Unusual Intracytoplasmic Inclusions in Metastatic Carcinoma*

Discussion of their Possible Significance

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SUMMARY
Unusual filamentous inclusion bodies in the cytoplasm of metastatic tumor cells are described. Their presence (intermingled with zymogen granules) seems rather restricted to cells of primary or metastatic acinar cell carcinoma of the pancreas, acinic carcinoma of salivary gland (parotid gland) and Paneth cells (neoplastic or in zinc deficiency state). For the time being, the real nature of these inclusions (deranged zymogen granules?) is unclear.

This case also emphasizes the value of electron microscopy in solving the problem of the occult primary tumor and avoiding the misdiagnosis of an endocrine tumor (e.g. islet cell tumor or carcinoid), or a duct cell tumor with eosinophilic granular cytoplasm or in recognizing the foci of acinar cells in a mixed variant of carcinoma of the pancreas.

Introduction
Correct pathological diagnosis is the most important cornerstone in patient therapy and management. However, metastatic tumors of occult primary origin is a repeatedly occurring challenge for the clinician or pathologist. In the last decade enormous progress has been achieved in resolving those diagnostic problems with the advent of immunohistochemistry, but the results are not always straightforward or conclusive. In this paper we present and discuss the diagnostic value and significance of peculiar cell organelles in metastasis detected by electron microscopy.

Results

Histopathology
On light microscopy the lymph node was almost completely replaced by predominantly solid cords and sheets of tumor cells with eosinophilic granular cytoplasm and centrally located mildly pleomorphic nuclei with small single nucleoli (Fig. 1). Mitoses were numerous. A glandular or ductular growth pattern was noted in different areas (Fig. 2). Alcian blue and mucicarmine stains were slightly positive in the lumina but intracellular mucin was never detected. Small PAS positive granules resistant to diastase digestion could be observed in scattered individual neoplastic cells. There was no necrosis nor lymphocytic infiltrate in the metastatic areas.

Immunohistochemistry
The cytoplasm of numerous tumor cells exhibited positive staining for polyclonal cytokeratin (Fig. 3).
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Fig. 5. An intense diffuse cytoplasmic reaction (arrows) could be observed for muramidase. × 410.

CAM 5.2 was generally more positive on the apical cell membranes than in the cytoplasm (Fig. 4). No immunoreactivity was identified for vimentin, NSE, CEA, PSA, S-100 protein, chromogranin and synaptophysin. Muramidase was locally strongly positive in the cytoplasm (Fig. 5). EMA decorated especially the apical sides of the tumor cells comparable to CAM 5.2 (Fig. 4).

Electron Microscopy

On ultrastructural examination, dark and clear neoplastic cells showed mostly a tubular arrangement and exhibited inter- as well as intracellular luminal spaces with numerous apical blunt microvilli. Each tumor cell contained a large number of homogenous electron-dense spherical granules resembling zymogen granules that were often crowded within the apical cytoplasm and under the lateral cell membranes (Fig. 6). In addition, many cells displayed variable numbers of pleomorphic but mostly oval or slightly curved inclusions with electron-dense fine filamentous internal structure with no apparent periodicity (Fig. 7). These inclusions were not or only partly surrounded by a membrane, and lay free in the cytoplasm outside the parallel arrays of well-developed rough endoplasmic reticulum (Fig. 8) and between the numerous mitochondria. Cells with endocrine features were not identified.

Discussion

When these electron microscopical findings were presented years ago as a case for the panel in Ultrastructural Pathology6 we suspected metastases of a poorly differentiated adenocarcinoma with zymogen granules and abundant developed rough endoplasmic reticulum. Therefore, the primary site or origin was considered to be acinar cells of the pancreas, of the salivary gland or of Paneth cells in the intestine. At that time, and to the best of our knowledge, the association of filamentous inclusions or bodies, which constitute the second most important component and finding in the tumor cells of our case, with acinar cell carcinoma was never explicitly reported. Several studies have recently described pleomorphic, membrane bound inclusions that contain filaments in this respect: nine cases of acinar cell carcinoma of the pancreas11,18, in cases of malignant mixed exocrine-endocrine tumor of the pancreas8,12, in the neoplastic Paneth cells of a carcinoma of the ascending colon but not in the adjacent non-neoplastic Paneth cells19 and in altered zymogen granules in acinar cells of human parotid glands17.

For those who have written-off the discipline of TEM as a diagnostic technique (for discussion see Erdland 199411), this current case proves the contrary. Ultimately, the ultrastructural analysis of the metastasis of a clinically occult primary tumor provided the final or prompt diagnosis of an unusual acinar cell carcinoma. In addition, on the basis of the partition and the polarity and size of the zymogen granules within the tumor cells, the distinction from mixed acinar-endocrine carcinoma (MAEC), mixed pancreatic tumor with ductal differentiation and mucin production, islet cell tumor, or carcinoid, can be made by the electron microscopical study11,12,16,14,8. Moreover, filamentous inclusions, as found in our case, have not been identified in the latter two forms of tumors19.
× 18,700

Electron micrograph showing the internal structure of a part of a mucous cell. The cell displays many nerve endings and fine structural features (arrows) are connected by short, fine processes. The apical luminal (L) side of the glandular structure is shown. The microscopic appearance of part of a mucous cell is also shown.

Pig. 6. Electron micrograph.
The significance and genesis of the dense fibrillar inclusions has been a subject of debate. One could speculate that the polymerization of proteins form either filamentous or crystalline structures of various resident or secretory long-chain molecules (proteins or mucin) lying free in the cytoplasm. Many examples of such secretory products have been described in various normal and neoplastic tissues. If so, this would cast doubt on the specific character of these inclusions. The assumption of crystallized proteins seems less probable if, as stated by Tucker et al. and Hassan et al., the inclusions are totally or at least partly membrane bound. There is no indication that the structures are located within the cisternae of the rough endoplasmic reticulum. Do they represent products of degeneration (e.g., of keratin filaments) or perhaps the products of an abnormal synthesis of keratin and keratohyaline as is suggested for the filamentous bodies described in squamous cell carcinoma? Tucker et al., with well-illustrated ultrastructural findings, stress the suggestion of an apparent transition from zymogen granules to those inclusions, or that the latter represent abnormal or deranged zymogen granules. The latter opinion is favoured by the rare labeling of the large irregular fibrillar granules with a chymotrypsin antibody as shown by immuno-gold electron microscopy. On the other hand, how could this explain the view of the great variability in size or form of the bodies observed in different cells? The filamentous instead of lamellar or microtubular ultrastructure on cross-sections clearly demonstrated by Tucker et al., speaks against the origin of so-called angulate lysosomes (formerly called the angulate bodies) as originally described by Dingemans et al. As it is known, the angulate bodies are an integral part of the ultrastructural appearance of so-called granular cell tumors and their presence is of major importance in the diagnosis of this tumor. In our case there is no indication of metastases of a granular cell tumor as the primary tumor.

Unfortunately, autopsy was not permitted in our case excluding a more thorough examination of the pancreas. Theoretically, we should consider intestinal Paneth cells as the second best possibility for the cell of origin of this malignant tumor. This presumption stems from the fact that alongside zymogen type granules, neoplastic Paneth cells can contain bizarre cytoplasmic fibrillar inclusions. Moreover, their similarity to those associated with zinc deficiency or zinc metabolism disorder has been reported in normal intestinal Paneth cells in cases of acrodermatitis enteropathica in (metaplastic?) Paneth cells in chronic inflammatory bowel disease (e.g., Crohn’s disease) and in experimental zinc deprivation. In our patient there was no direct clinical evidence of abnormal zinc metabolism. No further examination in this concern was performed after the diagnosis of metastatic disease was made.
Materials and Methods

A 65-year-old man was presented with vague pain in the right upper abdomen for approximately one month. Palpation, x-ray examination and computed tomography revealed a large retroperitoneal mass measuring 10 × 13 cm localized behind the stomach and duodenum and infiltrating the mesentery, right colon and inferior vena cava; serum lipase: 90 (nl. value: 10–190 U/l); amylase: 115 (nl. value: max. 65 U/l). At surgery, this tumor appeared to be irresectable. The pancreas was described as normal intraoperatively. A biopsy was taken of one of the enlarged para-caval lymph nodes. The patient died shortly after the operation. Autopsy could not be performed.

Tissue obtained at surgery was fixed in 10% neutral buffered formalin and embedded in paraffin for light microscopy. Sections were stained with hematoxylin-eosin, periodic acid-Schiff (PAS) with and without diastase pretreatment, mucicarmine and alcian blue. Immunohistochemical staining was performed on the sections of the same paraffin-embedded material for polyclonal cytokeratin, CAM 5.2, vimentin, CEA, NSE, PSA, S-100 protein, EM A, chromogranin, synaptophysin and muramidase. Small fragments were fixed in glutaraldehyde (2.5%), postfixed in osmium tetroxide (1%) and procesed routinely for electron microscopy. The ultra-thin sections were stained with uranyl acetate and lead citrate and examined using a Siemens Elmiskop 101.

References


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