ATYPICAL ALVEOLAR HYPERPLASIA: RELATIONSHIP WITH PULMONARY ADENOCARCINOMA, p53, AND c-erbB-2 EXPRESSION

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SUMMARY

Atypical alveolar hyperplasia (AAH) has recently been described in human lungs in association with primary lung cancer, particularly adenocarcinoma. Unlike proximal bronchogenic carcinoma, peripheral (parenchymal) adenocarcinoma of the lung does not have a well-recognized progenitor lesion. Epidemiological, morphometric, and cytofluorometric data in the literature suggest that AAH is a candidate premalignant entity. In this study, 97 AAH lesions were found in lungs resected from 29 patients (1–13 lesions per case, mean 3.5) being treated for presumed carcinoma (25/29 had adenocarcinoma). From a study case-load of 285 adenocarcinoma-bearing lungs, the AAH incidence was 8.8 per cent. Sections of 67 AAH lesions from 19 patients were stained using monoclonal antibodies against Ki67 (MIB1), pS3 (D07), and c-erbB-2 (NCL-CB11). Ki67 was expressed in up to 10 per cent of AAH nuclei. Thirty-nine lesions (58 per cent) showed stainable p53 protein, while five (7 per cent) expressed membrane c-erbB-2 oncoprotein. These latter five lesions were all strongly positive for p53, and both p53 and c-erbB-2 staining was associated with increased cellular crowding and pleomorphism in AAH. These data demonstrate that AAH exhibits some genetic changes associated with malignancy and thereby support the hypothesis that AAH is premalignant.

KEY WORDS—Lung, atypical alveolar hyperplasia, adenocarcinoma, oncogenes, p53, c-erbB-2, premalignancy.

INTRODUCTION

Primary adenocarcinoma of the lung is a common neoplasm with a suggestion in some series of cases that its incidence may be increasing. This tumour shows some epidemiological differences from other primary lung cancers, so that, for example, it is relatively more common in non-smokers and in some ethnic populations.1 Study of the pathogenesis of pulmonary adenocarcinoma has been significantly hampered in the past by the lack of an identifiable precursor lesion. Although originally described in the English literature by Meyer and Liebow in 1965,2 the nature of atypical alveolar (or bronchioloalveolar) hyperplasia (AAH) and its possible role as a progenitor lesion of adenocarcinoma of the lung have only recently been more thoroughly explored.3,4

Atypical alveolar hyperplasia is characterized by variable interstitial expansion, mild lymphocytic infiltration, and alveolar spaces lined by cuboidal or columnar cells varying in shape, size, and degree of nuclear atypia (Figs 1 and 2). Architectural atypia also varies, with the most atypical lesions exhibiting cell crowding and papillary outgrowths.5

These lesions are principally associated with adenocarcinoma of the lung, although they are described in association with other tumour types and have been found in lungs resected for
metastatic disease. In our own preliminary series, all ten patients had lung resection for adenocarcinoma. The evidence that the lesions of AAH are indeed truly premalignant is based on epidemiological, morphological, morphometric, and cytofluorometric analyses of the lesions and associated cancers.

Over recent years, a variety of immunohistochemical probes have been used to demonstrate aberrant expression of oncogene and tumour suppressor gene products in a number of clinical situations. The protein products of the p53 gene are thought to have an important role in the control of cell proliferation and therefore in normal cell homeostasis. Mutations of the p53 gene are one of the commonest genetic changes found in human tumours. Gene mutation leads to intranuclear accumulation of non-functional, stabilized p53 protein which reaches levels detectable using anti-p53 antibodies and immunohistochemistry. Alternatively, p53 protein may be inactivated by binding to another protein, again leading to its stabilization and accumulation. Demonstration of nuclear p53 protein accumulation may therefore both indicate genetic or epigenetic changes predisposing to malignancy and act as a biomarker of malignancy. Such changes have been sought and found in a number of situations where premalignant lesions and their malignant counterparts are recognized, including squamous dysplasia and carcinoma of the lung.

In carcinoma of the lung, other alterations associated with malignant transformation include detectable expression of proto-oncogenes. In adenocarcinomas including those of the lung, activation of the c-erbB-2 proto-oncogene is well recognized and can be detected immunohistochemically.

We have previously published a series of ten patients with AAH associated with peripheral adenocarcinoma of the lung. The present study examines these cases together with 19 more. We describe the proliferative activity, p53 protein, and c-erbB-2 proto-oncogene product expression in AAH lesions and their associated carcinomas.

**Fig. 1** A well-defined focus of atypical alveolar hyperplasia surrounded by relatively normal lung. (H & E)
IS ATYPICAL ALVEOLAR HYPERPLASIA PREMALIGNANT?

Fig. 2—AAH showing thickened alveolar walls and cuboidal/columnar alveolar lining cells. There is mild cytological atypia. Some cells have a hobnail appearance. (H & E)

MATERIALS AND METHODS

Case material was derived from the University Departments of Pathology in Aberdeen and Edinburgh. In both centres, lung resection specimens were inflated with 10 per cent neutral buffered formalin, cut into parasagittal 1 cm thick slices, and blocks taken for microscopy. As well as sampling tumour, visible focal parenchymal abnormalities and random blocks of macroscopically normal lung were also taken. Areas of obstructive pneumonitis or other obvious inflammatory disease, emphysema, or scarring were avoided. All cases were reviewed and all parenchymal blocks were examined for foci of AAH. In some cases, if lesions of AAH were found and the gross specimen was available, it was re-examined and more blocks were taken.

One or two blocks of tumour from each case together with all blocks containing foci of AAH were selected and serial sections were cut, mounted on APES-coated slides, and dried at 56°C for 30 min. After dewaxing and blocking endogenous peroxidase, sections were rinsed in water and then placed in 10 mm citric acid at pH 6.0. These sections were then microwaved (800 W for 20 min), while ensuring they were covered by liquid throughout. Following the microwave heating, the sections were left in hot buffer for 20 min, after which they were rapidly transferred to 0.005 M Tris-buffered saline, pH 7.6 (TBS).

Sections were stained with proliferation marker anti-Ki67 (MIB1, Immunotech, Marseilles, France); monoclonal antibody DO7 (Dakopatts, Copenhagen, Denmark), which recognizes the p53 gene product; and a monoclonal antibody against human c-erbB-2 oncoprotein (NCL-CB11, Novocastra, Newcastle, U.K.), using dilutions of 1:150, 1:200 and 1:100, respectively. Primary antibody bound to antigen was detected using a standard sABC technique, visualized with DAB.

Appropriate positive controls were used for all immunohistochemical staining procedures and where nuclear staining was being sought (Ki-67 and p53), a very light haematoxylin nuclear counterstain was employed to avoid masking weak
positive staining. For each tumour and AAH lesion, an estimate of the number of positive cells was made. Staining was graded as follows: focal (<5 per cent of cells stained), moderate (5–70 per cent), abundant (>70 per cent), and staining intensity was recorded.

All patients’ case-notes were examined for occupational and smoking history, as well as any increased family incidence of malignancy.

RESULTS

In total, we have detected 29 cases showing foci of AAH in the parenchyma of lungs resected for presumed carcinoma. All of our originally reported ten cases and 15 of 19 more recent resections were from patients having a peripheral (parenchymal) adenocarcinoma. The remaining cases were resected for large cell undifferentiated carcinoma, carcinoma showing mixed differentiation, metastatic osteosarcoma, and pleural fibroma, respectively. These cases represent the findings from a total of 760 resection specimens (of which 175 were for adenocarcinoma) in Edinburgh and 360 resections in Aberdeen (110 adenocarcinomas). AAH was thus found in 5.7 per cent of patients with resected adenocarcinoma in Edinburgh but in 13.6 per cent in Aberdeen. In Edinburgh the mean number of parenchymal blocks taken was 1.5 (range 1-5), while in Aberdeen the figure was nearly 6 (range 2–14). Some of the larger lesions (up to 10mm) were visible to the naked eye as soft pale foci but many lesions were found only after microscopy. Lesion numbers per case ranged from 1 to 13 (mean 3.5). A total of 97 AAH lesions were found in 29 lung resections.

Eighteen of the 29 patients were women (62 per cent); all were cigarette smokers, often heavy and lifelong, or had smoked up to 2 years before surgery. None had a family history of excessive cancer incidence and there was nothing to suggest occupational carcinogen exposure.

Material for immunohistochemistry was available in only 19 cases (17 primary lung carcinomas, 67 AAH lesions). AAH lesions are often of small size and the material from the original ten cases was exhausted in our previous study. Staining of AAH foci for Ki67 antigen was variable and patchy with lesions showing from 2 to about 10 per cent of cells positive. Positive cells sometimes occurred in clusters within lesions. The surrounding morphologically normal alveolar epithelium showed minimal Ki67 positivity. The associated carcinomas showed high levels of Ki67 expression with up to 50 per cent of cells positive. Allowing for the known differences in performance between the two antibodies, this is a similar result to that obtained with anti-PCNA (PC10) on the original ten cases.7

Of the 17 primary lung carcinomas stained for p53 protein, nine showed moderate/abundant staining (53 per cent), six focal/weak staining (35 per cent), and two were negative (12 per cent). Nineteen of the 67 foci of atypical alveolar hyperplasia showed moderate/abundant staining (28 per cent) (Fig. 3), 20 scored focal/weak (30 per cent), while in 28 (42%) AAH lesions p53 protein was undetectable. There was no relationship between the results for tumour and its associated AAH or amongst AAH lesions in the same lung or tissue block.

Usually only membrane staining is accepted as a true positive result with antibodies to the c-erbB-2 oncogene product. The significance of cytoplasmic staining is uncertain.21 In this study, both membrane and cytoplasmic staining was found. Eleven out of 17 tumours (65 per cent) showed widespread cell membrane staining of variable intensity together with cytoplasmic positivity in some cases. Two carcinomas (12 per cent) showed abundant cytoplasmic staining only, while in three (17 per cent) cytoplasmic staining was focal and weak. One tumour (6 per cent) was negative. Of the 67 AAH lesions, five (7 per cent) showed cells with clear evidence of membrane staining (Fig. 4). Only cytoplasmic staining was seen in 40 lesions [23 (34 per cent) abundant/moderate, 17 (25 per cent) focal]. No staining was seen in 22 AAH lesions (33 per cent). As with p53, there was no correlation between c-erbB-2 positivity in carcinomas and associated foci of atypical alveolar hyperplasia.

Seven carcinomas showed abundant strong staining for p53 protein and c-erbB-2 protein. All five AAH lesions which showed membrane c-erbB-2 positivity also had many cells with stainable nuclear p53 protein. In AAH lesions, p53 and c-erbB-2 positivity was associated with greater cellular pleomorphism and cell crowding.

DISCUSSION

The pathogenesis of proximal bronchogenic carcinoma, principally squamous carcinoma, is associated with a recognized premalignant phase of bronchial epithelial dysplasia.22,23 A progenitor
Fig. 3—Atypical alveolar hyperplasia with many cells exhibiting strong nuclear staining for p53 protein. (DO7/sABC with haematoxylin counterstain)

Fig. 4—Membrane staining of cells in AAH to demonstrate c-erbB-2 oncoprotein. (NCL-CB11/sABC with haematoxylin counterstain)

lesion for adenocarcinoma, and in particular peripheral so-called parenchymal adenocarcinoma, is, however, not well recognized. Some authors have proposed an origin in pulmonary scar, but others have questioned this. Miller et al. suggested that some peripheral adenocarcinomas of the lung may be derived from foci of atypical alveolar hyperplasia, an idea which has been supported by epidemiological, immunohistochemical, morphometric, and cytofluorometric analyses of patients, tumours, and associated AAH lesions.
The data presented here further strengthen the association between peripheral 'parenchymal' adenocarcinoma and associated foci of atypical alveolar hyperplasia in the cancer-bearing lung. These AAH lesions are morphologically and immunohistochemically quite distinct from their associated tumours; i.e. they are not intrapulmonary metastases. Foci of AAH do show increased proliferative activity when compared with the surrounding lung. The expression of p53 protein and c-erbB-2 oncoprotein suggests that at least some of the genetic changes leading to malignant transformation are exhibited by AAH.

The discovery of AAH with 5-7 per cent of adenocarcinomas in Edinburgh but with 13.6 per cent of such cases in Aberdeen reflects changing practice, with more blocks being taken per case and, with experience, more AAH foci being identified at macroscopic examination. Our study was not designed to study the incidence of AAH. This has been best done by Weng et al., who examined a mean of 51 blocks from each case and found AAH in 25 per cent of patients with adenocarcinoma but also in 10 per cent of patients with squamous carcinoma. Miller, who processed less tissue than Weng et al. but more than us, found AAH with 15 per cent of adenocarcinomas and 3 per cent of squamous carcinomas. Although different tissue sampling procedures may well explain these differences, it is interesting to note that peripheral adenocarcinoma is particularly common in the Far East1 and most of the literature on AAH originates from Japan. Although the association between AAH and parenchymal adenocarcinoma is an attractive one, the association may be biased since AAH cannot be found easily in severely emphysematous lung or with proximal cancers showing distal obstructive pneumonitis.

The staining of our original cases by PC10 has been discussed elsewhere. The findings in the more recent cases using anti-Ki67 (MIB1) support our original data indicating that there is an increase in proliferative activity in AAH lesions even though mitoses are rarely found. Although the high levels of PCNA expression in sections of paraffin-embedded tumours may not be an accurate indicator of proliferative activity and may indicate deregulation of the PCNA gene, others have shown a correlation between anti-PCNA staining and both mitotic index and anti-Ki67 staining.

The proposed roles of the p53 and c-erbB-2 genes in oncogenesis have been well discussed elsewhere. Mutations of the p53 gene and overexpression of p53 protein5 have been described in adenocarcinoma of the lung. This latter study showed p53 protein overexpression in 56 per cent of adenocarcinomas resected from active smokers. Two recent studies17,18 have demonstrated a high incidence (up to 94 per cent) of p53 protein accumulation in premalignant bronchial lesions as well as in the associated invasive squamous carcinoma, indicating that abnormal p53 expression may occur even in the mildest forms of bronchial dysplasia and predate the development of invasion in a neoplastic cell population. Similar results have been reported in premalignant/dysplastic lesions outside the lung.14-16 Comparison of results between studies and with ours must, however, be made with caution, given the range of anti-p53 antibodies used and the different antigen unmasking and visualization techniques employed.15 Bearing in mind the wise council of Hall and Lane, especially since we used microwaves to unmask antigen, we may regard only our moderate/abundant staining cases as truly positive. The problem of result comparability could, to some extent, be addressed by the widespread use of standard positive control material, perhaps derived from cell lines, where cells contained a known amount of the antigen under study. Our data still compare favourably (28 per cent of AAH lesions stained) with those derived from premalignancy at other sites and provide strong evidence that AAH is indeed the result of genetic damage and is likely to be strongly implicated in the genesis of adenocarcinoma in the lung.

c-erbB-2 protein expression has been previously described in up to 57 per cent of adenocarcinomas of the lung.19,26 The strong association of c-erbB-2 with adenocarcinoma, as opposed to squamous carcinoma, presumably explains the lack of data on the expression of this oncoprotein in bronchial dysplasia. In our series, 67 per cent of adenocarcinomas showed positive membrane staining for c-erbB-2, this slightly higher figure possibly being explained by our use of microwaves, which has been reported to enhance immunoreactivity for c-erbB-2 oncoprotein in breast cancer.37 It is therefore of interest that in five examples (7 per cent) of AAH we could demonstrate positive membrane c-erbB-2 staining. Kamel et al. failed to obtain any staining in three cases of dysplasia in the gall bladder.16 A recent paper provided some evidence that p53 mutation occurs early in the development of
adenoacanthoma of the lung and speculated that it may be present in small preneoplastic lesions.38 These authors suggested, however, that such a premalignant lesion was still a hypothetical rather than an established, recognized entity and may only be expected to show p53 mutation once over 3 mm in size. We suggest that atypical alveolar hyperplasia is such a preneoplastic entity and have provided evidence of abnormal p53 protein expression even in lesions less than 1 mm in size. Abnormal p53 expression may increase with lesion atypia and, perhaps, later in the carcinogenic sequence, abnormal c-erbB-2 proto-oncogene expression may occur. All these events occur before a lesion is such a preneoplastic entity and have abnormal c-erbB-2 proto-oncogene expression may be present in small preneoplastic lesions.38 Only be expected to show p53 mutation once over provided evidence of abnormal p53 protein expression even in lesions less than 1 mm in size. Abnormal c-erbB-2 proto-oncogene expression may increase with lesion atypia and, perhaps, later in the carcinogenic sequence, abnormal c-erbB-2 proto-oncogene expression may be present in small preneoplastic lesions.38

The work done on bronchial dysplasia and the development of bronchial squamous carcinoma demonstrate a correlation between p53 expression and the degree of dysplasia, a situation mirrored in the cervix.15 Using published criteria to grade atypia in AAH lesions,3 we observed a tendency for p53 expression to be greater in the more atypical foci.

The findings presented here add further weight to the accumulating evidence of a novel pathway in the morphogenesis of the most common variant of primary pulmonary adenocarcinoma. The results have implications for histogenetic theories of lung cancer and for the further study of epidemiological and genetic associations.

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REFERENCES


