Diagnostic Value of Electron Microscopy on Paraffin-Embedded Cytologic Material
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Transmission electron microscopy (TEM) is an important adjuvant to light microscopy, but is underutilized in cytopathology because of technical obstacles. One should attempt to obtain properly fixed material for TEM whenever possible. If that is not available, TEM on the cell block requires no preplanning or additional needle passes, and avoids problems of inadequate tissue for diagnosis. However, cell blocks are often not examined because of the perception that their poor ultrastructural preservation precludes their utility. We describe our experience in performing TEM on cell blocks from 15 cytologic specimens. In 13 of 15 cases, the cell block material was adequate for ultrastructural evaluation, and it clarified or extended the diagnosis in seven of these cases. TEM is a useful adjuvant technique to cytodagnosis and can be successfully performed on cell blocks when gluteraldehyde-fixed material is not available. Diagn Cytopathol 1993;9:282–290 © 1993 Wiley-Liss, Inc.

Key Words: Cell blocks; Ultrastructure; Cytology

Transmission electron microscopy (TEM) is a valuable diagnostic adjuvant to cytology. The literature describes various methods for processing cytologic material for diagnostic TEM. These approaches all require forethought and special preparation at the time of specimen procurement. There are several techniques available for performing electron microscopy (EM) on paraffin-embedded tissue and such methods are used for surgical pathology material. However, we are unaware of any reports describing the use of such techniques for cell blocks. From our own cases we illustrate the value of retrieving cytologic material from cell blocks for diagnostic TEM.

Materials and Methods

Only cases that had an accompanying cell block were eligible for the study. Archival material from our files, as well as current cytologic material, was used for our study. Two types of cases were chosen for the study: those that were a diagnostic dilemma, and those that might have distinguishing ultrastructural features demonstrable on the cell block preparation.

The cytologic material sent to our laboratory was preserved by adding an equal volume of fixative (Saccamano; Lerner Laboratories, Pittsburgh, PA) and spun at 2,000 rpm for 15 min. The pellet was smeared if judged grossly cellular or resuspended and used to prepare cytospins (Shandon Southern Instruments, Pittsburgh, PA) at 1,100 rpm for 4 min if the material was sparse. A cell block was prepared from the remaining material by resuspending the pellet in 10% buffered formalin and centrifuging at 2,000 rpm for 15 min. Sections from the cell block were stained with hematoxylin-eosin (H&E).

The H&E slides were reviewed to determine what area of the cell block to study and the corresponding area was cut out of the paraffin block with a razor. The tissue was deparaffinized in xylene for 2 hr, then rehydrated by placing in decreasing ethanol concentrations of 100, 95, 75, and 50% for 10 min each. The tissue was then placed in phosphate buffered saline (PBS) for 1 hr followed by 1% OSO4 (Electron Microscope Sciences, Fort Washington, PA) for 30 min. The tissue was washed three times in PBS for 5 min each, dehydrated in concentrations of 50, 75,
Table I. Ultrastructural Features Seen in Cell Blocks

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Source/history</th>
<th>Cytologic diagnosis</th>
<th>Ultrastructural diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Abdominal mass</td>
<td>Poorly differentiated CA vs. lymphoma</td>
<td>Too necrotic to assess; no diagnosis possible; lymphocytes and unidentifiable cells</td>
</tr>
<tr>
<td>2</td>
<td>Abdominal mass</td>
<td>CA vs. sarcoma</td>
<td>Poorly preserved carcinoma; golgi; RER; vacuoles; abundant cytolasm; cell jxns; no desmosomes</td>
</tr>
<tr>
<td>3</td>
<td>Pancreatic mass, ZE syndrome</td>
<td>Islet cell tumor</td>
<td>Well preserved neuroendocrine tumor; epithelial/endocrine cells: rounded nuclei; rich in organelles; dense core secretory granules</td>
</tr>
<tr>
<td>4</td>
<td>Liver mass</td>
<td>Hepatocellular CA; Poorly differentiated</td>
<td>Poorly preserved anaplastic tumor; irregular nuclei; abundant euchromatin; nucleoli prominent, irregular with vesicles; high N/C ratio</td>
</tr>
<tr>
<td>5</td>
<td>Liver mass in patient with cecal mass on BE. Colon primary?</td>
<td>Metastatic CA; Favor colon primary</td>
<td>Poorly preserved carcinoma (S/O of GI primary); irregular nuclei; prominent nucleoli; closely apposed cells; desmosomes; no mucin (but poorly preserved)</td>
</tr>
<tr>
<td>6</td>
<td>Liver mass</td>
<td>Hepatocellular CA; Well differentiated</td>
<td>Moderately well-preserved C/W hepatocellular carcinoma; full of mitochondria, RER, SER</td>
</tr>
<tr>
<td>7</td>
<td>Liver mass. No other documented lesions</td>
<td>Poorly differentiated CA; Metastatic small-cell CA?</td>
<td>Poorly preserved neuroendocrine carcinoma; high N/C ratio; sparse organelles; membrane-bound granules</td>
</tr>
<tr>
<td>8</td>
<td>Lung mass</td>
<td>Bronchoalveolar carcinoma</td>
<td>Moderately well preserved; C/W, but not diagnostic of bronchoalveolar carcinoma; intracellular lipid; alveolar pattern; pleomorphic nuclei; no myelinosomes identified</td>
</tr>
<tr>
<td>9</td>
<td>Pleural effusion</td>
<td>Adenocarcinoma vs. mesothelioma</td>
<td>Moderately preserved adenocarcinoma; glandular arrangement; few cytoskeleton fibrils; short microvilli around lumen; cytoplasm rich in organelles</td>
</tr>
<tr>
<td>10</td>
<td>Pleural effusion</td>
<td>Adenocarcinoma vs. mesothelioma</td>
<td>Well-preserved mesothelioma; abundant long microvilli around periphery of cell; bundles of cytoskeleton fibrils</td>
</tr>
<tr>
<td>11</td>
<td>Peritoneal effusion; known history of mesothelioma</td>
<td>Mesothelioma</td>
<td>Well-preserved mesothelioma; groups of cells without lumens; organelles not abundant; numerous long microvilli around periphery of cells</td>
</tr>
<tr>
<td>12</td>
<td>Adrenal mass</td>
<td>Lymphoma</td>
<td>Poorly preserved lymphoma; relatively regular, oval nuclei; prominent nucleoli; separate cells; scant cytoplasm with mostly ribosomes, occasional mitochondria</td>
</tr>
<tr>
<td>13</td>
<td>Retroperitoneal mass</td>
<td>Malignant neoplasm; favor sarcoma, not lymphoma</td>
<td>Poorly preserved lymphoma; small single cells; immature nuclei; limited cytoplasmic organelles; normal adrenal medulla cell identified</td>
</tr>
<tr>
<td>14</td>
<td>Pleural effusion; history of amelanotic melanoma</td>
<td>Spindle cell tumor; C/W metastatic melanoma</td>
<td>Poorly preserved spindle cell tumor; spindle cells; no melanosomes found; neutrophils; monocytes; fibrin</td>
</tr>
<tr>
<td>15</td>
<td>Lung mass</td>
<td>Carcinoid tumor</td>
<td>Well-preserved carcinoid tumor; nests of cells contain numerous dense core neurosecretory granules</td>
</tr>
</tbody>
</table>

CA = carcinoma; RER = rough endoplasmic reticulum; jxns = junctions; ZE = Zollinger Ellison; N/C = nuclear/cytoplasmic; BE = barium enema; S/O = suggestive of; GI = gastrointestinal; C/W = consistent with; SER = smooth endoplasmic reticulum.
and 95% ethanol for 5 min each, and then three times in 100% ethanol for 8 min each.

The tissue was infiltrated with spurr (Electron Microscope Sciences) for 30 min with a 1:1 dilution of spurr to alcohol followed by 30 min in a 1:3 (alcohol to spurr) dilution, and then a 1-hr incubation in pure spurr. The tissue was placed in fresh spurr in beem capsules (Electron Microscope Sciences) and placed in a 70°C oven overnight.

Three-micron-thick sections were cut with a glass knife and stained with Toluidine blue. Thin sections (600–1000 Å) were cut using a diamond knife, then placed on a copper grid and stained with 5% uranyl acetate in 50% ethanol for 10 min followed by lead citrate for 5 min. Sections were examined and photographed by TEM (Zeiss EM 10).

Results
Table I lists the 15 cases chosen for this study. Cases 1–8, 12, 13, and 15 were fine-needle aspirates (FNA); cases 9 and 10 were pleural fluids; and case 11 was a peritoneal fluid. Cases 1, 2, 4, 5, 7, 9, 10, and 13–15 were diagnostically difficult, and TEM was used to characterize the lesions better. TEM was performed on cases 2 and 15 at the time of the initial evaluation to extend the cytologic diagnosis. Cases 3, 6, 8, 11, and 12 were diagnostically obvious

Fig. 1. Liver aspirate: undifferentiated tumor cells (Pap stain, ×400).

Fig. 2. A few dense core neurosecretory granules (arrows) confirm the diagnosis of metastatic small cell carcinoma (uranyl acetate and lead citrate stain, ×40,000).
by light microscopy and were studied retrospectively only to assess preservation of ultrastructural features after cell block preparation.

In seven of the 10 diagnostically challenging cases, TEM clarified or extended the diagnosis. Case 2 was an aspirate of an abdominal mass in a 76-yr-old female. The mass was so poorly differentiated that it was unclear whether the tumor was a carcinoma or a sarcoma. The mucicarmine stain was negative and the immunoperoxidase stains against epithelial membrane antigen (EMA), low and high molecular weight keratins, carcinoma embryonic antigen (CEA), actin, desmin, and S-100 protein were negative. Only stains for vimentin showed reactivity. TEM, although poorly preserved, demonstrated abundant cytoplasm, cytoplasmic vacuoles, golgi, rough endoplasmic reticulum (RER), and junctions (although no desmosomes). These ultrastructural features were more indicative of a carcinoma than a sarcoma. The patient died 1 mo later with no further biopsies.

In case 5, TEM helped confirm the cytologic impression. The liver aspirate was on a patient with a cecal mass on barium enema. For unclear reasons the liver mass was aspirated before the cecal mass was biopsied. The cytologic features demonstrated an adenocarcinoma in which a colon primary was favored. TEM also suggested a gastrointestinal primary by the close apposition of the cells and the presence of desmosomes. Rootlets and mucin were not seen in this poorly preserved specimen. A subsequent hemicolectomy revealed adenocarcinoma.

In case 7, TEM was extremely helpful in clarifying the diagnosis. The patient was a 71-year-old male with a liver mass and no known primary lesion. The clinicians were strongly entertaining the diagnosis of hepatocellular carcinoma. CT scans of the lung and abdomen were reportedly negative. Fine-needle aspiration of the liver revealed an undifferentiated neoplasm that resembled small-cell carcinoma (Fig. 1). TEM confirmed the diagnosis by revealing scattered membrane-bound granules (Fig. 2). A follow-up liver core biopsy also demonstrated small-cell anaplastic carcinoma.

TEM extended the diagnosis of a pleural effusion (case 9) by demonstrating cytoplasm rich in organelles and an absence of long microvilli suggestive of adenocarcinoma rather than mesothelioma. A follow-up right neck lymph node biopsy revealed necrotic tumor, and a lung mass was identified on chest radiograph. The patient died without an autopsy but one can presume the patient had a primary lung carcinoma.

Abundant long microvilli around the periphery of the cells and bundles of cytoskeletal fibrils characteristic of mesothelioma were evident in the papillary groups from the case 10 pleural fluid (Figs. 3 and 4). A subsequent pleural biopsy was interpreted as mesothelioma. The patient died and no autopsy was performed.

Case 13 was an FNA biopsy on a retroperitoneal mass from a 51-year-old mentally retarded man. The cell block was retrieved from archival material for this study. The tumor involved both the retroperitoneum and abdomen. The cytologic diagnosis was: “Scant material. Malignant tumor. Not lymphoma, favor sarcoma” (Fig. 5). Immunoperoxidase staining for vimentin and epithelial and melanoma markers were negative. Leukocyte common antigen was not performed at the time. No other material for diagnosis was obtained and the patient died shortly after the cytologic diagnosis was made. The material was scant, but what was present was retrieved from the cell block and scanned by TEM. Poorly preserved single cells with immature nuclei and limited cytoplasmic organelles highly suggestive of malignant lymphoma were seen (Fig. 6). In addition, a few cells with mature nuclei and dense core secretory granules probably representing adrenal medulla were identified as part of the normal cellular component from the retroperitoneum. Background debris in the cytologic material resembling cytoplasm may have accounted
for the impression that the tumor was not a lymphoma. No further diagnostic procedures were performed and the patient died without an autopsy.

Case 14 was a pleural fluid diagnosed as “spindle cell neoplasm consistent with metastatic malignant melanoma” in a 75-year-old female with a history of amelanotic melanoma and new onset of bilateral lung lesions. The immunoperoxidase stains were inconclusive. Unfortunately, no melanosomes were identified by TEM as a result of either poor preservation or sampling artifact. Follow-up surgical biopsy of the lung showed conclusive metastatic malignant melanoma.

Case 15 was a lung aspirate of a coin lesion that had not changed for 1 yr. The radiographic impression was a hamartoma. Cytology revealed small uniform cells in a ribbon pattern suggestive of carcinoid tumor (Fig. 7). Immunoperoxidase stains for cytokeratin, neuron specific enolase, chromogranin, and Leu-7 were positive. EM was done prospectively on the cell block and confirmed the diagnosis by revealing dense core neurosecretory granules (Fig. 8). We have no additional follow-up on this patient.

In cases 3 and 6, the diagnosis was made by light microscopy but the ultrastructural features were well preserved even after cell block preparation. Case 3 was a pancreatic islet cell tumor with many dense core secretory granules (Figs. 9 and 10). Case 6 was a well-differentiated hepatocellular carcinoma in which the cells were full of mitochondria, RER, and smooth endoplasmic reticulum (SER), characteristic of these cells.

In cases 8 and 12, the diagnosis was made by light microscopy. TEM agreed with the diagnoses of bronchoalveolar carcinoma and lymphoma, respectively, but was not able to extend or add to the diagnoses. Although rudimentary myelin figures were seen, classic myelinosomes were not identified in the bronchoalveolar carcinoma.

In two of the seven cases, a diagnosis was not possible by TEM. Case 1 (poorly differentiated carcinoma vs. lymphoma) was uninterpretable because of necrosis and sampling error. Case 4 (poorly differentiated hepatocellular carcinoma) was poorly preserved. Anaplastic features such as irregular, enlarged nuclei, abundant euchromatin,
TEM on paraffin-embedded material

and prominent nucleoli were demonstrated but no other differentiating features were identified.

Only five of our cases, 3 and 6, and 9–11, were well preserved or moderately well preserved. These were all well-differentiated neoplasms. However, all but two cases were interpretable despite poor preservation.

Discussion

TEM is recognized as an important adjuvant to light microscopy in tumor diagnosis, but most of its applications have been in surgical pathology. Even recent articles have described the usefulness and cost effectiveness of TEM in FNA biopsy, but many cytotology laboratories underutilize this technique. Immunohistochemical stains have eliminated the need for TEM in certain cases, but the staining may be equivocal or their sensitivity and/or specificity insufficient to increase diagnostic accuracy.

An important reason for the underutilization of diagnostic TEM in cytopathology is the technical obstacles. Obviously properly fixed material should be obtained for TEM whenever possible. Direct injection of aspirated material into standard EM fixative at the time of the procedure is the ideal method, but is not always practical. First, the aspirator must have the forethought to submit the material. It is often only after examination by light microscopy that the need for TEM is realized. In the lung, where there is a concern about pneumothorax after serial aspirations, one is reluctant to repeat aspirations routinely for gluteraldehyde fixation. A number of combined EM/FNA studies have reported a high percentage of specimens as having inadequate material for diagnosis. A pathologist is often grateful to get enough material for a light microscopic diagnosis and may be reluctant to give up additional material for EM. These same obstacles occur in surgical pathology, but there is usually more tissue to work with.

Methods for examining formaldehyde-fixed and paraffin-embedded tissues by TEM have been developed. Although it is preferable by far to use optimal techniques, experienced electron microscopists are familiar with the artifacts of formaldehyde fixation and paraffin embedding. Despite poor preservation, many ultrastructural markers
Fig. 7. Lung aspirate: carcinoid tumor with small uniform cells in a ribbon-like pattern (Pap stain, $\times 400$).

Fig. 8. Carcinoid tumor: numerous dense core neurosecretory granules (uranyl acetate and lead citrate stain, $\times 4,600$).

Fig. 9. Pancreatic islet cell tumor (Pap stain, $\times 200$).
of cellular differentiation can still be identified for diagnostic purposes. We are unaware of any reports describing the use of TEM on paraffin-embedded cell block preparations from FNA biopsies. Cell blocks should be particularly suitable for diagnostic TEM. A retrospective and prospective study by Wang and Minassian evaluated paraffin-embedded tissues for diagnostic TEM. They found that paraffin embedding caused little alteration of the fine structure of well-fixed tissues regardless of the initial fixative used. However, poorly preserved tissues appeared to be altered further by paraffin embedding. Formaldehyde forms less strong and stable bonds between proteins and other molecules than does gluteraldehyde. Membranes and loose structures already damaged at the time of fixation are particularly vulnerable to the dehydrating and clearing agents used in paraffin embedding. Since FNA biopsies are generally fixed immediately and penetration by fixative is optimal, cell blocks on these specimens might be even better than surgical blocks for TEM retrieval.

We were surprised by how much information TEM yielded on our cell block preparations. Ultrastructural detail was better preserved in the well-differentiated lesions, but we were able to obtain useful information even in poorly differentiated tumors, and only two cases were uninterpretable. The dense core granules in cases 3, 7, and 15 were beautifully preserved, as were the microvilli in cases 10 and 11. In seven of 15 cases, the ultrastructural features were helpful in further characterizing the lesions. In one case, a possible diagnostic error was identified in which lymphoma was suspected by TEM but not by cytology. If TEM had been performed at initial diagnosis, a different conclusion may have been reached. Note that if there is a significant discrepancy between TEM and immunoperoxidase findings, it should be resolved by properly processed TEM. Serious mistakes can be made by relying on a tiny sample which is poorly preserved.

There is often reluctance to perform TEM on cell block preparations, perhaps for fear that it will be a waste of
time and money. It is preferable to submit material in gluteraldehyde at the time of specimen procurement, but in cases in which properly fixed material is not available, TEM on cell blocks can be rewarding by providing additional information that might clarify the diagnosis or eliminate the need for further diagnostic procedures.

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References