Estrogen and Progesterone Receptors in Bronchogenic Carcinoma

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ABSTRACT

Although the lung is not usually considered a major target organ of sex hormones, epidemiological observations, studies of pulmonary neoplasms in laboratory animals, and investigations of carcinomas derived from other "nontarget" organs suggest that sex hormones may have a role in the pathogenesis of bronchogenic carcinoma. To confirm that estrogen (ER) and progesterone receptors are present in human lung cancers, 19 resected lung cancers were examined for receptors using a prelabeled sucrose gradient method. Three squamous cell carcinomas were positive for ER (>6.9 fmol/mg cytosol protein). Three squamous cell carcinomas, two adenocarcinomas, and one small cell carcinoma were positive for progesterone receptors (>6.9 fmol/mg cytosol protein). One tumor, a squamous cell carcinoma arising in a woman who smoked, had an ER level of 30 fmol/mg, a highly positive level even for breast tumor, a squamous cell carcinoma arising in a woman who smoked, had ER level of 30 fmol/mg, a highly positive level even for breast cancer patients might be designed for selected lung cancer patients as well. Improved survival of even a minority of lung cancer patients would be a significant gain.

The sex hormones exert their influence via specific hormone receptors. We examined 19 resected lung cancers for estrogen and progesterone receptors using a prelabeled sucrose gradient method to confirm whether or not these receptors are present in bronchogenic carcinomas.

INTRODUCTION

Bronchogenic carcinoma is the leading cause of cancer mortality in the United States with 142,000 deaths expected this year (1). Although small cell carcinoma may respond initially to chemotherapy or radiotherapy, cure is rare. Among non-small cell cancers (adenocarcinoma, squamous cell carcinoma, and large cell carcinoma) cure is occasionally achieved by surgical resection in patients with limited disease. No reliable adjuvant therapy is yet available for those with metastatic disease which make up the majority of patients. The overall 5-year survival rate for lung cancer is less than 10% in most series, a figure which has remained unchanged for decades (2, 3).

Although cigarette smoke is well established as a risk factor for bronchogenic carcinoma, most people who smoke do not develop lung cancer, while about 10% of lung cancers occur in people who have never smoked. Therefore, factors other than cigarette smoke must also have a role in the pathogenesis of lung cancer. Such factors may be environmental (air pollution, occupational exposures) or host related (genetic, nutritional).

Sex hormones may be host-related factors that influence the development of lung cancer. The lung is not usually considered a target organ of sex hormones and, therefore, it is not intuitively apparent that bronchogenic carcinomas would be responsive to these hormones. However, the epidemiology of lung cancer differs between the sexes and it has been proposed that these epidemiological findings might be explained in part by differing sex hormone levels in men and women (2-9). There is evidence that estrogen has an influence on the growth of carcinomas derived from other "nontarget" organs including the pancreas (10-14), liver (15-20), stomach (21, 22), and colon (22). Additionally, several studies have demonstrated that sex hormones influence the incidence of pulmonary neoplasms in laboratory animals (23-26).

MATERIALS AND METHODS

Tumor tissue was obtained from 19 lung cancer resection specimens at the time of frozen section from the surgical pathology service of The Methodist Hospital, Houston, TX. In each case, the specimens represented the primary resection of the tumor subsequent to diagnosis by biopsy or cytology. None of the patients had received previous adjuvant therapy for their lung cancer. In addition, normal lung, uninvolved with tumor or other processes, was obtained from 5 of the specimens. Approximately 0.4-1 g of tissue was snap frozen and stored at —70°C for use in estrogen and progesterone receptor studies.

Steroid-binding Protein Assays. Estrogen- and progesterone-binding proteins were quantified by a prelabeled sucrose gradient method which has been previously described (30). This technique permits analysis of smaller volumes of tissue than the dextran-coated charcoal method. The tissue was pulverized at a temperature of <60°C, suspended in 5 volumes of buffer (1 mM monothioglycerol in 0.005 M sodium phosphate plus 10% glycerol, pH 7.4, at 4°C), and homogenized. After centrifugation at 105,000 x g for 30 min, aliquots of the cytosol were set aside for protein determination by the Bio-Rad protein assay (31). To determine cytoplasmic estrogen-binding proteins, aliquots were incubated for 18 h with either 2 nM 17β-estradiol (New England Nuclear, Boston, MA) or 2 nM 17β-estradiol plus 200 nM diethylstilbestrol. The reaction mixtures were then transferred to test tubes containing a sedimented pellet derived from 0.5 ml of a dextran-coated charcoal suspension [0.025% dextran:0.25% Norit A in buffer (1 mM monothioglycerol in 0.005 M sodium phosphate plus 10% glycerol, pH 7.4)]. The tubes were vortexed, incubated for 5 min, and centrifuged at 2000 x g for 1 min. Aliquots of the supernatant were layered onto 10-30% sucrose gradients (prepared in 0.001 M EDTA-0.01 M Tris HCl-1 mM monothioglycerol, pH 7.4, at 4°C) and centrifuged for 3.5 h at 220,000 x g. The gradients were collected in 0.2-ml fractions and counted on a gamma counter. Specific binding was obtained by subtracting nonspecific binding in the presence of diethylstilbestrol from total binding.

To determine cytoplasmic progesterone-binding proteins, aliquots of the cytosol were incubated for 18 h with either 10 nM [3H]R5020 (New England Nuclear, Boston, MA) or 10 nM [3H]R5020 plus a 400-fold excess of unlabeled R5020. The reaction mixtures were incubated with a dextran-coated charcoal suspension and separated on a sucrose gradient as described above. Radioactivity was determined on a scintillation counter. Specific progesterone binding was determined by subtracting nonspecific binding from total [3H]R5020 bound in the absence of the nonradioactive R5020. Results were expressed as fmol of receptor/mg of cytoplasmic protein. Possible binding of R5020 by glucocorticoid receptors was not excluded by this technique. Studies to determine whether or not glucocorticoid receptors are present in lung cancers are underway.


Table 1: Estrogen and progesterone receptor levels in 19 primary pulmonary carcinomas

<table>
<thead>
<tr>
<th>Case</th>
<th>Cell type</th>
<th>Stage</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Smoking history</th>
<th>ERa</th>
<th>PRa</th>
</tr>
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<tr>
<td>1</td>
<td>Squamous</td>
<td>I (T2N0M0)</td>
<td>M</td>
<td>65</td>
<td>15</td>
<td>9</td>
<td>2</td>
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<tr>
<td>2</td>
<td>Squamous</td>
<td>I (T2N0M0)</td>
<td>M</td>
<td>66</td>
<td>50</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Squamous</td>
<td>I (T2N0M0)</td>
<td>M</td>
<td>78</td>
<td>50</td>
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<td>1</td>
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<tr>
<td>4</td>
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<td>I (T1N0M0)</td>
<td>M</td>
<td>64</td>
<td>60</td>
<td>0</td>
<td>1</td>
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<td>5</td>
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<td>M</td>
<td>74</td>
<td>20</td>
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<td>6</td>
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<td>II (T2N1M0)</td>
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<td>58</td>
<td>60</td>
<td>1</td>
<td>2</td>
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<td>Squamous</td>
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<td>F</td>
<td>61</td>
<td>0</td>
<td>1</td>
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<tr>
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<td>F</td>
<td>68</td>
<td>90</td>
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<td>7</td>
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<tr>
<td>9</td>
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<td>III (T3N2M0)</td>
<td>M</td>
<td>55</td>
<td>35</td>
<td>3</td>
<td>11</td>
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<td>M</td>
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<td>0</td>
<td>0.9</td>
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<tr>
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<td>M</td>
<td>53</td>
<td>40</td>
<td>3</td>
<td>6</td>
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<td>12</td>
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<td>F</td>
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<td>9</td>
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<td>Large cell</td>
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<td>F</td>
<td>65</td>
<td>30</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>19</td>
<td>Small cell</td>
<td>II (T1N0M0)</td>
<td>M</td>
<td>59</td>
<td>70</td>
<td>4</td>
<td>16</td>
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</tbody>
</table>

* Pack-yr.  
* fmol/mg of cytoplasmic protein.

RESULTS

The results are summarized in Table 1. The cancers included 9 squamous cell carcinomas, 8 adenocarcinomas, 1 large cell carcinoma, and 1 small cell carcinoma. At The Methodist Hospital, small cell carcinoma is usually treated by chemotherapy and radiotherapy and we receive resections of these tumors only rarely. In our laboratory, human breast carcinomas are applied these criteria to our lung cancer cases, then 3 tumors receptor concentration exceeds 6.9 fmol/mg cytosol protein. If only rarely. In our laboratory, human breast carcinomas are summarizing in Table 2. The identification of these receptors in (as well as androgen and glucocorticoid receptors in some cases) have previously reported ERs and PRs in human lung cancers have previously reported ERs and PRs in human lung cancers (27-29). Their findings (with regard to ER and PR only) are summarized in Table 2. The identification of these receptors in a portion of human lung cancers suggests the possibilities that

DISCUSSION

Nearly half of the lung cancers in our study had positive ER or PR status and in one case the ER level was strikingly high. Our findings confirm the observations of other who have previously reported ERs and PRs in human lung cancers (as well as androgen and glucocorticoid receptors in some cases) (27-29). Their findings (with regard to ER and PR only) are summarized in Table 2. The identification of these receptors in a portion of human lung cancers suggests the possibilities that

(a) sex hormones may have a role in the pathogenesis of lung cancer in some patients and (b) selected cases of lung cancer may respond favorably to adjuvant hormonal therapy. This latter possibility is particularly provocative since lung cancer patients generally have a dismal prognosis and relatively few therapeutic options.

Sex hormones influence the development and progression of carcinomas in a number of organs which are considered major target organs of these hormones, including androgens in prostate cancer and estrogens and progestins in breast and uterine cancer. Well-established antihormone therapies, such as treatment of prostate cancer with the antiandrogen flutamide and treatment of breast cancer with the antiestrogen tamoxifen are based on this observation. For example, approximately 2 of 3 of all primary breast cancers contain detectable ERs and, of these, about 60% will respond to hormonal therapy. In contrast, <10% of ER-negative tumors will respond (32).

Although the lung is not usually considered a major target organ of the sex hormones, evidence suggests that estrogen may have a role in the development of the normal lung. For example, Khosla and Rooney (33) found that the surfactant content of fetal rabbit lungs was increased 4-fold when pregnant rabbits were given 17β-estradiol. It is possible that some lung cancers may retain this ability to respond to the sex hormones.

The epidemiology of lung cancer differs between men and women. Adenocarcinoma is the most frequent cell type among women, while squamous cell carcinoma is the most frequent among men. In particular, adenocarcinoma accounts for >75% of the lung cancers occurring in nonsmoking women. (Although nonsmoking men have an increased incidence of adenocarcinoma compared to smoking men, it is less than that seen in nonsmoking women, about 40%). It has been proposed that these epidemiological findings might be partly explained by differing sex hormone levels in men and women (2-9).

There is evidence that the sex hormones influence the growth of carcinomas arising in other nontarget organs which, like the lung, are derived embryologically from the endodermal tube. For example, ERs have been reported in 10 of 11 human pancreatic adenocarcinomas (10, 11). Growth of the ER-positive human pancreatic cancer line MiaPaCa is stimulated 40% above control by exposure to nanomolar concentrations of estradiol and is inhibited significantly by 1 μM tamoxifen (12). Administration of hypothalamic hormone analogues which suppress sex hormone production results in inhibition of growth of pancreatic adenocarcinomas in Wistar/Lewis rats and Syrian hamsters (13). Preliminary studies of tamoxifen therapy in 14 patients with unresectable pancreatic adenocarcinoma showed an increase in median survival from 2.5 to 8.5 months (14).

In addition, exogenous steroids induce benign and malignant hepatic neoplasms in humans (15-18) and repeated estra-
diol injections increase the incidence of hepatic neoplasms from 29 to 69% in acetylaminofluorene-treated Sherman rats (26).

Progestin therapy resulted in partial tumor regression in 2 of 5 patients with hepatocellular carcinoma as documented by computed tomographic scan (20). Kitaoka (21) reported a 43.3% three-year survival rate in 21 patients with gastric carcinoma who received tamoxifen in addition to chemotherapy after gastrectomy. This contrasted with a 5.6% three-year survival rate in 23 patients receiving only chemotherapy after gastrectomy. Estradiol stimulates growth in gastric cancer cell lines and in colorectal cancer cell lines. 5-Dihydrotestosterone inhibits growth in gastric cancer cell lines and has mixed effects in colorectal cancer cell lines (22).

In laboratory animals, the sex hormones affect the incidence of pulmonary neoplasms. In NZR/Gd rats, 70% of males but only 16% of females developed alveologenic lung carcinoma after a single dose dimethylnitrosamine (23). Lifetim administration of testosterone increased the incidence to 63% in females (24). In male Cri/CDF rats the incidence of alveologenic lung cancer after a single dose of dimethylnitrosamine was reduced from 60 to 17% and the number of tumors per tumor-bearing rat reduced from 3.2 to 1.6 after estrogen administration (25). In a similar study, stilbestrol administration reduced the incidence of lung adenomas from 30 to 10% in acetylamino fluorene-treated rats (26).

We have confirmed that some human lung cancers possess sex hormone receptors. We do not yet have enough cases to address specific epidemiological questions; for example, whether the predominance of adenocarcinomas in nonsmoking women is related to hormone receptor levels. Regardless of whether or not future studies demonstrate a unique relationship between receptors and subpopulations of patients, our findings indicate that the potential for hormonal responsiveness is not restricted to either sex, to one cell type, or to a negative smoking history.

The majority of patients with lung cancer are smokers and male and this is reflected in our series. Most of the patients in our series with positive receptor levels are also smokers and male, although our most impressive receptor level was in a woman. In other malignancies, male sex does not preclude positive receptor levels in both squamous cell carcinomas and adenocarcinomas (27–29). Bronchogenic carcinomas of all major cell types are thought to arise from a common precursor cell and share certain genetic abnormalities (i.e., deletions or rearrangements of the short arm of chromosome 3) (2, 3, 36, 37). Therefore, it is not surprising that lung cancers of different cell types share a property like positive sex hormone receptor status.

Assuming that the sex hormones influence the natural history of human lung cancer, elevated receptor levels are only one possible mechanism by which this influence may occur. Mutations in receptor genes may produce structurally altered receptors which have a higher affinity for the hormone or remain stable in an activated state. Sex hormones may activate oncogenes. For example, estradiol increases transcription of c-myc 10-fold in estrogen-responsive breast cancer cell lines. This effect is inhibited by tamoxifen (38, 39). We are investigating the possible role of these mechanisms in lung cancer.

The presence of ERs or PRs in some lung cancers does not prove that sex hormones have a specific role in the pathogenesis of lung cancer. However, we do not need to assume such a role to justify investigation of hormonal therapy in these tumors. Lung cancer patients currently have few therapeutic options. Even if the presence of receptors is fortuitous, further studies are needed to determine whether these receptor-positive tumors respond to therapeutic hormonal manipulation. Potentially, these studies may provide a basis for a new type of adjuvant therapy in selected lung cancer patients.

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