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Predicting non-small cell lung cancer expression of epidermal growth factor receptor and matrix metalloproteinase 9 from immunohistochemical staining of diagnostic biopsy samples

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Abstract

Epidermal growth factor receptor (EGFR) and matrix metalloproteinase 9 (MMP9) expression in resected non-small cell lung cancer (NSCLC) has been associated with a poor prognosis. Specific inhibitors have been developed to these molecules and have entered into clinical practice. We performed immunohistochemical staining on a series of 36 resected cases of NSCLC in parallel with the associated preoperative diagnostic biopsies in order to determine whether expression of these markers in the tumour could reliably be predicted from the result obtained with the small diagnostic biopsy. The results demonstrated considerable intratumour heterogeneity of expression for both markers in the resected tumours, and this was associated with a poor negative predictive value for the result obtained with the diagnostic biopsy. We conclude that, staining small diagnostic biopsies for EGFR and MMP9 is unlikely to be helpful in defining tumour status for these markers and allowing targeted therapy.

Keywords: Non-small cell lung cancer; Epidermal growth factor receptor; Metalloproteinase; Immunohistochemistry

1. Introduction

Non-small cell lung carcinoma (NSCLC) is one of the most common forms of primary malignancy in the Western world [1]. The overall prognosis remains very poor and the majority of patients are inoperable at presentation [2]. Whilst chemotherapy for small cell carcinoma has been accepted as routine for many years, its use in non-small cell carcinoma is less well established [3]. Recently, improvements in our understanding of tumour cell biology and the interaction of cells with the surrounding tissues have suggested possible new therapeutic targets [4,5].

Epidermal growth factor receptor (EGFR) is a growth factor receptor tyrosine kinase with a number of ligands including EGF, and its expression has been identified in a wide number of cancer types [6]. Gene amplification and autocrine stimulation may be an important factor in tumour growth and invasive behaviour, providing cells with growth and survival advantages [7]. Immunohistochemical expression of EGFR is present in the majority of NSCLC [8], although the association between expression and poorer prognosis is less clear-cut than for primary tumours at other sites [6,9].

Matrix metalloproteinase 9 (MMP9) is a gelatinase, a member of a family of enzymes that degrade extracellular matrix, and is thought to be important in facilitating tumour cell invasion and angiogenesis [4,10]. MMP9 degrades collage IV, a major component of the basement membrane, and increased amounts have been detected in NSCLC [11–13]. Varying results of studies linking expression to prognosis have been reported [11,12], with one group finding that coexpression with EGFR may be an independent prognostic factor [12].

Much of the recent interest in these molecules has arisen because specific inhibitors are available; these have entered clinical practice as part of the chemotherapeutic armoury used to treat NSCLC but the results have been

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variable [5,6,14–16]. Not all NSCLC express EGFR and MMP9, some studies finding positivity in only 50–60% of resected tumours [12], and the relation between expression and response is unclear. If these agents are to be used in the majority of patients who have inoperable tumours, then assessment of these for the expression of EGFR and MMP9 using small diagnostic biopsies may be desirable in targeting therapy. In order to assess whether the expression of EGFR and MMP9 could be predicted from small biopsies we studied matched pairs of specimens from patients diagnosed with NSCLC by small endobronchial or transthoracic needle biopsies who underwent subsequent resection.

2. Materials and methods

2.1. Patients

Ethical approval for the study was obtained from the local research ethics committee. Eighteen consecutive cases each of squamous carcinoma and adenocarcinoma (19 lobectomies and 17 pneumonectomies) were identified in the departmental archives for which preoperative (22 bronchial biopsies and 14 transthoracic needle biopsies) specimens were available and sufficient material was left in the tissue block to allow further sections to be taken (18 males, median age 72.5 years, range 55–81 years).

2.2. Immunohistochemistry

Sections (3 μm) were cut from each block with tumour in it from the resection specimens (median 3, range 1–4) and from the diagnostic preoperative biopsies. The sections were stained following antigen retrieval in citric acid buffer with antisera to EGFR (clone EGFR.113 40; Novocastra Laboratories, UK) diluted 1:150 and MMP9 (clone 56–2A4; Chemicon International, UK) diluted 1:40, using a standard immunoperoxidase method and an automated staining machine (TechMate; Dako Corp., UK) with appropriate positive and negative controls.

2.3. Scoring

Each slide from every resection specimen was scored separately. Only positive staining of tumour cells was scored, and this was recorded as 100%, 75%, 50%, 25% or 0% of the tumour present. Following scoring of the resected tumours the small preoperative biopsies were assessed and scored as positive or negative without knowledge of the results obtained for the resections.

2.4. Statistical Analyses

The results were analysed with Mann–Whitney tests and 2×2 contingency tables.

3. Results

3.1. EGFR

The results obtained for staining both the resected tumour and the preoperative diagnostic biopsies are shown in Table 1. Five (14%) of the tumours were entirely positive (4 squamous, 1 adenocarcinoma) and five (14%) completely negative (1 squamous and 4 adenocarcinoma), with the remaining 26 cases showing variable staining within individual blocks and between different blocks. Twenty-six (72%) of the diagnostic biopsies were positive and 10 negative.

Separating the cases into two groups on the basis of the positive and negative result obtained for the small diagnostic biopsies showed that the scores obtained for the staining of the resection specimens was significantly higher ($P < 0.01$, Mann–Whitney) in the cases with positive small biopsies.

The resection cases were then classified as positive (those showing at least focal positive staining) or negative (those entirely negative), and the results were entered in a 2×2 contingency table (Table 2) to assess the value of the immunohistochemical result obtained for the small biopsies in assessing the status of the resected tumour. This demonstrated a predictive value for a positive result obtained with the small biopsy of 0.92 (95% confidence interval (CI) 0.82–1.03) but the predictive value of a negative result was only 0.3 (95% CI 0.02–0.58).

3.2. MMP9

The results obtained for staining both the resected tumour and the preoperative diagnostic biopsies are shown in Table 1. Nine (25%) of the tumours were entirely positive (7 squamous, 2 adenocarcinoma) and none completely negative, with the remaining 27 cases showing variable staining within individual blocks and between the different blocks. Thirty (83%) of the diagnostic biopsies were positive and six negative.

Separating the cases into two groups on the basis of the positive and negative result obtained for the small diagnostic biopsies showed that the scores obtained for the staining of the resection specimens were significantly higher ($P < 0.01$, Mann–Whitney) in the cases with positive small biopsies.

The resection cases were then classified as positive (those showing at least focal positive staining) or negative (those entirely negative), and the results were entered in a 2×2 contingency table (Table 3) to assess the value of the immunohistochemical result obtained for the small biopsies in assessing the status of the resected tumour. This demonstrated a predictive value for a positive result obtained with the small biopsy of 1.0 (95% CI 1.0–1.0) but the predictive value of a negative result was 0 (95% CI 0.0–0.0).

Table 1
Results obtained staining the resected tumour and corresponding diagnostic biopsies for epidermal growth factor receptor (EGFR) and matrix metalloproteinase 9 (MMP9)

Cell type	Scores for EGFR on resection specimens	EGFR small biopsy	Scores for MMP9 on resection specimens	MMP9 small biopsy
SQUAM	100,100,75	+	75,100,50	+
SQUAM	100,100,75,100	+	100,100,100,100	+
SQUAM	25,100,50,75	+	100,75,50,100	+
SQUAM	0,25,50,25	+	100,100,100,100	+
SQUAM	50	+	50	+
SQUAM	50,25,25	+	75,75,75	+
SQUAM	25,0,50,25	+	75,100,75,75	+
SQUAM	100,100,100,100	+	75,50,50,100	−
SQUAM	100,100,100	+	75,100,100	+
SQUAM	50,75,50,75	+	100,100,100,100	+
SQUAM	25,50,100,100	+	100,100,100,100	+
SQUAM	100,100,100,100	+	100,100,100,100	+
SQUAM	100,100,100	+	100,100,100	+
SQUAM	100,25,75	+	100,100,100	+
SQUAM	50,100,50	+	75,50,75	−
SQUAM	25,25	+	50,75	+
SQUAM	25,0,0,	+	100,75,100	+
SQUAM	0,0,0	+	100,75,100	+
ADENO	25,100,100,100	−	75,75,100,100	+
ADENO	25,75,50	−	100,100,100	+
ADENO	0,25,0	−	75,25,25	−
ADENO	0,0,25,0	+	100,100,50,100	+
ADENO	50,50,25,25	−	100,75,100,75	+
ADENO	0,0,0,0	−	75,100,75,75	+
ADENO	100,100,100,100	+	100,100,100,100	+
ADENO	100,75,75,75	+	100,100,75,100	+
ADENO	0,25,25,0	+	100,75,100,75	+
ADENO	25,0,0,	−	100,100,75	+
ADENO	50,50,100,75	+	100,75,100,100	+
ADENO	100,75,75,75	−	75,100,100,100	+
ADENO	25,50,25	−	100,100,75	−
ADENO	0,0,0	−	75,100,100	−
ADENO	0,25	+	100,75	+
ADENO	0,0	+	50,75	+
ADENO	50,75,0	+	100,75,75	+
ADENO	0,0,0	−	25,25,25	−

The results for the resection specimens are expressed as the percentage of the tumour in the slide from each block expressing the antigen. The diagnostic biopsies were scored as positive (+) or negative (−).

Table 2
2 × 2 Contingency table summarising the results obtained for epidermal growth factor receptor (EGFR) staining

EGFR		Resection specimen		
		Negative	Positive	Total
Small biopsy	Negative	3	7	10
	Positive	2	24	26
	Total	5	31	36

Positive predictive value 0.92 (95% CI 0.82–1.03), negative predictive value 0.3 (95% CI 0.02–0.58).

Table 3
2 × 2 Contingency table summarising the results obtained for matrix metalloproteinase 9 (MMP9)

MMP9		Resection specimen		
		Negative	Positive	Total
Small biopsy	Negative	0	6	6
	Positive	0	30	30
	Total	0	36	36

Positive predictive value 1.0 (95% CI 1.0–1.0), negative predictive value 0.

4. Discussion

Immunohistochemical testing of small biopsies to determine the expression of prognostic factors has become established in some areas of pathology, particularly the breast to guide therapeutic strategies [17]. Lung tumours are, however, well recognised to show marked intratumour heterogeneity at the cellular [18] and genetic [19] levels, as well as in the expression of cellular proteins [20]. Previous studies have indicated that the expression of EGFR and MMP9 in resected NSCLC may have prognostic significance, but the possibility of

using small diagnostic biopsies from patients with NSCLC to predict the status of the tumour for EGFR and MMP9 expression has not been addressed.

Our results indicate that EGFR was expressed by most of the resected NSCLC (86%), although there was considerable variability in the proportion of the tumour staining between cases and marked heterogeneity even within sections from the same tumour. All the resected tumours in our series showed at least focal staining with MMP9, although there was a similar variability in the proportion of tumour staining within and between cases. The percentage of cases positive in this study is higher than in others and this may reflect the fact that we stained a median of three tumour blocks per case. Given the variability in staining observed, staining fewer blocks is likely to result in a higher numbers of negative cases. In keeping with this, two of the diagnostic biopsies were positive for EGFR but the resected blocks were all entirely negative. This is likely to reflect the fact that not all the resected tumour was processed and focal positive areas were not sampled when the specimen was dissected. Not surprisingly, as small biopsies represent a random sample of the main tumour, cases in which the small biopsy was positive with EGFR or MMP9 showed a statistically significant higher proportion of the resected tumour to be positive compared to those that were negative ($P < 0.01$).

There are as yet no data on the clinical significance of the proportion of the tumour staining positively with these markers, but is possible that patients with even focal positivity might benefit from therapy with specific inhibitors. The majority of patients with NSCLC are not suitable for surgery and prediction of the expression of these markers by the tumour might therefore, aid selection of patients who might benefit from these new drugs. We therefore, classified the resected cases as negative (entirely negative) or positive (any degree of positive staining) and examined the ability of the small biopsies to predict the presence of the positive staining in the resected specimen. This demonstrated a positive predictive value of 0.92 for EGFR and 1.0 for MMP9 but negative predictive values of only 0.3 and 0, respectively, indicating that whilst a positive result might be useful a negative result is more than likely to be false. This result is an inevitable consequence of the degree of intratumour heterogeneity we observed.

We conclude on the basis of these results that there is marked intratumour heterogeneity of expression of EGFR and MMP9 by NSCLC, and that immunohistochemical staining of small routine diagnostic biopsies is unlikely to be helpful in defining patients who may be suitable for therapy with specific inhibitors.

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