

# Primary Adenoid Cystic Carcinoma of the Lung

## *Absence of KIT Mutations*

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**BACKGROUND.** Primary pulmonary adenoid cystic carcinomas (ACCs) are rare lung neoplasms that are challenging to completely resect and can exhibit poor survival. Adjuvant therapy is often ineffective and identification of a targeted novel therapy would be useful. The objective of the current study was to evaluate KIT expression and *KIT*-activating mutations.

**METHODS.** Primary salivary gland-type tumors of the lung diagnosed between 1972 and 2002 at the Mayo Clinic were identified and the subset of primary pulmonary ACCs were reviewed. Immunohistochemical study for KIT expression and *KIT* gene mutations in exons 9, 11, 13, and 17 were performed on paraffin-embedded tissue.

**RESULTS.** Forty-nine patients were diagnosed with primary pulmonary ACC. The majority of ACC cases were predominantly the cribriform type (74.4%). KIT immunoreactivity was evaluated in 34 cases and was found to be present in all but 1 case (97%). No mutations were detected in *KIT* gene exons 9, 11, 13, and 17 in a subset of 12 cases.

**CONCLUSIONS.** Although KIT expression was found frequently in primary pulmonary ACC, a correlation with *KIT*-activating mutations was not observed. *Cancer* 2007;110:2507–10. Published 2007 by the American Cancer Society\*

**KEYWORDS:** adenoid cystic carcinoma, *KIT* mutation, adenoid cystic carcinoma, lung.

**P** primary salivary gland-type tumors of the lung are uncommon, representing <1% of all lung tumors.<sup>1–4</sup> Adenoid cystic carcinoma (ACC) represents 1 of the most common subtypes, usually occurring in the fifth decade of life and demonstrating no sex predilection.<sup>1–4</sup> Although salivary gland-type tumors generally have a good prognosis, ACC tend to present at a higher stage and are often unresectable or, if resected, often have positive surgical margins and subsequent local recurrences.<sup>1–5</sup> Survival has been difficult to assess because most series are comprised of a small number of patients. In general, reported survival has been poor. An initial report from our institution showed that 10 of 13 patients (79%) died of extensive local or metastatic ACC, with average survival of 5.2 years.<sup>5</sup> This was followed by another study in which 2 patients (29%) died over a 5-year period, suggesting improved survival over time, perhaps related to earlier detection or improved treatment.<sup>2</sup> However, a recent study from our institution showed 5-year and 10-year survival rates of 55% and 39%, respectively, in 40 patients diagnosed with ACC.<sup>6</sup> This poor survival does not appear to be affected by traditional chemotherapy and radiotherapy. Therefore, there appears to be a potential use for targeted novel therapies in the care of these patients.

KIT (CD117) expression is commonly detected by immunohistochemistry in ACC of the head and neck,<sup>7,8</sup> but to our knowledge has

not been examined in primary pulmonary tumors of this type. Moreover, analysis of *KIT* gene mutations, which in gastrointestinal stromal tumors (GISTs) are correlated with response to the tyrosine kinase inhibitor imatinib, has not been performed in patients with primary pulmonary ACC. The goal of the current study was to evaluate *KIT* expression and screen for mutations of *KIT* in ACC of the lung.

## MATERIALS AND METHODS

### Case Selection

The group of patients with ACC represents a subgroup of patients from a retrospective review of primary salivary gland tumors treated at the Mayo Clinic.<sup>6</sup> The institutional pathology database was searched for patients who underwent biopsy or surgery for salivary gland tumors of the tracheobronchial tree and lung between 1972 and 2002. All cases were reviewed for confirmation of the diagnosis of ACC. Metastatic disease from a head and neck primary malignancy was ruled out on the basis of clinical and radiologic data. ACCs were graded based on their predominant architectural pattern as in the head and neck: tubular (grade 1), cribriform (grade 2), and solid (grade 3). Tumors were classified as solid type if >30% of one cross-sectional tumor area was comprised of the solid growth pattern. This study was reviewed and approved by the Mayo Foundation Institutional Review Board.

### Immunohistochemical Study

Immunohistochemical stains were performed on representative 4- $\mu$ m, formalin-fixed, paraffin-embedded tissue sections from the tracheobronchial and lung specimens using a polyclonal rabbit antibody to *KIT* (Dako Corporation, Carpinteria, Calif) (1:1000). Antigen retrieval was performed by steam ethylenediamine tetraacetic acid (EDTA), which consists of graded alcohol deparaffinization and methanol/hydrogen peroxide block, followed by pressure treatment in buffered EDTA at 100°C for 30 minutes. Immunostaining was performed using the Advance platform (Dako Corporation) with the Dako Autostainer (Dako Corporation). Appropriate positive and negative controls were employed. Membranous staining was scored as follows: 0 if <5% of cells were positive, 1+ if 5% to 25% of cells were positive, 2+ if 26% to 50% of cells were positive, and 3+ if >50% of cells were positive.

### *KIT* Mutation Analysis

A small subset of tumors from recent surgical resections was used for this part of the study. Tumor-rich

**TABLE 1**  
**Results of Immunohistochemical Study**

Results	0	1+	2+	3+	Total +
No. (%)	1 (3)	7 (21)	8 (23)	18 (53)	33 (97)

0 indicates <5% of the cells stained positive; 1+, 5-25% of the cells stained positive; 2+, 26-50% of the cells stained positive; 3+, > 50% of the cells stained positive.

areas of unstained, 5- $\mu$ m sections were manually dissected using a fresh, sterile scalpel blade for each case. DNA was extracted using a QIAMP mini-kit (Qiagen, Chatsworth, Calif; #51,306) in accordance with the manufacturer's instructions. Polymerase chain reaction (PCR) amplicons of *KIT* gene exons 9, 11, 13, and 17 were generated and screened for mutations by high-performance liquid chromatography (HPLC) (Transgenomic WAVE system, Omaha, Neb) exactly as previously described.<sup>9</sup> The WAVE system allows rapid screening of amplicons for the presence of size-altering mutations (deletions or insertions) in exons 9 and 11, as detected at a non-denaturing temperature of 50°C. When operated at higher temperatures that result in partial denaturation of PCR amplicons, the system also allows the detection of single nucleotide substitutions (exon 11, 56°C; exon 13, 59°C; and exon 17, 58°C). Based on prior studies in our laboratories of *KIT* mutations in GISTs, the WAVE system is more sensitive than standard DNA sequencing.<sup>9</sup> Therefore, DNA sequencing was not performed.

## RESULTS

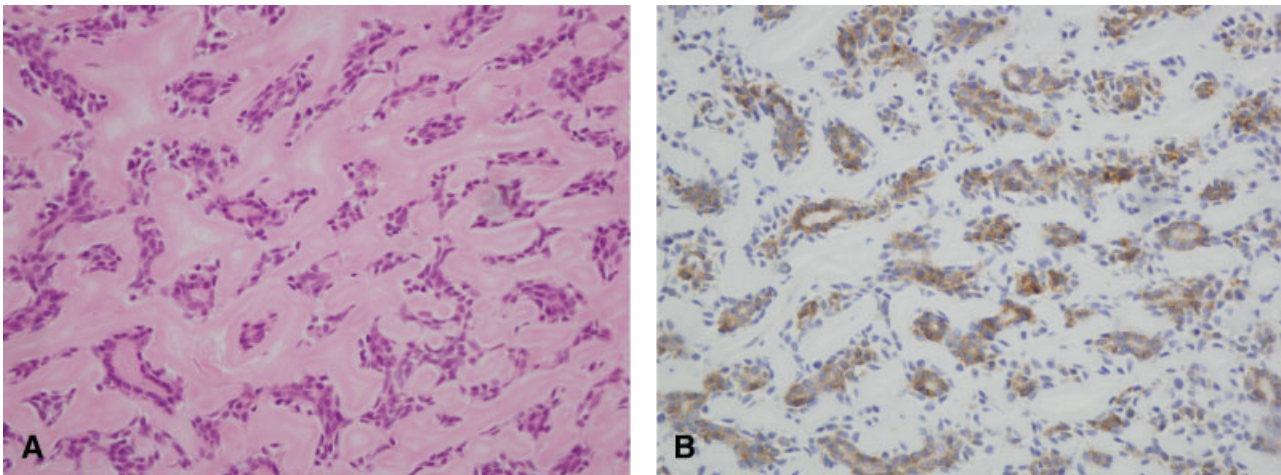
Forty-nine patients, including 25 women (51%), with an overall mean age of 53 years were diagnosed with primary pulmonary ACC of the lung at the Mayo Clinic during the stated 31-year period. The majority of ACC were cribriform (74.4%), with rare tubular (17.9%) and solid (7.7%) types noted.

Thirty-four cases had sufficient tissue for immunohistochemical study and *KIT* immunoreactivity was present in all but 1 case (97%) (Table 1) (Fig. 1). The majority of cases were scored as 3+ (53%).

A subset of 12 cases was selected for DNA extraction and *KIT* gene mutation screening. No mutations were detected in *KIT* gene exons 9, 11, 13, and 17 (Fig. 2).

## DISCUSSION

The results of the current study demonstrate *KIT* expression in primary pulmonary ACCs. Similar rates of immunoreactivity have been reported in ACCs of



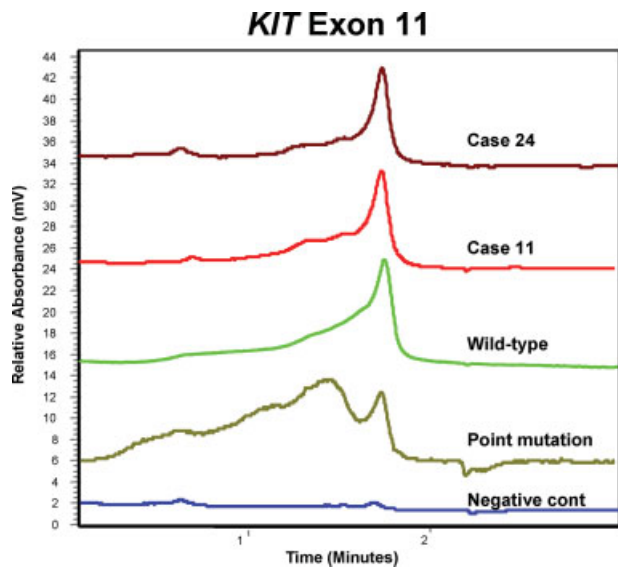
**FIGURE 1.** High-power photomicrograph of a grade 1 adenoid cystic carcinoma demonstrating immunoreactivity for KIT (A: H & E,  $\times 400$ ; B: KIT immunostain,  $\times 400$ ).

the head and neck. In 1 large study, KIT was positive in 90% of ACC cases.<sup>7</sup> Another series evaluating KIT in neoplasms of the head and neck reported c-kit positivity in 62% of ACCs, which was in sharp contrast to all other common head and neck neoplasms.<sup>8</sup> Indeed, KIT immunoreactivity was found to be present in <5% of all of the other 13 tumor types evaluated, both benign and malignant. The authors concluded that KIT could be used as a diagnostic tool to distinguish ACC from other tumors of the head and neck region, in particular in small biopsy specimens.<sup>8</sup> The same authors reported a differential expression among the different histologic subtypes, with the lowest expression observed for the cribriform subtype. Indeed, KIT expression was noted in 61% of cribriform ACC, in contrast to 75% of solid ACC and 89% of tubular ACC.<sup>8</sup> This pattern was not observed in our series of primary lung ACC.

To our knowledge to date, a wide variety of tumors types have been evaluated for KIT immunoreactivity in the hopes of identifying tumors that might respond to inhibitor treatment. Thus, KIT positivity has been observed in mesenchymal tumors, and reported in rare cases of synovial sarcoma (up to 11%)<sup>10</sup> and in >65% of Ewing sarcoma,<sup>11</sup> but it is otherwise uncommon outside of GISTs.<sup>12</sup> KIT expression is also present in mast cells and their tumors,<sup>13</sup> often in nevi and some types of malignant melanomas,<sup>14</sup> and in the majority of seminomas,<sup>15</sup> but is rare in carcinomas,<sup>12</sup> with the exception of small cell lung carcinoma<sup>16</sup> and ACC.

*KIT* mutation was not identified in a subgroup of primary pulmonary ACCs. Although our number of cases is small, the results of the current study support other studies that have shown a lack of correla-

tion between KIT expression and *KIT*-activating mutations because activating mutations have been described only rarely.<sup>12</sup> Indeed, in 1 large series examining 11 different tumor types (579 exons examined), only 12 tumors, all of which were GISTs, harbored mutations. No *KIT* mutations in exons 11 and 17 were found in ACC of the salivary glands.<sup>7</sup> These



**FIGURE 2.** Denaturing high-performance liquid chromatography (HPLC) profiles of *KIT* exon 11 amplicons. Polymerase chain reaction (PCR) amplicons of *KIT* gene exon 11 were run at a partially denaturing temperature of 56°C and the resulting peaks were detected by absorbance at 260 nanometers (nm). The profiles of the cases shown (Case 11 and Case 24) match that of the wild-type control amplicon and not that of a point mutation (V560D) control amplicon. Negative control refers an amplification without template DNA, which was performed to rule out contamination.

data suggest that, with the exception of GISTs, activating *KIT* mutations are uncommon in cancers and are not correlated with *KIT* overexpression. Several clinical trials have suggested that response to *KIT* tyrosine kinase inhibitors such as imatinib correlates with the presence of mutational activation of the gene and not with the protein expression.<sup>16-19</sup>

In the case of ACC, there are conflicting data regarding the response to treatment with the *KIT* kinase inhibitor imatinib. In 1 trial, there were no responses noted among 15 treated patients with ACC of the salivary gland.<sup>20</sup> Conversely, there is the report by Hotte et al.<sup>20</sup> regarding a multicenter phase 2 trial of imatinib for patients with recurrent or metastatic ACC strongly overexpressing *KIT* using CD117 immunohistochemistry (50-100% staining in tumor cells). Of 6 patients assessable for evaluation after 3 months of treatment, 3 patients had stable disease and 1 patient achieved a partial response. *KIT* mutations were not assessed. The authors suggested that the benefit of imatinib may be most pronounced in patients with accelerated tumor growth and strong overexpression of *KIT*. It is possible that these criteria explain the apparent benefit of imatinib described in 2 patients treated by Alcedo et al.<sup>21</sup> These exceptions notwithstanding, however, the finding that clinically significant, sustained responses to imatinib among ACC patients are uncommon is consistent with the absence of *KIT* gene mutations in these tumors.

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