Rhabdomyomatous Differentiation in Wilms Tumor Pulmonary Metastases: A Case Report and Literature Review

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Abstract. While sparsely reported in the literature, Wilms tumor may differentiate into more mature mesenchymal tissue types, such as skeletal muscle, following chemotherapy. The frequency of this event is unknown. Chemotherapy and radiation may induce cytodifferentiation of Wilms tumor cells or select for the survival of less mitotically active cells. In follow-up biopsies, the presence of rhabdomyomatous differentiation can confound the histologic diagnosis. Furthermore, these differentiated tumors appear to be more resistant to chemotherapy, thus biopsy and positron emission tomography scans following chemotherapy and radiation may prevent unnecessary treatment. We report an unusual case of Wilms tumor in a 21-year-old man with rhabdomyomatous differentiation of pulmonary metastases after chemotherapy, which presented a challenge during frozen section diagnosis.

Key words: Wilms tumor, pulmonary metastases, differentiation, rhabdomyomatous, case report

Introduction

Wilms tumor is the most common primary renal malignancy of childhood and 98% of the disease occurs in children under the age of 10 years. It is a genetically and histologically diverse entity. Wilms tumor is classically described as having triphasic histology including blastemal, epithelial, and stromal components, which are felt to arise from multipotent mesenchymal stem cells that fail to undergo normal differentiation. Skeletal muscle is the most common heterologous, stromal cell type [1]. Wilms tumor spreads hematogenously from the abdomen and its metastases are typically restricted to the regional lymph nodes, lungs and liver. While it has been sparsely reported, Wilms tumor may undergo differentiation to mature-appearing skeletal muscle following chemotherapy and radiation. These post-therapy changes can make histologic diagnosis difficult in follow-up biopsies. We report a Wilms tumor with rhabdomyomatous differentiation of pulmonary metastases in a 21-year-old man that presented a challenge during intraoperative pathologic consultation.

Materials and Methods

The wedge resection of the right lower lobe of the lung was received fresh for frozen in the gross laboratory. The nodule in question was palpated and serially sectioned. An optimal representative section of the lesion was chosen, frozen along with the bronchial margin in OCT medium, and cut in 5-micrometer slices on a LEICA CM1900 cryostat. The specimens were then transferred to separate glass slides and stained in hematoxylin and eosin (H&E) for the evaluation by the pathologist.
After frozen section diagnosis, the wedge resection specimen was formalin fixed overnight in 10% formaldehyde. Representative sections were again selected for and embedded in paraffin, transferred to glass slides, and subsequently stained via H&E. To ensure accurate diagnosis, a battery of immunostains was performed utilizing commercially available antibodies, a steam-induced epitope retrieval process, and a Ventana-platform (Tucson, AZ) on paraffin-embedded sections. The antibodies included the following: WT1 (6F-H2, dilution 1:25; DAKO), CK8/18 (B22.1, dilution 1:100; Cell Marque [CM]), pancytokeratin (AE1/AE3, Ready to use [RTU]; Ventana [VMS]), NSE (E27, RTU; VMS), CD56 (1B6, RTU; Leica), EMA (E29, RTU; VMS), Ki-67 (30-9, RTU; VMS), CD99 (O13, dilution 1:100; Covance), desmin (NCL-DE-R-11, RTU; VMS), smooth muscle actin (HHF35, RTU; VMS), synaptophysin (SP11, RTU; VMS), and vimentin (3B4, RTU; VMS). Adequate negative and positive controls were included. The immunostain pattern of the tumor was then assessed by an AP/CP certified pathologist.

**Case Report**

The following case was evaluated and reported in compliance with the University of South Florida’s Institutional Review Board Policy #311.

A 21-year-old Hispanic male presented with right flank pain that was initially attributed to muscle strain. The pain continued and plain X-rays were performed, revealing multiple, bilateral pulmonary nodules too numerous to count (Figure 1). The largest was noted in the right middle lobe, with the majority of nodules ranging between 3 and 5 mm. Further workup demonstrated a unilateral, complex, solid, right upper pole renal mass measuring 10 cm on both computed tomography (CT) and magnetic resonance image (MRI) of the abdomen. Although possible involvement of the inferior border of the liver with evidence of internal necrosis and hemorrhage was noted, there was no discernible renal vein invasion or retroperitoneal lymphadenopathy. Subsequent tumor staging revealed no evidence of visceral metastasis other than to the lungs and an unremarkable contralateral kidney.

CT guided biopsy of the right lower lung nodule revealed a triphasic neoplasm suggestive of Wilms tumor. A subsequent flexible bronchoscopy and left thoracoscopy with wedge excision of a lingular nodule also demonstrated a triphasic Wilms tumor with areas resembling immature tubules and glomeruloid bodies, but lacked areas of anaplasia (see Figure 2).

Immunohistochemistry revealed the epithelial and blastemal components to be positive for pancytokeratin, CK 8/18, NSE, CD56, focally positive for EMA and CD99, but negative for WT1, desmin, smooth muscle actin and synaptophysin. The blastemal component was also focally positive for vimentin, while the stromal component expressed both vimentin and actin (focally). The pulmonary nodules were thought to be metastases from the right renal mass, establishing a diagnosis of stage IV Wilms tumor of favorable histology.

![Figure 1. Chest X-ray at presentation revealing multiple bilateral pulmonary nodules.](image-url)
A right radical nephrectomy with en bloc excision of the right adrenal gland, portion of the liver and portion of the right hemidiaphragm, with repair, was performed. Intraoperatively, it was difficult to determine if there was tumor infiltration of either the liver or diaphragm. There was significant reaction and obliteration of surgical resection planes around the upper extent of the tumor, more so than what would normally be encountered with other primary renal tumors. Final pathology revealed an 11 cm Wilms tumor composed of epithelial, blastemal and stromal components, without evidence of anaplasia. Six mitoses per high-powered field and 10% necrosis were noted along with vascular and perineural invasion. All surgical margins were free of tumor except for a minute focus (< 0.1 mm) invading through the anterior aspect of Gerota’s fascia. The regional lymph nodes, ureter, liver and diaphragm showed no evidence of invasion and the adrenal parenchyma was also spared, however tumor was present in surrounding adrenal vessels and soft tissue. Immunohistochemistry for WT1 showed a predominantly cytoplasmic staining pattern (Figure 3). Polymerase chain reaction (PCR) analysis performed to evaluate for loss of heterozygosity (LOH) on chromosome bands 1p36 and 16q22 was negative, which is associated with good prognosis [2].

The patient was not eligible for the current Children’s Oncology Group therapeutic study of Wilms tumor. Therefore, adjuvant chemotherapy and whole lung irradiation was given as per standard of care. This involved 12 Gy of radiation therapy in 8 daily fractions and National Wilms Tumor Study Group (NWTSG) regimen DD-4A chemotherapy, including dactinomycin, vincristine, and doxorubicin. The total duration of therapy was 28 weeks.

Seven months later, surveillance CT scan performed at the conclusion of chemotherapy noted remarkable improvement in the patient’s lung metastases. All but two nodules in the right lower lobe and a small hazy density in the
right upper lobe had resolved. A flexible bronchoscopy and right thoracoscopy with wedge excision of the 2 right lower lobe lesions was performed. One nodule measured 1.7 cm and the other 0.5 cm. Frozen sections of the lung nodules revealed changes consistent with “rhabdomyoma” with no overt evidence of residual triphasic Wilms tumor (Figure 4). This was an unexpected histologic finding during intraoperative pathologic consultation. In order to accurately diagnose the newly resected lung nodules, the histology of both the original nephrectomy and pre-treatment lung specimens were reviewed and compared. In addition, a literature review was conducted. Immunohistochemical staining with Ki-67 was performed on both the renal primary and the post-treatment lung biopsy. Nuclear Ki-67 staining in the Wilms primary was seen in 90% of blastemal cells and 15-20% of stromal cells. There was less than 1% nuclear Ki-67 staining observed in the stromal and tubule cells of the post-treatment lung biopsy and the skeletal muscle component was completely negative. These efforts resulted in a final diagnosis of “metastatic Wilms tumor with extensive therapy changes and rhabdomyomatous differentiation and maturatio.” It was noted that nephrectomy and pre-treatment lung metastases exhibited some rhabdomyomatous differentiation in addition to abundant viable triphasic tumor cells.

Because of the benign and mature nature of the post-treatment lung nodules, the patient was observed with surveillance imaging every three months. Unfortunately, the CT scan done six months after the completion of therapy revealed an increase in size and number of pulmonary nodules and NWTSG V relapsed protocol therapy was re-instituted using carboplatin, cyclophosphamide, and etoposide.

**Discussion**

Morphologically, classical Wilms tumor may be divided into 2 subtypes: one is associated with intralobular nephrogenic rests (ILNRs), early onset, a predominant stromal component, and syndromes such as WAGR and Denys-Drash [3]. ILNRs’ stromal element often includes rhabdomyomatous features [4]. Another subtype is associated with perilobular nephrogenic rests (PLNRs), late onset and overgrowth syndromes such as Beckwith-Wiedemann syndrome [4]. PLNRs tend to have more predominant epithelial or blastemal components [4]. Fetal rhabdomyomatous nephroblastoma (FRN) is a variant of Wilms tumor. It is monophasic and composed almost entirely of skeletal muscle, although it has been reported to contain nodular blastemal elements, which may represent spontaneous differentiation of an earlier, more
malignant lesion [5,6]. While FRN is usually larger than classical Wilms tumors, it is typically less aggressive but is consequently more resistant to chemotherapy [6,7]. Thirty percent of FRNs are reported to occur bilaterally and FRNs have not been reported in patients over four years of age [5,6].

Immunohistochemistry for WT1 is frequently performed on Wilms tumors, yet only approximately 15-20% of Wilms tumors actually contain a WT1 mutation [3]. The wild type WT1 gene product appears to function in the epithelialization of mesenchymal lineage cells (See Figure 5A) [1]. Using an antibody to the N-terminus of WT1, it has been shown that, in stromal predominant Wilms tumors with mutations in WT1, the WT1 gene product was aberrantly localized to the cytoplasm, rather than the nucleus, indicating that the mutant protein may not be able to promote mesenchymal to epithelial transformation (Figure 5B) [8]. The mutant WT1 product may lack an intact zinc finger preventing its activity as a transcription factor [1]. This alteration may explain the lack of epithelial differentiation. In the current case, the studies for WT1 mutation illustrated predominantly cytoplasmic immunoreactivity consistent with the aberrant localization of WT1 to the cytoplasm as described by Schumacher, et al.[8].

It has been noted that Wilms tumors with loss of imprinting (LOI) at the Igf2 gene on 11p15 have normal to increased nuclear expression of wild type WT1 and CTNNB1 and have epithelial predominant rather than stromal predominant histology (Figure 5C) [1]. This paternal uniparental disomy is most frequently seen in PLNR associated classic Wilms tumors [1,3,4]. However, in some cases of LOI CTNNB1 mutation may still occur, leading to stromal predominant histology instead. This further supports the relationship of WT1 and CTNNB1 in epithelial maturation. It has also been suggested that most WT1 mutations may induce a “gain-of-function” and the observation of mesenchymal lineage cell apoptosis seen in WT1 knock-out mice, supports this view. In fact, apoptosis is not typically seen in Wilms tumor except focally in the blastemal component [4].

Classic Wilms tumors with ILNRs have been associated with WT1 mutations [1,3,4], unfortunately the prevalence of specific WT1 aberrations within this subtype has not been defined because tumor WT1 analysis is not a common component of larger studies [3]. WT1 mutations may be spontaneous or inherited, and Knudson's two-hit hypothesis has been illustrated in Wilms tumor [1,8]. However, germ-line WT1 mutations have not been associated with increased risk for bilateral disease in the absence of WAGR and Denys-Drash syndrome [3]. Despite the observation that WT1 and CTNNB1 mutations are associated with stromal histology and ILNRs, it has been reported that some Wilms cases with ILNRs can have the genetic profile of PLNR associated Wilms cases: LOI of 11p15 and wild type WT1. These cases had mutations in CTNNB1 and had a predominantly rhabdomyomatous stromal component much like “typical” ILNR associated Wilms tumor with WT1 mutations [4]. This observation suggests that mutant CTNNB1 may be involved in the formation of ILNRs with rhabdomyomatous features independently of WT1 mutations [4]. It was reported that approximately 50-75% of ILNRs containing WT1 mutations have CTNNB1 mutations in their associated tumors [4], and this may even be an underestimation [1]. CTNNB1 of chromosome 3p21 encodes beta-catenin. These CTNNB1 mutations prevent beta-catenin from being phosphorylated, as normal, which results in its stabilization and migration to the nucleus, where it activates the Wnt signal pathway [1]. Wnt pathway inactivation is required for normal nephrogenesis.

Interestingly, the ILNR cells with mutant WT1 and wild type CTNNB1 had associated Wilms tumor cells with mutant CTNNB1, indicating that CTNNB1 mutation may be acquired in the transition from nephrogenic rest to tumor [4]. It appears that Wilms tumorigenesis can have a variety of genetic origins, yet CTNNB1 mutation is frequently reported in cases of stromal predominant Wilms tumor. The use of CTNNB1 as a marker for this type of Wilms tumor should be explored further.
### Table 1. Case reports of Wilms tumor with mesenchymal differentiation after therapy.

<table>
<thead>
<tr>
<th>Author</th>
<th>Tumor</th>
<th>Therapies</th>
<th>Description of post-therapy tumor and patient’s course</th>
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<tr>
<td>Shimmoto, K.,</td>
<td>Stage IV Wilms tumor with primarily rhabdomyoblastic histology</td>
<td>•Actinomycin-D •Vincristine •Radiotherapy</td>
<td>Multiple pulmonary nodules were discovered 9 years after treatment. Biopsy revealed dense collagen and mature skeletal muscle.</td>
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<td>1991 [9]</td>
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<tr>
<td>Rao, S.P., 1993</td>
<td>Stage III Wilms tumor without anaplasia.</td>
<td>•Actinomycin-D •Vincristine •Radiotherapy •Adriamycin •Cyclophosphamide</td>
<td>Multiple recurrences over the course of 13 years following initial treatment. Recurred in the gastrocnemius muscle, which is an unusual site, in addition to liver and lungs. Each recurrence was sephosphamide rated by at least 3 years. All recurrent tumors displayed well differentiated mesenchymal histology with rare blastemal elements.</td>
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<td>[10]</td>
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<tr>
<td>Seemayer, T.A.,</td>
<td>Stage IV Wilms tumor.</td>
<td>•Vincristine •Actinomycin-D •Doxorubicin •Dacarbazine •Radiotherapy</td>
<td>Lung nodules remained radiographically stable for 13 years. Subsequent biopsy revealed bland, non-proliferative, neoplasm of epithelium, tubules and smooth muscle. Lung nodules were highly resistant to chemotherapy.</td>
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<tr>
<td>Pinarli, F. G.,</td>
<td>Stage III, favorable histology, Wilms tumor.</td>
<td>•Vincristine •Actinomycin-D •Radiotherapy •Carboplatin •Cyclophosphamide •Etoposide</td>
<td>Unresectable abdominal recurrence 9 years after therapy with primarily rhabdomyomatous histology. The mass effect of the tumor ultimately caused renal failure and death despite aggressive chemotherapy.</td>
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<td>2009 [12]</td>
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<td>Chao, Y. H.,</td>
<td>Stage IV, favorable histology, Wilms tumor.</td>
<td>•INF-a •All-trans-retinoic acid •Vincristine •Actinomycin-D •Epirubicin •Cyclophosphamide •Etoposide •Carboplatin •Radiotherapy</td>
<td>Radiologically stable pulmonary and hepatic metastases with rhabdomyomatous differentiation that persisted for 9 years after cessation of therapy</td>
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<td>2011 [13]</td>
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<td>Ishikawa, K.,</td>
<td>Bilateral FRN with rhabdomyoblastic features.</td>
<td>•Vincristine •Dactinomycin •Etoposide •Carboplatin</td>
<td>Histology of resection included mesenchymal components such as skeletal muscle, osteoid and ganglion cells. Highly resistant to chemotherapy.</td>
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<td>2011 [5]</td>
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<td>Current Case</td>
<td>Stage IV Wilms tumor without anaplasia, 21 year old male</td>
<td>•Dactinomycin •Vincristine •Doxorubicin •Radiotherapy •Carboplatin •Cyclophosphamide •Etoposide</td>
<td>Two pulmonary nodules remained after remarkable improvement following chemotherapy and radiation. The remaining nodules contained mostly mature skeletal muscle. Unfortunately, six months after the biopsy of the rhabdomyomatous nodules the size and number of pulmonary nodules increased and chemotherapy has been reinstated.</td>
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Chao, Y. H., 2011 [13] Stage IV, favorable histology, Wilms tumor. •INF-a •All-trans-retinoic acid •Vincristine •Actinomycin-D •Epirubicin •Cyclophosphamide •Etoposide •Carboplatin •Radiotherapy Radiologically stable pulmonary and hepatic metastases with rhabdomyomatous differentiation that persisted for 9 years after cessation of therapy

Ishikawa, K., 2011 [5] Bilateral FRN with rhabdomyoblastic features. •Vincristine •Dactinomycin •Etoposide •Carboplatin Histology of resection included mesenchymal components such as skeletal muscle, osteoid and ganglion cells. Highly resistant to chemotherapy.

Current Case Stage IV Wilms tumor without anaplasia, 21 year old male •Dactinomycin •Vincristine •Doxorubicin •Radiotherapy •Carboplatin •Cyclophosphamide •Etoposide Two pulmonary nodules remained after remarkable improvement following chemotherapy and radiation. The remaining nodules contained mostly mature skeletal muscle. Unfortunately, six months after the biopsy of the rhabdomyomatous nodules the size and number of pulmonary nodules increased and chemotherapy has been reinstated.
While it is not commonly known, there are cases of Wilms tumor that show differentiation after chemotherapy and/or radiation (see Table 1). It is well known that neuroblastomas may spontaneously (without chemotherapy) mature into benign ganglioneuroma, but this has not been reported in Wilms tumors [7,11]. In Wilms tumors, this differentiation is often rhabdomyomatous, although many other mesenchymal cell types have been described in post-chemotherapy biopsies, further illustrating the multi-potent nature of Wilms tumor [3, 5-8, 11-13]. These differentiated tumors tend to have more benign histology, with very low proliferative indices on immunohistochemistry [5,6]. While the induction of differentiation of Wilms tumor cells in culture has been attempted, it has not been attempted using typical chemotherapeutic agents or their analogs [1].

The chemotherapy agents reported to induce cell maturation in Wilms include vincristine, doxorubicin, dactinomycin, and dacarbazine, however the association between agent and rate of rhabdomyomatous/mesenchymal differentiation remains unknown [3,5,6,11,12]. Chemotherapy and/or radiation therapy may induce Wilms tumor cells to undergo a programmed maturation response, or it may facilitate survival of well differentiated cells over immature cells in the original tumor population [11]. More genetic analysis is required, particularly of lesions biopsied prior to the initiation of radiation and/or chemotherapy. Chemotherapy may also permit differentiation of Wilms tumor metastases, however this is difficult to ascertain because these are not often subject to biopsy [3,11].

Implications for Clinical Practice

Overall, treatment of Wilms tumor, even relapsed Wilms tumor, has improved much over the past 30 years. The estimated 4-year overall survival in patients with relapsed Wilms tumor is 81.8% [14]. However, both primary Wilms tumors that possess a large skeletal muscle component and FRN’s appear to show poor volumetric response to chemotherapy [3,7]. Brisigotti, et al, reported a series of 61 Wilms tumors in which rhabdomyoblastic stromal components appeared to be more resistant to chemotherapy than other components [15]. It was also noted that only 6 of the 27 untreated Wilms tumors in this study showed mature skeletal muscle differentiation, further implicating chemotherapy as an facilitator of cell differentiation [15]. In a study of 26 Wilms tumor patients who underwent pre-surgical and post-surgical chemotherapy it was shown that tumors with rhabdomyomatous differentiation after pre-surgical chemotherapy, had poor volumetric response to post-surgical chemotherapy [6]. However, lack of volumetric response to chemotherapy does not necessarily represent treatment failure or tumor aggression [3,6,7,13].

In conclusion, we report rhabdomyomatous differentiation in a case of Wilms tumor metastatic to the lung. One must consider the possibility of rhabdomyomatous differentiation in a Wilms tumor following neo-adjuvant chemotherapy. This is important both diagnostically and clinically, as it may spare the patient further cycles of treatment and allow for close observation or surgical management to be employed instead.

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References


