**ABSTRACT**

New strategies are needed for the detection and treatment of lung cancer and must derive from a fuller understanding of lung carcinogenesis. Frequent molecular genetic abnormalities occur in non-small cell lung cancer (NSCLC), but little is known about which of these precede an invasive carcinoma. We examined the expression of p53, epidermal growth factor receptor (EGFR), and transforming growth factor α, the most common molecular genetic abnormalities in NSCLC, in preneoplastic bronchial lesions. Primary NSCLC and associated bronchial lesions were identified by retrospective review of resected tumors at this center. Expression in the invasive carcinomas, the associated bronchial lesions, and normal lung were contrasted using immunohistochemistry. Thirty-four NSCLC associated with 62 bronchial lesions were identified. The invasive tumors included 15 squamous cell carcinomas (SCCs) and 19 non-SCCs. Bronchial lesions included areas of squamous metaplasia (n = 14), inflammatory atypia (n = 19), dysplasia (n = 17), and carcinoma in situ (n = 12). Nineteen (56%) NSCLC and 10 (16%) bronchial lesions exhibited aberrant p53 immunostaining, whereas 18 (53%) NSCLC and 30 (48%) bronchial lesions showed abnormal EGFR immunostaining. Positive staining for transforming growth factor α was seen in 16 (47%) NSCLC but occurred inconsistently in the bronchial lesions and in normal bronchial epithelium. Only bronchial lesions associated with squamous cell carcinomas exhibited staining for p53. Aberrant EGFR expression was not associated with a specific type of invasive carcinoma or with specific preneoplastic lesions, although there was a trend toward increased expression in dysplasia and carcinoma in situ relative to metaplasia and atypia. All but one of the NSCLC simultaneously showing aberrant p53 and EGFR staining were SCC. We conclude that: (a) transforming growth factor α is variably expressed in normal respiratory epithelium as well as reactive and preneoplastic bronchial lesions; (b) p53 expression is seen in preneoplastic bronchial lesions but is not present in reactive or metaplastic epithelium; (c) aberrant EGFR expression occurs in both reactive and preinvasive bronchial lesions and may be an early marker of neoplastic transformation; and (d) the simultaneous aberrant expression of EGFR and p53 occurs predominantly in SCC and their associated bronchial lesions. These findings indicate that aberrant expression of p53 or the EGFR is frequent in bronchial neoplasia, and coexpression may predispose to the development of squamous cell carcinomas of the lung.

**INTRODUCTION**

Lung cancer is a formidable clinical problem. There are now over 170,000 cases annually in the United States, and lung cancer is the most common cause of cancer-related deaths in both men and women. Despite improvements in patient care, the overall 5-year survival rate for lung cancer remains a dismal 10% (1). Although the clinical behavior and histological features of lung cancer have been exhaustively characterized, much less is known about the basic biology of lung cancer. A better understanding of the steps involved in lung tumor initiation and progression is needed to develop innovative approaches for detection and treatment that will improve the survival of patients with lung cancer.

Cytogenetic studies reveal that numerous chromosomal abnormalities are present in overt lung cancers and underscore the genetic complexity contributing to the genesis of these tumors (2). The biological role of specific chromosomal abnormalities, the sequence in which they occur during lung tumorigenesis, and the nature of the associated molecular genetic changes are largely undefined. Of those molecular genetic abnormalities already identified, lung cancers, like other solid tumors, are characterized by the activation of oncogenes (3), the expression of growth factor loops (4–6), and the inactivation of tumor suppressor genes (7, 8). To date, in NSCLC, the two most frequently identified abnormalities are deregulation of the tumor suppressor gene p53 (7, 9) and aberrant expression of the EGFR and of one of its ligands, TGFα (5).

Determining which of these molecular genetic abnormalities occur frequently in bronchial neoplasia could identify genetic changes that predispose lung cancers to develop. The systematic study of the genetic changes in other solid tumors and their premalignant lesions, most notably in colon cancers and colonic polyps, has led to an improved understanding of the steps in carcinogenesis. An analogous study in lung cancer and its premalignant lesions has considerable clinical relevance, because therapeutic interventions that potentially prevent or reverse preinvasive lesions (smoking cessation, treatment with retinoids, photodynamic therapy) now exist. The histopathological changes thought to precede invasive lung cancers include metaplasia, dysplasia, and CIS.

Several studies suggest that inactivation of p53 occurs in selected cases of bronchial dysplasia and CIS (10–13). However, early bronchial neoplasia has not been studied extensively or systematically because there is no routine way to detect this clinically. Most patients are diagnosed when they have advanced primary tumors and the associated early bronchial lesions are no longer present or may not be biopsied. Previous studies either were performed using small numbers of preinvasive bronchial lesions, or examined these lesions without comparison to the genetic changes present in the invasive NSCLC (10–13). This study was performed to examine systematically whether those frequent abnormalities found in NSCLC (i.e., altered expression of p53, EGFR, and TGFα) also occur in early bronchial neoplasia.

**MATERIALS AND METHODS**

**Pathological Criteria for Specimen Selection and Classification.** Reactive and preinvasive bronchial lesions occurring in association with invasive NSCLC were identified by a retrospective review of the Department of Pathology (Memorial Sloan-Kettering Cancer Center) records pertaining to pulmonary resections performed for primary NSCLC. The cell type of the invasive NSCLC was classified according to standard criteria (14). Each
patient from whom specimens were obtained for this study had a complete mediastinal lymph node dissection (15, 16) and was staged according to the International Staging System for non-small cell lung cancer (17).

Bronchial lesions were selected for this study when they occurred within the resected lung or lobe but were anatomically separate from the invasive NSCLC. Premalignant lesions included areas of dysplasia or CIS, whereas foci of squamous metaplasia and inflammatory atypia were considered reactive. Squamous metaplasia included areas of bronchial mucosa exhibiting replacement of the ciliated respiratory epithelium by mature or immature stratified squamous epithelium which lacked cytological atypia. The basal cell layer was generally well defined and uniform, and the maturation of more superficial cells appeared orderly (Fig. 1a). A category of inflammatory atypia was used for areas with or without squamous metaplasia which exhibited acute or chronic inflammatory cell infiltrates accompanied by mild cytological atypia (Fig. 1b). In areas of dysplasia, the metaplastic squamous epithelium exhibited mild to marked nuclear atypia (hyperchromasia, irregularity, clumping of chromatin) and an increased nucleus:cytoplasm ratio. The organization of the basal layer was disrupted, and the more superficial cells were arranged haphazardly. Mitotic figures were increased and often present above the basal layer (Fig. 1c). Varying degrees of dysplasia (mild, moderate, and severe) were recognized but were not included in the data analysis due to limited numbers in each group. CIS exhibited more marked cytarchitectural atypia than dysplasia. Extreme nuclear atypia, very high nucleus:cytoplasm ratio, and numerous mitotic figures at all levels of the epithelium were present. There was loss of the normal organization of the epithelium, and little maturation was seen (Fig. 1d).

Methods of Tissue Preparation and Immunohistochemical Staining. Five-mm sections were cut from the archival paraffin blocks of the invasive carcinomas and associated bronchial lesions and were placed on superfrost/plus microscope slides. The slides were baked at 60°C for 2 h immediately prior to staining. The sections were then deparaffinized in xylene and rehydrated through graded alcohols to distilled water. For staining of p53 and TGFα, the sections were placed in a citrate buffer solution (2.1 g of citric acid in 1 liter of distilled water, buffered to pH 6.0 with NaOH) in a microwavable container. The slides were microwaved in a citrate buffer at 560 W for two 5-min cycles, with addition of distilled water if necessary to correct for evaporation. The sections were allowed to cool in the solution for 20 min at room temperature, rinsed in distilled water, and transferred to a bath of PBS. For staining of EGFR, the sections were incubated for 10 min in 0.05% pepsin in 0.01% HCl preheated to 37°C, rinsed in distilled water, and transferred to PBS. For TGFα and EGFR, endogenous peroxidase activity was then quenched with a 5-min incubation in 3% hydrogen peroxide, followed by rinsing in PBS. The sections were then placed in a bath of 0.05% BSA/PBS for 1 min, followed by application of normal horse suppressor serum diluted 1:5 in 2% BSA/PBS for 10 min. The normal horse suppressor serum was removed, and the sections were incubated overnight with the primary antibody (Table 1) diluted with 2% BSA/PBS at 4°C. The following day the primary antibody was removed and the slides were rinsed in three changes of PBS. The secondary antibody (biotinylated horse anti-mouse diluted 1:500 in 1% BSA/PBS) was applied for 60 min. The sections were again washed in PBS followed by application of horseradish peroxidase-conjugated streptavidin diluted 1:500 in 1% BSA/PBS for 60 min. Diaminobenzidine was used as the chromogen. The slides were counterstained with hematoxylin, dehydrated, and coverslipped. The mAbs used in this study included pAb1801 recognizing p53 (Oncogene, Uniondale, NY) in a dilution of 1:500, 100701 anti-EGFR antibody (Trition Diagnostics, Alameda, CA) in a dilution of 1:200, and Ab-2, an anti-TGFα antibody (Oncogene, Uniondale, NY) in a dilution of 1:500.

Negative controls for each of the antibodies were performed using nonimmune serum instead of the primary antibody. Positive controls consisted of pulmonary squamous cell carcinomas known to exhibit nuclear overexpression of p53 or cytoplasmic overexpression of EGFR or TGFα, respectively. The uniform presence of EGFR staining in the basal layer of the bronchial epithelium and its absence in other normal pulmonary tissues served as additional internal positive and negative controls, respectively. The uniform staining of nerves and submucosal salivary glands for TGFα served as an internal positive control. To confirm the specificity of positive immunostaining for TGFα, sections of a TGFα-positive squamous cell carcinoma and adjacent nonneoplastic pulmonary tissues were stained for TGFα following overnight preincubation of the primary antibody (diluted 1:500 in 2% BSA/PBS) with equal volumes of either appropriately diluted TGFα, EGF, insulin, or PBS. The preincubated antiserum was applied in the usual manner, and the remainder of the above protocol was followed.

Criteria for Evaluation of Immunostaining Results. For p53, microscopic examination for the nuclear reaction product was performed in the invasive carcinomas and bronchial lesions and scored as follows: negative = <5% of cells staining; + = 5-30% of cells staining; ++ = 30-70% of cells staining; and +++ = 70-100% of cells staining. For EGFR and TGFα, the same scale was applied to the invasive carcinomas for the cytoplasmic reaction product. For EGFR, the bronchial lesions were scored as follows: negative = no staining or staining of basal cell layer only (i.e., similar pattern to adjacent histologically normal bronchial epithelium); + = staining of cells in basal and suprabasal regions (up to one-third of the epithelial thickness); ++ = staining of cells up to two-thirds of the epithelial thickness; and +++ = staining of full thickness of epithelium. The presence of TGFα positivity in bronchial lesions relative to the adjacent histologically normal bronchial epithelium was assessed semiquantitatively as increased, similar, or decreased.

---

**Fig. 1.** Histological appearance of the bronchial lesions. (a) Squamous metaplasia. The ciliated respiratory epithelium is replaced by a stratified squamous epithelium. The cells exhibit orderly maturation, uniform nuclei, and a well defined basal layer. (b) Inflammatory atypia. The ciliated respiratory epithelium shows loss of mucus cells and disorganization. Nuclear hyperchromasia and scattered prominent nucleoli are present. The submucosa exhibits a lymphocytic infiltrate, while neutrophils are scattered within the epithelium. (c) Dysplasia (moderate). The squamous epithelium shows disorganization, with some variation in nuclear size and shape. Some superficial maturation is present, the basal layer is still evident, and the degree of nuclear pleomorphism is moderate. (d) Carcinoma in situ. There is haphazard arrangement of the cells and mitoses are present throughout the full thickness of the epithelium. Extreme nuclear irregularity is present, with clumping of chromatin and marked variation in shape and size of the nuclei.
ABERRANT EXPRESSION OF p53 AND EGFR IN BRONCHIAL NEOPLASIA

Because differential immunostaining for TGFα was not observed in normal epithelium versus the bronchial lesions, only the results in the invasive carcinomas are shown.

### Table 1 Results of immunostaining for p53, EGFR, and TGFα

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>TN Status</th>
<th>Stage</th>
<th>Invasive Cancer</th>
<th>Metaplasia</th>
<th>Atypia</th>
<th>Dysplasia</th>
<th>CIS</th>
<th>EGFR</th>
<th>Metaplasia</th>
<th>Atypia</th>
<th>Dysplasia</th>
<th>CIS</th>
<th>TGFα</th>
<th>Metaplasia</th>
<th>Atypia</th>
<th>Dysplasia</th>
<th>CIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCC</td>
<td>T2N0</td>
<td>I</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>T2N0</td>
<td>I</td>
<td></td>
<td>+</td>
<td>0</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>T2N0</td>
<td>II</td>
<td>++</td>
<td>0</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>T2N0</td>
<td>II</td>
<td>+</td>
<td>0</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>T2N0</td>
<td>I</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td></td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>T2N0</td>
<td>I</td>
<td>IIIA</td>
<td>++</td>
<td>0</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>0</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>T2N0</td>
<td>IIIA</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td></td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>I</td>
<td>+</td>
<td>0</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>II</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>IIIA</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>I</td>
<td>+</td>
<td>0</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>II</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>IIIA</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>I</td>
<td>+</td>
<td>0</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>II</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>IIIA</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>I</td>
<td>+</td>
<td>0</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>II</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>IIIA</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>SCC + foc</td>
<td>T2N0</td>
<td>I</td>
<td>0</td>
<td>*</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>SCC + foc</td>
<td>I</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td></td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>SCC + foc</td>
<td>IIIA</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td></td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>SCC + foc</td>
<td>T2N0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td></td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>No. of lesions</td>
<td>(+)/total #</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCC + foc</td>
<td>19/34</td>
<td>0/13</td>
<td>0/19</td>
<td>2/17</td>
<td>8/12</td>
<td>18/34</td>
<td>5/19</td>
<td>10/17</td>
<td>8/12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

As noted in "Materials and Methods," +, ++, and +++ proportion of cells exhibiting stain; 0, bronchial lesions present on histological examination but did not show staining for p53 or EGFR. Blank spaces, not present on histological examination; *, areas of metaplasia and dysplasia seen in association with invasive carcinoma were so small that insufficient tissue was available to perform staining for both p53 and EGFR.

## RESULTS

### Pathological Features of the Invasive Carcinomas and Associated Bronchial Lesions

A total of 34 primary NSCLC having at least 1 associated bronchial lesion were identified from retrospective review of 150 archival specimens of resected NSCLC resected at this center. The invasive carcinomas included 15 squamous cell carcinomas, 15 adenocarcinomas, 1 adenosquamous carcinoma, 1 adenocarcinoma with small foci of squamous cell carcinoma, 1 tumor that contained small foci of small cell carcinoma within a predominantly squamous cell carcinoma, and 1 mucoepidermoid carcinoma. Sixteen tumors were stage I, 10 were stage II, and 8 were stage IIIA (Table 1). A total of 62 associated bronchial lesions were identified and included 14 areas of metaplasia, 19 areas of atypia, 17 areas of dysplasia, and 12 CIS. These occurred in association with all stages and cell types of NSCLC. In 21 cases (62%), there were multiple types of bronchial lesions.

### Immunohistochemical Staining

Immunostaining for TGFα was consistently seen in nerves and submucosal salivary glands, and variable staining was observed in histologically normal bronchial epithelium (Fig. 2, a-c) without a consistent pattern associated within the epithelium. This immunostaining was antagonized by the addition of excess TGFα protein but not by epidermal growth factor (EGF) or insulin (data not shown), demonstrating the specificity of this antibody-antigen reaction. The p53 immunostaining was distinguished by its characteristic nuclear localization and was never detected in normal bronchial epithelium or other normal tissues (Fig. 3, a–e). EGFR immunostaining was uniformly present in the basal cell layer but not in the more superficial layers of histologically normal bronchial epithelium (Fig. 4, a–d). No consistent staining of other normal tissues was observed.

To summarize the results of this study in the bronchial lesions, staining was considered positive for p53 or aberrant for EGFR if any one of the lesions associated with a particular invasive carcinoma stained specifically. In general, TGFα expression was noted in both metaplastic and atypical lesions, as well as in dysplastic lesions and CIS. The variable staining of normal bronchial epithelium for TGFα precluded quantification of the staining of the bronchial lesions. In CIS there was a trend toward increased staining relative to the adjacent normal epithelium. This trend was not seen for dysplasia; in fact, some dysplastic lesions exhibited reduced staining relative to normal epithelium (Fig. 2, a, b, and c). For these reasons, the analysis is confined to the results of staining for p53 and EGFR.
ABERRANT EXPRESSION OF p53 AND EGFR IN BRONCHIAL NEOPLASIA

Fig. 2. Immunohistochemical staining for TGFα. Invasive carcinomas were positive in 16 of 34 cases (a). In normal bronchi, there was consistent staining in submucosal salivary glands (SG) and nerves (N), with patchy staining of the overlying respiratory epithelium (b). While some areas of CIS were intensely stained (c), the variable positivity in normal and reactive epithelia made quantitation difficult.

CIS (8 of 12) than in dysplastic lesions (2 of 17) (Fig. 3, b and c). In one case, pagetoid spread of individual cells of CIS beneath the ciliated respiratory epithelium was recognized by the positivity of the CIS elements for p53 (Fig. 3d). There were 10 cases in which the invasive carcinoma showed p53 staining and the associated areas of dysplasia or CIS did not. All of these invasive carcinomas were adenocarcinomas. However, the converse did not occur. All areas of dysplasia or CIS that showed p53 staining were associated with an invasive carcinoma that was also p53 positive.

Eighteen (53%) of the invasive carcinomas showed positive staining for EGFR (Fig. 4a). Thirty (48%) of the bronchial lesions showed the characteristic aberrant pattern of staining for EGFR. In addition to positivity of the basal cell layer, staining was also observed in the superficial layers of the bronchial epithelium (Fig. 4b). In contrast to the p53 staining, aberrant EGFR staining occurred as frequently in metaplasia and atypia as it did in dysplasia and CIS (Table 1). However, the staining was more intense and involved more of the superficial layers of the epithelium in areas of dysplasia and CIS than in metaplasia and atypia (Fig. 4, c and d). Of note, in 11 cases the bronchial lesions showed an aberrant EGFR staining pattern when the associated invasive carcinoma did not stain for EGFR. In 5 of these cases, the preinvasive lesion was a CIS, while in the other 6 it was an area of metaplasia, atypia, or dysplasia.

Ten (29%) of the invasive carcinomas showed staining for both p53 and EGFR. Seven of the 34 (21%) cases had bronchial lesions with both positive immunostaining for p53 and the described abnormal staining pattern for EGFR (Fig. 5a). For both the invasive carcinomas and the bronchial lesions this combined abnormality of p53 and EGFR occurred almost exclusively in squamous cell carcinomas as compared to non-squamous cell tumors. Only 1 of the 20 non-squamous tumors and none of the associated bronchial lesions showed simultaneous positive immunostaining for both p53 and EGFR (Fig. 5b).

DISCUSSION

Understanding the molecular events involved in lung carcinogenesis is essential to developing strategies for the prevention and treatment of this common and lethal malignancy, yet relatively little is known about those genetic steps which precede or cause NSCLC to form or to become invasive. Squamous cell carcinomas are thought to arise as a progression from metaplasia to dysplasia, CIS, and finally invasive cancer. It is unclear at what stage in this progression the lesion should be regarded as "preneoplastic." It is known that some of the earlier steps in this process, particularly metaplasia and atypia, can revert to a normal appearing bronchial epithelium (18). Possible precursor lesions for adenocarcinomas and other types of NSCLC are less well established. Preneoplastic changes similar to those seen in squamous cell carcinoma may be present, perhaps reflecting multicentric carcinogenic foci. Determining which of the common molecular genetic abnormalities known to occur in primary NSCLC also occur frequently in early bronchial neoplasia, and correlating them with pathological and clinical features, could define more precisely the critical events responsible lung tumorigenesis. This knowledge would allow patients at risk for progression to invasive carcinomas to be identified and offered effective chemoprevention. This approach is now clinically relevant because of the recent advent of autofluorescence bronchoscopy, which permits targeted biopsies of areas of early bronchial neoplasia not visible by standard white light bronchoscopy (19), and because of potentially effective methods of chemopreven-
ABERRANT EXPRESSION OF p53 AND EGFR IN BRONCHIAL NEOPLASIA

Fig. 3. Immunohistochemical staining for p53 protein. Invasive carcinomas exhibited nuclear immunoreactivity in 19 of 34 cases (a). Positivity was also seen in areas of CIS (b), while adjacent normal respiratory epithelium was invariably negative. In rare instances, foci of dysplasia were positive (c). In one case there was pagetoid spread of CIS within the ciliated respiratory epithelium (d), and only the CIS cells were p53 positive (e).

The molecular genetic changes already identified in primary NSCLC include the activation of oncogenes, the expression of growth factor loops, and the inactivation of tumor suppressor genes. Cytogenetic abnormalities including 3p deletion also occur commonly in NSCLC, but the gene lost at this site has not yet been identified. K-ras is the most frequently activated oncogene in NSCLC. Its activation by point mutation may play a role in tumor progression, since this abnormality is correlated clinically with a short overall survival rate (3). However, K-ras activation almost always occurs in adenocarcinomas and not in other tumor types. The most frequent genetic alterations reported in NSCLC include inactivation of the tumor suppressor gene p53 (7, 9) and overexpression of EGFR and of one its ligands, TGFα (5). These abnormalities are found in approximately 50% of NSCLC and are seen in all cell types and tumor stages. The frequency of these three molecular abnormalities in invasive carcinomas prompted us to examine the incidence of aberrant expression in bronchial lesions that are thought to precede the development of overt NSCLC.

Both p53 protein overexpression and p53 mutation are reported to occur in bronchial dysplasia and CIS (10–13, 20). They are also seen in premalignant lesions associated with other tobacco-related tumors including head and neck and esophageal squamous cell carcinomas (21, 22). However, p53 expression has been examined either in bronchial preneoplastic lesions in the absence of associated invasive carcinomas or in small numbers of selected cases with associated squamous cell cancers (10–13, 20). The expression of p53 in bronchial lesions associated with other NSCLC histologies has not yet been reported. EGFR and TGFα expression in early bronchial neoplasia have not been systematically analyzed previously. Therefore, p53, EGFR, and TGFα expression in bronchial lesions associated with invasive carcinomas of different histologies and tumor stages were examined in this large series of cases.

Specimens for this study were drawn from patients who theoretically might be at increased risk for preinvasive lesions of the bron-
chial epithelium because they already had an established NSCLC. The small number of preinvasive lesions found in a large number of archival specimens may reflect the limited sampling of bronchial epithelium which has historically been performed and which does not permit examination of all of the epithelium at risk for malignant transformation. Despite these sampling limitations, it is notable that over 60% of the specimens in this series had multiple types of bronchial lesions in association with an invasive carcinoma.

Immunohistochemical staining was selected as the method of choice for determining abnormal expression of p53, EGFR, and TGFα because it is accurate and is easily applied to small paraffin-embedded specimens. Positive p53 immunostaining is thought to provide a more complete assessment of p53 abnormalities than sequence analysis because it reflects both the presence of most mutant p53 protein product and the accumulation of wild-type p53 protein products which may relate to other deregulated cellular functions (9). We have shown previously that immunohistochemical staining in NSCLC and in adjacent benign lung for EGFR and TGFα correlates with overexpression at the level of total cellular RNA (5).

This study highlights several notable findings. Although TGFα is frequently intensely expressed in invasive NSCLC, it is often expressed in histologically normal bronchial epithelium and is inconsistently expressed in early bronchial neoplasia. The use of positive and negative controls in this study indicates that these staining patterns for TGFα are specific and not artifactual. TGFα expression can occur early in the process of malignant transformation but may also reflect a nonspecific response to cellular injury. Our findings do not suggest that TGFα expression is useful as a marker of early lung tumorigenesis.

This study confirms four previous reports indicating the occurrence of p53 expression by immunohistochemical staining in dysplastic bronchial epithelium and CIS (10–13, 20). p53 expression has rarely been reported to occur in metaplasia (13) but was not found in metaplastic or atypical lesions in this study. Our findings also confirm a previous study showing that p53 protein expression occurs in dysplasia or CIS when the associated invasive carcinomas also exhibit p53 positivity by immunohistochemistry (12). However, some p53-positive invasive carcinomas may be associated with dysplastic lesions or CIS that do not show p53 staining. These results suggest that p53 abnormalities occur early in the sequence of carcinogenesis but are not always necessary for invasion to occur. p53 staining was not seen in any of the bronchial lesions associated with invasive tumors of histologies other than squamous cell carcinoma even when the invasive carcinoma was p53 positive. However, this could reflect the fact that only one area of CIS associated with adenocarcinoma was available for analysis. Although the sequence of metaplasia, dysplasia, CIS, and invasive carcinoma is thought to be the process through which squamous cell carcinomas develop, it is not yet known whether a similar progress occurs in the more distal airways in adenocarcinomas. Since adenocarcinomas arise in the pulmonary parenchyma from cells unrelated to bronchial epithelial cells, it is possible that preneoplastic bronchial lesions might not fully reflect this process of peripheral lung tumorigenesis. In accordance with the concept of “field cancerization,” these bronchial lesions could represent changes that are occurring simultaneously with peripheral lung carcinomas but are not biologically related to them, yet the striking difference in p53 expression in the bronchial lesions associated with squamous versus nonsquamous tumors suggests otherwise. Our results indicate that
ABERRANT EXPRESSION OF p53 AND EGFR IN BRONCHIAL NEOPLASIA

**ACKNOWLEDGMENTS**

We thank Melody Owens for her expert assistance in the preparation of this manuscript.

**REFERENCES**

ABERRANT EXPRESSION OF p53 AND EGFR IN BRONCHIAL NEOPLASIA


Aberrant Expression of \( p53 \) or the Epidermal Growth Factor Receptor Is Frequent in Early Bronchial Neoplasia, and Coexpression Precedes Squamous Cell Carcinoma Development

Valerie Rusch, David Klimstra, Irina Linkov, et al.


Updated version  Access the most recent version of this article at: [http://cancerres.aacrjournals.org/content/55/6/1365](http://cancerres.aacrjournals.org/content/55/6/1365)

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.