Pediatric Spindle Cell, Fibroblastic and MyofibroblasticTumors

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Spectrum of Fibroblastic and Myofibroblastic Tumors

When one considers soft tissue tumors in pediatrics, tumors of vascular (29%), neurogenic (15%), and myogenic (striated muscle, 14%) origin occur more often than fibroblastic-myofibroblastic tumors (12%). The spectrum of fibroblastic/myofibroblastic tumors is quite divergent from both clinical and histopathologic viewpoints (Table 1). These tumors are most commonly benign, but may follow an aggressive course that mimics a malignant process. The only "true" pediatric fibroblastic malignancy is infantile fibrosarcoma that has a characteristic tumor-defining translocation in most cases. Other fibroblastic/myofibroblastic tumors may be associated with congenital syndromes (inclusion body fibromatosis and fibrous hamartoma of infancy) and a predisposition to adenomatous polyps and colon cancer (Gardner-associated fibromas and desmoids). The major problem for the surgical pathologist is the similarity between myofibromas and infantile fibrosarcomas and the vast difference in treatment of myofibromas versus infantile fibrosarcoma. This presentation will be limited to infantile fibrosarcoma, myofibroma/myofibromatosis, a newly recognized entity - pericytic tumor with t(7;12), inclusion body fibromatosis, fibrous hamartoma of infancy, and juvenile hyaline fibromatosis.

Triaging of Fibroblastic and Myofibroblastic Tumor Tissue

A general schema for triaging tissue from fibroblastic/myofibroblastic tumors is presented in Table 2. Paramount to evaluation of these tumors is providing adequate tissue for intraoperative interpretation and final diagnosis. Perhaps, it should be emphasized that glutaraldehyde-fixed tissue should be set aside for electron microscopy. Often times with soft tissue tumors due to nonspecific, aberrant and spurious immunocytochemical findings, ultrastructural examination will provide the clues necessary to provide a definitive diagnosis. Once it has been determined that adequate tissue has been obtained for diagnosis, residual tissue may be designated for cytogenetics, molecular analysis and biologic study. In general, submission of fresh tissue for cytogenetics, and retention of the frozen section tissue block at -70^{O}C for molecular, RT-PCR, biochemical and microarray gene product analyses will allow for appropriate assessment and further characterization of the tumor. The preparation of cytologic imprints from fresh tissue can be performed prior to submitting the tissue for other protocol studies. Cytologic imprints allow for fluorescent in situ hybridization (FISH) evaluation of mutated genes, tumor-defining translocations, increased oncogene copy number, and other cytogenetic abnormalities.

There are several recently initiated protocols by the Children's Oncology Group dealing with infantile fibrosarcoma and various fibroblastic/myofibroblastic tumors. Communication with the local or regional pediatric oncologist and tumor protocol coordinator should ensure submission of the tissue required for protocol enrollment based upon the biopathology of the tumor.

Infantile Fibrosarcoma (congenital fibrosarcoma, congenital infantile fibrosarcoma)

This malignant fibroblastic tumor presents during the first year of life, or even at birth, in the majority of cases and may be detected *in utero* in some instances. The trunk and extremities are most often involved. Unlike adult fibrosarcoma, this tumor usually involves the distal portions of the extremities. The infant presents with an asymptomatic, rapidly growing, bulky tumor that is located in the deep soft tissues. These tumors tend to be locally invasive and metastasize infrequently. Conservative resection with negative surgical margins is the key to avoiding recurrence. Outcome is dependent upon the site of the tumor with low survival rates for retroperitoneal tumors and high survival rates for extremity tumors. With large nonresectable tumors, chemotherapy based upon rhabdomyosarcoma protocols has proven beneficial in reducing tumor size and allowing for adequate resection. Many pediatric oncologists now consider chemotherapy for infantile fibrosarcoma in order to reduce tumor volume and limit the need for more radical surgery, especially when amputation may be a concern prior at initial evaluation.

Gross examination reveals an infiltrative fleshy tumor that lacks a well-defined border. The cut surface of the tumor tends to be lobulated with a myxoid to mucinous character with areas of necrosis, cystic degeneration and hemorrhage. The tumor is a highly cellular neoplasm with a herring-bone pattern. The cells are spindled in outline, but have a high nuclear to cytoplasm ratio. There is considerable nuclear hyperchromasia, tumor necrosis and frequent mitoses scattered throughout the tumor. The tumor cells are closely packed and overlap one another. Focal hemangiopericytoma-like areas with increased vascularity may be seen. These tumors typically are diffusely positive for vimentin and focally reactive for actin (Table 3). Electron microscopy provides evidence for fibroblastic differentiation (Table 3). The ultrastructural features of lack of to extremely rare extracellular banded collagen, rare to absent basal lamina, branching and markedly dilated rough endoplasmic reticulum, irregular nuclear membranes, and extracellular fibrillogranular material are helpful in separating this malignant fibroblastic tumor from myofibromas. These features are also more characteristic of origin from a primitive mesenchymal spindle cell, representing a precursor cell in the fibroblastic/myofibroblastic cell line. Electron microscopy plays a critical role in distinguishing this malignant tumor from spindle cell rhabdomyosarcoma, infantile rhabdomyosarcoma, undifferentiated sarcoma and benign fibroblastic/myofibroblastic neoplasms. Ultrastructural examination is particular important with the approximately 20 to 30% of infantile fibrosarcomas that immunoreact with desmin and/or muscle specific actin, and may result in confusion with a spindle cell rhabdomyosarcoma. An extensive search for striated muscle differentiation (well-formed external lamina, monoparticulate glycogen, myofilaments and Z-band material) should be undertaken with a spindle cell tumor possessing a predominance of banded collagen and absence of extracellular matrix fibrillogranular material. In addition, the herring-bone pattern, nuclear hyperchromasia, necrosis and cystic degeneration distinguish this tumor from a benign myofibromatous tumor. An unusual childhood tumor that may be confused with infantile fibrosarcoma is the infantile rhabdofibrosarcoma. The identification of rhabdomyoblasts by immunocytochemistry, or more likely by electron microscopy, and chromosomal 19 monosomy define the high-grade infantile rhabdofibrosarcoma. Infantile fibrosarcoma shares a tumor-defining translocation (ETV6-NTRK3) with cellular mesoblastic nephroma (Table 3). In addition, it may have other cytogenetic abnormalities, such as trisomy of certain chromosomes (Table 3).

Infantile Myofibromatosis (myofibromatosis, myofibroma)

Although initially described some 5 decades ago as congenital fibrosarcoma, this entity was quickly reclassified as congenital generalized fibromatosis in I954 when it was

recognized that this spindle cell tumor lacked malignant potential. A systematic review of a large number of cases in 1981 resulted in recognition of the myofibroblastic nature of the tumor and the tumor was renamed infantile myofibromatosis. There are 3 forms: solitary (most common > 50%), multicentric (less common, 33%) and multicentric (uncommon, < 15%) with visceral involvement. The solitary form is usually cutaneous with dermal involvement and extension into the underlying subcutis, muscle and even bone. Several soft tissue sites may be involved alone (multicentric) or concomitantly with lung, cardiac, gastrointestinal or even central nervous system involvement (multicentric with visceral involvement). Solitary or multicentric bone lesions may also be present as the sole type of lesions. The prognosis for solitary and multicentric forms is excellent; whereas, > 75% of infants with visceral involvement die of disease. Many of the lesions without visceral involvement stabilize and may undergo spontaneous regression. Some of the solitary lesions may become locally aggressive with unremitting gradual destruction of normal tissues. Particularly aggressive tumors in non-resectable sites may require chemotherapy, similar to that for infantile fibrosarcoma in order to allow for resection or alleviate dysfunction. The head and neck followed by the extremities and trunk are the most common sites of involvement.

The histopathology of myofibromas is characterized a nodular or multinodular proliferation with a zoning phenomenon, characterized by peripheral spindle-shaped cells organized into fascicles. These tend to merge and blend with centrally placed sheets of less differentiated ovoid to polygonal shaped cells. A prominent pericytoma architecture may be seen throughout the tumor, but more prominently within the center of the tumor. The recognition of this pattern has resulted in inclusion of the tumor previously considered to be infantile hemangiopericytoma into the infantile myofibromatosis category. This is appropriate because these tumors lack the cytogenetics features and other features of true adult hemangiopericytoma (solitary fibrous tumor, as reclassified by the WHO). Myofibromas with this pattern are often referred to as myofibromas with a hemangiopericytoma-like pattern. Myofibromas may have a relatively high mitotic rate without atypical mitotic figures, areas of necrosis and calcifications, stromal hyalinization, nuclear atypia and even subendothelial ‘‘intravascular’’ tumor growth. These histopathologic features have no bearing on the clinical outcome of myofibromas. Immunocytochemical staining of the tumor cells reveals vimentin and alpha-smooth muscle actin reactivity, with lack of immunoreactivity with S100 protein, epithelial membrane antigen, keratin and desmin (Table 3). Ultrastructural examination shows myofibroblastic differentiation with prominent dilated rough endoplasmic reticulum, longitudinal filaments with dense bodies, and focal basal lamina. Fibronexus structures may be found in a limited number of cases. It should be emphasized that intercellular junctions, pinocytotic junctions and basal lamina are found in myofibroblastic tumors; however, these are found much more commonly in rhabdomyosarcomas. This may give the false sense of a benign fibroblastic lesion when striated muscle differentiation (well-formed external lamina, monoparticulate glycogen, myofilaments and Z-band material) is expressed ultastructurally in infrequent to rare tumor cells. Immunocytochemical studies should be performed in those cases that do not provide convincing evidence of myofibroblastic differentiation (peripheral cytoplasmic filament arrays).

Cytogenetic analyses of myofibromas have shown nonspecific findings (Table 3) with chromosome 8 abnormalities. A small number of reports of familial myofibromatosis have implicated an autosomal dominant inheritance pattern. It must be emphasized that the vast majority of myofibromas are sporadic, isolated occurrences. Most importantly, these tumors lack the tumor-defining translocation (ETV6-NTRK3) found with infantile

fibrosarcoma and cellular mesoblastic nephroma. In particularly troublesome cases, RT-PCR or FISH for the ETV6-NTRK3 translocation should be performed to eliminate infantile fibrosarcoma as a consideration.

Pericytic Tumor with t(7;12)

A distinct pericytic tumor with a recurrent novel translocation involving the short arm of chromosome 7 and the long arm of chromosome 12 has recently been recognized. This tumor may be mistaken for a myofibroma with a prominent hemangiopericytoma-like pattern or a true adult hemangiopericytoma (solitary fibrous tumor, as reclassified by the WHO). These tumors have involved a spectrum of ages (11 to 65 years) and sites (tongue, stomach, lower leg). These tumors were characterized by multilobulation and infiltrative growth of spindle-shaped tumor cells arranged around thin-walled small vessels. The tumor cells were subendothelial in location. Cytologic atypia and pleomorphism were lacking. Mitoses were infrequent. Immunocytochemistry revealed smooth muscle actin, laminin, and type IV collagen. The tumor cells were not immunoreactive with S100 protein, keratin, desmin or CD34. Ultrastructural features supported a pericytic origin with incomplete basal lamina, subplasmalemmal thickenings, and thin filaments with focal dense bodies along the periphery of the cytoplasm (Table 3). Considering the overall features, this tumor will most likely be classified within the myopericytoma category, according to the WHO soft tissue guidelines.

A novel translocation associated with this tumor (Table 3) involves the beta-actin gene (ACTB) and GLI oncogene [t(7;12)(p21-22;q13-15)]. This fusion gene may result in overexpression of GLI by the inclusion of a promoter region from ACTB. GLI is essential in the sonic hedgehog signaling pathway which participates in cell cycle regulation, cell adhesion, apoptosis, signal transduction and cell proliferation.

These pericytic tumors are limited in number; however 3 of the 5 tumors were resected with negative margins and have not recurred. Two of the tongue tumors required chemotherapy in order to decrease tumor size and to allow for gross total resection. No recurrences or metastatic disease were reported over a mean follow-up period of 24 months.

Inclusion Body Fibromatosis (congenital infantile digital fibromatosis)

Although only identified as a distinct entity in 1965, there are case reports that describe such lesions are early as 1924. This lesion occurs almost exclusively on the dorsal aspect of digits in young children (< 3 years of age) with about one-third of cases being diagnosed shortly after birth. However, cases in adults have also been reported. This typically red to pink lesion is comprised of a firm broad-based, nontender nodule that stretches the overlying skin. The clinical course is a gradual enlargement of the nodule, which may lead to interphalangeal joint deformity. The lesion may erode the underlying bone. Treatment is complete surgical excision. Local recurrence happens in about 50 to 60% of cases.

The tumor has typical features of a spindle cell proliferation with the neoplastic cells arranged into fascicles and sheets, and embedded in a variable amount of collagen matrix. The tumor cells infiltrate the adjacent deep dermis and subcutis. There is lack of encapsulation, and the tumor is poorly defined. The spindle cells are fibroblastic/myofibroblastic in origin with eosinophilic inclusions that are negative for PAS, while being positive for trichrome, phosphotungstic acid-hematoxylin, and iron hematoxylin. Immunocytochemistry finds the inclusions to be reactive with smooth muscle actin and vimentin. Ultrastructural examination illustrates the fibroblastic/myofibroblastic nature of

the tumor. The spindle cells possess dilated rough endoplasmic reticulum, cytoplasmic thin filaments with dense bodies, and large ovoid aggregates with a granular to filamentous character (inclusions). The cytoplasmic thin filaments extend into the large aggregates.

A final consideration with this entity is the recent report of infants with digital fibromas comprising one component of a syndrome. Digital fibromas have been associated with facial pigmentary dysplasia, focal dermal hypoplasia, metacarpal and metatarsal disorganization, and limb malformations. The association with these malformations may lead to new insight into the molecular genetics and mechanisms behind infantile digital fibroma formation.

Fibrous Hamartoma of Infancy

This unique tumor occurs primarily in the first year of life (90%) and there is a male predilection (2.4M:1.0F). The most common sites for this tumor are axillary regions, upper arms, upper trunk, inguinal regions and external genitalia. The typical lesion is a rapidly growing, painless nodule that is freely movable, but poorly circumscribed. Most are single lesions. The tumor is comprised of three components that form organoid structures: 1) well-defined bundles (trabeculae) of dense intertwining fibrous tissue that extends into subcutis adipose tissue; 2) primitive mesenchyme arranged in nests, whorls and bands in a myxoid matrix; and 3) mature adipose tissue admixed with the other components. The fibrous tissue is composed of myofibroblasts and fibroblasts separated by variable amounts of collagen. The spindle cells react primarily with vimentin and to a lesser extent with smooth muscle actin. Ultrastructural examination reveals an admixture of fibroblastic and myofibroblastic cells. The primitive mesenchymal cells embedded in the myxoid stroma show slender cell processes and a paucity of organelles. Cytogenetic characterization data are lacking. Treatment is surgical excision with recurrence rates relatively low (12%).

Juvenile Hyaline Fibromatosis

This hereditary disorder was first described in 1873 as molluscum fibrosum. In the mid-1960's, it became known as juvenile hyaline fibromatosis. This autosomal recessive disorder is characterized by aberrant collagen synthesis with deposition of hyaline material in the supporting tissues of the skin, gingiva, bone and joints. It has been divided into 2 separate categories, a severe form – infantile systemic hyalinosis and a mild form – juvenile hyaline fibromatosis. The majority of individuals present with soft tissue nodules during infancy or early childhood; however, adult onset cases have been reported. Typically, there is a progressive increase in the number and size of subcutaneous and deep nodules, leading to deformity and dysfunction. With infantile and early childhood onset, survival may be into adulthood. The face and neck are frequently involved, as well as the gingiva, skull, long bones and phalanges. The involvement of periarticular areas results in joint contractures; whereas bony lesions cause osteolysis and osteoporosis. Perianal lesions may resemble genital warts.

The nodules are formed by plump fibroblasts embedded in an abundant non-fibrillary, eosinophilic hyaline matrix. In active lesions or in younger patients, the nodules may be quite cellular. The fibroblasts have a relatively amphophilic cytoplasm and an indistinct fascicular arrangement. In longstanding lesions, the cellularity is markedly decreased and the fibroblasts appear compressed by the matrix. Some areas of the nodule may have a chondroid character. PAS stain tends to be strong and diastase resistant. Immunocytochemical studies show only vimentin reactivity. Ultrastructural examination reveals the fibroblastic nature of the lesion (Table 3). The fibroblasts have a unique appearance with frequent markedly dilated membrane-bound vesicles containing granular

and filamentous (fibrillogranular) material. The contents of these vesicles are similar in morphology to that for the surrounding ground substance. Occasionally, the vesicles will be in direct continuity with the surrounding ground substance.

Abnormalities on the long arm of chromosome 4 (4q21) in juvenile hyaline fibromatosis has been known for sometime (Table 3). Recently, the mutated gene associated with juvenile hyaline fibromatosis has been identified. Capillary morphogenesis gene-2 (CMG2) is affected in both the mild and severe forms of juvenile hyaline fibromatosis. This gene encodes a protein that is upregulated in endothelial cells during capillary formation. The resulting protein binds laminin and collagen IV via a von Willebrand factor type A domain. It is believed that a mutation that causes complete interference with binding by the von Willebrand factor A results in the severe form of the disease – infantile systemic hyalinosis. The milder form of the disease – juvenile hyaline fibromatosis – may occur when there is an in-frame mutation that affects a highly conserved cytoplasmic domain with retention of some binding function. Fibroblasts derived from affected patients indicate that CMG2 mutations abrogate normal cell interaction with the extracellular matrix. It is possible that this condition represents a perturbation of the basement membrane matrix assembly apparatus culminating in excessive hyaline deposition. CMG2 also functions as a cellular receptor for anthrax toxin. The role for this anthrax toxin receptor function in juvenile hyaline fibromatosis is unclear.

Table 1: Tumors of Fibroblastic and Myofibroblastic Origin in Children and Adolescents

Infantile Fibrosarcoma (uncommon)
Myofibroma/Myofibromatosis (common, includes ''infantile hemangiopericytoma'')
Pericytic Tumor with t(7;12) (rare, most likely will be classified with myopericytoma)*
Inclusion Body Fibromatosis (rare)
Fibrous Hamartoma of Infancy (rare)
Desmoid (uncommon)
Fibromatosis Coli (uncommon)
Gardner Fibroma (uncommon)
Inflammatory Myofibroblastic Tumor (uncommon)
Nodular Fasciitis (uncommon)
Calcifying Aponeurotic Fibroma (rare)
Calcifying Fibrous Tumor (rare)
Juvenile Hyaline Fibromatosis (rare)
Lipofibromatosis (rare)
Solitary Fibrous Tumor (rare, includes ''adult hemangiopericytoma'')

* = recently described entity with features mimicking hemangiopericytoma-like myofibroma

Table 2: Triaging of Fibroblastic and Myofibroblastic Tumors in Children and Adolescents

- Frozen Tissue with Cryopreservative for Intraoperative Diagnosis
- Formalin-Fixed Tissue for Routine Histopathology, Immunocytochemistry, *In Situ* Hybridization and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) Evaluation
- Glutaraldehyde-Fixed Tissue for Electron Microscopy
- Fresh Tissue in Tissue Culture Media for Cytogenetics and Molecular Studies, and Tissue Cultures
- Frozen Tissue without Cryopreservative for Molecular Studies, Gene Rearrangement, and Microarray Gene Analysis
- Cytologic Imprints of Neoplastic Tissue
    - Cytogenetic Interphase Studies
    - Fluorescent *In Situ* Hybridization for Cytogenetics (FISH)
    - Special Stains and Immunocytochemical Phenotyping
- Alcohol-Fixed Tissue for Improved Cytoplasmic Glycogen Preservation, Immunocytochemistry (requiring such fixation) and Microarray Gene Analysis

Table 3: Tumors of Fibroblastic and Myofibroblastic Origin in Children and Adolescents: Immunocytochemical, Ultrastructural and Cytogenetic Features

Immunocytochemical Features

Vimentin	vast majority
Smooth Muscle Actin	most
Muscle Specific Actin	infrequent to occasional
Desmin	infrequent to occasional
bet-catenin	rare (Desmoid, Gardner's Fibroma)
CD34	rare (Gardner's fibroma)
S100 Protein	rare
Neuron specific enolase	rare
Epithelial Membrane Antigen	rare
Cytokeratin	rare
Factor XIIIa	rare
ALK-1	negative (except Inflammatory Myofibroblastic Tumor)
CD68	rare
CD57	rare
CD31	rare
STAT6	rare (Solitary Fibrous Tumor)

Ultrastructural Features

Infantile Fibrosarcoma
- Extracellular Banded Collagen, None to Extremely Rare
- Intermediate Filaments
- Primitive Intercellular Junctions, Occasional
- Basal Lamina, Rare
- Dilated Rough Endoplasmic Reticulum with Branching and Fibrillogranular Material
- Nucleoli, Increased in Number
- Irregular Nuclear Membrane
- Lysosomal Granules, Common
- Intercellular Junctions, Rare to Absent

Myofibroblastic Tumors
- Extracellular Banded Collagen, Infrequent
- Peripheral Cytoplasmic Myofilaments and Cytoplasmic Filaments
- Intercellular Junctions, Occasional
- Pinocytotic Vesicles, Occasional
- Basal Lamina, Occasional
- Dilated Rough Endoplasmic Reticulum

Pericytic Tumor with t(7;12)
- Incomplete Basal Lamina
- Subplasmalemmal Thickening
- Thin Filaments with Dense Bodies

Inclusion Body Fibromatosis
- Dilated Rough Endoplasmic Reticulum
- Thin Filaments with Dense Bodies
- Large Ovoid Aggregates with Granular to Filamentous Character (Inclusions)

Fibrous Hamartoma of Infancy
- Trabecular Areas with:

Juvenile Hyaline Fibromatosis
- Cystically Dilated Membrane-Bound

Dilated Rough Endoplasmic Reticulum
Well-Developed Golgi
Pinocytotic Vesicles
Extracellular Collagen
Thin Filaments with Dense Bodies
Basal Lamina
Vesicles with Granular and Filamentous Material Similar to Extracellular Ground Substance (fibrillogranular material)
Continuity Between Vesicles and Extracellular Space

Myxoid Areas:
Stellate to Spindle Cells
Slender Cytoplasmic Processes
Paucity of Cytoplasmic Organelles

Cytogenetics Features

	Abnormality	Gene Affected or Fusion Product
Infantile Fibrosarcoma	t(12;15)(p13;q25) Trisomy 8, 11, 17, 20	ETV6-NTRK3
Myofibroma	Chromosome 8	Non-Specific
Pericytic Tumor with t(7;12) (Myopericytoma)	t(7;12)(p21-22;q13-15)	ACTB-GLI
Juvenile Hyaline Fibromatosis	4q21	ANTXR2 (CMG2)
Inflammatory Myofibroblastic Tumor	2p23 t(2:1)(p23;q25) t(2;2)(p23;q13) t(2;11)(p23;p15) t(2;17)(p23;q23) t(2;19)(p23;p13.1) Chromosome 12	ALK Rearrangements ALK-TPM3 ALK-RANBP2 ALK-CARS ALK-CLTC ALK-TPM4 HMGIC (HMGA2)
Solitary Fibrous Tumor/ Hemangiopericytoma	t(12;19)(q13;q13) t(13;22)(q22;q13.3) Loss 3p, 12q, 13q, 17p. 17q, 19q, 10 (entire) Gain 5q inv12(q13q13)	Not Known Not Known Not Known *NAB2–STAT6*
Desmoid	Chromosomes 8 & 20 5q 3p21	Not Known APC beta-catenin (CTNNB1)
Gardner-Associated Fibroma	5q 3p21	APC beta-catenin (CTNNB1)
Nodular Fasciitis	Rearrangement (3q21;2) t(2;15)	Not Known Not Known

	Loss of 2 and 13	
	t(17;22)(p13;q13)	MYH9-USP6
	17;p13	USP6(TRE17) Rearrangements
Dermatofibrosarcoma Protuberans and Giant Cell Fibroblastoma	t(17;22)(q22;q13) Ring chromosome	COL1A1-PDGFB