed to epithelial cells is not a specific by-product of them, but can be secreted by a variety of cell types like smooth muscle, skeletal muscle, Schwann cells, myofibroblasts, or even histiocytes. On the other hand, infiltrating ductal carcinoma with tubular pattern lacks basement membrane.

7) The presence of long spacing collagen in the stroma suggests neural tumor. Amyloid, bone, and cartilage and helpful stromal findings; otherwise, the stroma is not useful diagnostically.

Some Common Questions Which Diagnostic Electron Microscopy May Be Able to Answer:

1) Is it anaplastic carcinoma, lymphoma, or undifferentiated malignant neoplasm ?

Carcinoma	Lymphoma	Undifferentiated Malignant Neoplasm
Numerous cell to cell attachments, almost always primitive; rarely desmosomes; mucin	None; antileukocyte antigen commonly positive	None

2) Is it melanoma ?

Unequivocal premelanosomes of the striated type.
Attachments between cells rare

3) What type of small round cell tumor of childhood is this ?

Neuroblastoma	Rhabdomyosarcoma, Alveolar Embryonal	Undifferentiated and Ewings	Lymphoma, Leukemia
Neurosecretory granules concentrated in dendritic processes neurotubules Intermediate filaments 8-17nm(neurofilaments) Basal lamina absent	Glycogen; thick and thin filaments or Z-bands Basal lamina occas. present	Glycogen Basal lamina absent	Surface markers

Note: treatment groups are: 1) neuroblastoma; 2) rhabdomyosarcoma, Ewing's, and small cell undifferentiated;

3) lymphoma/leukemia. A combination of light microscopy, EM, and surface markers will allow placement into proper treatment groups.

4) Is this spindle cell neoplasm : carcinoma, leiomyosarcoma, neurofibrosarcoma, synovial sarcoma, or malignant fibrous histiocytoma ?

Carcinoma	Leiomyosarcoma	Synovial sarcoma
Numerous Primitive cell junction	At least numerous microfilaments with contractile bodies, sometimes cell margin plaques and pinocytotic vesicles	Spindled component has features of fibroblasts, occasional junctions can be found; when biphasic the glandular elements have epithelial features

MFH	Neurofibrosarcoma
None	Elongate, interdigitating cell processes Basal lamina

5) Is this an endocrine neoplasm ? Neurosecretory granules

6) What is this mediastinal tumor ?

Thymoma	Spindle cell Carcinoid(Thymic carcinoid)	Carcinoma
Desmosomes and tonofilaments Basal lamina	Neurosecretory granules	Numerous primitive and poorly formed cell junctions (Neurosecretory granules if oat cell)

Lymphoma	Germ cell tumor
None except nuclear projections	Variable EM findings

7) Is this an oat cell carcinoma ?
Neurosecretory granules, scattered junctions

8) Is this a smooth muscle or stromal uterine tumor ?

Smooth muscle	Stromal
As a minimum, numerous Microfilaments with contractile bodies	No specific differentiating features

9) Finally, in desperation, does this undifferentiated anaplastic neoplasm by light microscopy have any differentiating feature by EM ?

In most instances, a tumor undifferentiated by light microscopy will be undifferentiated by electron microscopy, but there are enough exceptions to deep many, including ourselves, examining such tumor on occasion.