

**Slide Seminar
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Case 1: Three year old male with a 3cm right testicular mass.

Diagnosis: Spindle Cell Rhabdomyosarcoma

Rhabdomyosarcoma is the most common soft tissue sarcoma of childhood. There are two major histologic subtypes of rhabdomyosarcoma, embryonal and alveolar, and each has its own variants. The key differences in these two subtypes are summarized below:

	Subtype		Genetic Changes	Survival	Location	Patient Age
Alveolar RMS	<i>PAX3;FKHR</i>	16%	t(2;13)	Poor	Extremities and trunk	Adolescents and young adults
	<i>PAX7;FKHR</i>	6%	t(1;13)	Intermediate		Younger patients, lower stage
	Fusion negative	6%	a. Variants b. Low-express c. None	Variable		
Embryonal RMS	classic	55%	11p15 LOH	Intermediate	Head & neck, retroperitoneum	Young children
	botryoid	6%	11p15 LOH	Excellent	GU tract	Young children
	spindled	5%	?	Excellent	paratesticular	Young children

Alveolar RMS is composed of nests of primitive round blue cells separated by fibrous septae. The tumor cells are discohesive within the nests, while the cells at the periphery adhere to the fibrous septae, resulting in a cystic, alveolar appearance. Minimal evidence of myogenous differentiation is often present by H&E. Some tumors lack the fibrous septae and remain cohesive, forming a solid pattern of ARMS. Other ARMS, even those that contain fusion transcripts, may demonstrate an embryonal pattern mimicking the histologic appearance of ERMS. A defining genetic feature of most ARMS is the presence of cytogenetic translocations. *PAX3* and *PAX7* are transcription factors involved with early skeletal muscle development. *FKHR* (*FOXO1A*) encodes a transcription factor that regulates metabolic, cell proliferation, and apoptosis pathways. The fusion product functions as a potent transcription factor that transactivates a number of critical downstream targets. It should be noted that several other *FKHR* members (*FOXO4* and *FOXO3A*) are fused to the *MLL* gene in small subsets of acute leukemia. Despite the substantial increase in knowledge regarding their underlying pathogenesis, the clinical outcome of ARMs has remained poor throughout the last two decades. However, tumors with *PAX7* fusion transcripts appear to present at a younger age, at lower stages, and to have a somewhat better prognosis. There are no reliable histologic features that distinguish the three molecular subtypes of ARMS.

Embryonal RMS is composed of alternating loose and compact aggregates of primitive spindle cells embedded in a myxoid stroma. Varying cellularity and degree of myogenesis may be seen. No clinically useful genetic features diagnostic of ERMS have been described, although they show loss of heterozygosity at 11p15, an imprinted locus. Occasional ERMS are quite cellular, lack the myxoid stroma, and closely resemble solid variants of ARMS. The clinical outcome for embryonal rhabdomyosarcoma has improved significantly over the last decade,

particularly those of the botryoid subtype. Botryoid rhabdomyosarcomas (defined by the presence of a cambium-like layer) are now treated differently from other ERMS.

Spindle Cell Rhabdomyosarcoma: Many have observed that embryonal rhabdomyosarcomas in the paratesticular regions in young children have a particularly favorable prognosis. Leuschner et al reviewed 173 cases of paratesticular rhabdomyosarcoma to define histological features that might explain this favorable outcome. Almost all cases were of embryonal histology, and a large number of these demonstrated a spindle-cell appearance with a storiform growth pattern and abundant collagen between the tumor cells, often resembling leiomyosarcoma. Overt skeletal muscle differentiation was identified by histology, immunohistochemistry and electron microscopy. At this location, the outcome for patients with spindle cell histology was distinctly better than those with the classic embryonal histology, with decreased lymph node metastasis and a 95% 5-year survival compared with 80% survival in patients with the classic ERMS. The spindle cell subtype may also be found in the head and neck, extremities, and orbit, but 31% are located in the paratesticular area. Those tumors in non-paratesticular sites do not show the same favorable outcome. For these reasons, in the current protocols, paratesticular RMS (a category based on site and not histology) receive less aggressive therapy. It should be noted that no biologic studies have been performed of this spindle cell variant to demonstrate an association with (or difference from) classic ERMS.

Diagnostic Challenges of RMS: ARMS and ERMS have different prognoses and are treated differently, therefore their histologic distinction is critical. However, tumors continue to exist that show features of both ARMS and ERMS. This results in diagnostic discrepancies within RMS of up to 40%. In addition, the criteria for diagnosis of ARMS have changed with time. Currently, a minimum of 50% alveolar histology must be present to qualify for classification as ARMS. These criteria are likely to change with the next protocol. Immunohistochemical markers available for the diagnosis of RMS have evolved. While actin, myosin, myoglobin, and desmin have been useful in the diagnosis of RMS, they lack in sensitivity or/or specificity. More recently, two diagnostically useful nuclear transcription factors that function in the regulation of myogenesis have been shown to be quite useful: MyoD1 (sensitivity 97%, specificity 91%) and myogenin (sensitivity 97%, specificity 90%). Both may be focally positive in pleuropulmonary blastomas, Wilms tumors, and germ cell tumors showing muscle differentiation. They may also be positive in reactive or regenerative myocytes, including those non-neoplastic myocytes within a surgical field following rhabdomyosarcoma surgery. The quality of staining of myogenin is often superior to that of MyoD1. In particular, unstained slides kept more than a few days at room temperature prior to staining with MyoD1 showed marked reduction in staining. ARMS tend to show greater and more diffuse staining for both markers when compared with ERMS, whose staining is often patchy. However, this is not diagnostically reliable. It should be noted that several non-muscle markers may be positive in RMS, including WT1, NSE, ALK, PLAP.

Selected References

1. Parham DM, Qualman SJ, Teot L, et al. Correlation between histology and PAX/FKHR fusion status in alveolar rhabdomyosarcoma. *Am J Surg Pathol* 31:895, 2007.
2. Morotti RA Nicol KK, Parham DM et al. An immunohistochemical algorithm to facilitate diagnosis and subtyping of rhabdomyosarcoma. *AJSP* 30:8:962, 2006.
3. Mercado GE, Barr FG. Fusions involving PAX and FOX genes in the molecular pathogenesis of alveolar rhabdomyosarcoma: Recent advances. *Current Molecular Medicine* 7:47-61, 2007.
4. Leuschner I, et al. Spindle cell variants of embryonal rhabdomyosarcoma in the paratesticular region. A report of the Intergroup Rhabdomyosarcoma Study. *Am J Surg Pathol* 1993, 17:858.
5. Parham DM. Pathologic Classification of rhabdomyosarcomas and correlations with molecular studies. *Modern Pathol* 2001 14:506-514.

CASE 2: 5 month old underwent biopsy of a liver mass. He then received chemotherapy with little response, and a right lobectomy was performed for a 13 cm mass.

Diagnosis: Malignant Rhabdoid Tumor of Liver

Malignant rhabdoid tumor (MRT) was initially described in 1978 as a variant of WT. Since this original description, MRTs have been reported throughout the body, including the brain, liver, soft tissues, lung, skin, and heart. The median age at diagnosis is 11 months; >95% of patients are under 3 years of age. This diagnosis should be considered carefully over the age of 5 years. The recognition that MRTs of all sites contain deletions and mutations of the *SMARCB1/INI1* gene supports the hypothesis that MRTs at different sites represent identical or very closely related entities. In particular, central nervous system tumors are observed in approximately 15 percent of patients with renal MRT. Most of these brain tumors morphologically resemble primitive medulloblastoma or primitive neuroectodermal tumor yet contain the same genetic changes seen in MRT. The current information suggests that most patients with both renal and central nervous system MRTs have constitutional abnormalities in their INI-1 gene.

Infants with embryonal tumors of the liver are most commonly diagnosed as hepatoblastoma. Hepatoblastomas vary from well-differentiated hepatocytes resembling normal fetal liver to small cell undifferentiated types (formerly referred to as anaplastic). While the latter is usually seen as a small population within an otherwise typical hepatoblastoma, it may comprise the entire tumor. In recent years, the outcome for low stage, completely excised hepatoblastoma has been demonstrated to be quite good, and to not require chemotherapy. However, those low-stage tumors classified as small cell undifferentiated type have not shown this good prognosis. This led to an in-depth histologic evaluation of lesions classified as small cell undifferentiated hepatoblastoma. All such patients lacked significant elevation of AFP, all showed homozygous deletion of INI-1, and none survived. Therefore, many undifferentiated tumors in the infantile liver represent MRT, and protocols are being changed to reflect this. A handful of liver tumors appear to represent composite hepatoblastoma/rhabdoid tumors; their pathogenesis has not been elucidated.

Histology: MRTs are composed of loosely cohesive, jumbled cells with distinct cell borders *sometimes* containing large whorls of intermediate filaments resulting in acidophilic cytoplasmic inclusions. Cells *sometimes* have large nuclei with prominent, centrally placed nucleoli, although the size of the nuclei and the nucleoli vary. The cells with characteristic cytoplasmic inclusions tend to be clustered rather than uniformly distributed and not all cells contain these inclusions. Many MRTs are simply small blue cell tumors. The growth pattern is typically quite infiltrative with frequent invasion of local blood vessels. A number of pattern variations may be seen, including sclerosing, epithelioid, spindled, vascular and lymphomatoid, and these can simulate other neoplasms. Cutaneous MRTs in particular often lack the histologic hallmarks of rhabdoid tumors and may appear bland.

Genetic Changes: MRTs characteristically demonstrate loss or mutation of the *SMARCB1/INI1* gene located at chromosome 22q11.2. The INI-1 protein functions by altering the conformation of the DNA-histone complex so that transcription factors have access to target genes. Of tumors with documented *SMARCB1/INI1* abnormalities, approximately half have shown homozygous deletions and half have contained mutations. In addition, germ line mutations have been demonstrated. Currently available data suggests that patients with both CNS and renal rhabdoid tumors have germ line mutations involving one copy of the *SMARCB1/INI1* gene, with separate alterations involving the second copy confined to the separate tumors. Recent information demonstrates a much higher prevalence of germline *INI-1* abnormalities in patients having a single MRT than was previously expected.

While MRT predisposition results from germline mutations of *SMARCB1/INI-1*, a subset of patients with familial schwannomatosis likewise have germline mutations of *SMARCB1*. Indeed, families have now been described in which both MRTs and schwannomatosis are present. These schwannomas are mostly cutaneous without visceral involvement or neural deficits. It has been proposed that an early developmental window exists during which an *SMARCB1* mutation carrier is predisposed to MRT: survivors may subsequently develop schwannomatosis.

Immunohistochemistry. MRTs are characterized by a polyphenotypic immunoreactive pattern. Vimentin immunoreactivity is uniform and intense in all tumors. Tumor cells may react simultaneously with a variety of markers including (but not limited to) cytokeratin, EMA, desmin, and neurofilament. The reaction pattern is characteristically patchy and strong, with small clusters of cells having intense positivity. One particularly helpful pattern of reaction is the presence of scattered clusters of intensely EMA or cytokeratin positive cells in a background of non-reactive tumor cells. This pattern is seen in >90% of MRTs while few other pediatric tumors react in this distinct fashion. Rare MRTs with the characteristic *INI-1* mutation may have either no or diffuse epithelial immunoreactivity. The BAF47 antibody to the INI-1 protein has been demonstrated to be quite useful in the diagnosis of MRT. This demonstrates strong nuclear positivity in other pediatric tumors and normal tissues, but MRT show no nuclear staining.

Diagnostic Criteria: Because of the variability in the histologic appearance and immunoprofile within MRTs, diagnostic criteria are utilized by the Children's Oncology Group. In order to be treated on CNS or non-CNS MRT protocols, at least 2 of the 3 below features must be present: (1) tumor histology consistent with rhabdoid tumor, (2) polyphenotypic immunohistochemical staining profile consistent with RT, (3) INI1 loss (loss of BAF47 expression or other evidence of INI1 deletion or mutation).

Differential Diagnosis: The diagnosis of MRT is complicated by the fact that a wide variety of tumors may have pseudo-rhabdoid features. Neuroblastoma may offer a particular challenge due to the common presence of nuclei with large nucleoli and cells with abundant cytoplasm with ganglionic differentiation. Renal medullary carcinoma accounts for many of the cases previously reported as MRT in patients over the age of 5 years. This highly lethal tumor is virtually restricted to patients with sickle cell hemoglobinopathy and may show loss or attenuated expression of INI-1. In some patients, the distinction between epithelioid sarcoma and MRT may be difficult. Over 90% of both proximal and distal type epithelioid sarcomas show loss of INI-1 by BAF47 staining. Of note, 50% of MPNST also showed negativity for BAF47.

Selected References

1. Biegel JA, Zhou JY, Rorke LB, Stenstrom C, et al. Germ-line and acquired mutations of INI1 in atypical teratoid and rhabdoid tumors. *Cancer Res* 1999; 59(1):74-79.
2. Burger PC, Yu IT, Tihan T, Friedman HS, et al. Atypical teratoid/rhabdoid tumor of the central nervous system: a highly malignant tumor of infancy and childhood frequently mistaken for medulloblastoma. *Am J Surg Pathol* 1998; 22(9):1083-1092.
3. Parham DM, Weeks DA, Beckwith JB. The clinicopathologic spectrum of putative extrarenal rhabdoid tumors. *Am J Surg Pathol* 1994; 18(10):1010-1029.
4. Hoot AC, et al. Immunohistochemical analysis of hSNF5/INI1 distinguishes renal and extra-renal malignant rhabdoid tumors from other pediatric soft tissue tumors. *AJSP* 28:1485-1491, 2004.
5. Hornick JL, Dal Cin P, Fletcher CD. Loss of INI-1 expression is characteristic of both conventional and proximal-type epithelioid sarcoma. *AJSP* 33:542, 2009.
6. Trobaugh-Lotrario AD, et al. Small cell undifferentiated variant of hepatoblastoma: adverse clinical and molecular features similar to rhabdoid tumors. *Pediatric Blood Cancer* 52:328-334, 2009.
7. Swensen JJ, Keyser J, Coffin CM, Biegel JA et al. Familial occurrence of schwannomas and malignant rhabdoid tumor associated with duplication in *SMARCB1*. *J Med Genet* 46:68, 2009.
8. Cheng JX, Tretiakova M, Gong C, et al. Renal medullary carcinoma: rhabdoid features and the absence of INI1 expression as markers of aggressive behaviour. *Mod Pathol* 21:647, 2008.

Case 3: A one month old male presented with a mass in the lower pole of the left kidney. The nephrectomy specimen weighed 59 grams, and a 3.0cm white mass was present.

DIAGNOSIS: Metanephric Stromal Tumor

Stromal tumors comprise approximately 15% of all primary pediatric renal neoplasms. Congenital mesoblastic nephroma (CMN), rhabdoid tumor of the kidney (RTK), and clear cell sarcoma of the kidney (CCSK) account for the majority of these tumors. In 1992, Dr. Beckwith described five cases of a biphasic neoplasm of the childhood kidney, the nephrogenic adenofibroma. This tumor, subsequently renamed metanephric adenofibroma (MAF), combines a bland embryonal epithelial component (similar to that seen in metanephric adenoma) with a bland spindle cell stroma. Rare cases were then recognized that contained only stroma, which were called Metanephric Stromal Tumor (MST). This completed the spectrum of the group of lesions that include metanephric adenoma on one end and metanephric stromal tumor on the other. Thirty one cases of MST have been identified in the files of the National Wilms Tumor Study Pathology Center. The majority of these were identified during a retrospective review of cases previously classified as CMN, 3 cases were previously classified as CCSK, and 7 cases were identified prospectively after MST was recognized as an entity.

Clinical Features: Patients range in age from newborn to adulthood, with a median age of 13 months and a mean age of 24 months. The most common presentation is that of an abdominal mass, often cystic, localized to the kidney. Hypertension is present in a minority.

Gross Pathologic Features: MST are commonly tan, lobulated, partially cystic, and fibrous masses located in the renal medulla. Reported tumors have ranged in size from 3-10cm. Rare cases show polypoid protrusions into the renal pelvis.

Microscopic Features: MSTs are unencapsulated stromal proliferations that superficially infiltrate the native renal parenchyma. The tumor is composed of spindled or stellate cells with thin, tapering nuclei and indistinct cytoplasmic extensions. The juxtaposition of hypocellular myxoid areas and more cellular stromal areas create a characteristic **nodular appearance on low power**. Occasional tumors will demonstrate areas composed of epithelioid cellular stroma that may cause diagnostic confusion. Mitotic activity may be brisk in cellular regions. The stromal component may show a number of variant architectural patterns, including a palisading, storiform, and hemangiopericytomatous patterns. Tumors may infiltrate the soft tissue of the renal sinus, occasionally entrapping nerves, however no MST has shown vascular invasion.

A characteristic feature of MST is the presence of spindled cells surrounding entrapped renal tubules or blood vessels, forming concentric “onion skin” rings or **collarettes** around these structures. Native renal tubules surrounded by the stromal proliferation may also show cystic dilation (accounting for the cystic gross appearances), embryonal hyperplasia (creating the appearance of superimposed papillary renal cell carcinoma or Wilms tumor), and an intracanalicular pattern of growth (reminiscent of mammary fibroadenomas). Another diagnostically useful feature of MST is the presence of **angiodysplasia**, characterized by epithelioid transformation of medial smooth muscle and myxoid stromal change. This may result in enlarged serpentine channels with disorganized walls. These changes are similar to those reported in association with neurofibromatosis. Three patients with MST suffered sequelae from angiodysplasia of extrarenal vessels.

In a minority of cases, **juxtaglomerular cell hyperplasia** of entrapped glomeruli is present, resulting in nodules of polygonal cells with minimal clear cytoplasm at the vascular pole. These changes have been documented in the tumors of patients who present with hypertension, at times associated with increased serum renin levels. **Overt heterologous differentiation** is not uncommon, and consists of glial, and chondroid differentiation as well as neuroblastic rosette-like structures similar to those of PNETs. Neither adipose or rhabdomyoblastic differentiation have been described in MST.

Immunohistochemical Features: Immunohistochemical stains demonstrate reactivity for WT-1, vimentin and CD34, although the latter is often variable and patchy. Tumor cells are only rarely, and then only focally, immunoreactive for muscle specific actin, smooth muscle actin, and desmin. The epithelioid cells in the dysplastic arterioles are consistently immunoreactive for actin and desmin. Stains for AE1/AE3 and EMA are negative in the spindle cells, even in the epithelioid stromal areas.

Clinical Course: The vast majority of reported cases have been treated by nephrectomy alone without adjuvant chemotherapy, as the diagnosis of CMN was rendered. No evidence of recurrence or metastasis has been documented, including a case with a positive medial margin and a case with abdominal rupture treated by nephrectomy alone. Therefore, recognition of this entity by pathologists can spare a child toxic therapy.

Selected References

1. Beckwith JB. Metanephric stromal tumor (MST): a new renal neoplasm resembling mesoblastic nephroma but related to metanephric adenofibroma. *Mod Pathol* 1998;11:1P.
2. Westenend PJ, Smedts F, de Jong MC, Lommers EJ, Assmann KJ. A 4-year-old boy with neurofibromatosis and severe renovascular hypertension due to renal arterial dysplasia. *Am J Surg Pathol* 1994;18:512-6.
3. Arroyo MR, Green DM, Breslow NE, Perlman EJ, Beckwith JBB, Argani P. The spectrum of metanephric adenofibroma and related lesions: clinicopathologic study of 25 cases from the National Wilms Tumor Study Group Pathology Center. *Am J Surg Pathol*. 25:433-44, 2001.
4. Argani,P.; Beckwith,J.B. Metanephric stromal tumor: Report of 31 cases of a distinctive pediatric renal neoplasm *Am J Surg Pathol* 24:917, 2000.

Case 4: Three month old male with a 9cm mass of the left kidney. Two months following nephrectomy pulmonary masses were identified.

Diagnosis: CELLULAR CONGENITAL MESOBLASTIC NEPHROMA

Congenital mesoblastic nephroma is virtually confined to infancy. The median age at diagnosis is 2 months and over 90% of cases have appeared within the first year of life. The diagnosis should be questioned when applied to individuals over 2 years of age. Rare cases have been reported in older children and adults, but these tumors have been subsequently reclassified, most commonly as cystic hamartoma or metanephric stromal tumor.

The molecular pathogenesis of cellular mesoblastic nephroma grows clearer with time. Initially, the most consistent genetic change in mesoblastic nephroma was trisomy for chromosome 11, which was confined to the cellular subtype, and which has also been reported in infantile fibrosarcoma. The potential linkage between infantile fibrosarcoma and cellular mesoblastic nephroma was then established when both were shown to contain the same t(12;15)(p13;q25) chromosomal translocation. The cloning of the genes involved in this translocation has allowed more reliable molecular detection assays to be applied to archival material. The *ETV6* gene on 12p13, which belongs to the ETS transcription factor family, is fused to the neurotrophin-3 receptor (*NTRK3*) gene on 15q25, a membrane-bound protein with tyrosine kinase activity. The consensus is that cellular congenital mesoblastic nephroma represents infantile fibrosarcoma arising within the kidney. In contrast, no consistent genetic abnormalities have been identified in the classic type of congenital mesoblastic nephroma. In approximately 20% of CMNs, cellular and classic patterns coexist, and such cases have been classified as *mixed mesoblastic nephroma*. The majority of mixed CMNs have multiple foci of cellular histology amidst a background of classic histology, suggesting a "field effect". In some cases, a rim of cells consistent with classic CMN may be associated with a single nodule of cellular CMN. Hypotheses to explain the occurrence of mixed CMNs are two-fold. The first is the possibility that at least some cellular mesoblastic nephromas arise within regions of classic morphology. Alternatively, the morphologic spectrum of both classic and cellular CMN may significantly overlap one another, resulting in tumors that appear to be mixed yet are biologically either classic or cellular.

Of interest, in recent years, the same t(12;15) translocation has been shown to be the initiating event in the pathogenesis of secretory breast cancer as well as a subset of acute myelogenous leukemia.

Histology: Cellular mesoblastic nephromas are composed of spindled mesenchymal cells of fibroblastic or myofibroblastic lineage. These are characterized by increased cellular density and a high proliferative rate, imparting a sarcomatous appearance to the tumor. Plump cells with large, vesicular nuclei and a moderate amount of cytoplasm are characteristic. Lesions are commonly sharply circumscribed grossly without the interdigitating margins of classic lesions. Nevertheless, a peritumoral fibrous capsule is seldom present and microscopically the tumor subtly infiltrates the adjacent normal renal parenchyma, resulting in entrapped normal renal elements. Slight to moderate nuclear pleomorphism may be present and the cells often grow in sheets of somewhat elongated cells. Rare tumors may contain cells with prominent nucleoli as well as areas of necrosis, very closely resembling the features of a rhabdoid tumor. In some cellular mesoblastic nephromas, a prominent capillary vasculature that mimics the vasculature of a clear cell sarcoma of the kidney (CCSK) may be present.

Immunohistochemistry. CMNs are consistently positive using antibodies directed toward myofibroblasts. In addition to vimentin, desmin, and actin, reactions for fibronectin have been positive. CMNs are negative for epithelial markers, laminin, and S100 protein. WT-1 positivity has been reported in CMNs, but this has not been the experience of all observers, and may reflect misdiagnosis of metanephric stromal tumors (which are WT1 positive).

Molecular pathology: RT-PCR for the t(12;15) fusion transcript is extremely valuable as a positive marker for cellular CMN, as no other sensitive and specific marker exists. However, it has been our experience that RT-PCR is rather unreliable for this entity, requiring primers recognizing ultra-short targets and requiring excessive cycle numbers. This suggests that most cellular CMNs may be low expressors of the fusion transcript. FISH provides a promising tool, particularly since the ETV6 (TEL) gene is also translocated with other partner genes resulting in leukemia, and is therefore available in cytogenetics laboratories.

Therapy and Prognosis: CMNs are treated by complete surgical excision without adjuvant chemotherapy unless gross residual tumor remains. The majority of CMNs are predominantly localized to the kidney and perinephric or hilar soft tissue at the time of detection. Recurrences and metastases have occurred in approximately 5-10 percent of cases overall and are confined to tumors containing cellular histology. Of 415 CMNs evaluated by the Pathology Center, 29 patients have had documented recurrences. Local recurrence was present in 18/29 recurrences, and was associated with positive surgical margins at the time of original resection. This underscores the need for wide surgical resection and careful evaluation of the medial margin. An additional 11/29 patients developed distant metastases, most commonly involving the lung. The most significant factors associated with local recurrence and metastases are: 1) the presence of cellular histology; 2) stage III or greater; and 3) involvement of intrarenal or sinus vessels. Virtually all recurrences of CMNs occurred during the 12 months following diagnosis, with several occurring within one month following negative ultrasound surveillance. As a result, it is recommended that patients with cellular CMN and either stage III or vascular invasion be screened via abdominal ultrasound monthly for one year. When gross residual tumor is left behind following surgery, adjuvant chemotherapy is indicated.

References

1. Argani P, Fritsch M, Kadkol SS, Schuster A, Beckwith JB, Perlman EJ. Detection of the ETV6-NTRK3 chimeric RNA of infantile fibrosarcoma/cellular congenital mesoblastic nephroma in paraffin-embedded tissue. *Mod Pathol* 2000; 13(1):29-36.
2. Beckwith JB, Weeks DA. Congenital mesoblastic nephroma. When should we worry? *Arch Pathol Lab Med* 1986; 110(2):98-99.
3. Knezevich SR, Garnett MJ, Pysher TJ, Beckwith JB, Grundy PE, Sorensen PH. ETV6-NTRK3 gene fusions and trisomy 11 establish a histogenetic link between mesoblastic nephroma and congenital fibrosarcoma. *Cancer Res* 1998; 58(22):5046-5048.
4. Lowery M, Issa B, Pysher T, Brothman A. Cytogenetic findings in a case of congenital mesoblastic nephroma. *Cancer Genet Cytogenet* 1995; 84(2):113-115.
5. Rubin BP, Chen CJ, Morgan TW, Xiao S, Grier HE, Kozakewich HP et al. Congenital mesoblastic nephroma t(12;15) is associated with ETV6-NTRK3 gene fusion: cytogenetic and molecular relationship to congenital (infantile) fibrosarcoma. *Am J Pathol* 1998; 153(5):1451-1458.
6. Li Z, Tognon CE, Goninho FJ et al. ETV6-NTRK3 fusion oncogene initiates breast cancer from committed mammary progenitors via activation of AP1 complex. *Cancer Cell* 12:542, 2007
7. Eguci M et al. Fusion of ETV6 to Neurotrophin-3 receptor TRKC in acute myeloid leukemia with t(12;15). *Blood* 93:1355, 1999.

Case 5: Sixteen month old female with thoracic and abdominal aortic aneurisms, abnormal renal findings, and suspicious findings on imaging studies of the brain. A nephrectomy was performed.

Diagnosis: TSC2 tuberous sclerosis complex with angiomyolipomas and angiodyplasia.

Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disorder with incomplete penetrance. It is associated with hamartomatous tumors in many organs, particularly the kidney. TSC is caused by abnormalities in one of two genes. TSC1 (hamartin, chromosome 9q34) and TSC2 (tuberin, chromosome 16p13) are each responsible for approximately 50% of familial cases. TSC2 is responsible for the majority of sporadic cases, which comprise the majority of all TSC cases. These two proteins form a complex that regulates cellular proliferation, cell adhesion, migration, and protein trafficking. There are phenotypic differences associated with the two genes. Renal cysts occur exclusively in TSC2 patients, which is in some cases due to loss of the contiguous PKD1 gene. TSC2 mutations lead to more severe clinical manifestations, the most common being renal angiomyolipomas (AML), which tend to be larger, bilateral, multifocal, and present at a younger age in patients with TSC compared with sporadic AMLs.

Renal manifestations of TSC are seen in 60-75% of patients with TSC, and are diverse, including cysts, AML, polycystic kidney disease, oncocytomas, and renal cell carcinomas. AMLs are by far the most prevalent, and are seen in 34-80% of patients with TSC. They are also the greatest source of morbidity due to spontaneous hemorrhages. AMLs are benign mesenchymal tumors composed of smooth muscle, fat, and dysmorphic blood vessels. There is a growing body of literature indicating that AMLs show perivascular epithelioid cell (PEC) differentiation, and therefore belong to the PEC-oma tumor family. Variants of AML include those with oncocytic, cystic, and epithelioid predominance. Epithelioid AMLs are characterized by polygonal cells with clear to eosinophilic cytoplasm and round to oval nuclei that may show varying degrees of nuclear atypia. EAML are positive for Melan A and/or HMB45, and SMA is positive in 89%. Classification difficulties arise when a lesion otherwise quite typical of EAML is positive for epithelial markers. Malignant behavior, including local recurrence and distant metastasis has been reported in EAML.

Renal AML in TSC is associated with the development of **pulmonary lymphangiomyomatosis (LAM)**. LAM is characterized by the proliferation of abnormal smooth muscle cells in the interstitial spaces of the lung and affects female subjects almost exclusively. AML is observed in ~88% of patients with TSC and LAM, and usually predates the onset of pulmonary disease. One hypothesis suggests that LAM arises through the non-malignant metastasis of AML cells from the kidney. This is supported by evidence that LAM can recur following lung transplant. Furthermore, identical LOH loci have been observed in AML and LAM cells in patients with sporadic disease, suggesting a common cell of origin.

Lesions classified as renal cell carcinoma occur in only 2-3% of TSC patients; such lesions have been observed in the TSC population as young as 2 years of age. Such tumors are usually heterogeneous, and are frequently multifocal. But debate continues over whether TSC mutations increase susceptibility to RCC, and whether TSC-related AML can progress to carcinoma, or whether some cases of EAML are misclassified as RCC. Some authors have suggested that certain variants of EAML are capable of malignant behavior. However, this issue remains controversial. Lastly, within children with TSC, multifocal oncocytic lesions are seen that lack the typical nested appearance, the uniform round nuclei, and the positivity for EMA that is characteristic of oncocytoma. However, these show focal positivity for a number

of epithelial markers and often negativity of melanocytic markers. Such lesions may be quite large, multifocal, and bilateral, and are quite difficult to classify and to treat.

Therapy: While no therapy has been available historically to address the lesions that develop in patients with TSC, recent advances may change this. The TSC1/TSC2 protein complex has recently been shown to inhibit the mammalian target of rapamycin pathway, mTOR. The discovery of upregulation of the mTOR pathway in TSC-associated tumors presents new possibilities for treatment strategies. Interferon gamma and interferon alpha interact with mTOR. In cells that lack either TSC1 or TSC2, Sirolimus normalizes the dysregulated mTOR pathway. Several studies suggest that sirolimus or its analogues might be effective in the treatment of various manifestations of TSC. It diminishes the volume of AMLs. However, these lesions increase in volume upon discontinuation of therapy. At this point, therefore, the serious adverse side effects of sirolimus precludes its application for most AMLs.

Selected References

1. Rakowski SK, Winterkorn EB, Paul E et al Renal manifestations of tuberous sclerosis complex: Incidence, prognosis, and predictive factors. *Kidney Internat.* 70:1777 2006.
2. Armah HB, Parwani AV. Perivascular Epithelioid Cell Tumor. *Arch Pathol Lab Med* 133:648, 2009.
3. Au K, Williams AT, Gambello MJ, Northrup H. Molecular Genetic Basis of Tuberous Sclerosis Complex: From Bench to Bedside. *J. Child Neurology* 19:699, 2009.
4. Aydin H, Magi-Galluzzi C, Lane BR et al. Renal Angiomyolipoma: Clinicopathologic Study of 194 cases with emphasis on the epithelioid histology and tuberous sclerosis association. *AJSP* 33:289, 2009.
5. Henske EP. Metastasis of benign tumor cells in tuberous sclerosis complex. *Genes Chromosomes Cancer* 38:376, 2003.
6. Wienecke R Fackler I, et al. Antitumoral activity of rapamycin in renal angiomyolipoma associated with tuberous sclerosis complex. *A J Kidney Disease* 48:e27, 2006.

Case 6: Three year old male with a 2.5cm renal mass

Diagnosis: TFE3 translocation-associated Renal Cell Carcinoma

Malignant renal epithelial tumors of children account for more than 5% of new pediatric renal tumors, and are therefore more common than either CCSK or rhabdoid tumor. Pediatric renal cell carcinomas differ in their histologic appearance from those of adulthood and comprise a heterogeneous group of malignancies. Approximately 25% of pediatric renal cell carcinomas are unable to be classified due to atypical histologic features. However, the majority can be conceptually divided into two subgroups based on histology, as recently reviewed (Bruder et al)

Clear Cell Lesions: The first subgroup contains lesions that have a clear cell appearance. This is a genetically heterogeneous subgroup that includes 1) extremely rare tumors that are true conventional adult-type clear cell renal cell carcinoma, complete with 3p25 (*VHL* locus) abnormalities, and 2) exceedingly rare tumors of patients with tuberous sclerosis.

By far the most common tumor with clear cell features to arise in children are the **translocation-associated RCC**. These tumors have histologic appearances that may closely resemble conventional RCC, and they often have a papillary architecture. These features often result in alternative classifications, including clear cell RCC and papillary RCC. While many translocation-associated RCC have clear cell features, others contain a variable population of cells with eosinophilic cytoplasm. Translocation-associated RCC preferentially affect children and young adults, and some arise as second malignancies following chemotherapy.

The most common translocations involve the *TFE3* gene at Xp11 and the *PRCC* gene of chromosome 1. There are many other variant translocations that also involve *TFE3*, all of which result in over-expression of TFE3 at the protein level. *TFE3* encodes a transcription factor, and the genes that partner with *TFE3* in the many variant translocations are involved with RNA splicing. Of note, the balanced translocation involving *TFE3* and the *ASPS* gene at 17q25 is the same translocation that is detected in alveolar soft part sarcoma, although in the latter it is unbalanced. Alveolar soft part sarcoma is itself a close mimic of conventional renal cell carcinoma. The histologic features of these translocation-associated RCCs do not appear to greatly differ depending on the translocation variant. Grossly, the tumors are unencapsulated and often infiltrate the surrounding kidney. Microscopically they demonstrate nests or papillary fronds of large, clear cells with voluminous cytoplasm and distinct cell borders separated by thin fibrovascular septa. Small calcifications are often present. An infiltrative border is present in most cases, with entrapment of native renal tubules and vascular invasion. Lymph node metastasis is common, although this finding does not appear to have the same prognostic impact as in adult RCCs. The full histologic spectrum of this category of tumors is not known. In addition to TFE3- translocation-associated RCC, there are other translocation-associated RCCs that involve different genes in the same transcription factor family. In particular, rare tumors show t(6;11) translocations that involve the *TFEB* gene on chromosome 6 and the *Alpha* gene on chromosome 11 (an intronless gene of unknown function). These tumors have a similar histologic appearance to the TFE3-translocation tumors, however also show rare clusters of smaller cells clustered around hyaline nodules that on EM are composed of basement membrane material.

TFE3 and TFEB are members of the microphthalmia transcription factor family that also includes MiTF itself (which is upregulated in melanomas and PEC-omas) and TFEC, which has not yet been shown to be involved in cancer.

Translocation-associated RCCs have a similar immunohistochemical profile that differs from that of adult-type RCCs. They are negative or only focally positive for epithelial membrane antigen, cytokeratin Cam5.2, and cytokeratin 7, cytokeratin AE1/AE3, and vimentin. Tumors may be focally positive for CD10. TFE3 cases tend to be positive for RCC antigen, whereas TFEB cases are negative, however the number of tumors that has been examined is small in each case. Nuclear expression of either TFE3 or TFEB may be documented by immunohistochemistry, although these antibodies are not robust.

Papillary basophilic renal cell carcinoma: The second subgroup of pediatric RCC is classic papillary renal cell carcinoma. These show the same genetic features as those found in adults (gains of chromosomes 7 and 17) and likewise show characteristic positivity for cytokeratin 7, which is of diagnostic utility for this tumor. Papillary renal cell carcinoma may also arise in the setting of Wilms tumor, metanephric adenoma, and metanephric adenofibroma. The differential diagnosis includes differentiated epithelial nephroblastoma and metanephric adenoma. The most valuable histologic clues for distinguishing these similar lesions are: 1) the presence of a peritumoral pseudocapsule which is present in epithelial nephroblastomas and papillary renal cell carcinomas and absent in metanephric adenomas; 2) evidence of proliferation is present in most nephroblastomas whereas mitotic figures are virtually absent in metanephric adenoma, and are often quite rare in papillary renal cell carcinoma; 3) the cells of nephroblastoma are generally columnar with large, overlapping nuclei and finely dispersed chromatin whereas the cells of metanephric adenoma contain oval, quiescent nuclei that lack prominent nucleoli and papillary renal cell carcinomas commonly have prominent nucleoli. Aggregates of foamy macrophages are seen in over 80% of papillary renal cell carcinomas, but may also be seen focally in epithelial predominant Wilms tumor as well as in metanephric adenoma. Calcospherites may be a prominent feature in each of these tumors.

Treatment and outcome of pediatric renal cell carcinomas: While it is difficult to compare the outcomes of childhood RCC to those in adults, they appear to be similar stage-for-stage. An important difference is the prognostic significance of local lymph node involvement. Adults presenting with RCC with involved lymph nodes have a 5-year overall survival of approximately 20% whereas the literature would suggest that 72% of children with RCC and local lymph node involvement at diagnosis (without distant metastases) survived their disease. Little is known about the treatment of childhood RCC. Neither chemotherapy nor radiation therapy have demonstrated activity in adult or pediatric patients with metastatic RCC. The relatively good survival for children with localized RCC combined with the relative inefficacy of the known adjuvant treatments support treating children without adjuvant therapy. However, the provision of adjuvant chemotherapy is at the discretion of the local physicians.

Selected References

- 1) Bruder E, Passera O, Harms D, Leuschner I et al. Morphologic and molecular characterization of renal cell carcinoma in children and young adults. *Am J Surg Pathol* 2004; 28:1117-1132.
- 2) Argani P, Lae M, Ballard E, et al Xp11-Translocation Carcinomas of the Kidney as Chemotherapy-Induced Secondary Malignancies. *Mod Pathol* 18:303a. 2005.
- 3) Argani P, Hawkins A, Griffin CA, Goldstein JD, Perlman EJ. A distinctive pediatric renal neoplasm characterized by epithelioid morphology, basement membrane production, focal HMB45 immunoreactivity, and t(6;11)(p21.1;q12) chromosome translocation. *Am J Pathol* 2001; 158:2089.
- 4) Davis IJ, Hsi BL, et al. Cloning of an Alpha-TFEB fusion in renal tumors harboring the t(6;11)(p21;q13) chromosome translocation. *Proc Natl Acad Sci U S A* 2003; 100:6051-6056.
- 5) Renshaw AA. Basophilic Tumors of the Kidney. *Journal of Urologic Pathology* 1998; 8:85-102.
- 6) Geller JI, Dome JS. Local lymph node involvement does not predict poor outcome in pediatric renal cell carcinoma. *Cancer* 2004; 101:1575-1583.