Genomic Alterations in Pulmonary Adenocarcinoma In Situ in an Adolescent Patient

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- Lung cancer is a rare event in the pediatric and adolescent population. To date, only a few case reports and small case series have been published, and little is known about the risk factors associated with this entity in children and adolescents. We describe a case of adenocarcinoma in situ in a 15-year-old adolescent girl with previous surgical treatment for malignant melanoma. We provide a detailed genomic characterization of this neoplasm by comparative genomic hybridization, genome-wide single-nucleotide polymorphism array, and fluorescence in situ hybridization analyses. We identify chromosomal regions with copy number changes and correlate the corresponding genes within these regions with the available literature in the area.


Primary epithelial lung malignancies are extremely rare in the pediatric and adolescent population, with an incidence of less than 0.08 per 100,000 cases.1 Lung adenocarcinoma in situ (AIS, previously classified as bronchioloalveolar carcinoma) and invasive adenocarcinoma with mucinous histology have been classically described in the setting of congenital pulmonary airway malformation, primarily in type 1 malformations.2,3 However, pulmonary nodules with predominantly nonmucinous AIS morphology have also been described in pediatric patients without congenital pulmonary airway malformation.4 These lesions were named “pulmonary nodules resembling bronchioloalveolar carcinoma” because the neoplastic nature of the process was uncertain at the time. Recently, Kayton et al5 reported 8 cases of AIS and early invasive adenocarcinomas in children and adolescents, all of whom had a history of other malignancies. Additionally, this study demonstrated KRAS and epidermal growth factor receptor (EGFR) alterations in a subset of cases, confirming their neoplastic nature. We describe a case of solitary nonmucinous AIS in an adolescent patient with malignant melanoma. This is the first report defining genetic abnormalities in adolescent AIS by both comparative genomic hybridization and genome-wide single-nucleotide polymorphism (SNP) array analyses, allowing comparison with existing data on lung adenocarcinoma.

REPORT OF A CASE

This is the case of a 15-year-old adolescent girl who never smoked with a previously resected malignant melanoma of the left fourth toe. A sentinel lymph node biopsy performed at the time of resection demonstrated microscopic tumor metastasis. Subsequent left inguinal radical lymph node dissection was negative. A subsequent computerized tomography scan of her chest revealed an ill-defined, semisolidsolid, and ground-glass nodule measuring 0.6 cm, located in the superior segment of the left lower lobe. The remaining lung was normal. Whole-body positron emission tomography scan showed no evidence of fluorodeoxyglucose uptake in the lesion. The patient subsequently underwent thoracoscopic lung wedge resection. Following lung resection, the patient was treated with interferon therapy and is disease-free after a 4-year follow-up.

MATERIALS AND METHODS

Immunohistochemistry

Immunohistochemistry for cytokeratin (1:500; AE1/AE3, 35BH11, Millipore, Billerica, Massachusetts), thyroid transcription factor 1 (clone 8G7G 3/1, Dako, Carpinteria, California), cytokeratin 7 (1:200; clone OV-TL, Dako), cytokeratin 20 (1:200; clone KS20.8, Dako), S100 (1:1000; Dako), HMB-45 (1:100; Dako), and tyrosinase (1:80; clone T311, Cell Marque, Rocklin, California) was performed on Ventana Ultra instruments (Ventana Systems, Tucson, Arizona).

Comparative Genomic Hybridization Analysis

A sample of frozen tumor tissue was used for these studies. Frozen sections were cut at a thickness of 8 μm and stained with cresyl violet, and tumor cells were isolated with PALM Zeiss laser-capture microdissection (Zeiss, Germany). Whole-genome amplification using the GenomePlex Whole Genome Amplification kit (Sigma-Aldrich, St Louis, Missouri) was used to obtain adequate amounts of tumor DNA. Comparative genomic hybridization (CGH) was performed as previously described using normal diploid DNA hybridized to normal male metaphase spreads.6

SNP Oligonucleotide Microarray Analysis

A sample of frozen tumor tissue was used for these studies. Frozen sections were cut at 8-μm thickness, and manual needle dissection of tumor-rich areas was performed with an 18-gauge needle on an Olympus microscope (Olympus, Center Valley, Pennsylvania).
Pennsylvania) at ×40 magnification and collected by vacuum suction into a pipette tip. A total of 500 ng of DNA was collected for SNP oligonucleotide microarray analysis (SOMA). Copy number analysis was performed using the Affymetrix Genome Wide Human SNP Array 6.0, which includes more than 906 600 SNPs and more than 946 000 probes for the detection of copy number variation (http://www.affymetrix.com; accessed on June 30, 2011). Sample preparation, hybridization, and scanning were performed using GeneChip Instrument System hardware according to the manufacturer’s specifications (Affymetrix, Santa Clara, California). The intensities of both SNP and copy number variation probes were used to determine segments that varied in copy number. Single-nucleotide polymorphism and copy number intensities were normalized against an internally derived normal reference set of 48 samples.

Data analysis was performed using Nexus Copy Number Software Version 6.0 (BioDiscovery, Hawthorne, California). The FASST2 Segmentation algorithm was used to make copy number calls (a hidden Markov model approach). The significance threshold for segmentation was set at 5 × 10⁻⁶, also requiring a minimum of 3 probes per segment and a maximum probe spacing of 1000 between adjacent probes before breaking a segment. The log ratio thresholds for single-copy gain and single-copy loss were set at 0.1 and −0.15, respectively. The log ratio thresholds for 2 or more copy gain and homozygous loss were set at 0.7 and −1.1, respectively. Minimal loss of heterozygosity length was 500.

**Mutation Testing, Fluorescence In Situ Hybridization, and Silver In Situ Hybridization**

Mutation testing for *EGFR* was performed on DNA obtained from needle-microdissected tumor tissue from paraffin sections and analyzed by Sanger sequencing of exons 18 to 21. Mutation testing for *KRAS* was performed using similarly obtained DNA using a *KRAS* exon 12/13 ARMS Scorpions Assay (Qiagen, Valencia, California).

Fluorescence in situ hybridization (FISH) for *EGFR* was performed on paraffin sections using Vysis probe locus-specific identifier (LSI) EGFR 7p12 Spectrum Orange probe and Spectrum green–labeled centromeric CEP7 using standard protocols (Abbott Diagnostics, Abbott Park, Illinois). Fluorescence in situ hybridization for HER2 was performed on paraffin sections using Vysis probe LSI Her2/neu 17q11.2-q12 Spectrum Orange probe and Spectrum green–labeled centromeric CEP17 using standard protocols (Abbott Diagnostics). Silver in situ hybridization (SISH) for HER2 was performed using the Ventana Inform Dual ISH Her2 assay on a Ventana Benchmark XT instrument following the manufacturer’s protocol (Ventana Systems).

**PATHOLOGIC FINDINGS AND STUDIES OF GENETIC ALTERATIONS**

**Pathology Findings**

Gross examination of the wedge lung resection specimen demonstrated a white, rubbery nodule with ill-defined borders measuring 1.1 × 0.6 × 0.5 cm, without pleural or surgical margin involvement. Histologic evaluation of the entire lesion revealed an epithelial proliferation of cuboidal to low-columnar cells growing along thickened alveolar walls and showing cellular crowding, nuclear pleomorphism, and hyperchromasia, and scattered mitoses, consistent with nonmucinous AIS (Figure 1). The alveolar architecture was preserved throughout the entire lesion, with no evidence of stromal invasion. By immunohistochemistry, the tumor cells expressed cytokeratin 7 and TTF-1, and were negative for cytokeratin 20, S100, HMB-45, and tyrosinase, confirming a diagnosis of lung carcinoma rather than metastatic melanoma (data not shown).

**Detection of *EGFR* and *KRAS* mutations**

*EGFR* and codon 12/13 *KRAS* mutations were not identified in the needle-microdissected tumor cells. In addition, no *EGFR* gene amplification was identified by FISH using LSI *EGFR* and CEP7 probes.

**CGH Analysis**

Comparative genomic hybridization was performed to further characterize copy number alterations in the tumor. Figure 2 summarizes the results. Briefly, copy number gains were identified in regions of 1p, 1q, and 4q. In addition, most of chromosome 17 was overrepresented. Underrepresentation of chromosomal regions in 3p, 3q, 5p, 5q, 7q, 11p, 12p, 16p, 16q, 19p, and 19q was also detected.

**SNP Oligonucleotide Microarray Analysis**

Single-nucleotide polymorphism oligonucleotide microarray analysis was performed, and the results are summarized in Figure 3. High copy number gains were noted in 17q21.31, corresponding to a region containing genes NBR1 and TMEM106A. No significant homozygous deletions were identified. Copy number gains were identified in chromosomes 1, 4, 8, and 17. A list of the corresponding relevant genes in these regions is shown in the Table.

**Validation of Chromosome 17 Overrepresentation Using FISH and SISH**

To confirm chromosome 17 copy number gains, we performed FISH and SISH analysis using locus-specific probe LSI Her2/neu located in 17q, and a control probe to the centromere of chromosome 17 (CEP17). The FISH and SISH results suggest aneuploidy of chromosome 17 (Figure 4). Overall, LSI:CEP17 ratio equal to 1.9 was seen on FISH, and HER2:chromosome 17 ratio of 1.02 was obtained by SISH. The increased ratio did not meet criteria for HER2/neu amplification but supported the CGH and SNP array results of polysomy 17. These abnormalities were not present in this patient’s uninvolved lung parenchyma on SISH testing. Following these studies, we performed a literature search and obtained a list of genes previously reported as altered in AIS, invasive lung adenocarcinoma, and malignant
melanoma.10 This list of genes was then compared to our list of amplified loci, corresponding to a total of 2580 genes. Among the most relevant genes identified (Table), ERBB2, ARNT, and NKX2-1 were described in a large-scale study of lung tumors by SNP array analysis.9 Similarly, SNRPE and CRABP2 were previously identified in CGH analysis of stage I non–small cell lung cancer.7 Other genes, such as ASTN2 and SERPINF1, have been associated with increased risk of lung adenocarcinoma in never-smokers.8 Among the melanoma-related alterations, we identified KIT amplification, well described in acral melanoma.10

COMMENT

The exceedingly low incidence of AIS and pulmonary adenocarcinoma in the adolescent population has limited our ability to better characterize these lesions. To our knowledge, this is the first comprehensive genome-wide analysis of AIS in a pediatric patient. Stacher et al11 previously reported CGH analysis of 22 patients with congenital pulmonary airway malformation showing gains in chromosomes 2 and 4; however, only 1 patient with nonmucinous AIS was included.

In our case, CGH and SOMA identified subtle copy number abnormalities in the tumor cells. Most copy number gains were in chromosome 17, findings that were detected by both CGH and SOMA, and supported by HER2/neu SISH and FISH. In part, this was due to polysomy 17.

High copy number alterations were identified in one region corresponding to 2 genes, NBR1 and TMEM106A, located in chromosome 17. TMEM106 encodes transmembrane protein 106A, a protein of unknown function and not implicated in human malignancy. NBR1, or neighbor of Figure 2. Comparative genomic hybridization. Ideogram showing DNA copy number changes in tumor cells. Green lines indicate copy number gains, whereas red lines represent losses. Chromosomes 1 and 17 showed the most significant alterations.

Figure 3. Overview of all genetic aberrations found with single-nucleotide polymorphism oligonucleotide microarray analysis. Chromosomal gains in chromosomes 1, 4, 8, and 17 represent most alterations.
BRCA1 gene 1 protein, has been reported as an ovarian tumor antigen (CA125) and contains a protein motif reported in genes with transformation potential. Among the lower-level alterations in chromosome 17, we identified copy gains in the \textit{ERBB2} gene. Interestingly, Weir et al\textsuperscript{9} performed large-scale SOMA of lung adenocarcinomas and identified amplifications in chromosome 17 corresponding to this gene.

Low copy number gains in the \textit{KIT} gene locus on chromosome 4 were also present. \textit{KIT} mutations and copy number gains have been described in both acral malignant melanomas and stage I lung adenocarcinomas,\textsuperscript{12,13} observations that are particularly interesting in light of this patient’s history of melanoma.

Our case showed amplification of 1p36 and 1p12, both previously described.\textsuperscript{9} Alterations in the short arm of chromosome 1 (1p) are a common event in lung adenocarcinoma. For instance, genomic analysis of pulmonary adenocarcinomas with bronchioloalveolar features detected low copy gains at 1p. Moreover, amplification and upregulation of \textit{NOTCH-2}, a transcription factor located in 1p12, have been described recently in minimally invasive lung adenocarcinomas and seem to correlate with more aggressive tumor behavior.\textsuperscript{14} Interestingly, 1p36 has also been described as a susceptibility locus in a subset of familial melanoma cases.\textsuperscript{15} In fact, a small proportion of malignant melanomas are linked to familial genetic syndromes with increased susceptibility to other malignancies, including primary lung adenocarcinomas.\textsuperscript{16} These findings are relevant because they suggest a possible relationship between these two malignancies in our patient.

### Summarized List of Relevant Genes Obtained by Single-Nucleotide Polymorphism Oligonucleotide Microarray Analysis

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene Description</th>
<th>Chromosomal Location</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genes with high copy number gains:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NBR1</td>
<td>Neighbor of BRCA1 gene 1</td>
<td>17q21.31</td>
</tr>
<tr>
<td>TMEM106A</td>
<td>Transmembrane protein 106A</td>
<td>17q21.31</td>
</tr>
<tr>
<td><strong>Genes with copy number gains:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAX7</td>
<td>Paired box 7</td>
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<td>STMN1</td>
<td>Stathmin 1</td>
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<td>NOTCH2</td>
<td>Notch 2</td>
<td>1p12</td>
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<tr>
<td>CRABP2</td>
<td>Cellular retinoic acid binding protein 2</td>
<td>1q22 - q23.1</td>
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<td>KISS1</td>
<td>KISS-1 metastasis-suppressor</td>
<td>1q22.1</td>
</tr>
<tr>
<td>KCN212</td>
<td>Regulator of G-protein signaling 12</td>
<td>4p16.3 - p16.2</td>
</tr>
<tr>
<td>KIT</td>
<td>V-kit H-Z 4 feline sarcoma viral oncogene homolog</td>
<td>4q12</td>
</tr>
<tr>
<td>PDGFRL</td>
<td>Platelet-derived growth factor receptor-like</td>
<td>8p22</td>
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<tr>
<td>WISP1</td>
<td>WNT1 inducible signaling pathway protein 1</td>
<td>8q24.22</td>
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<td>SERPINF1</td>
<td>Serpin peptidase inhibitor, clade F, member 1</td>
<td>17p13.3</td>
</tr>
<tr>
<td>ERBB2</td>
<td>V-erb-b2 erythroblastic leukemia viral oncogene homolog 2</td>
<td>17q12 - q21.2</td>
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<tr>
<td><strong>Genes with LOH:</strong></td>
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<tr>
<td>ARNT</td>
<td>Aryl hydrocarbon receptor nuclear translocator</td>
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<tr>
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<td>ERBB4</td>
<td>V-erb-a erythroblastic leukemia viral oncogene homolog 4</td>
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<td>9q33.1</td>
</tr>
<tr>
<td>NKX2-1</td>
<td>NK2 homeobox 1</td>
<td>14q13.3</td>
</tr>
</tbody>
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Abbreviation: LOH: loss of heterozygosity.

Figure 4. Fluorescence in situ hybridization (FISH) and silver in situ hybridization (SISH) identification of HER2 copy number in the tumor cells. A, FISH shows aneuploidy, but no definite HER2 amplification was identified. Fluorescent red signals indicate HER2/new copies, and green corresponds to chromosome 17 centromeres; 4’,6-diamidino-2-phenylindole (DAPI)–stained nuclei appear in blue (original magnification ×60). B, SISH shows similar findings. Black signal indicates HER2, and red signal represents chromosome 17 centromere (original magnification ×40).

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Significantly increased risk of lung cancer has been reported in rare genetic syndromes, including an autosomal recessive MUTYH mutation in an adolescent with multifocal lung AIS.\(^1\)\(^7\) Although a genetic factor was not identified in our case, the possibility of an inherited risk of lung cancer cannot be entirely excluded.

Our case shares clinical and morphologic similarities with previous reports.\(^4\)\(^5\) In the most recent case series of pediatric and adolescent lung adenocarcinomas,\(^5\) more than 50% of patients showed AIS histology, and all 8 patients had a history of prior malignancy. It is well known that pediatric patients diagnosed with a malignant tumor are at increased risk of developing other malignancies. The incidental discovery of pediatric and adolescent AIS during cancer staging and their subsequent resection makes it difficult to comment on metastatic potential, including latency toward developing invasion. Furthermore, a direct association between these primary lung tumors and other malignancies has not been established.

Some reports suggest that these primary lung carcinomas could be secondary to chemotherapeutic treatment of other malignancies.\(^2\)\(^5\) It is true that chemotherapy is associated with tumorigenesis; nevertheless, our case and other published cases are not associated with prior cancer treatment. Further studies are required in order to accurately determine the risk factors and the distinct alterations involved in these rare pediatric tumors.

In summary, we present a case of a 15-year-old adolescent girl with AIS. Comprehensive copy number analysis revealed alterations on chromosomes 1, 4, 8, and 17. Some of these alterations have been described in other malignancies, including lung adenocarcinoma.

References