Morphoproteomic Study of Primary Pleural Angiosarcoma of Lymphangioendothelial Lineage: A Case Report

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Abstract. An unusual case of bilateral primary pleural angiosarcoma with an immunophenotype of lymphangioendothelial lineage is described. Pleural angiosarcoma is a highly malignant neoplasm for which there is currently no standard of care. A comprehensive immunophenotypic characterization established a lymphangioendothelial lineage. A morphoproteomic analysis was also performed to identify the proteins and corresponding molecular pathways activated in the patient’s tumor. The information derived from the morphoproteomic studies provides insight into the biology of the tumor and may be useful in formulating therapeutic alternatives.

Key Words: Morphoproteomics; Pleural Lymphangiosarcoma

Introduction

Primary pleural angiosarcoma remains a very rare and highly malignant neoplasm of endothelial cell origin. Angiosarcomas account for approximately 1-2% of all soft tissue sarcomas (STS). Among these, pleural angiosarcoma is rarer with around 50 cases reported in the literature [1-9]. The prognosis is dismal, with very limited palliative therapeutic alternatives, and the vast majority of patients die within months of diagnosis. The diagnosis of this entity is made difficult due to its non-specific clinical and radiologic findings, similar to mesothelioma, pulmonary carcinoma, or metastatic adenocarcinoma [10,11]. Occasionally, angiosarcomas may also be positive for epithelial markers such as cytokeratin (CK) and CK7, which can impede an accurate diagnosis. Immunostains specific for endothelial components such as CD31, CD34, Factor VIII, and FLI-1 are particularly useful in the identification of these tumors. Moreover, the platelet endothelial cell adhesion molecule (PECAM-1; CD31) has proven to be highly sensitive and specific for endothelial malignancies including angiosarcoma [12].

We report a case of primary pleural angiosarcoma of lymphangioendothelial immunophenotype. By using morphoproteomic analysis, we identify and characterize the molecular pathways driving the biological behavior of this tumor. No previous proteomic studies have been described in pleural angiosarcoma. This information may contribute to the development of targeted therapies for this otherwise fatal tumor.

Case Report

A 58-year-old Chinese woman, born in Shanghai and resident of the United States for 6 years, with a past medical history of asthma under good control is the subject of this report. She presented to an outside hospital for refractory dyspnea and subjective fever for ten days, prompting treatment with azithromycin and cefotaxime for a presumed community-acquired pneumonia. Ultrasound-guided right thoracentesis resulted in the removal of three liters of serosanguinous fluid. Failure to improve with antibiotics during the course of three weeks prompted the transfer of the patient to our institution for a higher level of care.

Upon admission to our hospital, the patient was in respiratory distress but did not require endotracheal intubation. She denied cough, hemoptysis, wheezing, or sputum production. Her physical examination after stabilization was noncontributory with the exception of decreased breath sounds bilaterally. There was no history...
of prior risk factors such as smoking, radiation or asbestos exposure, tuberculosis or pyothorax. Imaging of the chest revealed a large right-sided pleural effusion (Figures 1A and 1B). A thoracentesis was performed for management of recurrent pleural effusion.

A diagnosis of an angiosarcoma of the pleura with lymphangioendothelial lineage was established by histopathology and immunophenotype (vide infra). There was no clinical evidence of angiosarcoma elsewhere at the time of presentation. The patient received systemic therapy with four (4) cycles of paclitaxel every 3 weeks and daily sorafenib from November 19, 2011 through January 30, 2012. She was readmitted 3 weeks later with recurrent and progressive bilateral pleural effusions and dyspnea followed within 72 hours by hypotension and a decrease in hemoglobin to 7.7 g/dL (suspected to be due to pleural hemorrhage). As per the request of the patient and her husband, no further interventions were undertaken and end of life palliative measures were implemented until her demise. There was no consent for autopsy.

| Table 1. Immunophenotype of Patient’s Tumor Establishing A Lymphangioendothelial Lineage |
|---------------------------------------------|---------------------------------------------|
| POSITIVE                                   | NEGATIVE                                   |
| Vimentin                                   | Calretinin                                 |
| CD31                                       | CK 5/6                                     |
| D2-40                                      | MOC-31                                     |
| Nestin                                     | CD34                                       |
| VEGF-A                                     | CK7                                        |
| α-SMA (focally)                            | CK20                                       |
| Ki-67 (~30% positive nuclei)               | Bcl-2                                       |
| ---                                        | HMB45                                      |
| ---                                        | Melan-A                                    |
| ---                                        | Pancytokeratin                             |
| ---                                        | Factor 8                                   |
| ---                                        | Mart-1                                     |

Abbreviations: CD (Cluster of Differentiation); VEGF (vascular endothelial growth factor)-A (isoform); SMA (alpha-smooth muscle actin); CK (cytokeratin).
Materials and Methods

The general immunohistochemical and morphoproteomic procedures utilized in our laboratory have been previously described [13,14]. Positive controls using established immunoreactive tissues and negative controls shown to lack the antigen being tested or minus the primary antibody were run concurrently and noted to react appropriately. A panel of antibodies was assembled to detect various protein antigens in the patient’s tumor, in an effort to discern its immunophenotype and lineage and to define its biology.

The morphoproteomic process employed in the identification and quantification of protein analytes in the tumor cells in this case involved: their immunohistochemical detection; quantification of their signal intensity and/or percentage of tumoral nuclei with the diamino-benzidine (DAB) chromogen (brown) signal by visual analysis on a scale of 0 to 3+ and in terms of cell-cycle-related analytes by visual estimation; their subcellular compartmentalization (nuclear, cytoplasmic and/or plasma-memmal); and an assessment of their state of molecular activation to include phosphorylation (p), compartmental translocation, and functional grouping morphoproteomics [13,14].

Figure 2. Representative digital images of the microanatomical features of the pleural tumor showing spindle cell features (frame A) and a more epithelioid focus (frame B). Cell cycle progression of the tumor is reflected in the Ki-67 at ~30% which detects G1, S, G2 and M phases (frame C) and mitotic activity (frame D, arrow) with mitotic index of 8 mitotic figures per 10 high power fields. (original magnifications, x200 frames A and C and x400 frames B and D).

Figure 3. Digital images favoring a lymphangioendothelial lineage of the patient’s pleural tumor to include CD31 (frame A) and D2-40 (frame B) expressions and absence of CD34 (frame C) and pancytokeratin (frame D) expression (also see Table 1). Note expected, positive expression in intratumoral vascular endothelial cells for CD31 and CD34 (arrows VEC) (original magnifications, x400 A-D).
Results

All biopsies demonstrate a similar histologic appearance consisting of an admixed spindle cell /epithelioid neoplasm, scattered mitotic figures, and rare giant cells with an associated mixed inflammatory infiltrate (Figure 2). Additionally, there was focal infiltration of tumor cells into contiguous adipose and soft tissues, focal tumoral necrosis and slit-like vascular spaces within the lesions. The proliferation index was approximately ~30% as measured by the Ki-67 antigen expression and had a mitotic index of up to 8 mitotic figures per 10 high power fields.

Immunohistochemical staining revealed the tumor cells to be positive for vimentin and with focal positivity for alpha-smooth muscle actin. Epithelial and other markers including calretinin, cytokeratin (CK)5/6, CK7, MOC31, CD34, Bcl-2, HMB45, and Melan-A were not evident in the tumor cells. Notably, the tumor cells were diffusely positive for D2-40 and nestin but negative for factor VIII and panCK. These data are summarized in Table 1. The tumoral immunophenotype of vimentin+/CD31+/nestin+/D2-40+/CD34-/panCK- (Table 1 and Figure 3) coincides with its mesenchymal staining pattern, vascular architecture, and aggressive nature in supporting a diagnosis of pleural angiosarcoma of lymphangioendothelial lineage.

Figure 4. Morphoproteomic findings of constitutively activated pathways in patient’s pleural tumor to include: extracellular signal-regulated kinase (nuclear p-ERK 1/2 [Thr 202/Tyr 204], frame A); signal transducer and activator of transcription (nuclear p-STAT3 [Tyr 705], frame B); and mammalian target of rapamycin (p-mTOR [Ser 2448], predominantly nuclear favoring mTORC2, frame C). Contrast with absence of chromogenic signal in the overnight negative control (frame D). (Original magnifications, x400 A-D)

Figure 5. Morphoproteomic correlates of the hypoxia pathway in the patient’s pleural tumor, in addition to hypoxia-associated mTORC2 expression (see Figure 4, frame C), to include: nuclear hypoxia-inducible factor (HIF)-1 alpha (frame A); plasmalemmal-cytoplasmic vascular endothelial growth factor (VEGF)-A (frame B); and cytoplasmic secreted protein, acidic and rich in cysteine (SPARC, frame C). Morphoproteomic correlate of either the transforming growth factor (TGF)-beta [Smad3] or sonic hedgehog pathway is evident in the form of variable nuclear glioma-associated oncogene protein2 (Gli2) expression (frame D). (original magnifications x400 A-C, x200 D).
Morphoproteomic analysis of the tumor revealed the following: (1) Constitutive activation of the ras/Raf kinase/extracellular signal-regulated kinase (ERK) and signal transducer and activator of transcription (STAT)3 pathways as evidenced by expression with nuclear translocation of phosphorylated (p)-ERK 1/2 (Thr 202/Tyr 204) and of p-STAT3 (Tyr 705); (2) Activation of the hypoxia pathway supported by the correlative expression of the following protein analytes: (a) nuclear p-mammalian target of rapamycin (mTOR) [Ser 2448]; (b) nuclear hypoxia inducible factor (HIF)-1 alpha; (c) cytoplasmic-plasmalemmal expression and compartmentalization of vascular endothelial growth factor (VEGF)-isoform A; and (d) secreted protein, acidic and rich in cysteine (SPARC) [13-15]; (3) nuclear glioma-associated oncogene protein 2 (Gli2) indicative of either sonic hedgehog pathway or transforming growth factor (TGF)-beta [Smad] 3 signaling [16]. These are illustrated in Figures 4 and 5.

Discussion

Primary pleural angiosarcoma is a highly malignant and very rare neoplasm with nonspecific clinical or radiological manifestations and with similarities to more common entities including mesothelioma, metastatic, and invasive bronchogenic adenocarcinoma. Primary pleural angiosarcoma is most often diagnosed in advanced stages with very limited therapeutic choices [1-9]. Surgical intervention may provide palliation of recurrent pleural effusions, which are often hemorrhagic. Radiation therapy has a very limited role in localized disease and none for diffuse and bilateral pleural involvement [1-3].

The differential diagnosis in tumors with epithelioid features, as in this case, also includes epithelioid sarcoma, epithelioid hemangiomia, Kaposi sarcoma, and melanoma. Immunohistochemistry narrows the differential in defining the tumor lineage. We describe a patient with bilateral primary pleural tumors which exhibited the histopathologic and immunophenotypic features characteristic of angiosarcoma of lymphoendothelial lineage [17,18] (Table 1).

Pathogenetically, angiosarcomas seem to arise from endothelial cells, suggesting that pro-angiogenic proteins may be relevant to their growth. Specifically, the vascular endothelial growth factor (VEGF) pathway seems to play a dominant role in the pathogenesis and biology of angiosarcoma. Strong VEGF expression is often present in tumors rich in vasculature and epithelioid features. The VEGF downstream pathway involves PI3K and mitogen-activated protein kinase (MAPK), which can also be triggered by other growth signals, including VEGF-induced activation of PGLG1, which exerts pro-angiogenic effects via protein kinase C [19,20].

Morphoproteomic analysis provides insight into the biology of this patient’s tumor and highlights both existing therapeutic approaches and opportunities to improve upon such therapies. Specifically, the reported antitumoral activity of paclitaxel alone, paclitaxel with bevacizumab, or sorafenib and sorafenib in angiosarcomas of other sites of origin [21-25] is consistent with targets identified by morphoproteomic analysis in this patient’s angiosarcoma. Our findings support the use of paclitaxel, a microtubule-stabilizing agent, because it would target cells entering the mitotic phase, reduce the activation of STAT3 and hinder its translocation into the nucleus, and sequester Smad3, thereby reducing the TGF-beta[Smad3] transcriptional activation of Gli2, a sonic hedgehog pathway mediator [16,24,25]. In general, activation of the sonic hedgehog pathway has been shown to be relevant to the growth of soft tissue sarcomas [26], and platelet-derived growth factor’s pro-angiogenic pathway is reflected in the phosphorylation of Stat3 [27]. Additionally, the finding of SPARC expression in the tumor cells suggests possible enhancement of taxane therapy with nab-paclitaxel, whose efficacy is reportedly enhanced by the expression of this protein analyte [28]. We also found expression of VEGF-A and both phosphorylated ERK 1/2 and STAT3 in the tumor cells, supporting the use of sorafenib, which interferes with the VEGF, Raf kinase/ERK, and STAT3 pathways [29,30].

Finally, the aforementioned detection by morphoproteomics of hypoxia pathway analytes in this tumor supports a role for hypoxia in the pathogenesis of recurrent and chemoradio-resistant disease in angiosarcoma [15]. Biomedical analytics has confirmed the application of various inhibitors of this pathway that might be applicable to the primary treatment and maintenance therapy for angiosarcoma, including metformin, valproic acid,
melatonin, and doxorubicin [15]. Parenthetically, single-agent doxorubicin has been shown to act against angiosarcoma [31,32].

In summary, morphoproteomic analysis of this rare case of bilateral pleural angiosarcoma of lymphoendothelial lineage has provided insight into its biology, linking its pathogenesis to hypoxia and suggesting new therapeutic options for further consideration.

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References