Bronchial Carcinoid Tumor With Crystalloid Cytoplasmic Inclusions

Karen L. Grogg, MD; Chandrashekar Padmalatha, MD; Kevin O. Leslie, MD

- We report a bronchial carcinoid tumor with distinctive, cytoplasmic, rod-shaped crystalloid inclusions that were visible by light microscopy. These cytoplasmic structures were immunoreactive with antibodies against chromogranin A and synaptophysin in paraffin-embedded tissue. Ultrastructural studies showed them to be paracrystalline in nature and located within lysosomes. This case highlights an interesting, and potentially confusing, histologic manifestation in an otherwise typical bronchial carcinoid tumor.

(March Pathol Lab Med. 2002;126:93-96)

Since the initial description of bronchial carcinoid tumors by Hamperl in 1937, many histologic variations have been described, including a diversity of architectural forms, such as insular, trabecular, rosette-forming, pseudoglandular, solid, and papillary. Despite this growth variability, most of these tumors have in common cytological bland, round cells with stippled chromatin and a moderate amount of granular eosinophilic cytoplasm. 2-5

The granularity of the cytoplasm in carcinoid tumors in most cases is related to the presence of neurosecretory granules, although rare cases of oncocytic carcinoids with an abundance of mitochondria have been reported. 2-5 We describe an endobronchial tumor displaying the characteristic cytoplasmic granularity of carcinoid tumor, but additionally containing cytoplasmic eosinophilic crystalloid inclusions visible on light microscopy, which led to diagnostic confusion. Immunohistochemical and ultrastructural studies were used to further elucidate the nature of these structures.

REPORT OF A CASE

A 54-year-old white woman was discovered to have an endobronchial mass during workup for a pleural effusion. The etiology of the pleural effusion was thought to be nephrotic syndrome. Additional significant medical history included insulin-requiring diabetes mellitus, diabetic nephropathy, nonalcoholic steatohepatitis, obstructive sleep apnea, and systemic hypertension. She had been a smoker previously.

Bronchoscopy revealed an endobronchial mass in the left upper lobe bronchus. Initial frozen section analysis of a biopsy of this mass was considered to be consistent with carcinoid tumor; however, the diagnosis was questioned when permanent hematoxylin-eosin-stained sections highlighted the presence of unusual crystals in the cytoplasm of the tumor cells. The crystalline structures were believed to be reminiscent of Auer rods. A consultation at our institution, including immunohistochemical study, concluded that this mass represented a carcinoid tumor containing unusual cytoplasmic crystalline structures.

Subsequent left upper lobe resection in April 1999 demonstrated the tumor to measure 1.1 x 0.8 x 0.3 cm. Aortopulmonary window lymph nodes were negative for tumor. The patient recovered from lobectomy uneventfully and has had no recurrence of disease after 1 year of follow-up.

MATERIALS AND METHODS

Case Material

The hematoxylin-eosin–stained microscopic slides of the transbronchial biopsy were obtained in consultation at Mayo Clinic in Scottsdale, Ariz, for confirmation of diagnosis. At the time of lobectomy, tissue was obtained by the primary pathologist (C.P.) and fixed in glutaraldehyde for electron microscopic studies.

Immunohistochemical Studies

The following immunohistochemical stains were performed on a representative block of formalin-fixed, paraffin-embedded tissue using the avidin-biotin immunoperoxidase method: keratin AE1/AE3 (Roche, Indianapolis, Ind, clones AE1 and AE3, 1:400), CAM 5.2 (Becton-Dickinson, San Jose, Calif, clone CAM 5.2, 1:50), chromogranin A (Roche, clone LK2H10, 1:1000), synaptophysin (ICN, Costa Mesa, Calif, clone SY38, 1:40), neuron-specific enolase (Dako Corporation, Carpinteria, Calif, polyclonal, 1:1000), S100 (Dako, polyclonal, 1:800), HMB-45 (Dako, clone HMB-45, 1:100), neurofilament (Dako, clone 2F11, 1:75), and κ and λ light chains (Dako, polyclonal, 1:4000 each).

Electron Microscopy

Glutaraldehyde-fixed tissue fragments were embedded in Spurrs resin, sectioned at 1 μm, and stained with toluidine blue to assess tissue adequacy. Ultrathin sections were mounted on grids, counterstained with lead citrate, and examined using a Philips CM12 transmission electron microscope (Eindhoven, The Netherlands).

RESULTS

Histopathologic Findings

The endobronchial tumor displayed morphologic features characteristic of carcinoid tumor, with small, round, monomorphic cells arranged in nests, ribbons, and rosettelike structures. Nuclei were round, centrally placed, and had finely stippled chromatin with indistinct nucleoli. Cytoplasm was abundant, eosinophilic, and finely granular. The majority of tumor cells displayed large, eosinophilic, rod-shaped crystalloid structures within their cytoplasm. 

Accepted for publication June 8, 2001.

From the Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minn (Dr Grogg); the Department of Pathology, Memorial Hospital of Carbondale, Ill (Dr Padmalatha); and the Department of Pathology, Mayo Clinic Scottsdale, Scottsdale, Ariz (Dr Leslie).

Reprints: Karen L. Grogg, MD, Department of Pathology, Mayo Clinic, 200 First St SW, Rochester, MN 55905.

Arch Pathol Lab Med—Vol 126, January 2002 Bronchial Carcinoid Tumor—Grogg et al 93
Figure 1. A, Section showing typical morphology of carcinoid tumor. Linear arrays of darker eosinophilic crystals can be seen within otherwise granular tumor cell cytoplasm (hematoxylin-eosin, original magnification ×400; insert, original magnification ×800). B, Luxol fast blue-periodic acid-Schiff stain highlights crystalloids in tumor cell cytoplasm (original magnification ×400). C, Synaptophysin immunohistochemical stain decorates many of the cytoplasmic crystalloid structures, along with more diffuse punctate staining of the cytoplasm (original magnification ×400). D, One-micrometer-thick section stained with toluidine blue shows prominent cytoplasmic crystalloids (original magnification ×400).

toplasm, easily visible on hematoxylin-eosin stain by light microscopy (Figure 1, A). The crystalloids appeared randomly arranged in the tumor cell cytoplasm and were not found in the adjacent nonneoplastic cells.

Immunohistochemical Findings

Immunohistochemistry on paraffin-embedded tissue demonstrated a profile consistent with carcinoid tumor. The neoplastic cells reacted with antibodies against cytokeratin (AE1/AE3 and CAM 5.2) in a cytoplasmic distribution sparing the crystalloid structures. Inclusions were further highlighted by staining with the Luxol fast blue-periodic acid-Schiff method (Figure 1, B). The neuroendocrine immunohistochemical markers (chromogranin, synaptophysin, and neuron-specific enolase) showed cytoplasmic staining, including the crystalloid structures themselves (Figure 1, C). Staining with toluidine blue dramatically highlighted the inclusions (Figure 1, D). No immunoreactivity was seen with antibodies directed against S100 protein, HMB-45, neurofilament, or κ and λ immunoglobulin light chains.

Electron Microscopic Findings

The tumor cells were nonciliated, cuboidal, and frequently arranged in nests. In some areas, tumor cells were arranged around lumina with short apical microvilli. Abundant small, round, electron-dense neurosecretory granules were present in the tumor cell cytoplasm, some having the characteristic dense core morphology of these granules with surrounding halo and discernible single-layer membrane. In addition, larger membrane-bound vacuoles were identified, approximately 1.0 to 1.5 μm in diameter. The contents of these vacuoles were overall slightly less electron dense than the contents of the neurosecretory granules, and were inhomogeneous with occasional lipid droplets and electron-dense debris, morphologically consistent with secondary lysosomes. Some of the vacuoles contained crystalloid structures of varying sizes, ranging from diamond-shaped crystalloids approximately 200 nm in diameter to large rods measuring approximately 8 μm in length and 1 μm in diameter (Figures 2 and 3). An internal latticelike paracrystalline structure was discernible in the cross sections of some crystalloids.

Additional features of the tumor cells included moderately abundant mitochondria, stacks of rough endoplasmic reticulum, and polyribosomes.

COMMENT

The ultrastructural features of carcinoid tumors, at all sites of occurrence, have been extensively studied in the past because of the diagnostic significance of neurosecretory granules.8 The presence of these characteristic granules has been used to place neoplasms in the category of neuroendocrine tumors. Moreover, neurosecretory granule abundance has been used to further subclassify tumors within the spectrum of differentiation that exists in this category.8,9 Despite the numerous published reports on carcinoid ultrastructural morphology, to our knowledge, the unique crystalloid inclusions identified in this case have not been reported previously.

The presence of the crystalloid inclusions in this case led to diagnostic uncertainty, since this histologic manifestation has not been documented previously in carcinoid tumor. The possibility of extramedullary myeloid cell tumor was entertained on the original small biopsy owing to the resemblance of the inclusions to Auer rods. The growth pattern and immunohistochemical findings excluded this diagnosis. Another classic, albeit rare, tumor with crystalline inclusions is alveolar soft part sarcoma. Other entities in the differential diagnosis for oxyphilic endobronchial lesions include granular cell tumor, salivary gland-type tumors, paraganglioma, and metastases from thyroid or renal neoplasms.

The case we describe displayed histologic features and
immunohistochemical phenotype diagnostic for carcinoid
tumor. The tumor cells were positive for immunohisto-
chemical markers documenting the neuroendocrine differ-
entiation of the neoplasm. In addition, electron-dense neu-
rosecretory granules were demonstrated on ultrastructural
analysis.

The exact nature of the crystallizing material in the cells
of this carcinoid tumor is unclear. Absence of reactivity
for antibodies directed against S100 protein, HMB-45, and κ
and λ light chains argues against the crystallizing ma-
terial being composed of melanin or immunoglobulin pro-
teins. It is plausible that it represents some component of
the neurosecretory product, supported by immunoreactiv-
ity with antibodies directed against neuroendocrine mark-
ers (chromogranin and synaptophysin).

Crystalline inclusions, thought to be composed of pro-
teins or lipoproteins, have been found in a variety of cel-
lar compartments, including the nucleus, mitochondria,
endoplasmic reticulum, and cytoplasm of mammalian
cells. An intramitochondrial “paracrystalline” inclusion
was reported in a bronchial carcinoid tumor once previ-
ously by Ghadially and Block, in the setting of oncocytic
transformation. These inclusions were described as
“sheaves of filaments” in a curvilinear whorling arrange-
ment, unlike the straight rodlike crystalloids of the case
reported here. The cells of the current case contained
moderately abundant mitochondria, which along with the
crystalloids and lysosomes, contributed to its oxyphilic
granular appearance on light microscopy. However, the
cytoplasmic inclusions we describe appeared to be present
within lysosomes in some cells, rather than in the mito-
chondrial compartment. Smaller crystalloid structures
could be seen within vacuoles that also contained lipid
and electron-dense debris, consistent with secondary lys-
osomes.

Crystalline inclusions occurring in lysosomes have been
documented rarely within endothelial cells in the setting
of active angiogenesis, such as in fetal tissue and hem-
angiomas. More commonly, the crystals found in lys-
osomes are composed of inert material, such as metals or
minerals. Presumably, crystalline or paracrystalline inclu-
sions are composed of proteinaceous material that one
would expect to be subject to attack by acid hydrolases
present in the lysosome. As has been suggested regarding
lysosomal inclusions in other cells, the site of crystalli-
zation in this instance may be another cellular compart-
ment, prior to its fusion with a primary lysosome. Alter-
natively, the crystalloid structures could be in the process
of degradation within the lysosomes.

In summary, the illustrated case highlights an interest-
ing histologic feature in an otherwise typical bronchial
carcinoid tumor. In the setting of small transbronchial bi-
opsy specimens of oxyphilic tumors, for which the differ-

Figure 2. Carcinoid tumor cell containing electron-dense, rod-
shaped, crystalloid structures and dense core neurosecretory granules
(original magnification ×5000).

Figure 3. Paracrystalline internal structure of the cytoplasmic struc-
tures seen at higher magnification with adjacent dense core granules
(original magnification ×45 000).
ential diagnosis may also include granular cell tumor, salivary gland–type tumors, paraganglioma, and metastases from thyroid or renal neoplasms, awareness of the variant histologic possibilities may be critical in making a diagnosis with confidence. Recognition of rod-shaped crystalloid inclusions within a proven bronchial carcinoid tumor is therefore a useful addition to the pathologist’s diagnostic armamentarium.

The authors thank Ricardo Lloyd, MD, PhD, for reviewing the case and manuscript. We also thank the Electron Microscopy Laboratory, Mayo Clinic Rochester, and the Immunohistochemistry Laboratories of Mayo Clinic Rochester and Mayo Clinic Scottsdale for their invaluable technical support.

References