New approach to assessing lung tumours in man

KC GATTER,* MS DUNNILL,* J GERDES,† H STEIN,† DY MASON*

*From the *Nuffield Department of Pathology, University of Oxford, John Radcliffe Hospital, Oxford, and the †Institute of Pathology, Klinikum Steglitz, Berlin, West Germany*

SUMMARY One hundred and four surgically resected lung tumours were labelled in either cryostat or freeze dried sections with a monoclonal antibody (Ki67), which reacts with a nuclear antigen expressed by proliferating cells. The tumours were categorised semiquantitatively into four proliferative grades, a classification that can be performed rapidly and reproducibly by the pathologist. In keeping with previous cell kinetic studies all small cell carcinomas had high proliferation rates, whereas the carcinoid tumours were in the lowest grade. In contrast, the adenocarcinomas (27 cases) and squamous cell carcinomas (63 cases) varied widely in their proliferative state, in keeping with their heterogeneous, morphological, and clinical behaviour. Immunocytochemical labelling of lung tumour biopsy specimens with antibody Ki67 is a simple technique within the scope of routine surgical pathology laboratories, which might enable these tumours to be classified according to their proliferative status and treatment to be selected accordingly.

Although morphological classification of lung tumours is complex, several studies have indicated that the crucial distinction from a clinical point of view is between small cell carcinoma and other types of lung cancer.¹⁻³ This is based on the premise that small cell carcinoma is a distinct biological entity with a rapidly progressive course and early tumour dissemination.⁴⁻⁶ For these reasons it is, in contrast to other lung tumour types, rarely amenable to surgery, but it is sensitive to both radiotherapy and chemotherapy.

It has long been known, however, that tumours of the lung are histologically heterogeneous and that different morphological types may often be seen in the same tumour.⁷⁻¹⁰ Recent studies using electron microscopy and immunocytochemistry have emphasised this fact.¹¹⁻¹⁸ Clinical follow up studies have also consistently shown the unpredictable course of many lung tumours and have begun to question the wisdom of rigidly dividing them into two groups that require totally different strategies of treatment.¹⁹⁻²¹

The recent production of a monoclonal antibody (Ki67), which reacts with a nuclear antigen expressed by proliferating cells, prompted us to use this reagent to assess the growth pattern of a series of tumours in pulmonary resection specimens, using immunocytochemical techniques.^{22,23} Our aim was to see whether immunohistological assessment of the proliferative activity of lung tumours might show differences between individual neoplasms, which could help the oncologist to make his therapeutic decisions.

Material and methods

LUNG SPECIMENS

One hundred and four tumours were received as lung resection specimens from the operating theatre. Representative portions of tumour were removed for snap freezing and storage in liquid nitrogen. During the second half of this study techniques for preparing freeze dried paraffin embedded material became available, and portions of tumour were processed in this way using an Edwards-Pearse ETD4 tissue drier (Edwards High Vacuum, Crawley, United Kingdom), as detailed previously.^{24,25} Additional small samples from all specimens were fixed immediately in 4% glutaraldehyde and processed for electron microscopy. The remainder of the specimen was processed for conventional histological examination. The tumours were categorised on light microscopical appearances by two of the authors (KCG and MSD) as either squamous cell carcinoma (63), adenocarcinoma (27), small (oat) cell carcinoma (5), or carcinoid tumour (9).

IMMUNOCYTOCHEMISTRY

Sections (cryostat or freeze dried, or both) were stained with the monoclonal antibody Ki67^{22,23} either by a three stage immunoperoxidase procedure or by the APAAP immunoalkaline phosphatase technique, as detailed previously.^{26,27} The antigen recognised by Ki67 does not survive conventional fixation and is thus not suitable for retrospective studies on stored paraffin embedded tissue specimens.

Representative examples of proliferative grading system used in this study. Grade 1 is a carcinoid tumour (single labelled nucleus is arrowed), grades 2 and 3 are squamous cell carcinomas, and grade 4 is an oat cell carcinoma. All sections illustrated were stained by APAAP technique.

PROLIFERATION GRADE

The monoclonal antibody Ki67 reacts with a nuclear antigen (as yet unidentified) expressed by cells in all phases of the cycle except G_0 . Immunocytochemical labelling with this antibody has been shown to correlate well with conventional measures of cell proliferation using autoradiography and flow

cytometry²³ and therefore provides a reliable means of rapidly evaluating the growth fraction of normal and neoplastic human cell populations. Ki67 is now commercially available (as DAKO-PC) from Dakopatts a/s, Copenhagen.

In the present study the individual tumours were classified into four grades according to the proportion

Grade of proliferation assessed by immunohistological labelling

Tumour	Grade				Total
	1	2	3	4	
Squamous cell carcinoma	17	20	20	6	63
Adenocarcinoma	14	5	8	0	27
Small (oat) cell carcinoma	0	0	2	3	5
Carcinoid	9	0	0	0	9
Total	40	25	30	9	104

of labelled cell nuclei as follows: 0–10%, grade 1; 11–25%, grade 2; 26–50%, grade 3; and 51–75%, grade 4. These grades were initially established by counting the number of labelled and unlabelled nuclei at several sites throughout the section. It was found, however, that with experience this grading system could easily be reproduced without formal counting simply by visual inspection of the section. This was validated by reviewing all the tumours in this series blindly and comparing this visual estimate with the previously established percentages. Examples of the appearances seen in these four categories are shown in the Figure.

Results

The Table shows the grade of proliferation as assessed by immunohistological labelling with monoclonal antibody Ki67 in the series of 104 lung tumours.

All cases of small cell carcinoma showed a high grade of proliferation (3 or 4), whereas all the carcinoids were in the lowest grade (1). The results were much more variable for the adenocarcinomas and squamous cell carcinomas, which were found in all four proliferation categories.

The distribution patterns of labelled nuclei within individual tumours also varied between the different tumour types. Small cell carcinomas and carcinoid tumours showed an even distribution of labelled nuclei through the section. In contrast, labelled nuclei were distributed much more variably in adenocarcinomas and squamous cell carcinomas, individual foci within these tumours showing high or low numbers of Ki67 positive nuclei. In such cases the final grading represented an average of the labelled nuclei in all foci. Although this was a semi-quantitative estimate, it was reproducible on blind review—that is, tumours were consistently placed within the same proliferation category.

Discussion

Treatment of lung cancer is currently based on the concept that rapidly growing lesions should be

treated with chemotherapy or radiotherapy, or both, while surgery is appropriate for more slowly growing tumours. This view is supported by clinico-pathological studies^{1–3} and has led to lung tumours being divided, for therapeutic purposes, into two groups—small cell type and others.

The biological basis for this approach to lung cancer is based on studies of tumour growth rates. These have entailed serial radiology,²⁸ autoradiography (using tritiated thymidine), or flow cytometry.²⁹ Such studies are complex, subject to numerous technical difficulties and artefacts, and are often difficult to reproduce. They have, however, confirmed that small cell carcinoma of the lung generally has a high proliferation rate.^{30–32} Other lung tumour types vary widely in their growth rates, but it is conventionally accepted that all of these neoplasms should be treated surgically, provided that the neoplasm has not spread too extensively by the time of diagnosis.⁴ Although this is successful in some patients, others rapidly succumb to their disease, suggesting that preoperative selection of cases with low proliferative rates might substantially improve the results of surgery. In support of this concept Muggia *et al*³¹ found that there was a correlation between the growth rate of lung tumours and their response to chemotherapy.

The present study describes a means whereby the treatment of individual lung tumours may be matched to their biological behaviour. The validity of this approach is indicated by the finding that all the small cell carcinomas had high proliferation rates, whereas those of the carcinoid tumours were low. This suggests that the selection of cases for chemotherapy on the basis of Ki67 reactivity is likely to be of practical value. Immunocytochemical labelling of histological sections with antibody Ki67 is a simple technique and well within the scope of routine surgical pathology laboratories. The semiquantitative categorisation of neoplasms into four proliferative grades on the basis of the number of labelled nuclei is rapidly and reproducibly made by the pathologist; and it represents a very minor addition to the task of histologically categorising individual cases.

The results of the present study confirm that adenocarcinoma and squamous cell carcinoma of the lung vary widely from case to case in their proliferative state. Clinical follow up is necessary to establish whether or not there is a correlation between a low proliferative state and long term survival (and vice versa), and this is being undertaken for all the cases in this study. The gathering of this data and its analysis will take time, however, and there is an argument for other centres to collect comparable cases prospectively. Showing a correlation between the proliferative state of lung tumours, as determined immunocytochemically, and their clinical behaviour

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would have important implications in terms of the treatment of lung cancer, for example, selection of patients for operation or postoperative chemotherapy.

Data from several independent studies will be required if such a correlation is to be proved. It will also be important to try to adapt these techniques for use in preoperative specimens, such as bronchial biopsies or cytological specimens. Finally, the possibility should be noted that immunocytochemical investigation of proliferative rate by the method used in the present study may also prove to be of clinical importance in other types of human neoplasm, such as breast or gastrointestinal cancer.

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Requests for reprints to: Dr KC Gatter, Nuffield Department of Pathology, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, England.