Activities of L-Dopa Decarboxylase and Diamine Oxidase (Histaminase) in Human Lung Cancers and Decarboxylase as a Marker for Small (Oat) Cell Cancer in Cell Culture

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ABSTRACT

The neuroendocrine [amine precursor uptake (decarboxylase)] properties of small (oat) cell lung cancer (SCC) have suggested that this neoplasm may have a separate histogenesis from the other major types of human lung tumors. We now report that a key element of this concept, L-dopa decarboxylase activity, is present in surgical and autopsy tissues from all forms of lung cancer. Values are highest in SCC lesions; however, lung adenocarcinoma tissues can have considerable activity, and values overlap those for SCC and fall between values for SCC and large cell and squamous cell carcinoma. The distribution of diamine oxidase activity is different except that even more overlap occurs between the major tumor types. These data may provide further evidence that SCC and other human lung cancers could share a common origin in the bronchial mucosa. In cell culture, the distribution of the two enzyme activities is different. The average L-dopa decarboxylase activity is much higher (seven separate culture lines) than in the in vivo specimens, and it completely separates these cell lines from non-SCC lung tumors (four lines). Diamine oxidase is generally low in both SCC cells and non-SCC cells in culture and does not separate the various cell types. L-Dopa decarboxylase activity thus does appear to be a valuable marker for separating SCC cells from other lung cancer cells in vitro.

INTRODUCTION

SCC constitutes approximately 20% of the histopathological types of lung cancer in humans (28). Increased investigative attention has been focused upon this neoplasm not only because of this high clinical incidence but also because the biological properties of this tumor differ from those of other forms of lung cancer. (a) SCC is a relatively more virulent tumor and has a greater capacity for dissemination than squamous cell, large cell, or adenocarcinoma of the lung (21); (b) SCC has the greatest sensitivity among lung tumors to chemotherapy and radiation therapy (2, 26, 35); (c) because SCC has the above 2 features and also a propensity to produce endocrine syndromes secondary to secretion of small polypeptide hormones, it has been proposed that this neoplasm has a separate histogenetic origin from other lung cancers. This tendency to produce small polypeptide hormones, such as vasopressin and adrenocorticotropic, has led some workers to include SCC among the group of peptide hormone- and amine-synthesizing cells termed by Pearse as the "APUD" system (13, 31, 32). The proven embryological origin of some APUD cells in the neural crest (24, 33) has raised questions that SCC may arise in a bronchial cell with such a distinctive histogenesis; definitive proof for this has not been obtained, however (34).

Recently, the probability of a separate histogenesis for SCC has become less secure. It has been recognized, through the use of immunoassays, that all histological types of lung cancer may contain significant quantities of small polypeptide hormones (20, 36). Also, it is increasingly appreciated that the major histological forms of lung cancer may coexist in the same patient (1, 14). Such findings, plus a preliminary study of the quantities of adrenocorticotropic in all types of lung tumors, has led Yesner (37) to suggest that the different morphological forms of lung cancer represent a continuum of neoplastic differentiation occurring in transformed bronchial epithelial cells.

In the present study, we attempt to establish further the comparative biochemical properties of the major human lung neoplasms by studying the activity of 2 enzymes in tumor specimens and in tissue culture. We previously have reported that high activities of L-aromatic amino acid decarboxylase (L-dopa decarboxylase, EC 4.1.1.28) and histaminase (diamine oxidase, EC 1.4.3.6) separate SCC from surrounding nonmalignant lung parenchyma and bronchus (4, 8). L-Dopa decarboxylase is a key enzyme step in the synthesis of biogenic amines and as such plays an integral role in the APUD concept (31) (the "D" stands for this decarboxylase activity). Histaminase, on the other hand, is an amine-metabolizing enzyme not restricted to endocrine or neural tissues (3) but which is found in relatively high amounts in such endocrine tumors as medullary thyroid carcinoma (5, 6) and ovarian cancer (16, 25). We now report that the differences between L-dopa decarboxylase and histaminase activities in the major forms of human lung cancer are quantitative rather than qualitative ones; we discuss the importance of this finding for an understanding of the histogenesis of lung tumors. We also demonstrate the value of L-dopa decarboxylase activity as a marker for identification of SCC tumor cells in culture.

MATERIALS AND METHODS

Tumor Tissues and Cell Culture Samples. Individual tumor specimens were obtained at time of surgery or autopsy from 21 patients with SCC (46 separate lesions collected), 12 patients with lung adenocarcinoma, 5 patients with squamous cell...
lung carcinoma, and 5 patients with large cell lung carcinoma. All autopsy tissues were obtained within 6 to 12 hr of death; as previously described, within this time range, no relationship between delay from time of death and levels of enzyme activity could be appreciated (8). Surgical specimens were frozen within minutes of removal, as were the cultured cells and athymic mouse transplants described below. The patient specimens represent either a sample of the primary lung tumor or a metastatic deposit in a thoracic cavity lymph node. As in previous studies (4, 8), each lung tumor was classified historically by Drs. Joseph Eggleston and Geoffrey Mendelsohn according to WHO guidelines (23).

In the cell culture studies, 7 established and well-characterized lines of SCC and 4 similarly characterized lines of non-SCC (2 adenocarcinomas and 2 large cell carcinomas) lung tumors were examined. All of the cell culture procedures and a complete characterization of the established lines will be detailed in a separate report.4 Briefly, cells from suspensions of mechanically disrupted tissue specimens or pellets from pleural fluids were seeded as single-cell suspensions in 75-sq cm flasks (Falcon) in Roswell Park Memorial Institute Medium 1640 or modified F-12 medium with 10 to 20% fetal calf serum and containing 50 units penicillin per ml and 50 μg streptomycin per ml (all reagents were from Grand Island Biological Co., Grand Island, N. Y.); the cells were then grown at 37° in 5% CO₂:95% air. The non-SCC tumors generally grew as monolayers and were detached for studies with either trypsin or EDTA. The SCC tumors usually grew as floating aggregates which could be centrifuged directly. All cell cultures were characterized as being visually free of fibroblasts and as containing no Mycoplasma infection at time of study.4 The SCC cells were examined by electron microscopy and contained neurosecretory granules.5

The tumorigenicity of most of the cell types was indicated by the growth of simultaneously initiated heterotransplants of the starting cell preparations in athymic mice. The cells were injected into 4- to 6-week-old athymic nude (nu/nu) mice of BALB/c background (ARS/Sprague-Dawley, Madison, Wis.). Detailed review of the histology of these tumors confirmed the type of lung cancer in each case. The nude mouse specimens were established for at least 2 heterotransplant passages prior to analyses of enzyme activity; in one case, as noted in the text, heterotransplants were established from the cells already passaged in cell culture.

Tissue Preparation and Assay Procedures. The patient specimens and athymic mouse explants were prepared, using a Brinkman Polytron, as 1:10 (w/v) homogenates in 0.1M phosphate buffer, (pH 6.8), exactly as in earlier studies (4, 8). For the tissue culture studies, cells from one 75-sq cm culture flask were pelleted, washed twice in the phosphate buffer, and finally suspended in 1.0 ml. Prior to assay the cells were sonicated with 2 to 3 ten-sec bursts at a setting of 4 on a Model W-220F sonicator from Ultra Sonics, Inc.

L-Dopa decarboxylase activity was determined on 80 μl of the homogenates or the cell sonicates using our previously described modifications (7) of the method of Beaven et al. (10). Activity is expressed as nmol 14CO₂ released per hr incubation per mg tissue or cell protein; in some cases, results are given as units per mg protein, where 1 unit is 1 nmol 14CO₂ released per hr incubation. L-Dopa decarboxylase (histaminase) activity was determined on 100 μl of the same homogenates or sonicates, using the modified method of Beaven et al. (9) as in our previous work (4, 8). [β-3H]Histamine (kindly provided by Dr. Michael A. Beaven, NIH) was used as substrate; results are expressed as units per mg protein, where 1 unit is 1 pmol of substrate deaminated per hr incubation. Protein determinations on all samples were by the method of Lowry et al. (27).

RESULTS

The L-Dopa decarboxylase and histaminase activities in all of the surgical and autopsy tissues studied are shown in Chart 1. Both enzyme activities were measurable in the majority of the specimens for all histological types; much overlap is apparent between the groups, especially for histaminase activity. Two aspects of the findings are most important for considering the histogenesis of lung cancer: (a) the L-Dopa decarboxylase activity is measurable in lung tumors other than SCC; (b) although the values of both enzyme activities do not qualitatively separate one tumor group from another, a distinct distribution pattern is present among the histological types. Highest L-Dopa decarboxylase activity occurred in SCC, although a wide range of values was obtained between each of the individual lesions examined as previously reported (8). Although the median value for SCC was above the highest value recorded in a non-SCC lung tumor, values for lung adenocarcinoma were also considerable. The population of values for adenocarcinoma fell clearly between SCC and the large cell and squamous cell tumors, with the median value being 13-fold lower than that

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Chart 1. L-Dopa decarboxylase and histaminase activities in homogenates of surgical and autopsy specimens of human lung cancer (note log scale). Each value represents activity in a portion of an individual tumor lesion removed from the thoracic region; in the non-SCC group, each tumor was taken from a separate patient, and in the SCC group, 46 separate lesions from 21 patients were studied. A, Squamous cell carcinoma, surgical; B, large cell carcinoma, surgical; C, large cell carcinoma, autopsy; D, adenocarcinoma, surgical; X, small cell carcinoma, autopsy; & small cell carcinoma, surgical. --- median value; h, hr.
for SCC and 7-fold higher than that for the large and squamous cell cancers. The distribution of histaminase activities is also not random and was very similar to that for L-dopa decarboxylase. The median value for SCC lesions was 3-fold higher than that for adenocarcinoma, which, in turn, was 4-fold higher than that for large and squamous cell tumors.

Both enzyme activities were also measured in the 4 established lines of non-SCC lung tumors, 7 lines of SCC tumors, and athymic mice heterotransplants of most of these same neoplasms. L-Dopa decarboxylase activity separated all of the SCC tumors from the others in cell culture (Chart 2A); the median level in the SCC cells was 8600-fold greater than that for the cultured non-SCC tumors, 25-fold higher than that for the SCC autopsy specimens (Chart 1A), and 1.5-fold higher than that for the 4 freshly removed surgical specimens of SCC (Chart 1A). Histaminase activity, on the other hand, did not separate the SCC and the non-SCC cultures (Chart 3A); the median value for the SCC lines was essentially equal to that for the non-SCC cells and 3-fold lower than that for the autopsy and surgical specimens of SCC (Chart 1B). Only one SCC line (Chart 3A, x²) had appreciable histaminase activity. The data for both enzyme activities could not be explained on differences in stage of cell growth at the time cell culture samples were taken. High L-dopa decarboxylase activity was maintained across the growth curve for the SCC lines; high L-dopa decarboxylase activity did not appear at different stages during the growth of the non-SCC cells or high histaminase activity during the growth of SCC or non-SCC cells.

In the athymic mouse heterotransplants, the L-dopa decarboxylase (Chart 2B) and histaminase (Chart 3B) activities were generally similar to those measured in the cell culture lines. The L-dopa decarboxylase activities were somewhat higher in the non-SCC heterotransplants than in the corresponding non-SCC cultures; however, the high decarboxylase activities again separated 4 of the 5 SCC tumors from all but one of the non-SCC tumors (Chart 2B, Transplant A₁ to correspond to its companion culture in Chart 2A). This one non-SCC tumor demonstrated the close relationship between the different forms of lung cancer and between small cell morphology and high L-dopa decarboxylase activity. The heterotransplant was derived by implantation of cells from pleural fluid taken from a patient whose lung tumor had mixed large and small cell histology; this initial implant (Chart 2B, A₁) also had the same mixed histology and a relatively high decarboxylase activity. The cells simultaneously placed into culture established a line with large cell morphology only and had virtually no activity (Chart 2A, Culture A). A later transplant derived from this culture (Chart 2B, Transplant A₂) had large-cell morphology and a 30-fold lower decarboxylase activity than the original A₁ transplant. The relationships between histaminase activities in these specimens did not parallel those for L-dopa decarboxylase, and values were somewhat lower in the transplants (Chart 3B, A₁ and A₂) than in the cell culture line (Chart 3A, A₁).

Some differences were seen between the enzyme activities in cell culture lines and in the heterotransplants which could not be explained histologically. The L-dopa decarboxylase activity in one SCC culture (Chart 2A, x²) was much higher than in a simultaneously derived mouse transplant (Chart 2B, x') of the same tumor. Also, histaminase activity was even higher in the SCC transplant (Chart 3B, x³) of the one SCC tumor with a high activity in culture (Chart 3A, x²). Finally, one adenocarcinoma had no histaminase activity in culture (Chart 3A, b) but had relatively high activity in a simultaneously established transplant (Chart 3B, b).
DISCUSSION

The patterns of enzyme activity that we have obtained in the study of surgical and autopsy tissues are important for considering the histogenesis of human lung cancer. We now report that a key element of the APUD concept, L-dopa decarboxylase activity, is not restricted to SCC among the human lung cancers. Our data thus argue that the possession of APUD elements by SCC does not necessarily provide evidence for a unique histogenic origin of this neoplasm in the bronchial epithelium. Rather, the findings could suggest that the major histological forms of lung tumors, including SCC, may be related and that development of APUD characteristics and endocrine activity may be but one of multiple differentiation pathways available to a common maturing progenitor cell in the bronchial epithelium. The recent reports of increased circulating and/or tumor tissue concentrations of small polypeptide hormones in patients with all types of lung cancer would also be consistent with this possibility (20, 36). The level of APUD activity in bronchial neoplasms could then somehow reflect the relative degrees to which each major histological tumor type develops and/or retains this differentiation. The differentiation of the tumor might, in turn, reflect the level of bronchial epithelial maturation at which the neoplastic transformation occurred.

The concept of endocrine cells arising from nonneural progenitor cells in nondendocrine epithelial surfaces is now receiving attention. Chimera studies have failed to reveal a neural origin for endocrine (APUD) cells in the gastrointestinal tract (19). Cheng and Leblond (15) have proposed that basal or crypt cells in the intestinal mucosa differentiate into such endocrine cells as well as into the other nondendocrine differentiated cells of the gut epithelium. A similar situation may well exist in bronchial epithelium.

A proposal which links the major forms of lung cancer to one another through a process of differentiation has clinical as well as biological implications. Our work and that of others has recently emphasized that the different histopathological forms of lung cancer, including SCC, can coexist in the same patient and in the same tumor lesion (1, 14). Since each type carries a somewhat different prognosis and, more importantly, has different therapeutic implications, it is important to distinguish between them. Such distinction, on morphological grounds alone, can, on occasion, be difficult; subtle but important relationships may not be appreciated or even well categorized as yet. Biochemical indices, such as those in our present study, could be useful in this regard and come to play an important role in developing clinically meaningful biochemical classifications for lung cancer. Such subclassifications have already proven useful in characterizing other tumors (22, 29). Our ongoing investigations have developed a promising biochemical profile which encompasses the 2 enzyme activities currently discussed and the levels of small polypeptide hormones. Such an index may provide useful information for the therapeutic approach to human lung cancer.

Finally, the present results for L-dopa decarboxylase and histaminase activities have at least 2 important ramifications for future in vitro studies of human lung tumors. (a) Cell culture and heterotransplant systems of SCC cells have become increasingly important for such purposes as studying the biosynthesis and secretion of polypeptide hormones (11, 12), and developing in vitro conditions to test the sensitivity of this tumor to chemotherapeutic agents. A constant tumor product has not been identified previously for SCC which would biochemically distinguish these cells from other types of lung cancer. The demonstration of a high L-dopa decarboxylase activity would appear to have great promise for accomplishing this purpose; our data suggest that this finding should be an important criterion for characterizing culture lines of human SCC tumor cells. (b) Our biochemical data may closely relate to the recent emphasis placed on the existence and importance of heterogeneous cell populations within a single neoplasm (17, 18, 30). Our previous studies have indicated that SCC is a tumor composed of such heterogeneous populations of cells. Thus, biochemical profiles may differ greatly, even between different tumor lesions from the same patient, and the presence of cells with high and low amounts of histaminase in the same tumor deposit has been directly demonstrated (4, 8). The reasons for such in vivo heterogeneity may be closely linked to findings such as the retention and possible increase of L-dopa decarboxylase in SCC cells in culture but the generalized failure of these same cells to have high histaminase activity. In vivo, tumors contain cells with all stages of proliferative activity and maturation; these different cell stages may, in turn, dictate certain of the biochemical properties of a given cell. In studies of rat gastrointestinal epithelial cells, we have found high histaminase activity to be associated with mature, nonproliferating villus-tip cells rather than proliferative crypt cells; L-dopa decarboxylase activity, however, was higher in the crypt cells than in the villus-tip cells (7). Cells grown under routine culture conditions often retain a proliferative capacity and may not undergo maturation and differentiation. The possibility that such a difference between in vivo and in vitro conditions might account for different profiles of biochemical expression in tumor cells growing in culture versus growing in vivo merits careful attention. Alternatively, our results may stem from differences in cell populations which selectively metastasize to different areas (17); the biochemical data could thus reflect a selection of cell types which proliferate in pleural fluid, the source for most of our lung tumor cultures. The selection feature might also occur in the cell culture process which may favor the growth of some cells and loss of others. All of these above factors would be important to clarify; comparisons such as the present study, of biochemical properties in tissue samples versus cultured cells, may be one important approach for delineating further the types of cells which populate the different forms of lung cancer. Modulation of cell culture growth conditions and transfer of cells between culture and mouse heterotransplants with simultaneous monitoring of biochemical profiles may, in turn, provide essential information about factors governing the differentiation characteristics of these heterogeneous cell groups.

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S. B. Baylin et al.


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