Transition from adenocarcinoma in situ to invasive adenocarcinoma then to large cell lung carcinoma with rhabdoid phenotype, immunohistochemical and molecular findings; case report and literature review

Xiaoyong Zheng¹, Olena Ardacheva¹, Carmelo Puccio², John Fallon¹ and Liying Han¹
¹Department of Pathology, Westchester Medical Center/New York Medical College, USA, ²Department of Oncology, Westchester Medical Center/New York Medical College, USA.

Abstract

Background: Current cancer therapy of non-small cell lung cancer depends on histopathological classification. Large cell lung carcinoma with rhabdoid phenotype (LCC-RP) is a rare type of lung cancer with poor prognosis.

Methodology and principal findings: This is a case of a 64-year-old male patient with a lung mass composed of adenocarcinoma in situ (AIS), invasive adenocarcinoma with acinar predominant pattern and LCC-RP. The tumor consisted of 10% AIS, 30% invasive adenocarcinoma with acinar predominant pattern and 60% solid tumor with rhabdoid phenotype. Immunohistochemical study showed that all three components expressed thyroid transcription factor-1 (TTF-1), pan-cytokeratin AE1/3 and epithelial membrane antigen (EMA). In the transition areas from AIS to rhabdoid phenotype, the tumor cells expressed less TTF-1 and napsin A, completely lost E-cadherin expression and expressed the mesenchymal marker Vimentin. Molecular testing showed that both the adenocarcinoma and rhabdoid components had the same mutation Gly13Asp (GGC->GAC) in KRAS and no EGFR mutations or ALK gene rearrangements.

Conclusion: These findings suggest that LCC-RP is not an entity by itself but a dedifferentiated adenocarcinoma undergoing epithelial-to-mesenchymal transition (EMT).

Keywords: Large cell lung carcinoma, rhabdoid phenotype, lung cancer, KRAS, epithelial-to-mesenchymal transition

Introduction

Currently, large cell carcinoma (LCC) is a confusing concept. Most authors think that the histological diagnosis of LCC is based on exclusion of other types of non-small cell carcinoma (NSCLC). As a result, this subtype of NSCLC is highly heterogeneous in histopathology and clinical presentation. Large cell lung carcinoma with rhabdoid phenotype (LCC-RP) is a rare histological form of lung cancer. According to the WHO definition, “In large cell carcinoma with rhabdoid phenotype, at least 10% of the tumor cell population must consist of rhabdoid cells. Small foci of adenocarcinoma may be seen”. If lung cancer has a rhabdoid phenotype (>10%) as well as invasive adenocarcinoma components, how should we categorize it? Many authors still categorize this type of lung cancer as LCC-RP while some categorize it as rhabdoid tumor of the lung or lung cancer with rhabdoid phenotype. So the question should we categorize this as adenocarcinoma with rhabdoid phenotype or LCC-RP still remains.

The evolution of LCC-RP also remains controversial. Some have hypothesized that these tumors represent an admixture of a carcinoma and a mesenchymal neoplasm [1]. Whereas, others have proposed that these are dedifferentiated adenocarcinomas [2]. Epithelial-to-mesenchymal transition (EMT)

How to cite this article: Zheng, X., Ardacheva, O., Puccio, C., Fallon, J. and Han, L. (2015). Transition from adenocarcinoma in situ to invasive adenocarcinoma then to large cell lung carcinoma with rhabdoid phenotype, immunohistochemical and molecular findings; case report and literature review. Annals of Cancer Research, 2:2. Retrieved from http://www.vipoa.org/cancer
is a process, in which cells lose generally immotile epithelial characteristics and gain motile mesenchymal properties [3]. It is unknown whether this may also apply to LCC-RP, originating from a dedifferentiated pulmonary adenocarcinoma.

Materials and methods

Immunohistochemistry

The specimen was fixed in histology grade buffered 4% formalin and dissected according to our standard surgical pathology grossing protocol. Paraffin sections were stained with hematoxylin and eosin and immunohistochemical detection systems according the manufacturer’s protocols. We used the fully automated immunohistochemical system Ventana Bench Mark Ultra.

Microdissection and real-time PCR

AIS is a minor component (10% of total) which is growing tightly adjacent and even mixed with components of invasive adenocarcinoma, so we only microdissected the component of invasive adenocarcinoma with acinar predominant pattern and LCC-RP. Two components were microdissected from the corresponding unstained slide(s), lysed and the DNA extracted. Real-time PCR was used to analyze for mutations of KRAS gene and Epidermal Growth Factor Receptor (EGFR) gene. The region of the KRAS gene containing codons 12 and 13 was amplified. A set of eight probes were specifically used to detect the wild type and mutant sequences. Real-time PCR was used to evaluate specific mutations, deletions and insertions in the tyrosine kinase domain of the EGFR gene. Eight reactions containing 30 primer and probe sets were used to target specific regions of exons 18–21 as well as the wild-type sequence, which includes Exon 19 deletion, G719S, G713A, G719C, L858R, L861Q, S7681, T790M and Exon 20 Insertion.

FISH analysis

Both tumor areas—the adenocarcinoma part and rhabdoid part which were microdissected and were assessed for the ALK gene rearrangement utilizing the Vysis ALK Break Apart FISH Kit (Abbott Molecular). The identification probes for LSI ALK 5’ probe (Spectrum Green) and LSI ALK 3’ probe (Spectrum Orange) were applied, hybridized and assessed along with standard controls. At least 50 non-overlapping nuclei were analyzed and the localization of the LSI ALK 5’ probe (green) and LSI ALK 3’ probe (orange) signals were recorded and interpreted according to manufacturer’s guidelines.

Results

Clinical history

The patient is a 64-year-old female, who actively smokes (66 packs per year for 40 years) with a history of hypertension and dyslipidemia, who was hospitalized for acute decompensated systolic heart failure. She was found to have severe three-vessel coronary artery disease. A CT scan of the chest revealed a spiculated mass in the left upper lobe measuring 1.9x0.8 cm and suspicious for a primary lung neoplasm. She underwent urgent coronary artery bypass grafting and a lung wedge resection for the lesion. Pathology revealed a 1.6 cm adenocarcinoma with free surgical resection margins. A subsequent PET/CT scan revealed only postsurgical changes, no suspicious lesion or activity in the left upper lobe. Two months later, a new nodule measuring 2 cm was found in the same lobe. Left upper lobectomy was performed which revealed that the nodule arose from the wedge resection staple line. Grossly, the nodule showed a 2 cm poorly-defined pink/tan cut surface and invasion to the visceral pleura.

Microscopic findings

The tumor from the first resection displayed three components: 1) Adenocarcinoma in situ (AIS, comprising about 10% of the tumor mass); 2) Invasive adenocarcinoma with (30%); and 3) Solid area with rhabdoid phenotype (60%). In the solid area with rhabdoid phenotype, the cells were large and uniform, with abundant eosinophilic cytoplasmic inclusions, and include occasionally multinucleated giant cells. Transitional areas exhibited characteristics of AIS (Figure 1), invasive adenocarcinoma and solid area with rhabdoid phenotype (Figure 2). In transitional area, some glandular lining cells have rhabdoid features (Figure 2).

Immunohistochemistry

Immunohistochemical staining showed all three components strongly cytoplasmic positive on pan-cytokeratin AE1/3,
cytokeratin 7 (CK7), and epithelial membrane antigen (EMA), but negative for smooth muscle actin (SMA), desmin, Myo-D1, P63, CK20, synaptophysin, chromogranin A, and CD56. Napsin A is strongly positive in AIS, but moderately and weakly positive in adenocarcinoma and rhabdoid components respectively. Thyroid transcription factor (TTF-1) and E-Cadherin are strongly positive in AIS and adenocarcinoma component, but moderately expressed and focally lost in the rhabdoid component. (Figures 3A-3D). There was a loss of CD117 expression in the rhabdoid components, whereas expression was detected in the AIS and adenocarcinoma components. Immunohistochemical studies also show the expressions of hSNF5/INI1 gene are almost equally detected in all three components. More detailed results and the information of reagent are showed in (Table 1).

Molecular study and fluorescence in situ hybridization findings
The tumor areas, the adenocarcinoma components and rhabdoid components, were microdissected and analyzed for EGFR and KRAS mutation. Both tumor areas revealed the same molecular pattern: mutations of Gly13Asp (GGC->GAC) in KRAS and negative for EGFR alteration. ALK gene rearrangements were not detected using the Vysis ALK Break A components FISH Probe Kit in both tumor areas.

Discussion
The first rhabdoid tumor was described by Beckwith et al., as a renal tumor with a peculiar morphology, found in children and characterized by poor prognosis [4]. The hallmark of this tumor is rhabdoid cells with a globular eosinophilic cytoplasmic inclusion that displaces the nucleus to the periphery and sometimes causes nuclear indentation [4,5]. The first pulmonary composite malignant rhabdoid tumor was described in 1995 as a neuroendocrine carcinoma with rhabdoid phenotype [6]. The striking morphologic feature of this phenotype is large pleomorphic, sometimes multinucleated cells with eosinophilic globular inclusions that immunohistochemically stain positive for Vimentin [4]. Ultrastructurally, the eosinophilic inclusions are composed of aggregates of large intra cytoplasmic paranuclear type intermediate filaments. Cancer cells with a rhabdoid phenotype may be seen focally in other poorly differentiated NSCLC.

In our case, we found components containing rhabdoid phenotype within the adenocarcinoma. From the H&E staining, continuous changes from AIS to adenocarcinoma and then to the solid area with rhabdoid phenotype were observed. In some areas the tumor appeared to have a glandular architecture. However, the lining cells had rhabdoid features. Immunohistochemical staining also revealed continuous changes of the markers. The tumor cell started losing the marker of Napsin A in the early stage from AIS to invasive adenocarcinoma. The tumor cells expressed less TTF-1 from invasive adenocarcinoma to tumor with rhabdoid phenotype. The rhabdoid component expressed mesenchymal cell markers like vimentin and preserved different epithelial antigens like cytokeratin and epithelial...
Table 1. Immunohistochemical of the tumor (Alphabetical order).

<table>
<thead>
<tr>
<th>Antigen</th>
<th>AIS</th>
<th>Adenocarcinoma</th>
<th>Rhadoid</th>
<th>Source</th>
<th>Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actin</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>Dako</td>
<td>HHF-35 Mouse</td>
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<tr>
<td>CD117</td>
<td>+++</td>
<td>+++</td>
<td>--</td>
<td>Epitomics</td>
<td>YR145 R-McAb</td>
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<tr>
<td>CD56</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>Cellmarque</td>
<td>MRQ-42 R-McAb</td>
</tr>
<tr>
<td>CD68</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>Dako</td>
<td>KP-1 Mouse</td>
</tr>
<tr>
<td>Chromogranin A</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>Cellmarque</td>
<td>LK2H10 Mouse</td>
</tr>
<tr>
<td>CK7</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>Ventana</td>
<td>SP52 R-McAb</td>
</tr>
<tr>
<td>CK20</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>Ventana</td>
<td>SP33 R-McAb</td>
</tr>
<tr>
<td>Cytokeratin</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>Ventana</td>
<td>AE1/AE3 PCK26Mouse</td>
</tr>
<tr>
<td>Desmin</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>Ventana</td>
<td>DE-R-11 Mouse</td>
</tr>
<tr>
<td>E-Cadherin</td>
<td>+++&quot;</td>
<td>+++</td>
<td>++2%</td>
<td>Ventana</td>
<td>36 Mouse</td>
</tr>
<tr>
<td>β-catenin</td>
<td>+++M&quot;&quot;</td>
<td>+++M</td>
<td>++M</td>
<td>Ventana</td>
<td>14 Mouse</td>
</tr>
<tr>
<td>EMA</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>Ventana</td>
<td>E29 Mouse</td>
</tr>
<tr>
<td>Myo-D1</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>Dako</td>
<td>5.8A Mouse</td>
</tr>
<tr>
<td>Myogenin</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>Dako</td>
<td>F5D Mouse</td>
</tr>
<tr>
<td>Napsin A</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>Ventana</td>
<td>Polyclonal Rabbit</td>
</tr>
<tr>
<td>P63</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>Ventana</td>
<td>4A4 Mouse</td>
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<tr>
<td>Synaptophysin</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>Ventana</td>
<td>MRQ-40 R-McAb</td>
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<tr>
<td>TTF-1</td>
<td>+++</td>
<td>+++</td>
<td>++50%</td>
<td>Dako</td>
<td>8G7G3/1 Mouse</td>
</tr>
<tr>
<td>Vimentin</td>
<td>--</td>
<td>--</td>
<td>+++</td>
<td>Dako</td>
<td>V9 Mouse</td>
</tr>
</tbody>
</table>

*The evaluation of the intensity of staining refer to Her 2 staining
"M=Membrane stain; +++=Strong stain
R-McAB=Rabit-Monoclonal

due to. membrane antigen. Herbst et al., [7] found that mutations and amplifications in the epidermal growth factor receptor gene (EGFR) occur in patients with adenocarcinoma (mostly women, nonsmokers, and those of Asian origin). These mutations occur early in the development of adenocarcinoma. KRAS mutations are seen in lung adenocarcinomas and large cell carcinomas, but less frequently or not at all in other subtypes [8]. In this case there were similar molecular phenotypes in both the invasive adenocarcinoma and tumor with rhabdoid phenotype components. The same KRAS mutations were detected in these two components; however, EGFR alteration and ALK gene rearrangements were not detected. These results indicate that invasive adenocarcinoma and tumor with rhabdoid phenotype components have the same origin. The morphology, immunohistochemical staining and molecular analysis in this case supports that the tumor cells with rhabdoid phenotype may originate from the adenocarcinoma component.

Studies on pulmonary carcinosarcomas have also indicated that the sarcomatous component arises from dedifferentiation of the epithelial component in view of the fact that both components are clonally related [9]. EMT has gained attention as a critical phenotypic alteration of cancer cells to acquire invasive and metastatic abilities. EMT is mediated through several transcription repressors, such as Snail, Slug, Twist and ZEB1, mesenchymal markers Vimentin and N-cadherin, and these EMT inducers typically suppress the transcription of the E-cadherin gene, an epithelial cell marker and a potent suppressor of tumor cell invasion and metastasis [10,11]. In our study we found tumor cells with decreased expression of adenocarcinoma markers such as TTF-1 and sudden expression of a mesenchymal marker, Vimentin, within the transition from adenocarcinoma to rhabdoid phenotype. Additionally, these cells lost the epithelial marker E-cadherin. In human colorectal adenocarcinomas, nuclear accumulation of β-catenin, an indicator of EMT, is seen only at the invasive front of the tumor [12]. Nuclear accumulation of β-catenin was not observed in our case. These immunohistochemical results support that EMT may occur during the transition from classic adenocarcinoma to rhabdoid phenotype.

Pediatric malignant rhabdoid tumor of the kidney, which is a highly aggressive tumor characterized by cells that resemble rhabdomyoblasts and by genetic alterations involving chromosome 22, particularly the hSNF5/INI1 gene on 22q11.2. Similar to previous reports, [13] the hSNF5/INI1 gene is not inactivated in both the invasive adenocarcinoma and tumor with rhabdoid phenotype components. This finding also indicates that LCC-RP is different from rhabdoid tumor, may be a dedifferentiated tumor from other type of lung cancer.
Clinically, LCC-RP are aggressive tumors with most cases presenting at advanced stages [1]. In our case, the tumor grew from an invisible tumor to 2 cm in only two months. Approximately 47 cases of lung tumors with a rhabdoid component have been reported [3,6,12,14]. Various different types of lung cancer have reported the presence of rhabdoid phenotype: 19 cases of adenocarcinoma (40%), 10 cases of large cell carcinoma (21%), 3 cases of large cell neuroendocrine carcinoma, 4 cases of sarcomatoid carcinoma and 4 cases of poorly differentiated tumor. By applying special stains and electron microscopy, it has been shown that many cases of LCC are poorly differentiated adenoscarcinoma or SCC [15]. Combining our study and others [1], rhabdoid component in lung cancer may come from the dedifferentiated adenoscarcinoma, and proceed to the epithelial-to-mesenchymal transition.

Although in the WHO classification, LCC-RP is under the definition of a large cell carcinoma, Dr. Popper thinks that “LCC-RP as other LCC variants have nothing to do with LCC itself and should not be mixed” [16]. But many authors have published papers combining LCC and LCC variants. Due to the aforementioned reason and the bad prognosis, we suggest not categorizing LCC-RP as large cell carcinoma. Instead, these types of tumors should be placed into a different type of carcinoma category with rhabdoid phenotype. Some possible ones could be adenoscarcinoma with rhabdoid phenotype, small cell carcinoma with rhabdoid phenotype, or simply labeling these tumors as lung carcinoma with rhabdoid phenotype. We prefer to label our case as: adenocarcinoma with rhabdoid phenotype.

Conclusion
We report the first case with transition from AIS, invasive adenocarcinoma to a rhabdoid tumor component. This case provides supporting evidence that LCC-RP is not an entity by itself but a dedifferentiated adenoscarcinoma or other type of lung cancer undergoing epithelial-to-mesenchymal transition.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
XY. Z., J. F. and LY. H designed and performed the detailed morphological and molecular analysis. C. P. provided clinical evaluation and data related to clinical follow-up. O. A. performed immunohistochemistry analysis. All authors gave input to the manuscript.

Acknowledgement
This study is supported by Pathology resident research fund from Westchester Medical Center/New York Medical College.

References