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COMMENTARY

WHAT CAN THE BIOLOGY OF SMALL CELL CANCER OF THE LUNG TEACH US ABOUT THE ENDOCRINE LUNG?

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The normal lung is an extensive organ which contains a host of humoral substances [1]. Some of our knowledge concerning this organ was obtained by a study of the abnormal lung. Indeed, there is an old adage which urges researchers to study the abnormal so that they might better comprehend the normal. In this respect, discoveries of the past two decades have revealed that small cell cancer of the lung (SCCL) is a distinct biologic entity which teaches us much about the biology of the normal lung.

Carcinoma of the lung is the principal cause of cancer death in American men, and probably will shortly become the leading cause in women as well [2]. The great majority of lung cancers can be categorized into four histologic types: squamous cell (epidermoid carcinoma), adenocarcinoma, large cell carcinoma, and SCCL. Of the remaining rare tumors of the lung, the most frequent is the bronchial carcinoid [3].

SCCL comprises approximately 25% of all lung cancers. It is two to three times as frequent in males as in females. The tumor rate increases with age, becomes relatively common after the age of 50, and reaches its peak in the seventh decade [2]. Although the number of patients under the age of 40 are few, SCCL comprises a larger percentage of all lung cancers in these younger patients. Most SCCL tumors arise centrally, and are associated with the major bronchi, while the remainder arise peripherally. The lesions are infiltrative, are usually gray-white in color, and often exhibit large areas of necrosis. By light microscopy, SCCL cells are rounded or polygonal, or, occasionally, can be fusiform in shape. The nuclei are hyperchromatic, the nuclear chromatin is finely granular and uniformly distributed throughout the nucleoplasm, and the cytoplasm is relatively sparse. The cells occur in sheets, ribbons or trabeculae, and are supported by a thin fibrovascular stroma [4]. These tumors spread early and extensively via lymphatics and veins and, in most cases, obvious or occult metastases are present at the time of initial diagnosis. Because of this predilection for early spread, surgery is rarely curative. The prognosis for untreated SCCL is grim; most such patients will die within a few months. However, SCCL tumors often demonstrate a striking response to combination chemotherapy, either with or without local radiotherapy. Slightly more than half of those with limited stage disease and about one-quarter of those with extensive stage disease respond to therapy with a complete remission, and most of the others undergo partial regression. Unfortunately, most tumors relapse and are resistant to further therapy. Nevertheless, although the percentage of treated patients with extensive stage disease who have a 2-year disease-free survival is small, such a survival occurs in 17% of limited stage disease patients who are treated with chemotherapy plus radiotherapy [5].

The rare bronchial carcinoid tumor, as will be discussed later, shares many biochemical characteristics with SCCL. Bronchial carcinoids usually arise centrally in the submucosal glands of the major bronchi but may also arise peripherally from smaller bronchi and bronchioles [6]. In contrast with SCCL, it occurs with similar frequency in both sexes. The bronchial carcinoid has relatively low malignant potential. Although the age-specific rate for the bronchial carcinoid is highest in the decades between ages 60 and 79, the distribution of cases is more evenly distributed throughout adult life than is SCCL. In 90% of cases, this tumor does not metastasize. Although some carcinoid tumors metastasize locally, usually to the mediastinal lymph nodes, distant metastases are rare. There is a group of tumors, some of which are referred to as atypical carcinoids, that have morphological and biological features which are intermediate between the relatively benign carcinoid and the highly malignant SCCL. These tumors underline the interrelationship between the two major types of pulmonary endocrine tumors.

There is a very strong association between smoking and SCCL [2]. Indeed, this tumor seldom occurs in nonsmokers. It appears to be the heavy smokers (over ten cigarettes daily) who are the most prone to develop SCCL. As is the case for the other major cell types of lung cancer, the risk of SCCL declines after cessation of smoking. Ionizing radiation also predisposes to SCCL (e.g. uranium miners, atomic bomb exposure). There are several chemical agents which, alone or in combination with other agents,

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are known to predispose to lung cancer in the human, such as asbestos, arsenic, nickel and chromate [2]. The chloromethyl ethers are widely used in the manufacture of many organic chemicals, and workers who are exposed to these agents are particularly prone to develop SCCL. In contrast to SCCL, the bronchial carcinoid tumor has no known etiologic relationship with either smoking or other respiratory carcinogens. Unfortunately, there is no known experimental animal model for SCCL or for the bronchial carcinoid. Exposure to nitrosamine carcinogens causes hyperplasia of the pulmonary endocrine cells of the hamster, and, eventually, pulmonary carcinoma [7]. However, the cell type of the lung cancers so induced is different from SCCL.

In spite of their epidemiological and etiological differences, SCCL and the bronchial carcinoid tumor possess three striking similarities which distinguish them from all other lung cancers: (1) the presence of dense core secretion granules; (2) similar biochemical characteristics; and (3) a predilection to produce multiple polypeptide hormones, with or without obvious paraneoplastic syndromes.

Secretion granules

Electron microscopy of SCCL and of the bronchial carcinoid demonstrates cytoplasmic granules with electron-dense centers which are often surrounded by a lucent halo [8, 9]. In SCCL, these granules range from 80 to 200 nm in diameter, are not present in every cell, and tend to occur singly or in clusters adjacent to the cell border and also within cytoplasmic processes. In the carcinoid tumor, the granules are larger (100 to 290 nm), more numerous and distributed more evenly, and are present in nearly every cell. The granules of both of these tumors are quite similar to the secretion granules which occur in normal cells with known endocrine functions.

Biochemical characteristics

Our knowledge concerning the biochemical characteristics of SCCL is largely due to extensive studies of well-characterized, continuous cell lines which have been established *in vitro* from SCCL tumors and from xenografts heterotransplanted into athymic nude mice [10, 11]. These cell lines, which exhibit a comparatively long doubling time, usually demonstrate distinct aneuploidy. Cytogenetic analysis of both SCCL tumors and cultures have revealed a wide range of chromosome numbers and, very frequently, a deletion of the short arm of at least one chromosome 3 (3p-) [12]. SCCL cultures, which have specific growth factor requirements, replicate indefinitely in fully-defined, serum-free media. As is the case for SCCL *in vivo*, *in vitro* cultures demonstrate considerable sensitivity to radiation. Interestingly, there is an excellent correlation between the prior treatment status of the patient with SCCL and the *in vitro* sensitivity or resistance (i.e. cell lines established from patients with tumors that have relapsed following chemotherapy demonstrate *in vitro* resistance to the same drugs). The culture characteristics of the bronchial carcinoid are, as yet, unknown.

Both SCCL and the bronchial carcinoid have been shown to be classic endocrine tumors of the APUD type (*a*mine content, amine *p*recursor *u*ptake, amino acid *d*ecarboxylase) [3]. The amine precursor uptake and its subsequent decarboxylation is demonstrable by the formaldehyde-induced fluorescence technique: when cells are incubated with an amine precursor and exposed to hot formaldehyde vapor, the cytoplasm fluoresces. This reaction indicates the presence of biogenic amines (i.e. dopamine, serotonin). These biogenic amines, which are associated with many normal endocrine tissues, appear to play an important role in the formation, the storage and/or the release of polypeptide hormones.

The argyrophilic staining characteristic (staining with silver salts in the presence of a mild reducing agent), which is exhibited by SCCL, the bronchial carcinoid, and many normal endocrine tissues, may be related to the amine content of the cytoplasmic secretion granules. Interestingly, L-dopa decarboxylase, the enzyme which removes the carboxyl group from dopa to form dopamine, and from 5-hydroxytryptophan to form serotonin, is found in very high concentrations in SCCL tumors and cell lines, and in bronchial carcinoids [10, 13].

APUD cells and their tumors are characterized by high concentrations of a specific form of the glycolytic enzyme enolase known as neuron specific enolase (NSE). Previously, NSE had been believed to be a specific marker for neuronal cells. However, this isoenzyme also is made by tumors and continuous cell cultures of SCCL, where it is present at much higher levels than occur in cultures of non-SCCL [14]. It is also found in the bronchial carcinoid.

Creatine kinase BB (CK-BB) is an isoenzyme of creatine kinase that is found in most cells, but in particularly high concentrations in brain. Very large amounts of CK-BB are found in tumors and cultures of SCCL, indicating that this substance, which generates ATP, is crucial to the high energy requirements of this tumor [15].

It is of great interest that nervous system cells and APUD cells and their tumors (including SCCL) express a surface antigen, leu-7, previously believed to be a specific marker for natural killer (NK) cells [16].

Biogenic amine and polypeptide production, and NSE, CK-BB and leu-7 expression provide evidence for strong functional relationships between APUD cells and neurons and, for these reasons, the former are often referred to as neuroendocrine cells.

Polypeptide hormones

Production by the tumor. Immunologic staining and radioimmunoassay of SCCL or of bronchial carcinoid, and also of cultured cells of SCCL, have demonstrated the production of several polypeptide hormones. Nearly all SCCL tumors and cell lines contain bombesin-like immunoreactivity (BLI) [17]. Bombesin is a tetradecapeptide that was first isolated from amphibian skin; its carboxyl terminal end is very similar to gastrin releasing peptide (GRP), a 27 amino acid polypeptide found in the gastrointestinal tract of mammals. Most antisera to synthetic bombesin cross-react with synthetic GRP. Because the exact chemical composition of the cross-reacting peptide in SCCL is not known, it is referred to as BLI. Pharmacologically, both synthetic bombesin and

GRP stimulate the release of many gut hormones and, physiologically, BLI probably plays a peptidergic role within the nervous system.

Calcitonin, a 32 amino acid polypeptide which was originally detected in the C cells of the thyroid gland, is found in approximately half of SCCL cell lines and has frequently been localized by immunostaining or by radioimmunoassay to SCCL tumors and bronchial carcinoids [18, 19].

ACTH and the opioid enkephalins have been detected in many SCCL and bronchial carcinoids, as well as in continuous cultures of SCCL [18]. Although studies *in vivo* and *in vitro* demonstrate the apparent presence of other hormonal polypeptides in SCCL and the bronchial carcinoid tumor, many of these substances often are associated with non-SCCL lung cancers as well.

Elevated serum levels. Some of the hormones which have been found in SCCL or in the bronchial carcinoid can be greatly increased in the serum. Often, these excess serum levels produce no symptoms. However, sometimes they can cause paraneoplastic syndromes (distant effects mediated by substances released by the tumor). Thus, although nearly two-thirds of patients with SCCL have increased serum ACTH, only about 5% manifest symptoms of Cushing's syndrome (i.e. hypersecretion of adrenal corticosteroids). Perhaps the relative rarity of symptoms is because of the comparatively high proportion of the biologically inactive large precursor forms (pro-ACTH) which are secreted by this tumor.

Most patients with SCCL and the bronchial carcinoid tumor have high levels of immunoreactive calcitonin (iCT) in their serum [20, 21]. Pharmacologically, calcitonin decreases serum calcium. However, SCCL patients with hypercalcitonemia are not hypocalcemic. This hormone also has many other pharmacologic actions. Nevertheless, although it is conceivable that some of the symptomatology associated with SCCL is related to high levels of calcitonin (e.g. anorexia, depression), no specific calcitonin-related syndrome has been identified in patients with this neoplasm.

Many patients with SCCL and some with the bronchial carcinoid tumor have increased serum levels of vasopressin, which causes the syndrome of inappropriate secretion of anti-diuretic hormone (hyponatremia and an inappropriately concentrated urine) [22]. Interestingly, however, although vasopressin has been found in SCCL tumor tissue, its presence in continuous cell cultures of SCCL is relatively uncommon.

Many other polypeptides have been found to be increased in the serum of patients with SCCL or with the bronchial carcinoid, e.g. β-endorphin, β-lipotropin, the amino-terminal glycopeptide of pro-opiomelanocortin, somatostatin, carcinoembryonic antigen, etc. [18, 23]. The extent to which these and other substances are associated with SCCL more often than with non-SCCL remains to be determined.

SCCL, BRONCHIAL CARCINOID AND THE ENDOCRINE LUNG

Coexistent or often subsequent to the studies which have demonstrated the endocrine characteristics of SCCL and the bronchial carcinoid, many studies have revealed that the normal as well as the non-neoplastic diseased lung also has the capacity to synthesize and secrete hormones. Some of these hormonal substances are produced by the pulmonary endothelium (e.g. prostacyclin, angiotensin II), some from the platelets (e.g. serotonin, thromboxane), some from alveolar macrophages, lung fibroblasts, and bronchial smooth muscle (e.g. prostaglandins and other arachidonic acid metabolites), some from pulmonary mast cells (e.g. histamine, arachidonic acid metabolites), some from pulmonary nerves (e.g. vasoactive intestinal peptide, substance P), and some from the pulmonary endocrine cells.

The pulmonary endocrine cells are distinctive appearing solitary cells or groups of cells (neuroepithelial bodies, NEB) which are distributed throughout the bronchial epithelium of man and other animals [24, 25]. They have been described in the epithelium of the larynx, trachea, bronchi, bronchioles, and the acini and ducts of the parabronchial glands, as well as the alveoli. NEB often are found in close association with nerves. The pulmonary endocrine cells are particularly numerous in the fetus and newborn, and are very sparse in the adult. Many characteristics of these normal cells are similar or identical to those of SCCL and the bronchial carcinoid: they contain membrane-bound, dense core cytoplasmic granules surrounded by a halo, they demonstrate amine precursor uptake and decarboxylation characteristics, they are argyrophilic and contain serotonin and perhaps dopamine, they contain neuron specific enolase, and, lastly, they produce some of the very hormones which are most commonly encountered in continuous cultures of SCCL [3, 25]. Immunohistochemical studies of pulmonary endocrine cells of the normal human have demonstrated the presence of BLI, calcitonin, and leu-enkephalin; and β-endorphin and ACTH have been found in the pulmonary endocrine cells of fibrotic bronchioles [26–29].

Thus, it has become apparent that SCCL and the bronchial carcinoid are malignant counterparts of the normal pulmonary endocrine cell. Conceivably, because SCCL and bronchial carcinoids are interrelated but very different, one could postulate that they arise from different subtypes of pulmonary endocrine cells.

The physiologic function of the hormones produced by the normal pulmonary endocrine cell remains to be elucidated. They may have an *autocrine* function (i.e. self-regulation). For example, in studies of continuous cultures of SCCL, it has been found that bombesin stimulates the growth of the very cells which produce a similar peptide. These hormones may function in a *paracrine* manner (i.e. exerting an influence on the cells or tissues which are in their immediate environment). Lastly, hormones of the pulmonary endocrine cell might also function in a *hemocrine* manner (i.e. via the blood stream or lymph to a site which is relatively distant from its source).

Studies are underway to elucidate the physiologic and/or pharmacologic effects of the known or putative hormones of the pulmonary endocrine cell.

For example, it has been found that ACTH promotes fetal pulmonary maturation, and also is a non-competitive inhibitor of angiotensin-converting enzyme [30, 31]. Pharmacologically, bombesin causes bronchoconstriction and can constrict the pulmonary artery [32]. Calcitonin, which increases the synthesis of prostacyclin by endothelium, also inhibits the intrapulmonary synthesis of prostaglandins and thromboxane [33, 34]. In various experiments, enkephalins influence pulmonary artery pressure, stimulate pulmonary J receptors and induce apnea, and also may trigger bronchial asthma [35–37].

Patients with acute pneumonitis have been found to have high levels of iCT in their serum and urine, and these values normalize with recovery [38]. Patients with chronic obstructive pulmonary disease often have hyperplasia of the pulmonary endocrine cells, and frequently have high levels of serum iCT, ACTH, β-lipotropin, and carcinoembryonic antigen [39–42]. Chronic smokers have higher urine iCT than nonsmokers, and levels correlate with the number of pack-years. Patients with chronic lung disease associated with the genetic disease cystic fibrosis often have high serum and urine iCT levels [38].

The role of the high levels of these aforementioned hormones in acute and chronic lung disease remains to be determined. Whether they will prove to be beneficial to the patient or detrimental, it seems likely that their measurement will provide important information concerning the course and prognosis of these conditions with which they are associated.

Conceivably, the measurement of the hormones produced by the pulmonary endocrine cell might, in certain experimental models, provide insights into the transition from the normal to the malignant. For example, in hamsters treated systemically with the carcinogen nitrosamine, there is a progressive and parallel increase in pulmonary endocrine hyperplasia, pulmonary iCT concentrations, and serum levels of iCT [43].

CONCLUSIONS

SCCL and the bronchial carcinoid are tumors whose progenitor cell appears to be the pulmonary endocrine cell. As the pathobiology of these tumors has unfolded, it has become apparent that many of the "abnormal" characteristics of these malignancies are merely reflections of the "normal" characteristics of the cells from which they originate. In particular, the so-called "ectopic" secretion of several polypeptide hormones by these neoplasms has been shown to be "eutopic". These cancers are endocrine because their cells of origin are endocrine. Undoubtedly, the continued study of the behaviour of these lesions *in vivo* and *in vitro* will provide further insights into the physiology and pharmacology of the normal endocrine lung.

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