นิพนธ์ต้นฉบับ

The role of electron microscopy in the diagnosis of spindle cell tumors

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The electron microscopic features of selected cases of spindle cell tumors which were also observed by light microscopy are described. With the transmission electron microscope, various organelles such as lipid inclusions, cytoplasmic fine filaments, basal lamina, desmosomes, myofilaments, and secretory granules which are related to the specific nature of the tumor cells were directly visualized. Such ultrastructural features are useful in distinguishing between the types of spindle cell tumors. The definite diagnosis is important because each tumor has an entirely different outcome. However, we want to emphasize the necessity of correlating the ultrastructural findings with the clinical data, as well as gross and light microscopic features.

Key words: *Spindle cell tumors, Spindle cell lipoma, Fibrous mesothelioma, Rectal leiomyosarcoma, Small-cell neuroendocrine tumor, Electron microscope.*

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ได้บรรยายลักษณะต่าง ๆ ของเซลล์เนื้องอกชนิด สปินดัลเซลล์ โดยกล้องจุลทรรศน์อิเลคตรอน อาทิเช่น ไลปิด, เส้นไยฟิลาเมนท์, เบซาลลามินา,เดสโมโซม,มัยโอฟิลาเมนท์ และแกรนูลของสิ่งคัดหลั่ง ซึ่งช่วยในการแยกชนิดเฉพาะของเนื้องอกในกลุ่มนี้ เพื่อให้ได้การวินิจฉัยที่ถูกต้องเนื่องจากเนื้องอกแต่ละชนิดมีการพยากรณ์โรคแตกต่างกัน อย่างไรก็ตามคณะผู้รายงานยังคงเน้นถึงความจำเป็นในการผสานข้อมูลทั้งจากกล้องจุลทรรศน์ ข้อมูลทางคลินิค ลักษณะเนื้องอกที่เห็นด้วยตาเปล่า และการตรวจด้วยกล้องจุลทรรศน์อิเลคตรอนเข้าด้วยกัน

There are several different types of spindle cell neoplasms and the type is often difficult to verify on the basis of light microscopy or immunohistochemistry. During the past decade the technology involved in electron microscopy (EM) has rapidly progressed and this instrument has now become an important tool in many fields of medical science, particularly in providing diagnostic pathology. The high magnification and the capability to visualize various cellular organelles undoubtedly will allow us to identify the specific tumor types by disclosing information regarding the cytoplasmic structures which can not be detected by other routine procedures. The pathological approach to spindle cell tumors adopted in our laboratory is now based on the use of EM while light microscopy, in conjunction with immunohistochemistry, still remains the mainstay in tumor diagnosis. The purpose of this presentation is to display the role of EM in obtaining an accurate diagnosis of various types of spindle cell neoplasms which have occurred in various organs. However, the case selection here is not intended to be a comprehensive coverage of all types of neoplasms.

Materials and Methods

One each of the spindle cell tumors was obtained from the stomach, peritoneum, rectum, and cervix of patients during a period of one year (January to December 1994). The clinical records of these patients were studied after microscopic review of the samples. The surgical specimens were fixed in 10 per cent formalin, embedded in paraffin and stained with hematoxylin and eosin (H & E), phosphotungstic acid hematoxylin, Mayer's mucicarmine, and Gomori's silver impregnation for reticulin fibers. Sections of the paraffin-embedded tissue were further processed by the avidin-biotin-complex method using antibodies to S-100 protein, vimentin, keratin, neurofilament, neuronal specific enolase (NSE), actin, desmin, and chromogranin. Additionally, portions of tissues were washed, refixed in buffered 2.5 per cent glutaraldehyde solution, embedded in epoxy resin, and prepared for electron microscopy.

Results

The clinical data, pathological findings and immunohistochemical features are given in Table 1. Most tumors were solid, well circumscribed, often yellow-gray or pink-white and firm in consistency. Only the cervical mass in case 4 was friable. Microscopically, all of the tumors consisted of spindle cells which were arranged in sheet, parallel and palisade patterns (Fig 1-4). The neoplastic cells in case 4 also showed trabeculae and perivascular pseudorosettes. Mitoses were not seen in case 1, infrequent in case 2, but prominent in cases 3, and 4. Immunohistochemistry demonstrated the positive immunostaining for S-100 protein, vimentin in case 1, the positive reaction for vimentin in cases 2 and 3, and the positivity for keratin, and chromogranin in case 4. Ultrastructurally, various important cytoplasmic structures of the tumor cells were observed. In case 1, the spindle cells closely resembled a fibroblast (Fig 5 A). They were, however, surrounded by a continuous basal lamina. Several neoplastic cells displayed many intracytoplasmic lipid inclusions (Fig 5 B). A dilated endoplasmic reticulum was occasionally observed. The collagen filbrils were noted in the extracellular spaces. These cells were regarded as spindle-cell lipoma of the gastric wall. EM studies of the neoplastic spindle cells in case 2 revealed intracytoplasmic fine filaments,a prominent rough endoplasmic reticulum, and the presence of basal lamina, and desmosomes (Fig 6). Microvilli were rarely noted. The final pathological diagnosis in this example was solitary fibrous mesothelioma of the peritoneum. The fine structure of the tumor cells in case 3 was characterized by elongated cells with deep clefted nuclei and bundles of myofilaments (Fig 7). Dense cytoplasmic bodies and pinocytotic vesicles were observed as well as intercellular junctions. The basal lamina was incomplete. This was interpreted as a leiomyosarcoma of the rectum. The important ultrastructure in case 4 was the presence of dense-core secretory granules of 120-250 mm (Fig 8). Additionally, the tumor cells were united with desmosomes. Such features were regarded as small-cell neuroendocrine tumors of the cervix.

Table 1. Clinical and pathological features of spindle cell tumors.

Case No.	AGE (yrs) Sex	Location	Size (cm)	Symptoms and signs	Microscopic findings	Immunohistochemisty
1	60 F	Body of Stomach	6×4	Hematemesis, melena	Interlacing fascicles. No mitoses	Vimentin + S-100 protein +
2	58 F	Peritoneum	12	Abdominal mass	Bundles of spindle cells. Mitotic count 0-2/HP	Vimentin +
3	62 F	Rectum	7.5×7	Rectal bleeding	Interlacing fascicles, numerous mitoses	Vimentin +
4	27 F	Cervix	6×4	Vaginal bleeding and cervical mass	Hypercellularity of Small cells with hyper-chromatic nuclei, perivascular rosettes, many mitoses	Chromogranin + Keratin +

Note. F = female

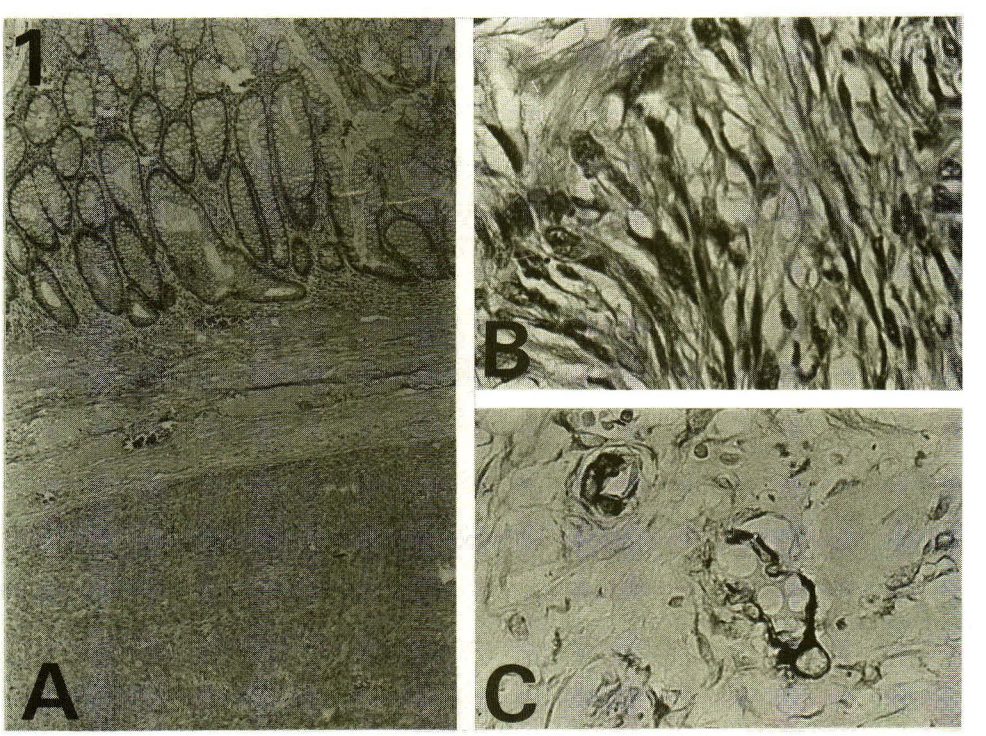

Figure 1. A. Photomicrograph of spindle cell lipoma showing interlacing pattern. Note the intact mucosa of stomach (case 1). H & E × 100
B. Higher-power view demonstrated vacuolated cells. H & E × 400
C. Positive immunostain for S-100 protein. Avidin-biotin × 400

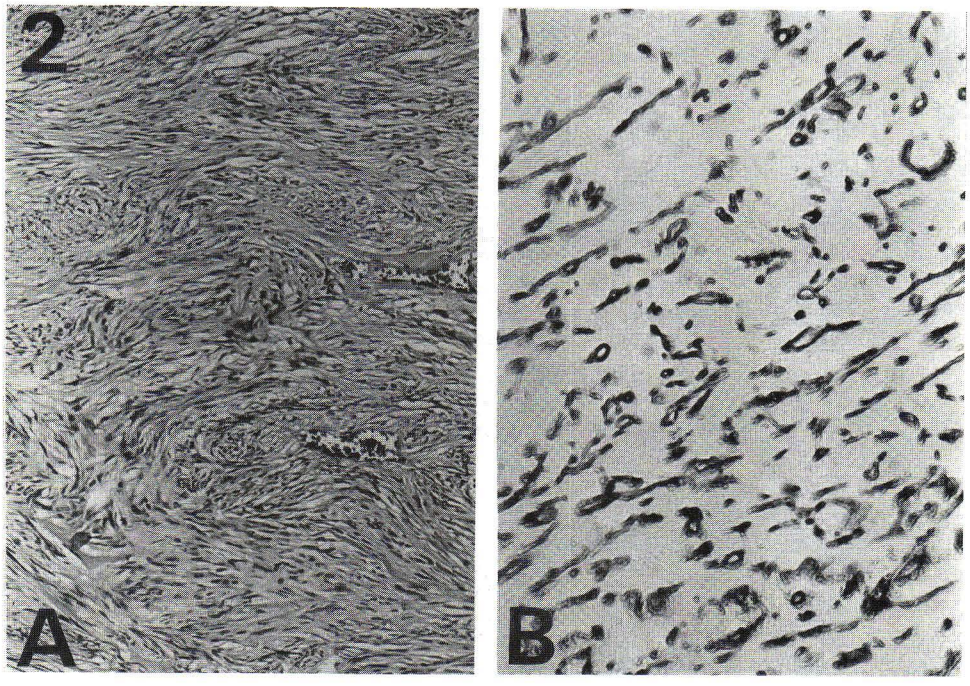

Figure 2. A. Microscopic feature of fibrous mesothelioma showing bundle of spindle cells (case 2). H & E × 200
B. Vimentin-positve cells are noted. Avidin-biotin × 200

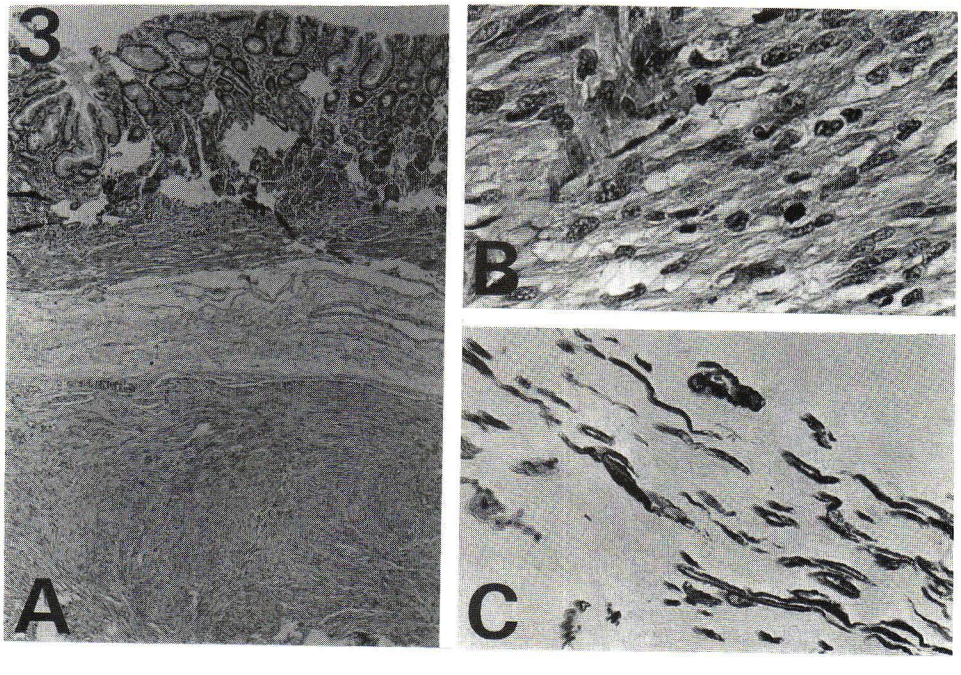

Figure 3. A. Leiomyosarcoma from rectum showing interlacing fascicles (case 3). H & E × 100
B. Higher-power view shows mitotic figures. H & E × 400
C. Immunoreactivity for vimentin. Avidin-biotin × 400

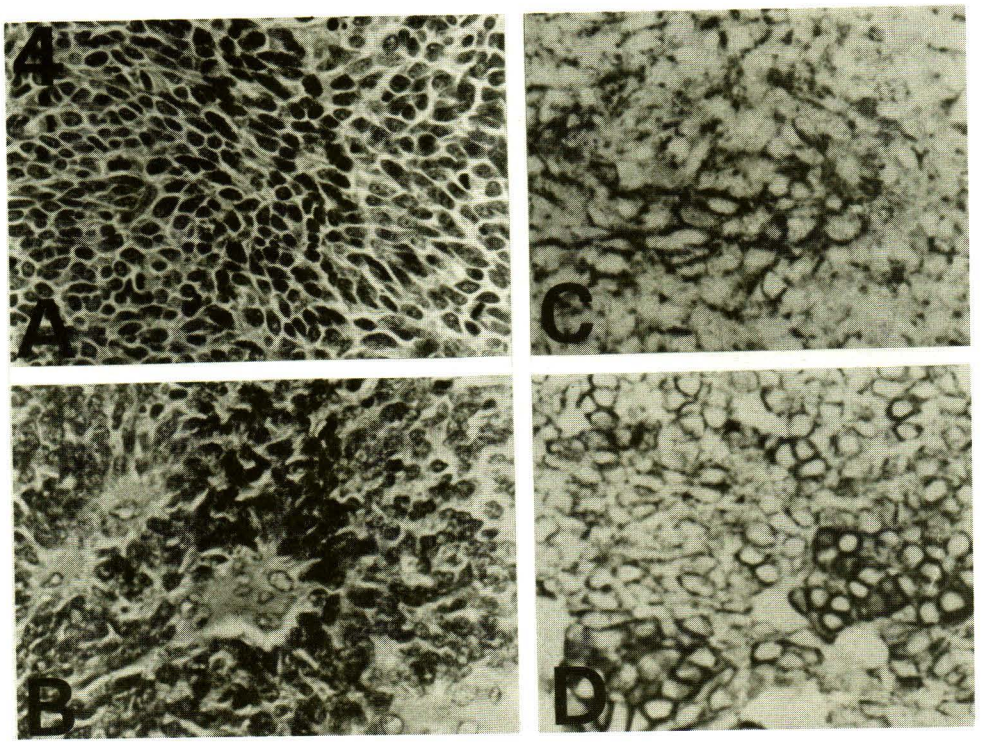

Figure 4. A. Photomicrograph of small-cell neuroendocrine tumor from the cervix. H & E × 200
B. Perivascular rossettes. H & E × 200
C. Chromogranin-positive cells. Avidin-biotin × 400
D. Keratin-positive cells. Avidin-biotin x 400

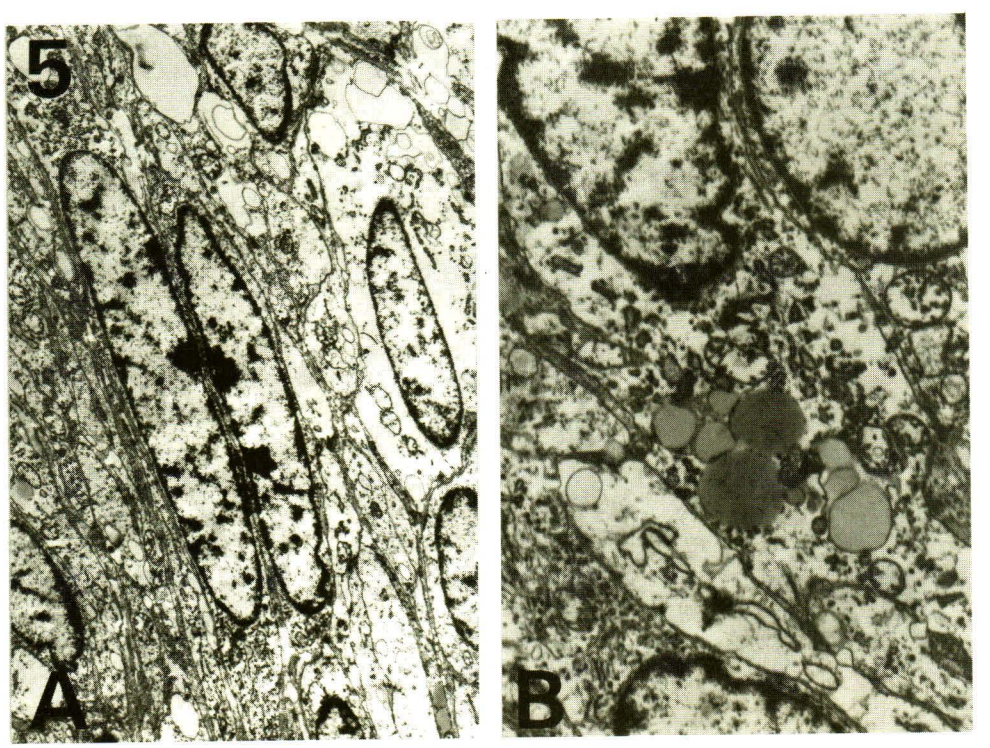

Figure 5. A. Electron micrograph of spindle cell lipoma showing elongated cells resembling fibroblasts which are surrounded by continuous basal lamina. × 4,500
B. Many intracytoplasmic lipid inclusions are noted. × 9,000

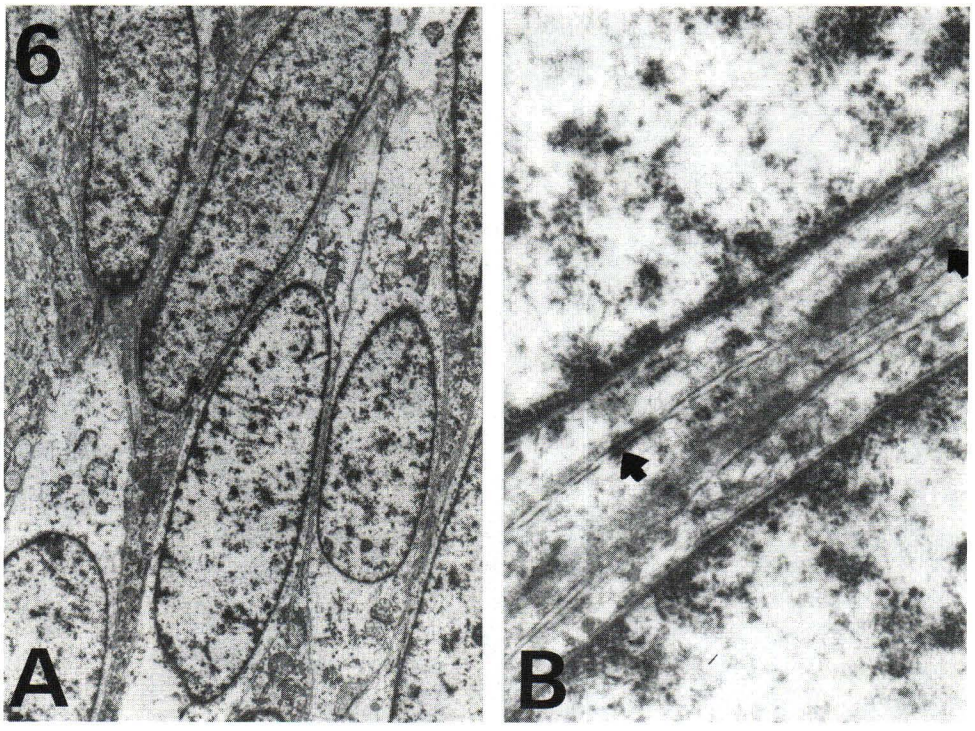

Figure 6. A. Fibrous mesothelioma showing spindle cells with a paucity of organelles. × 4,500
B. Higher-power view showing intracytoplasmic fine filaments and many desmosomes (arrows). × 30,000

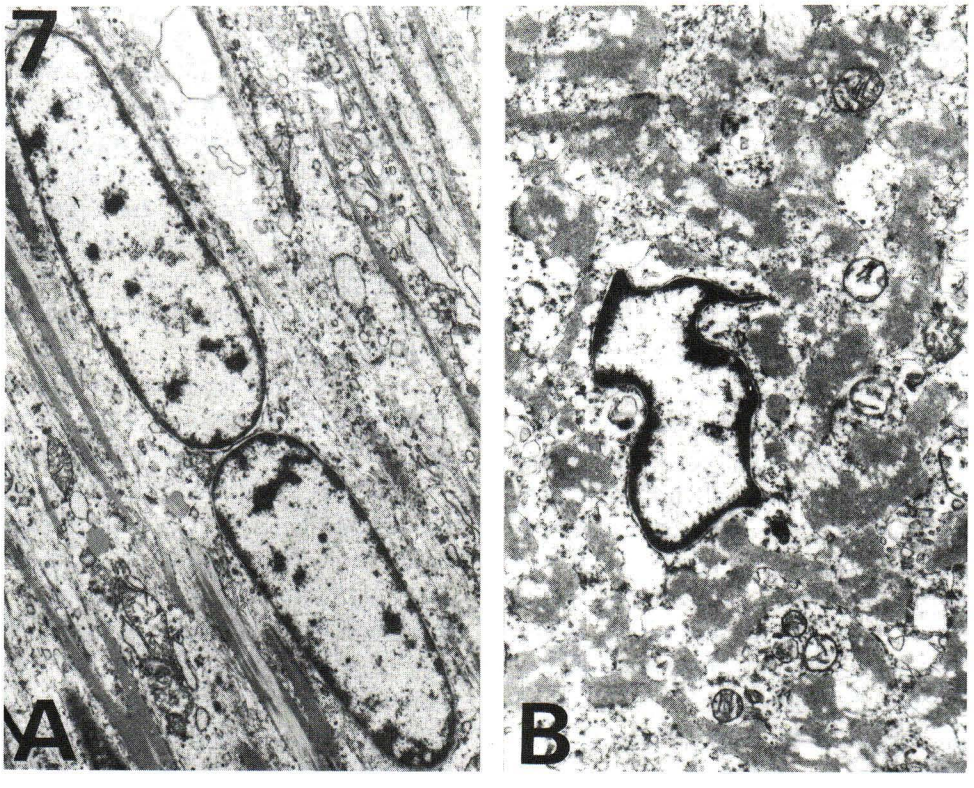

Figure 7. A. Leiomyosarcoma of rectum with characteristic myofilaments. X 6,000
B. Cross-section of myofilaments. × 9,000

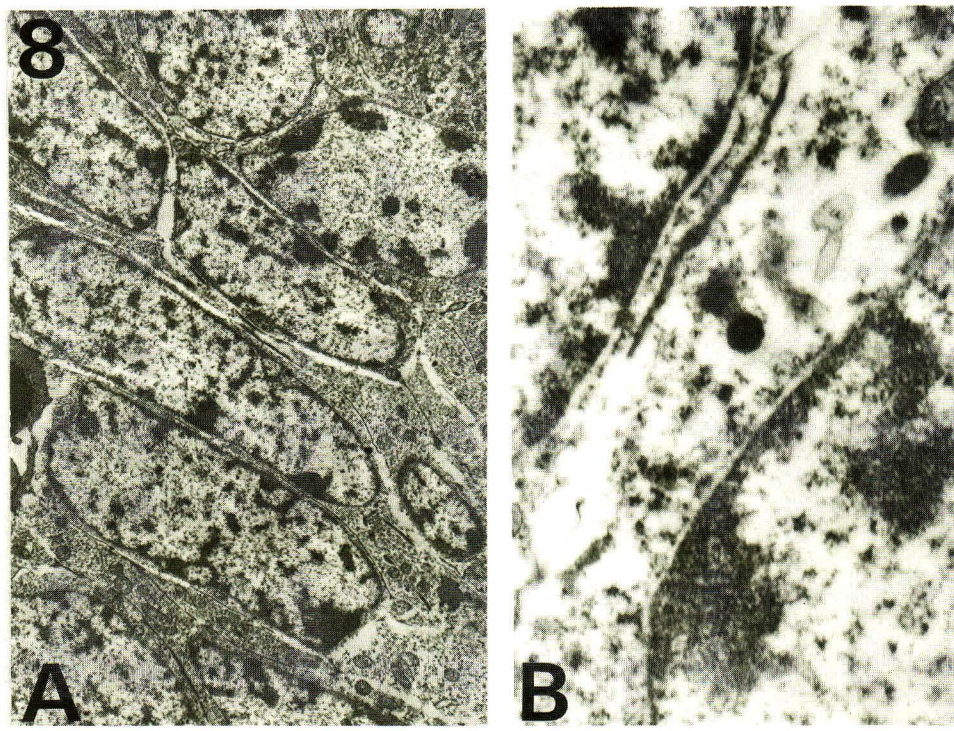

Figure 8. A. Small-cell neuroendocrine tumor showing spindle cells with dispersed chromatin and large nuclei. × 4,500
B. Dense-core secretory granules are shown. × 30,000

Discussion

The cases selected for illustration here displayed various types of spindle cell tumors which appeared similar when examined by morphological features by routine procedures. The basic nature was not readily identified at the light microscopic level. Although immunohistochemistry procedures has greatly facilitated the identification of cell products or surface markers, some tumor identities may not be apparent by this method. For example,vimentin, the predominant intermediate filament of mesen-chymal cells, is less specific because cettain epithelial tumors may coexpress both keratin and vimentin.[1] "False" negativity is an another factor which can limit the role of this technique. For instance, desmin which is specific for a neoplasm showing muscle differentation was not apparent in our patient with leiomyosarcoma.[1] On the other hand, we have clearly demonstrated that transmission EM, is a valuable methodology for direct identification of certain specific cells which are essential in distinguishing between the types of spindle cell neoplasms. A definite diagnosis is extremely necessary because each tumor has an entirely different outcome.

Generally, lipoma is a benign tumor that occurs in virtually every tissue but it is rarely found in the stomach.[2] Clinically, gastric lipoma is an important lesion for physicians because it may simulate malignancy and it causes massive gastrointestinal bleeding, abdominal pain, and obstruction.[2,3] Pathologically, the tumor is composed of masses of mature fat cells. The finding of spindle cell lipoma of the stomach in our example is unusual in our experience and we are not aware of its previous description.

Although few cases of gastric lipoma with adjacent adenocarcinoma of the stomach have been described, these are regarded as coincidence.[4]

Peritoneal mesotheliomas are uncommon tumors particularly the solitary fibrous type seen in our patient. According to Stout, there were only 25 cases of the fibrous type in a series of 114 peritoneal mesotheliomas the majority being either the papillary or mixed types.[5] Pathologically, the solitary fibrous mesothelioma can be easily mistaken by both surgeons and pathologists for one of the more common neoplasms of the abdomen. The ultrastructural findings of microfilaments and intercellular junctions aid in correct diagnosis.[6] This type of mesothe-

liomas, unlike the highly malignant diffuse type, occurs as solitary encapsulated mass as seen in our tumor.

Leiomyosarcoma of the rectum is of course a rare entity and accounts for less than 0.1% of colorectal malignancies.[7] About 150 cases have been described in the literature and most often are clinically apparent in patients aged between the fifth and sixth decades. It has rarely been described in children and infants.[8,9] The tumors occur predominantly in men. The usual clinical features are rectal bleeding and alterations in bowel habit as noted in our example. The prognosis is related to resectibility and the size of the tumor, as well as the histological-grade of the lesion.[10] Hence the gross and histological findings are important to determine the outcome of the patients while EM aids in diagnosis.

Histologically, small-cell neuroendocrine carcinoma simulates a number of cervical cancers such as lymphoma, stromal sarcoma, or metastatic diseases. The positivity of chromogranin, keratin and the demonstration of dense-core neurosecretory granules, as well as desmosomes, provides the definite diagnosis.[11] Most authors have considered this type of cervical cancer as an aggressive neoplasm often known with a fatal outcome.[11,12]

It should be noted that in this study we have limited our focus to only the ultrastructural features of various types of spindle cell tumors. Such a valuable role for EM can be easily expanded for the distinction of various other diseases in human beings. It is thus doubtless that EM is an essential tool for us in routine diagnostic pathology. However, we do not want to leave the false impression that such diagnoses can be made only with EM. For clinicians and pathologists, it is still necessary to correlate the ultrastructural findings with the clinical information, gross appearance and microscopic findings so as to arrive at an accurate tissue diagnoses and to provide proper management.

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