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ANATOMICAL PATHOLOGY

Loss of expression of BAP1 is very rare in non-small cell lung carcinoma

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Summary

Germline mutations of the *BAP1* gene have been implicated in a cancer predisposition syndrome which includes mesothelioma, uveal melanoma, cutaneous melanocytic lesions, renal cell carcinoma, and possibly other malignancies. Double hit inactivation of *BAP1* with subsequent loss of expression of the BAP1 protein also occurs in approximately 50% of mesotheliomas. The link between *BAP1* mutation and lung cancer is yet to be fully explored. We sought to assess BAP1 expression in a large cohort of lung cancers undergoing surgery with curative intent.

We searched the Anatomical Pathology database of our institution for lung cancer patients undergoing surgery with curative intent between 2000 and 2010. Immunohistochemistry for BAP1 was then performed in tissue microarray format.

Our cohort included 257 lung cancer patients, of which 155 (60%) were adenocarcinomas and 72 (28%) were squamous cell carcinomas, with no other subtype comprising more than 3%. BAP1 loss of expression was found in only one lung cancer.

We conclude that BAP1 mutation occurs very infrequently (0.4%) in non-small cell lung cancer. Given that the pathological differential diagnosis between lung carcinoma and mesothelioma may sometimes be difficult, this finding increases the specificity of loss of expression for BAP1 for the diagnosis of mesothelioma.

Key words: BAP1; Lung cancer; BRCA1 associated protein 1.

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INTRODUCTION

Lung cancer is amongst the most frequently diagnosed malignancies, as well as the leading cause of cancer related mortality, estimated to be responsible for 19.4% of cancer deaths worldwide.[1] The prognosis is poor and in the United States the 5-year survival rate is approximately 18%.[2] Whilst smoking is the leading cause of lung cancer,[3] a genetic basis for lung cancer has also been proposed, in view of the existence of familial clustering[4–6] and the variability of risk among smokers.[7,8] Given the role of modifiable risk factors in the pathogenesis of lung cancer and the need for early detection in effective treatment, a better understanding of the role of molecular genetics in this malignancy may result in improved prevention and prognosis.

The *BAP1* gene is located on the 3p21.1 chromosome and participates in cell cycle regulation.[9,10] It is a bona fide tumour suppressor protein,[11] and mutation of the gene is implicated in a germline cancer predisposition syndrome.[9] Somatic BAP1 inactivation has been reported to occur in approximately half of all mesotheliomas,[12] in which it appears to be associated with a survival advantage,[13] in up to a quarter of intrahepatic cholangiocarcinomas,[14,15] as well as in other malignancies such as uveal melanoma, cutaneous melanocytic neoplasms and clear cell renal carcinoma.[16–18] However, loss of BAP1 expression occurs infrequently in other malignancies, such as peritoneal and gynaecological serous adenocarcinomas[19] and pancreatic adenocarcinomas.[20]

The link between BAP1 mutation and lung cancer is yet to be fully explored, although there are several reasons to propose a potential connection. Firstly, the 3p21 gene region has a significant connection to lung cancer. 3p deletions are found in almost 100% of small-cell lung carcinoma (SCLC) and 90% of non-small cell lung carcinoma (NSCLC) cell lines,[21,22] and the 3p21 region houses a number of potential lung tumour suppressor genes, including *ARP*, *CACNA2D2*, *RASSF1A*, *HYAL1*, and *SEMA3F*, which have been frequently found to be inactivated in both SCLS and NSCLC.[21,23–25] Secondly, there have been occasional cases of lung cancer reported in patients with germline *BAP1* mutations, although it is not clear if these may be chance associations.[26–29] The range of tumours associated with the syndrome is yet to be fully defined, and the presence of occasional cases of lung carcinoma among patients with germline BAP1 mutations suggest that these tumours may also form part of the spectrum.

The pathological distinction between mesothelioma and non-mesothelial primary lung malignancies can be difficult in some cases even when multiple immunohistochemical

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markers are employed, particularly when the presentation is with pleural effusion. Given that BAP1 expression is lost in up to 50% of mesotheliomas,[13] it would be useful to have firm data on the incidence of BAP1 loss in lung cancers. That is, if BAP1 loss occurs rarely in lung cancer, loss of expression of BAP1 in a thoracic malignancy would provide strong support for the diagnosis of mesothelioma.

Therefore, we sought to assess BAP1 expression in a large cohort of lung cancers undergoing surgery with curative intent.

METHODS

We searched the database of the Department of Anatomical Pathology, Royal North Shore Hospital, for all lung cancers diagnosed between 2000 and 2010, which underwent resection with curative intent. The survival data, ALK gene rearrangement status and EGFR mutation status of this cohort have been previously reported.[30–33] All cases underwent independent pathological review to reclassify according to the World Health Organization (WHO) and International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society (IASLC/ATS/ERS) system,[34,35] and to restage according to the 7th edition 2009 Union for International Cancer Control/American Joint Committee on Cancer (UICC/AJCC) TNM staging system.[36]

Immunohistochemistry (IHC) was performed on a tissue microarray (TMA) of formalin fixed, paraffin embedded (FFPE) lung cancer specimens containing two 1 mm diameter cores from each tumour specimen. TMA sections were stained with a mouse monoclonal anti-BAP1 antibody (clone C-4, cat no ic-28383; Santa Cruz Biotechnology, USA) at a dilution of 1:100. An automated staining platform (the Leica Bond III autostainer; Leica Microsystems, USA), was used with heat induced antigen retrieval for 30 min at 97°C in the manufacturer's alkaline retrieval solution ER2 (VBS part no: AR9640; Leica Microsystems).

The BAP1 stain was interpreted by a single observer (AG) who was blinded to all clinical and pathological details at the time of scoring. The results were classified as negative, positive or indeterminate. Positive staining was defined as positive nuclear staining in any definite neoplastic cells (Fig. 1) while negative staining was defined as absent nuclear staining in all neoplastic cells in the presence of a positive internal control (Fig. 2). Indeterminate cases included cases where the tumour was negative but there was no internal positive control (that is the possibility of artefactually negative staining could not be excluded). Negative and indeterminate samples were repeated on whole mount sections and assigned a definitive positive or negative result. This study was approved by the local (NSHLD) human research ethics committee.

RESULTS

The database search identified 270 lung cancer patients who underwent surgery in the period 2000 to 2010. Thirteen patients had tumours which were unavailable for TMA construction or insufficient material present in the TMA for interpretation (Fig. 3). The remaining 257 patients formed the study cohort and comprised 155 (60%) adenocarcinomas and 72 (28%) squamous cell carcinomas, with no other subtype comprising more than 3%. The cohort was 57% male and had a mean age at diagnosis of 67 years. The clinical and pathological patient characteristics are presented in Table 1.

In the 257 patient cohort, diffusely positive BAP1 staining was found in all except one lung cancer, which showed negative staining on both TMA and the confirmatory whole mount section. One case was only very weakly positive on the TMA with no internal positive control, but confirmed to be strongly positive on whole sections. All other cases were strongly positive on the TMA.

The BAP1 IHC negative case was a 35 mm adenocarcinoma in the right middle lobe of a 58-year-old female. Additional IHC was also performed on the specimen, which confirmed the diagnosis of lung adenocarcinoma (TTF1,

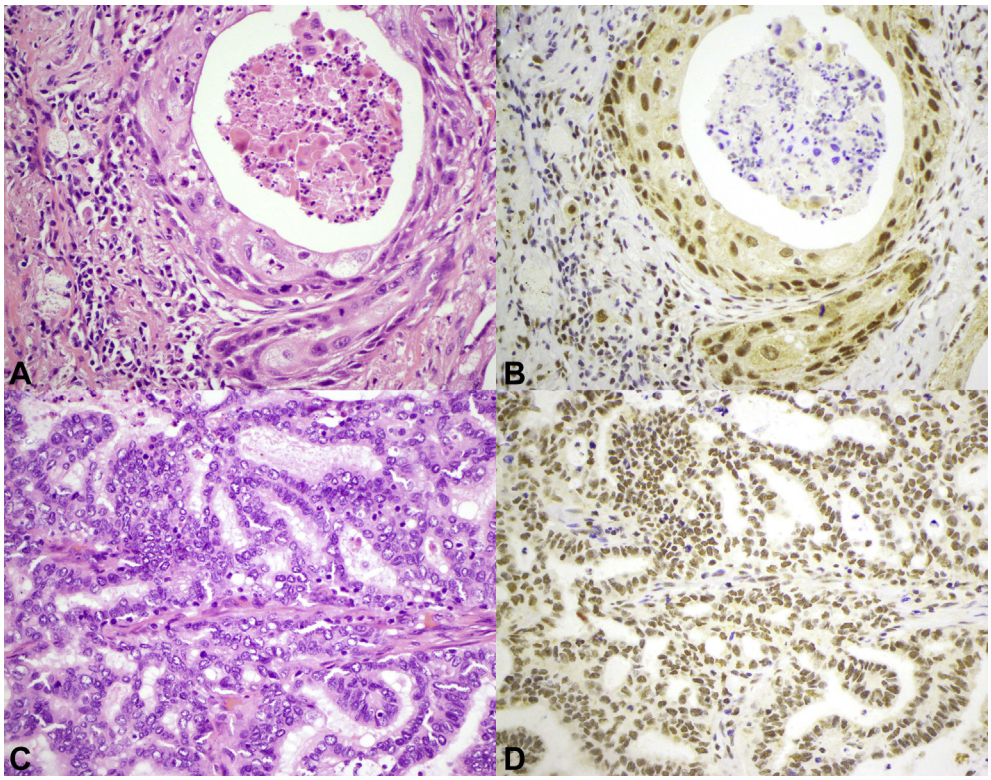

Fig. 1 Malignant cells with positive nuclear staining for BAP1 in both squamous cell carcinoma (A, B) and adenocarcinoma (C, D) of the lung. (A) Squamous cell carcinoma (H&E stain), (B) BAP1 immunohistochemistry. (C) Adenocarcinoma (H&E stain), (D) BAP1 immunohistochemistry.