THE HISTOGENESIS AND DEVELOPMENT OF PULMONARY TUMORLETS

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A lung, which was surgically removed from a patient who had oat cell carcinoma, contained multiple tumorlets and showed extensive Kultschitsky-type cell proliferation of bronchial and bronchiolar mucosa. On the basis of light and electron microscopic observations, it is shown that pulmonary tumorlets arise from focal areas of bronchial and bronchiolar Kultschitsky cell proliferation which may advance to luminal obliteration. Involvement of the pulmonary parenchyma takes place by extension of these newly proliferated cells along the terminal branches of the bronchiolar tree or by penetration of the bronchial or bronchiolar wall; the latter process evokes a striking proliferation of connective tissue which forms the matrix in which the cells of some fully developed tumorlets are embedded. Because of striking morphologic similarities between tumorlets and spindle cell carcinoid tumors, and the proven origin of tumorlets from pulmonary Kultschitsky-type cells, it is suggested that the more complete and histogenetically acceptable term "carcinoid tumorlet" be used to distinguish this lesion from other forms of epithelial proliferations in the lung.


**THE TERM “PULMONARY TUMORLET” WAS coined by Whitwell** for a minute epithelial lesion which is usually encountered as an incidental finding during microscopic examination of the lungs. Although this lesion has been interpreted as early carcinoma, more recent observers have considered it a benign proliferative process. Its light microscopic resemblance to carcinoid tumor was noted years ago and is reflected by Liebow’s reference to this lesion as “carcinoid atypical proliferations.” Recent electron microscopic studies have identified dense-core secretory granules within the cells of tumorlets, confirming its cytologic kinship to the carcinoid tumor. Although it is logical to assume that tumorlets arise from pulmonary Kultschitsky-type cells, this has not been conclusively demonstrated. The present study documents the genesis of tumorlets from bronchial and bronchiolar Kultschitsky-type cells and illustrates the various stages in the evolution of these lesions.

Case Report

A 61-year-old woman was noted to have a radiologically visible left pulmonary mass while being investigated for labile hypertension. The patient was asymptomatic except for a mild early morning cough. She had a smoking history of 1-1/2 packs of cigarettes daily for the past 40 years. Physical examination was unremarkable; blood pressure was 140/80 mm Hg. Bronchoscopy and bronchial brushings and washings revealed no abnormality; the left scalene lymph nodes were biopsied and showed no remarkable changes. A percutaneous needle biopsy of the mass showed the histologic features of oat cell carcinoma. Bone marrow biopsy, bone scan, liver scan and a computed tomographic brain scan were all within normal limits. A left pneumonectomy was performed; surgery was complicated by a wound infection and Staphylococcal empyema which responded to antibiotic therapy and drainage.

Four months after surgery, the patient developed liver metastases and clinical and biochemical evidence of Cushing’s Syndrome. Her condition deteriorated rapidly and resulted in her death two months later.

Materials and Methods

Light Microscopy

The lung was fixed in 10% phosphate-buffered formalin and tissue for light microscopy was prepared in the usual manner. Sections were stained with hematoxylin and eosin, elas-
tic-van Gieson, alcian blue, aldehyde fuchsin, Congo red, Gomori-Burtner argentaffin stain, Grimelius argyrophil stain, and Bodian's silver stain.

**Electron Microscopy**

After the lesions described below were discovered, two tumorlets and a bronchus and a bronchiole showing Kulitschtsky cell proliferation were excised from the paraffin blocks. These fragments of paraffin-embedded tissue were processed through xylene, decreasing concentrations of alcohol and then transferred to 0.2 M sodium cacodylate buffer (pH 7.4). Tissue for electron microscopy was also obtained directly from the formalin-fixed pulmonary tissue. Detailed examination of the lung revealed a 2 mm diameter pale, subpleural tumorlet; in addition, small bronchi and bronchioles were excised from the upper lobe with the help of a dissecting microscope. These fragments of tissue were fixed in a mixture of 2% paraformaldehyde and 2.5% gluteraldehyde buffered in 0.2 M sodium cacodylate (pH 7.4), post-fixed in 2% osmium tetroxide, counterstained with uranyl acetate, dehydrated in graded alcohols and embedded in Epon. The areas to be further studied were selected from toluidine blue stained 1 μm thick Epon sections. Thin sections were cut with a diamond knife and examined with a Hitachi HU 11 A electron microscope.

**Pathologic Findings**

**Gross**

The specimen consisted of the left lung which weighed 270 g. The pleural surface was unremarkable except for a single pale, firm, 2 mm diameter tumorlet which was found in the subpleural parenchyma of the upper lobe. Dissection of the bronchial tree showed a 2.5 cm diameter pale, soft neoplasm situated in the hilar region of the upper lobe. Two hilar lymph nodes were enlarged and replaced by tumor tissue. The remainder of the pulmonary parenchyma was grossly unremarkable.

**Light Microscopy**

Microscopy of the pulmonary neoplasm and lymph node metastases showed the features of oat cell carcinoma. Sections from other areas of the lung revealed 12 tumorlets in addition to the one observed grossly; they were all located in the upper lobe. In addition, the mucosa of many of the bronchioles and smaller bronchi unassociated with tumorlets showed increased numbers of "basaloid" cells which were interpreted as Kulitschtsky-type cells and later confirmed as such by electron microscopy. These cells were small and contained scanty eosinophilic cytoplasm with poorly demarcated cell membranes. The nuclei were most often oval or spindle in shape with a finely dispersed stippled chromatin pattern and a delicate nuclear membrane. Nucleoli were difficult to discern and mitoses were absent. These cells were located in the basal portion of the mucosa with the long axes most frequently oriented parallel or oblique to the long axis of the bronchus or bronchiole. They most often formed plaque-like lesions from one to several cell layers thick (Fig. 1) but occasionally there was focal nodular proliferation (Figs. 2,3). Where this Kulitschtsky cell proliferation was marked, they produced large plaques or polypoidal structures which narrowed or obliterated the bronchial or bronchiolar lumina. The surface epithelium overlying areas of Kulitschtsky cell proliferation showed varying degrees of atrophy (Fig. 3) and although these surface cells were markedly attenuated in areas, distinct mucosal ulceration was not seen.

A very striking connective tissue reaction was present within the walls of many of the bronchi and larger bronchioles harboring Kulitschtsky cell proliferation; fibroblasts, acid mucopolysaccharide and collagen, accompanied by a variable lymphocytic infiltrate and capillaries, were present between the epithelium and bronchial and bronchiolar elastic lamina. In addition, many of these foci contained masses of finely fibrillar elastic tissue which contained scattered fibroblasts (Fig. 4); this material stained positively with elastic van-Gieson (Fig. 1) and aldehyde fuchsin but did not stain with Congo red. The remarkable aspect of this connective tissue reaction was its segmental nature and its very definite relationship to areas of mucosal Kulitschtsky cell proliferation.

The pulmonary tumorlets varied in size from one high power field to 2 mm in diameter and were most often composed of clusters of oval or spindle cells which had cytologic characteristics similar to the Kulitschtsky cells described in the bronchial and bronchiolar mucosa. However, the peripheral cells of some clusters were cuboidal and showed a tendency to palisading and in one lesion, the cells were cuboidal and arranged in ribbons. In most tumorlets, these cells were centered around a bronchus or bronchiole which showed mucosal Kulitschsky cell proliferation, and with serial sections continuity between the mucosal Kulitschsky cells and the
extra-mucosal clusters of cells could be demonstrated.

Three main types of pulmonary tumorlets were observed. The first type was related to terminal bronchioles. In addition to the intraluminal proliferation of Kulitschitsky-type cells in the bronchiole, clusters of cells were present in the walls of adjacent respiratory bronchioles, alveolar ducts and alveolar septa (Fig. 5). The nodules located in the peripheral part of the lesion were covered by alveolar epithelial cells (mainly granular pneumocytes) and although they appeared isolated, serial sections showed that they were in continuity with the areas of Kulitschitsky cell proliferation in the associated terminal bronchiole. Only mild fibrosis was associated with this type of tumorlet, and it was confined to the central peri-bronchiolar part of the lesion.

The second type of tumorlet was seen in rela-
tion to small bronchi and proximal bronchioles. The extra-bronchial cells constituting these tumorlets were arranged in round nests which were embedded in dense fibro-elastic tissue. These cell nests were often located within a clear space (Fig. 6); a distinct endothelial lining to these spaces was not observed and they were interpreted as artifacts produced by retraction during fixation or processing. The connective tissue in the walls of some bronchi was exuberant and appeared to contribute to bronchial luminal narrowing and distortion.

The third type of tumorlet was composed of clusters of oval or spindle cells embedded in variable amounts of connective tissue. It differed from the second type in that a bronchus or bron-
Fig. 5. A tumorlet which has arisen in a terminal bronchiole. Nodules of Kultschitsky-type cells are present in the alveolar walls. Although these nodules appear to be independent, serial sections show that they are in continuity with areas of Kultschitsky cell proliferation in the bronchiole. (H & E, × 30).

Inset: A higher magnification of one of the alveolar wall nodules showing a surface covering of alveolar epithelial cells. (H & E, × 120).

Fig. 6. A tumorlet which has arisen in a bronchus. The mucosa of the bronchus shows Kultschitsky cell proliferation and islands of cells are embedded in connective tissue adjacent to the mucosa. The extra-mucosal cells lie in spaces which are interpreted as artifacts of fixation. (H & E, × 90).

chile could not be demonstrated within the lesion, but serial sections showed a normal bronchus or bronchiole leading to and from the lesion. Three tumorlets were of this type. All three were closely associated with a small pulmonary artery, and one of the tumorlets had fragments of cartilage embedded within it (Fig. 7). These findings strongly suggested that the segments of bronchi or bronchioles which gave rise to these tumorlets had been destroyed during the formation of these lesions.

None of the bronchi larger than 2 mm in
diameter showed evidence of Kulitschitsky cell proliferation. The Kulitschitsky-type cells in the mucosal lesions and in the tumorlets showed an absence of argyrophilia and argentaffinia. A number of otherwise unremarkable bronchioles in the upper lobe were dilated and plugged with mucus, and mild focal fibrosis was also observed in this lobe. It was felt that these changes could be a consequence of the bronchial and bronchiolar obstruction produced by the lesions described above and that significant pulmonary fibrosis did not antecede the development of the tumorlets.

Electron Microscopy

The tissue removed from paraffin blocks showed poor preservation, but dense-core cytoplasmic granules were readily recognized within the Kulitschitsky-type cells in the bronchial and bronchiolar mucosa and within the cells forming the tumorlets. The tumorlet and bronchial lesions obtained directly from formalin-fixed tissue showed much better preservation. The Kulitschitsky-type cells in the mucosal lesions and tumorlets were similar in appearance. The nuclei were round or oval in shape and the chromatin was arranged in clumps and strands and often concentrated peripherally along the nuclear membrane. Moderate numbers of desmosomes were present between these cells (Fig. 9,10). The cytoplasm contained the usual cytoplasmic organelles with the Golgi apparatus and rough endoplasmic reticulum appearing prominent in some cells. A few cells contained residual bodies (phagolysosomes). The most striking feature was the presence of variable numbers of dense-core secretory granules which were dispersed in the cytoplasm (Figs. 8–10); these granules were all spherical in shape, ranged in size from 900 Å to 1800 Å and had a centrally located dense core which was separated from the limiting membrane by a distinct electron-lucent zone (Figs. 8–10). The cores of these granules varied in density from cell to cell and at times within the same cell.
FIG. 8. Electron micrograph of a bronchiole showing ciliated surface epithelial cells with underlying Kultschitsky cell proliferation. (×6,000). Inset: Higher magnification of the cytoplasmic dense-core granules in the area marked by the square (×45,000).

DISCUSSION

The light microscopic resemblance of pulmonary tumorlets to carcinoid tumors was remarked upon more than two decades ago and this similarity of cell type has only recently been confirmed by ultrastructural studies. The present study provides further evidence that
the cells of pulmonary tumorlets contain dense-core granules of neuro-secretory type similar to those found in carcinoid tumors\(^3,16,40\) and normal pulmonary Kulitschitsky-type cells.\(^4,19,17,25\) In addition, evidence is provided for the origin of tumorlets from Kulitschitsky-type cells in the bronchial and bronchiolar mucosa.

On the basis of the observations made in this study, it is felt that the following sequence of events occurs in the formation of pulmonary tumorlets. The earliest stage is proliferation of Kulitschitsky-type cells in the mucosa of bronchi and bronchioles. The newly proliferated cells form plaques or nodular lesions which, as they increase in size, produce atrophy of the overlying columnar epithelium and, eventually, obliteration of the bronchial or bronchiolar lumen. Extension of Kulitschitsky-type cells into the adjacent pulmonary parenchyma may occur in one of two ways to produce tumorlets which differ somewhat in appearance. In lesions which arise in terminal bronchioles, the newly proliferated...
Kultschitsky-type cells extend into the walls of subtended respiratory bronchioles, alveolar ducts and alveoli, a process which is associated with only mild fibrosis. Lesions which arise in bronchi or proximal bronchioles extend into the adjacent alveoli in a different manner; the mucosal Kultschitsky-type cells penetrate the bronchial or bronchiolar wall, a process which evokes a striking connective tissue response. The extrabronchial and extra-bronchiolar cells of these tumorlets occur as clusters of spindle or spheroidal cells which are embedded within connective tissue and often appear to lie in spaces which have been interpreted by some observers as lymphatics. However, in agreement with Prior et al. and Kay, we feel that most of these spaces are artifacts produced by retraction of the cells from adjacent connective tissue and are presumably produced during fixation or processing for light microscopy. Further growth

![Fig. 10. A higher magnification of one of the cells in a tumorlet. The evenly distributed dense-core granules are readily evident. Scanty desmosomes are present at the cell membrane. (X 36,000).](image)
of the tumorlet results in obliteration of the parent bronchus or bronchiole, but its origin from these structures is recognizable by its location in the path of a bronchus or bronchiole, the presence of fragments of cartilage in some tumorlets and the close association of tumorlets with pulmonary arteries and arterioles.

The peculiar connective tissue reaction observed within bronchi and bronchioles in this case has not been previously commented upon. It occurs between the epithelium and the elastic lamina of bronchi and bronchioles and is composed of fibroblasts, collagen, acid mucopolysaccharides and a lymphoid infiltrate sometimes accompanied by thin walled capillaries; in some lesions, elastosis occurs in the outer zone of the newly formed connective tissue. The sharp segmental nature of this connective tissue reaction and its remarkably close association with areas of mucosal Kulitschitsky cell proliferation strongly suggests a causal relationship between these two. The prominence of connective tissue in bronchi and bronchioles in which Kulitschitsky cell proliferation appears to be confined to the mucosa suggests that penetration of the basement membrane by Kulitschitsky cells is the exciting factor and that this process stimulates inflammation and proliferation of fibroblasts, the latter being responsible for the synthesis of collagen, acid mucopolysaccharide and elastic fibers. The newly formed connective tissue probably forms the major part of the connective tissue matrix which is so characteristic of many tumorlets. This connective tissue reaction is quite different from the endocardial and intimal fibrosis seen in patients with the carcinoid syndrome; the latter lesions are composed of collagen and are characterized by an absence of elastic fibers. It is of interest that the elastosis does, however, have a striking resemblance to the elastosis seen around mammary ducts especially in areas harboring carcinoma. It has the same staining characteristics and a similar fine fibrillar pattern which, in the case of mammary elastosis, has been shown to be due to an abundance of immature elastic fibers.

Tumorlets closely resemble spindle cell carcinoid tumors both by light microscopy and electron microscopy, and the distinction between these two lesions is a clinicopathologic one. Spindle cell pulmonary carcinoid tumors may be single or multiple, are unassociated with underlying chronic lung disease, and present as a radiologically visible mass; pulmonary tumorlets, on the other hand, are usually multiple, measure 3 mm or less in diameter, are often associated with underlying pulmonary fibrosis and are found incidentally during microscopic examination of the lungs. Furthermore, while it is well known that pulmonary carcinoid tumors have a capacity to produce lymph node metastases and occasionally distant metastases, tumorlets only rarely metastasize and in no instance have the metastases been of clinical significance. Only two of the cases cited as tumorlets which have produced metastases are acceptable; in both cases, microscopic metastases were confined to regional lymph nodes.

Hage has recently described three ultrastructural varieties of Kulitschitsky-type cells in human fetal bronchi. Although existing ultrastructural studies do not show a significant difference in secretory granule morphology between tumorlets and the various histologic types of carcinoid tumors the possibility that they may arise from different pulmonary endocrine cells needs further investigation.

Tumorlets often occur in association with pulmonary fibrosis and they have to be distinguished from other types of epithelial proliferation which occur in injured lungs. Since pulmonary tumorlets are histogenetically distinct from focal cellular proliferations composed of granular pneumocytes, bronchiolar columnar cells and from foci of squamous metaplasia, it would lend specificity to the term if these lesions were referred to as "carcinoid tumorlets."

The occurrence of pulmonary tumorlets in association with oat cell carcinoma has not been previously reported. Since pulmonary tumorlets contain dense core granules similar to oat cell carcinoma and both are derived from the pulmonary Kulitschitsky-type cells, it is surprising that this association is not more frequent.

ADDENDUM

Since the preparation of this manuscript, Churg and Warnock (Cancer 37:1469-1477, 1976) have reported their findings on 20 cases of pulmonary tumorlets. These authors have also observed dense core secretory granules in the cells of tumorlets examined by electron microscopy.
REFERENCES