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ORIGINAL ARTICLE

Fine-needle aspiration of small pulmonary nodules yields material for reliable molecular analysis of adenocarcinomas

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Introduction Early molecular characterization with Kirsten rat sarcoma factor, epidermal growth factor, and anaplastic lymphoma kinase are critical to manage pulmonary adenocarcinoma. Fine-needle aspiration (FNA) of lesions <2 cm are routine in our institution and are used in molecular analysis. We report our experience.

Materials and methods We searched our databank for primary pulmonary adenocarcinomas diagnosed by FNA between January 2009 and April 2013. Size of the lesion aspirated, molecular results, and sample source (FNA versus surgical specimen) were recorded. We compared the frequency of mutations identified by FNA versus surgical specimens and the frequency of mutations in lesions by size (<1 cm, 1-2 cm, >2 cm).

Results We identified 397 primary pulmonary adenocarcinomas. Molecular studies were requested by the clinician in 89 (22%) of primary adenocarcinomas. FNAs were used in 55 cases; 51 (93%) yielded sufficient material for molecular studies; surgical tissue were used in 34 cases; 33 (97%) yielded sufficient material for molecular studies. The insufficient specimens came from 2 FNAs of 0.6 cm nodules, an FNA of a 2 cm nodule, and a core biopsy.

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Conclusions FNA was adequate for molecular analysis of small nodules. In nodules greater than 0.6 cm, the adequacy is comparable to surgical tissue. There was no statistically significant change in mutation rate by size (53%-58%). Importantly, FNA of small lesions for cytological diagnosis and molecular analysis is encouraged by our data and experience in order to provide early treatment.

Introduction

Molecular characterization of tumors is used clinically in several solid tumors. In the case of pulmonary adenocarcinomas, establishment of the epidermal growth factor (EGFR), anaplastic lymphoma kinase (ALK), and Kirsten rat sarcoma factor (KRAS), mutational status is becoming the standard of care.[1-6] The use of tyrosine kinase inhibitors such as gefitinib and crizotinib has revolutionized the care of non-small-cell lung carcinoma in patients with sensitizing mutations in EGFR and ALK, respectively. Patients are living longer without progression and with better quality of life.[3,7-10] Even without targeted treatment, patients with EGFR mutations in their tumors have a better prognosis than do patients with KRAS mutations.[11] Because of the vast difference in treatment and prognosis, it is ideal to characterize the tumor at the time of diagnosis. Multiple studies show that molecular diagnostics can be done on cytological specimens.[1,12-18] The adequacy of fine-needle aspiration (FNA) has been assessed based upon the number of cells examined but it has not previously been assessed by nodule size. In addition to the number of cells present in the nodule or mass, heterogeneity, fibrosis, and technical issues confound the picture. We hypothesize that, even in small nodules, adequate material for diagnosis and molecular studies can be reliably obtained by FNA.

Methods

A retrospective analysis was performed under an internal review board-approved protocol. We searched our database for adenocarcinomas of the lung diagnosed by FNA between January 2009 and April 2013. For all primary adenocarcinomas, we recorded the size of the lesion biopsied, the molecular results, and whether the diagnostic material was obtained from the diagnostic FNA or the subsequent surgical specimen (either resection or core biopsy). With exception of two cases in which molecular testing was done on a brain metastasis, all molecular testing was performed on chemo-naïve tissue. The specimens were then sorted by size of the lesion biopsied (<1 cm, 1-2 cm, and >2 cm) and by tissue source (surgical specimen vs fine-needle aspirate). We compared the adequacy and rate of mutation between FNA and surgical material from masses of the same size with a paired *t* test. We compared the adequacy and rate of mutation between smaller and larger masses with a paired *t* test.

All molecular testing, on both cytological and surgical specimens, was done in-house from archived formalin-fixed paraffin embedded tissue; molecular tests were performed on the cell blocks from cytological specimens. The tumor content was evaluated, on a hematoxylin and eosin (H&E)-stained sample to determine the adequacy. Adequacy was defined as 400 tumor cells, constituting at least 10% of the area microdissected. In cases of borderline adequacy, the DNA was extracted; a minimum of 30 ng/mL DNA is needed to run molecular tests. The tumor cells were microdissected from unstained slides for use in molecular studies.

DNA extraction method for EGFR and KRAS mutations

The tumor tissue section on each slide was removed with a sterile scalpel and digested with proteinase K (Roche). DNA was extracted as per QIAamp DNA blood Mini Kit and was eluted in 25-50 μL AE buffer. DNA was quantitated using optical density at 260 and 280 using the Nanodrop 2000 Spectrophotometer. A total of 10-500 ng of DNA was used for each reaction.

Detection of EGFR mutations

The DNA fragments containing E19del and E20ins mutations were amplified using conventional polymerase chain reaction (PCR). E18 G719S, G719C, and G719A, E21 L858R, and E20 T790M mutations were amplified and detected with Amplified Refractory Mutation System PCR (ARMS-PCR). All reactions were carried out with Qiagen Hotstar DNA polymerase (primers provided by Sigma Chemical) in an Applied Biosystems Thermocycler Gene Amp 9700. The E20ins mutation was detected by capillary electrophoresis with ABI 3130xl genetic analyzer. All other mutations (E19del, E21 L858R, E20 T790M, and E18 G719S, G719C, and G719A) were detected by electrophoresis on a 2.5% agarose gel and visualized by UV light. WT and positive controls were run with each reaction.

Detection of KRAS mutations

Screening for KRAS mutations in codons 12 and 13 was done by high resolution melting curve analysis using the Roche LC480. Positive controls and duplicate WT controls were run with each analysis. Specific mutations were identified using shifted termination assay (Applied Biosystems)