Abstract. Background: The 1999 WHO/IASLC histological classification of preneoplastic bronchial lesions has been shown to be reproducible, but little is known about its biological significance. The EGFR expression rate increases from normal epithelium to carcinoma in situ (CIS) with a significant difference between mild versus severe dysplasia. C-erbB-2, another member of the erbB family, is overexpressed in lung carcinomas, suggesting that this mechanism may play a role in carcinogenesis. We evaluated the correspondence between the morphological changes of the bronchial epithelium and the c-erbB-2 expression. Materials and Methods: Nine normal bronchial epithelia, 16 hyperplasia, 12 metaplasia, 12 mild dysplasia, 8 severe dysplasia, 11 CIS and 6 microinvasive tumours were evaluated. Immunostaining was performed using anti-c-erbB-2 antibodies (clone CB11). Results: No immunostaining was found whatever the bronchial lesions evaluated. Conclusion: C-erbB-2 does not seem to be involved in the first step of carcinogenesis of squamous cell carcinoma. These findings suggest that there is no place for chemoprevention by anti-c-erbB-2 drugs such as trastuzumab in lung cancer.

Lung cancer is the most common cause of death by malignancy in Europe and less than 10% of the patients can be cured. As tumour stage is the most significant prognostic factor for survival, there is considerable interest in early detection of the disease. It also seems important to detect bronchial mucosal abnormalities that could be precursors for lung cancer. The precursors of squamous cell lung carcinoma are well known: they include dysplasia and carcinoma in situ (CIS). Fluorescence bronchoscopy significantly increases the detection rate of these preneoplastic lesions and a WHO/IASLC consensus classification system of pre-invasive squamous lesions of the bronchial mucosa has recently become available (1).

Lung tumour development results from a multi-step accumulation of genetic and molecular alterations involving proto-oncogenes, tumour suppressor genes, proliferation factors and apoptosis. These biological alterations, mainly caused by inhaled carcinogens, are early events in carcinogenesis and can already be present in preneoplastic or early invasive bronchial lesions. Among the multiple biomarkers that have been implicated in bronchial carcinogenesis, the erbB family receptors could be particularly interesting. In a previous study, we showed that the EGFR expression rate changes with the stage of the bronchial lesion, increasing from normal epithelium to CIS and microinvasive tumours, with a statistically significant difference between mild versus severe dysplasia (2). In the present study, we evaluated another member of the erbB family, c-erbB-2 to assess whether it behaves as EGFR.

Our choice was made on the observation that c-erbB-2 (also called HER-2/Neu) is overexpressed in lung carcinomas, suggesting that its overexpression could play a critical role in the development and the progression of human cancers. Moreover, c-erbB-2 seems to be a prognostic factor of poor survival in non-small cell lung cancer (NSCLC) (3).

With this in mind, the aim of the present study was to evaluate the association between morphological changes of the bronchial epithelium and c-erbB-2 expression in order to better understand the WHO/IASLC classification from a biological point of view.
Materials and Methods

Study population. To be eligible, patients had to have a minimum smoking exposure of 30 cigarettes pack-years or and to have a history of lung or head and neck cancer. Fluorescence bronchoscopy was performed under local anaesthesia and any area that appeared as abnormal during fluorescence examination was biopsied (4). We reviewed 124 eligible consecutive biopsies from 124 different patients that had undergone laser-induced fluorescence (LIFE) bronchoscopy between February 1996 and October 2001. Ninety-eight were male and 26 female, with a median age of 63 years (range: 29-85). Thirty-six were current smokers, 67 former smokers, 3 non-smokers and for 18, smoking habits were unknown. Fifty-two had LIFE bronchoscopy for a follow-up of lung cancer (44 for NSCLC and 8 for SCLC), 26 for lung cancer screening, 22 for the pre-operative work-up of a lung (n=15) or head and neck cancer (n=7), 17 for the follow-up of a CIS of the lung and 7 for various other reasons. Several biopsies were performed for each patient. For statistical reasons, only the most severe lesion found in a given patient was evaluated and the patient was included only once in the study.

Sample preparation and selection. All the small biopsied tissues were routinely fixed in 10% neutral buffered formalin immediately following the bronchoscopic procedure and were embedded in paraffin. From each specimen block, 4-μm sections were cut from paraffin-embedded tissues and were deposited on SuperFrost Plus Slides (Menzel-Gläser, Braunschweig, Germany). All the haematoxylin eosin stained lesions were classified by one pathologist (JMV) according to the 1999 histological WHO/IASLC classification of pre-invasive squamous lesions of the bronchus (1).

Staining. All the reagents were of analytic quality and were used without any preliminary purification. Methanol, citric acid, sodium citrate, tris(hydroxymethyl)aminomethane (TRIS) and hydrochloridric acid were purchased from Merck (Darmstad, Germany). Immunohistochemistry was performed according to a standard avidin-biotin-peroxidase complex.

The slides were deparaffinised in xylene and rehydrated in ethanol. The slides were submitted to antigen retrieval in citric acid monohydrate buffer 0.01 M pH 6, consisting of 5 times 5-minute microwave treatments at 650 W. The slides were cooled for 25 minutes at room temperature and were rinsed twice in TRIS-HCl 0.005M, NaCl 0.9% pH 7.6 for 10 minutes. All the next steps were carried out automatically at 37°C in the NexES system (Ventana Medical Systems, Tucson, AZ, USA). The endogenous peroxidases were quenched by incubation in 0.3% hydrogen peroxide in methanol for 4 minutes at room temperature. The antibody anti-c-erbB-2, giving a membrane staining (clone CB11 from Novocastra Laboratories Newcastle Upon Tyne, UK) was used at a dilution 1/30; (final titration 0.9 μg/ml) and samples were incubated for 30 minutes. The complex between the protein and its antibody was carried out automatically at 37°C in the NexES system (Ventana Medical Systems, Tucson, AZ, USA). The endogenous peroxidases were quenched by incubation in 0.3% hydrogen peroxide in methanol for 4 minutes at room temperature. The antibody anti-c-erbB-2, giving a membrane staining (clone CB11 from Novocastra Laboratories Newcastle Upon Tyne, UK) was used at a dilution 1/30; (final titration 0.9 μg/ml) and samples were incubated for 30 minutes. The complex between the protein and its antibody was fixed with glutaraldehyde NaCl 0.9%. The secondary biotinylated antibody was incubated for 8 minutes. The slides were stained with diaminobenzidine (DAB) detection kit (Ventana Medical Systems) and counterstained with haematoxylin. Negative controls were carried out by omitting the primary antibody and also by substituting normal mouse immunoglobulin G1 for primary antibody. The positive controls were known positive breast cancer. Three observers (APM, BM, JMV) independently evaluated the slides. We used the classification applied in breast cancer according to the HercepTest® kit scoring guidelines approved by the Food and Drug Administration. Immunoreaction was considered as weakly positive (2+) if more than 10% of the tumour cells showed weak to moderate complete membrane staining, or as strongly positive (3+) if a strong complete membrane staining was observed in more than 10% of the tumour cells. All other staining patterns were interpreted as negative (0-1+).

Results

Among the 124 eligible patients, 74 patients (56 men and 18 women) provided sufficient large biopsies to allow c-erbB-2 expression assessment. The median patient age was 63 years (range: 29 – 76). Twenty patients were current smokers, 38 former smokers, 2 non-smokers and, for 14, smoking habits were unknown. The bronchoscopic procedure was performed in 15 patients for lung cancer screening, in 14 in the pre-operative work-up of a bronchial (10 patients) or a head and neck (4 patients) cancer, in 33 for follow-up of a previously treated lung cancer, in 8 for follow-up of CIS and in 4 for various reasons. Nine normal bronchial epithelia, 16 hyperplasia, 12 metaplasia, 12 mild dysplasia (MiD), 8 severe dysplasia (SD), 11 CIS and 6 microinvasive tumours were found evaluable.

No staining for c-erbB-2 was found in any of the 74 evaluated bronchial lesions (the positive control showing well a 3+ positive staining for c-erbB-2).

Discussion

This study has shown that c-erbB-2 is not expressed in pre-as well as early invasive bronchial lesions. These results are quite different from those reported in two previous studies. Piyathilake et al. (5) evaluated the expression of c-erbB-2, EGFR and TGF alpha in squamous cell carcinoma, in associated precancerous lesions and in normal bronchial epithelia. They found that the expression of c-erbB-2 was significantly higher in squamous cell carcinoma and associated precancerous lesions than in the normal bronchial epithelia and hyperplastic lesions of non cancer patients. However, for c-erbB-2, in contrast to EGFR, a significant increase was not observed from uninvolved bronchial mucosa (in cancer patients) to bronchial lesions and then to cancer. Moreover, the expression of c-erbB-2 was lower in basal cells compared with luminal cells in the same lesions, suggesting a lack of correlation with cell proliferation. This observation suggests that c-erbB-2 may not be involved in lung carcinogenesis. Hirsch et al. (6) reported, in a review article, that c-erbB-2 was expressed in 70% of 133 bronchial lesions without difference between low and high grade dysplasia, but without other information. All together, these results suggest that the c-erbB-2 is not or only minimally implicated in lung carcinogenesis and, in
by our population included only 14 patients with an active cancer. In contrast, our population included only 14 patients with an active cancer. Moreover, with LIFE bronchoscopy, we explored the cancerisation field whereas Piyathilake et al. (5) considered both membrane and cytoplasmic staining whereas Hirsch et al. (6) did not define its positivity rate. Whether cytoplasmic staining should be taken into account in lung cancer is still not clear, but interpreting the precise staining pattern against a background seems difficult. As recommended in the HercepTest® approved scoring system, we only took into account the membrane staining and not the cytoplasmic one. Moreover, the Piyathilake et al. population was quite different from the presently studied population. Indeed, they included 46 hyperplasia, 8 dysplasia, 4 metaplasia and 58 uninvolved bronchial epithelia in 60 patients with an active cancer. In contrast, our population included only 14 patients with an active cancer. Moreover, with LIFE bronchoscopy, we explored all the cancerisation field whereas Piyathilake et al. selected only one or some tissues blocks from each patient with cancer in order to provide sections that contained samples of bronchial lesions.

In a meta-analysis of the literature, we found that, according to the positivity threshold for c-erbB-2 expression as defined by the study authors, c-erbB-2 was expressed in 31% of the NSCLC patients (3). Moreover, in a previous work (7), we found, in 106 NSCLC, 22% positive (2+ and 3+) c-erbB-2 tumours. C-erbB-2 expression is thus less frequent than EGFR in lung cancer. Finally, c-erbB-2 was also evaluated in some lung cancers of the patients who benefited from LIFE bronchoscopy. We observed, in this population, that 25% of the tumours were positive for c-erbB-2. This less frequent expression could also, in part, explain the absence of c-erbB-2 expression in preneoplastic bronchial lesions. C-erbB-2 and EGFR are two growth factor receptors of the same family. However, c-erbB-2 is less expressed in preneoplastic bronchial lesions. C-erbB-2 expression is thus less frequent than EGFR in lung cancer. In contrast, our population included only 14 patients with an active cancer. Moreover, with LIFE bronchoscopy, we explored all the cancerisation field whereas Piyathilake et al. selected only one or some tissues blocks from each patient with cancer in order to provide sections that contained samples of bronchial lesions.

The role of c-erbB-2 could also be more important in the first steps of lung carcinogenesis and, if implicated, its expression occurs only late in the carcinogenic process. There then seems to be no role for anti-c-erbB-2 therapy (trastuzumab) in lung cancer prevention.

References


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