Contribution of Cytogenetics to the Management of Poorly Differentiated Sarcomas

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- Cytogenetic abnormalities in mesenchymal neoplasms can be divided into 2 groups: tumor-specific abnormalities and multiple, sometimes complex abnormalities that are rarely useful diagnostically.

- The identification of tumor-specific chromosomal abnormalities in mesenchymal neoplasms has added a new dimension to the formulation of a diagnosis complementing traditional light microscopic examination of hematoxylin-eosin stained slides, immunohistochemistry and electron microscopy.

- Detection of tumor-specific chromosomal aberrations by conventional cytogenetics, molecular cytogenetics (FISH) and/or RT-PCR analysis is especially useful in the management of poorly differentiated sarcomas and in confirming diagnostic impressions of lesions arising in rare anatomic locations or unusual age groups, or exhibiting atypical histopathologic, immunophenotypic or ultrastructural findings.

INTRODUCTION

In the present review, emphasis was placed on the contribution of cytogenetics to the management of poorly differentiated sarcomas. Certain case presentations are included to illustrate the integration of traditional histopathologic and genetic approaches and serve as useful paradigms. Cytogenetic abnormalities in mesenchymal neoplasms can be divided into **two major groups**: **(1)** A significant number of sarcomas are characterized by tumor-specific structural abnormalities. Most commonly, these are translocations that result in the production of chimeric genes encoding for abnormal, oncogenic proteins that are central to the causation of these tumors.[1-3] Other examples include supernumerary ring chromosomes that may lead to certain gene(s) amplifications.[4,5] Tumor-specific chromosomal anomalies serve as valuable diagnostic aids particularly in the differential diagnosis of those sarcomas of a confusing nature such as poorly differentiated sarcomas**. (2)** Other sarcomas are associated with **multiple and sometimes complex chromosomal changes** suggesting that tumor development in this subgroup requires a succession of changes. Although more difficult to appreciate the diagnostic value of these karyotypic changes, a pattern of chromosomal imbalances and/or recurrent breakpoints may be recognizable for some neoplasms such as embryonal rhabdomyosarcoma[6,7], malignant peripheral nerve sheath tumor[8,9] or conventional osteosarcoma.[10,11] These aberrant patterns, when viewed in association with other clinicohistopathologic features, may contribute to accurate nosology, but are not as useful as tumor-specific anomalies. Regrettably, for most sarcomas in this group however, the high degree of cytogenetic complexity (including large numbers of unidentifiable marker chromosomes and intratumoral heterogeneity) precludes its use as a discriminating tool.[12-14]

TUMOR-SPECIFIC CHROMOSOMAL ABNORMALITIES

Translocations, or exchange of chromosomal material between two or more nonhomologous chromosomes, are encountered as the most frequent tumor-specific anomalies in mesenchymal neoplasms. A tumor-specific translocation is considered a "primary" chromosomal abnormality. It is often present as the sole karyotypic aberration and is therefore likely to be etiologic. In contrast, "secondary" chromosomal abnormalities may be consistent in a particular neoplasm, but are also observed in other histologic tumor types, thereby lacking the specificity of the primary change. Secondary changes or additional genetic mutations are also thought to be essential in cancer development or contributory to tumor progression, but little is known about this group of changes in sarcomas.

Identification of nonrandom translocations in bone and soft tissue tumors has directed investigations of the underlying biological events (Table 1). Striking similarities among the translocations cloned in sarcomas are evident. Generation of a chimeric gene expressing an abnormal protein, a novel transcription factor that causes transcriptional deregulation, is the consequence of most sarcoma-associated translocations. Less commonly, sarcoma-associated translocations or other recurrent genetic alterations elicit signaling pathway deregulation.

Small Blue Round Cell Neoplasms

Ewing's sarcoma, rhabdomyosarcoma, neuroblastoma, desmoplastic small round cell tumor, Wilms' tumor, non-Hodgkin lymphoma, mesenchymal chondrosarcoma, small cell osteosarcoma, and poorly differentiated synovial sarcoma among others are members of a group of neoplasms categorized as small round cell tumors (SRCTs) because of their uniform small round cell microscopic appearance. This homogeneous light microscopic appearance often causes diagnostic difficulties. Establishing an accurate diagnosis requires studies beyond the routine hematoxylin and eosin-stained sections such as immunohistochemical and electron microscopic studies.[15,16] Cytogenetic and molecular genetic approaches are valuable in classifying these neoplasms.[3,12,17-24] This is particularly true when pathologists are confronted with poorly differentiated or undifferentiated SRCTs in which useful immunohistochemical or ultrastructural features are absent. Characteristic cytogenetic findings are not lost as a lesion becomes less differentiated or metastasizes. Moreover, detection of distinctive genetic alterations in small biopsies of SRCTs or SRCTs with unusual

immunohistochemical/ultrastructural features or with unexpected clinical presentations (ie. originating in an atypical anatomic location or uncommon age group) can also be instrumental in establishing a precise diagnosis.

The prototypical model of Ewing's sarcoma (ES) illustrates the value of cytogenetics in the management of poorly differentiated sarcomas. Approximately two decades ago, the observation of the nonrandom translocation t(11;22)(q24;q12) in ES, peripheral primitive neuroectodermal tumors (pPNET), Askin tumor, and other less frequent variants once considered unrelated neoplasms provided strong evidence for a common histogenesis and led to the conclusion that these lesions are members of the same family exhibiting a spectrum of differentiation.[25-27] Accurate diagnosis of the ES family of tumors is critical for ensuring optimal clinical care for these patients. The ES/PNET specific chromosomal translocations that result in the fusion of the *EWSR1* gene (22q12) with an *ETS* transcription factor family member [most commonly *FLI1* (90-95%) followed by *ERG* and others (5-10%)] can be identified by conventional cytogenetic, fluorescence in situ hybridization (FISH) or reverse transcription-polymerase chain reaction (RT-PCR) analysis.

CD99 (*MIC2* transmembrane glyoprotein product) immunoreactivity is present in virtually all ES/PNET and as such, is an important immunodiagnostic marker of these malignancies. Establishing a diagnosis of ES/PNET in the absence of CD99 immunoreactivity is more challenging. Conversely, it is important to recognize that CD99 expression is not specific for ES/PNET as originally considered and in fact, may be detected in a significant subset of other small, blue, round cell tumors.[28-33] Consider the following example:

> A 42-year-old male presented with right flank pain and gross hematuria. A large heterogeneous mass in the right kidney was detected by ultrasonography and magnetic resonance imaging (MRI). Additional radiographic studies revealed a right venal vein tumor thrombus, multiple liver lesions and possible bone (sacral) metastases. A provisional diagnosis of renal cell carcinoma was made and right radical nephrectomy performed. Grossly, the 9×7×6 cm neoplasm was focally necrotic and hemorrhagic. Histopathologic examination revealed sheets of small round cells with occasional rosette formation and vimentin, neuron specific enolase and synaptophysin immunoreactivity. The malignant cells were negative for CD99, but appeared weakly positive for FLI1. A diagnosis of ES/PNET was favored, but not expressed with certainty because ES/PNET is an extraordinarily rare primary tumor in the kidney and can be mistaken for a variety of other round cell tumors, including blastema-predominant Wilms' tumor, neuroblastoma, poorly differentiated synovial sarcoma, and undifferentiated neuroendocrine carcinoma. The diagnosis was subsequently confirmed by demonstrating a rearrangement of the *EWSR1* locus and the presence of an *EWSR1/FLI1* fusion transcript by FISH and RT-PCR, respectively.

This case demonstrates the value of genetic analysis in establishing a diagnosis of ES/PNET in the absence of CD99 immunoreactivity. Evaluation of this particular case was also compounded by the origin of the neoplasm in a rare anatomic location. Renal ES/PNET must be distinguished from blastema-predominant Wilms' tumor and other primitive renal tumors that require different therapy.[34-36] The immunohistochemical pattern of primary malignant neuroepithelial tumors of the kidney may be perplexing.[37] In general, FLI1 expression has been considered a more specific ES/PNET marker, however, it has also been detected in other neoplasms.[32,38]

An erroneous light microscopic interpretation may lead to inappropriate antibody selection.[16] In this event, a large (and expensive) battery of immunostains may be selected in an effort to identify a differentiating antigen. This is a potential danger in evaluating poorly differentiated sarcomas.

> A 76-year-old female presented with new onset of left ptosis, dysarthria, and headache (bilateral temporal). Radiographic studies revealed left ethmoid and sphenoid sinus opacification with contrast enhancement consistent with chronic sinus disease but concerning for neoplasm. Histopathologic evaluation of the surgically excised specimen revealed an infiltrative lesion composed of sheets of small round blue cells with focal fibrous tissue bands separating some cell aggregates. Examination of individual cells was limited by variable crush artifact and necrosis. The initial clinicohistopathologic impression was malignant undifferentiated neoplasm, favor small cell carcinoma. The following antibodies were examined: EMA, MAK6, AE1/AE3, CK7, CK20, CAM5.2, CD56, chromogranin, synaptophysin, GFAP, S-100, melan-A, CD45, EBV, and myeloperoxidase. The neoplastic cells were

positive for only CD56 and MAK6 (focal). A portion of the specimen was also submitted for cytogenetic analysis at the time of biopsy. Harvest and examination of the supernatant (first change of culture media performed within 48 hours), revealed a hypertetraploid complement featuring the t(2;13)(q35;q14) characteristic of alveolar rhabdomyosarcoma (ARMS). FISH analysis confirmed the presence of a rearrangement of the *FKHR* (*FOXOA1*) locus. Subsequently, immunoreactivity for desmin, myogenin and myoglobin were also demonstrated.

Initially, rhabdomyosarcoma (the most common soft-tissue sarcoma of childhood but rare in older adults) was not considered in the differential diagnosis for this case. The light microscopic appearance was not helpful because an alveolar pattern was not readily identifiable and many of the cell groupings were crushed or necrotic. An immunohistochemical panel for diagnostic consideration of small cell carcinoma, lymphoma and melanoma was explored. An advantage of conventional cytogenetic analysis is that knowledge of the histologic diagnosis or anticipated anomaly is not necessary. Cytogenetic analysis provides global information (primary and secondary aberrations) in a single assay and a quick turn-around-time can be achieved. A valuable diagnostic adjunct in ARMS is the identification of translocations t(2;13)(q35;q14) and t(1;13)(p36;q14), and the associated *PAX3-FOXOA1* and *PAX7-FOXOA1* fusion transcripts, respectively. In the absence of an alveolar pattern in the solid variant, with the low degree of differentiation in certain embryonal rhabdomyosarcomas (ERMSs) and with the increasing use of fine-needle aspiration biopsies, recognition of these specific translocations may be useful, if not essential, in establishing an accurate diagnosis and ensuring the correct therapy.[39-42]

Spindle Cell Neoplasms

Discrimination of sarcomas with predominantly spindle cell morphology can be difficult without ancillary immunohistochemical, ultrastructural or genetic techniques. There are advantages and limitations to each of these methods. Morphologic assessment of a spindle cell neoplasm can be complicated when the expected range of immunohistochemical markers or ultrastructural features are absent. Moreover, the immunohistochemical pattern for some spindle cell sarcomas, such as synovial sarcoma (SS) and malignant peripheral nerve sheath tumor (MPNST), can overlap with those of other neoplasms.[43-45] Detection of spindle cell sarcoma specific translocations such as the SS-associated X;18 translocation, low grade fibromyxoid sarcoma-associated 7;16 translocation or 2p23 (*ALK* gene) rearrangements in inflammatory myofibroblastic tumor (to name a few) may be necessary to confirm a diagnosis in difficult cases.

SS is an aggressive neoplasm arising most commonly in the extremities of young adults. By light microscopy, biphasic SS featuring morphologically distinct but histogenetically related epithelial cells and fibroblast-like spindle cells is less likely to pose diagnostic difficulties than monophasic fibrous, monophasic epithelial, or poorly differentiated variants. These latter variants may be confused with fibrosarcoma, leiomyosarcoma, MPNST, hemangiopericytoma, metastatic carcinoma, melanoma, and PNET among others. More than 95% of SSs, regardless of histology, exhibit the chromosomal translocation t(X;18)(p11.2;q11.2). This translocation results in the fusion of the *SYT* gene on chromosome 18 to either the *SSX1* or *SSX2* gene on chromosome X and can be detected by cytogenetic, molecular cytogenetic or molecular diagnostic means..

An 18-year-old male presented with right-sided chest pain. Radiographic studies showed an 8-cm diaphragmatic right pleural-based mass and associated pleural effusion. Exam was negative for extrapleural disease. Resection of the neoplasm revealed that it was composed of uniform spindle-shaped cells with scant cytoplasm and indistinct cell borders. The neoplastic cells were diffusely immunoreactive for vimentin and bcl-2 and focally for AE1/AE3, CAM5.2, and epithelial membrane antigen. The neoplastic cells were negative for S-100 protein, CD34, desmin, muscle-specific actin and α-smooth muscle actin. The diagnostic impression of monophasic fibrous SS was further validated by the karyotypic demonstration of the characteristic X;18 translocation. In addition, a ring chromosome was detected. The ring chromosome, considered a secondary change in this case, was shown to be composed of chromosome 8 material by subsequent spectral karyotyping and FISH studies.[46] An extra copy of chromosome 8 is one of the most frequent secondary numerical abnormalities in SS. Its presence may be associated with disease progression.[47,48]

Primary SSs of the pleura are rare. Monophasic fibrous SSs arising in this unusual site may be histologically indistinguishable from solitary fibrous tumor and sarcomatous malignant mesothelioma.[49,50] Moreover, recent studies have shown epithelial marker negativity and CD34 positivity in some pleuropulmonary monophasic fibrous SSs further complicating its distinction from solitary fibrous tumor and other spindle cell neoplasms.[51-53] Arriving at a correct diagnosis is crucial since these neoplasms show different prognoses and require varying treatment modalities. The SS-specific t(X;18) is considered one of the most reliable diagnostic criterion and its detection by cytogenetic or molecular genetic approaches may be necessary for definitive classification in exigent circumstances. This case also demonstrates the advantage of cytogenetic analysis in disclosing secondary changes that may contribute to neoplastic progression.

Adipocytic Tumors

Liposarcomas represent the single most common group of soft tissue sarcomas. Dedifferentiated liposarcoma (DDL) is a distinct subtype of liposarcoma showing transition into a nonlipogenic sarcoma of variable histologic grade, either in the primary tumor or in a recurrent tumor from a well-differentiated liposarcoma (WDL).[54] DDL may be difficult to distinguish from a high-grade pleomorphic sarcoma or other poorly differentiated sarcoma.[55] Cytogenetically, supernumerary ring chromosomes and/or giant rod-shaped marker chromosomes composed at least in part of chromosome 12 material accompanied by few or no other abnormalities are characteristic of atypical lipoma/WDL.[56]

> An 82-year-old female noted a non-painful medial left thigh mass approximately two months prior to seeking medical attention. Radiographic examination confirmed the presence of a mass with one portion demonstrating a signal intensity similar to that of subcutaneous adipose tissue and the other showing a darker signal intensity. The resected, grossly heterogeneous mass, measuring 5.3×6.1×10.5 cm, was histopathologically composed of a well-differentiated liposarcomatous component transitioning abruptly into a dedifferentiated one. The morphologic appearance of the latter resembled "MFH"-like pleomorphic sarcoma. Cytogenetic analysis of the WDL-component revealed the following abnormal complement: 47-48,XX,+1-2r,1-2dmin. Notably, cytogenetic analysis of the DDL-component revealed a similar, but slightly more complex complement: 42-45,XX,del(3)(p13),del(3)(q12q26),-4,-5,add(6)(p25),del(6)(p12),-7,add(9)(p24),-11,der(12)t(5;12)(q11.2;p11.2),add(13)(p12),-14,-16,-19,add(19)(p13.3),-21,i(22)(q10),+1-3r, +mar1,+mar2,+1-4mar.

A distinct advantage of cytogenetic analysis is that primary or characteristic chromosomal aberrations are present in all tumor cells and are expressed throughout the clinical course. These alterations are not lost as a neoplasm becomes less differentiated or metastasizes. Similar to WDL, DDL most often has rings or giant rod-shaped marker chromosomes and dmin, signifying a kinship to, if not in fact, derivation of these tumors from WDL.[56] FISH and genomic profiling studies have demonstrated that the ring/marker chromosomes in WDL/DDL consist chiefly of amplified 12q13-15 material, including the genes *MDM2* and *CDK4*. Cytogenetic or molecular demonstration of chromosome 12 comprised supernumerary ring/marker chromosomes or *MDM2*/*CDK4* amplification may serve to distinguish WDL/DDL from benign adipose tissue tumors and other poorly differentiated sarcomas respectively.[6,57-59]

In contrast to DDL, pleomorphic liposarcomas show high chromosome counts and complex structural rearrangements featuring numerous unidentifiable marker chromosomes, non-clonal alterations, polyploidy, and intercellular heterogeneity. The cytogenetic profile of pleomorphic liposarcoma appears therefore to be closer to other pleomorphic sarcomas than to WDL/DDL.[13] Perhaps correspondingly, the clinical behavior of DDL is less aggressive than in other high-grade pleomorphic sarcomas also.[60]

RECURRENT CHROMOSOMAL PATTERNS

Many sarcomas are associated with multiple and sometimes complex chromosomal changes suggesting that tumor development in this subgroup requires a succession of changes. Although more difficult to appreciate the diagnostic value of these karyotypic changes, a pattern of chromosomal imbalances and/or recurrent breakpoints may be recognizable for some neoplasms such as adamantinoma[61], neuroblastoma[62-64], embryonal rhabdomyosarcoma (ERMS)[6,7], malignant peripheral nerve sheath tumor[8,9] and conventional osteosarcoma.[10,11] These aberrant patterns, when viewed in conjunction with other clinicohistopathologic features, may contribute to accurate nosology, but are not as useful as tumor-specific anomalies.

> A 24-year-old female presented to her local primary care provider with a several month history of intermittent heavy bleeding. During this time-frame, the patient underwent an endocervical polypectomy and two D&C procedures with pathologic interpretation of each specimen as benign. Subsequent review of the latter D&C specimen at the University of Nebraska Medical Center revealed a malignant neoplasm interpreted as a mixed ERMS/ARMS with diffuse anaplasia. The patient subsequently underwent a hysterectomy. Angiolymphatic invasion with metastatic involvement of two of six lymph nodes was detected. RT-PCR studies were negative for *PAX/FOXOA1* fusion transcripts. Cytogenetic analysis revealed a near-tetraploid, complex karyotype with multiple numerical and structural abnormalities.

Although a specific chromosomal abnormality is not observed in ERMS, there is a pattern of recurrent imbalances that may contribute to its recognition. Specifically, gain of all or portions of chromosomes 2, 7, 8, 11, 12, 13, and 20, and loss of 22 are most frequent.[6,7,65] Mixed ERMS/ARMS lack the 2;13 or 1;13 translocations (as confirmed by conventional cytogenetic, FISH and/or RT-PCR studies) and appear to be more similar to ERMS cytogenetically.[42] ERMS occurs predominantly in children less than 15 years of age. The anaplastic variant of RMS features enlarged atypical cells with hyperchromatic nuclei and bizarre, multipolar mitoses.[66] Anaplasia may be seen in both embryonal and alveolar tumors, but is more prevalent in the former. Anaplastic features may be focal (single dispersed cells) or diffuse (clone-like cell clusters). Interestingly, genomic amplification as detected by comparative genomic hybridization (CGH) is common to both anaplastic ERMS and ARMS.[65] Likewise, karyotypic analysis of ERMS with anaplasia more frequently discloses the presence of double minutes than in ERMS without anaplasia. Importantly, changes such as double minutes and gene amplification in ERMS (and also in neuroblastoma) may be associated with tumor progression or prognosis.[62-65]

Regrettably for most sarcomas showing multiple chromosomal changes (predominantly high grade pleomorphic sarcomas), the high degree of cytogenetic complexity (including large numbers of unidentifiable marker chromosomes and intratumoral heterogeneity) precludes its use as a discriminating tool.[12,13,14]

CONCLUSIONS

Important and meaningful advances have been made in mesenchymal tumor cytogenetics during the last two decades. A number of bone and soft tissue tumors have been shown to have recurrent, if not specific, chromosomal changes, particularly translocations. The identification of these changes in mesenchymal neoplasms has added a new dimension to the formulation of a diagnosis complementing traditional light microscopic examination of hematoxylin-eosin stained slides, immunohistochemistry and electron microscopy. Detection of tumor-specific chromosomal aberrations is especially useful in the management of poorly differentiated sarcomas and in confirming diagnostic impressions of lesions arising in rare anatomic locations or unusual age groups, or exhibiting atypical histopathologic, immunophenotypic or ultrastructural findings.

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Table 1: Characteristic and Variant Chromosomal Aberrations and Associated Molecular Events in Bone and Soft Tissue Sarcomas

Neoplasm	Chromosomal aberration	Molecular event
Alveolar soft part sarcoma	der(17)t(X;17)(p11.2;q25.3)	*ASPL/TFE3*
Alveolar rhabdomyosarcoma	t(2;13)(q35;q14)	*PAX3/FOXOA1*
	t(1;13)(p36;q14)	*PAX7/FOXOA1*
	t(X;2)(q13;q35)	*PAX3/AFX*
	t(2;2)(p23;q35)	*PAX3/NCOA1*
Clear cell sarcoma	t(12;22)(q13;q12)	*EWS/ATF1*
	t(2;22)(q32;q12)	*EWS/CREB1*
Congenital fibrosarcoma[a]	t(12;15)(p13;q25)	*ETV6/NTRK3*
Dermatofibrosarcoma protuberans[b]	t(17;22)(q22;q13)[c]	*COL1A1/PDGFB*
Epithelioid hemangioendothelioma	t(1;3)(p36;q25)	?
Ewing sarcoma/pPNET	t(11;22)(q24;q12)	*EWS/FLI1*
	t(21;22)(q22;q12)	*EWS/ERG*
	t(7;22)(q22;q12)	*EWS/ETV1*
	t(17;22)(q21;q12)	*EWS/EIAF*
	t(2;22)(q33;q12)	*EWS/FEV*
	inv(22)(q12q12)	*EWS/ZSG*
	t(16;21)(p11;q22)	*FUS/ERG*
Extraskeletal myxoid chondrosarcoma	t(9;22)(q22-31;q12)	*EWS/NR4A3*
	t(9;17)(q22;q11)	*TAF2N/NR4A3*
	t(9;15)(q22;q21)	*TCF12/NR4A3*
Gastrointestinal stromal tumor	Monosomy 14, 22 1p deletion	*KIT* or *PDGFRA* mutation
Inflammatory myofibroblastic tumor	2p23 rearrangements	*ALK* rearrangement
Liposarcoma, well-differentiated	Ring/giant marker chromosome; 12q13-15 amplification	*MDM2, CDK4, HMGA2* amplification
Liposarcoma, poorly-differentiated	Ring/giant marker chromosome + more complex; 12q13-15 amplification	*MDM2, CDK4, HMGA2* amplification
Low grade fibromyxoid sarcoma	t(7;16)(q33;q37)[c]	*FUS/CREB3L2*
Malignant hemangiopericytoma	t(12;19)(q13;q13)	?
Myxoid/round cell liposarcoma	t(12;16)(q13;p11)	*TLS*[d]*/CHOP*
	t(12;22)(q13;q12)	*EWS/CHOP*
Parosteal osteosarcoma	Ring chromosome; 12q13-15 amplification	*CDK4, MDM2, SAS* amplification
Pericytoma	t(7;12)(p22;q13)	*AGTB-GLI*
Synovial sarcoma	t(X;18)(p11.2;q11.2)	*SYT/SSX1* *SYT/SSX2* *SYT/SSX4*
	t(X;20)(p11.2;q13.3)	*SS18L1/SSX1*

[a]This translocation is seen also in congenital mesoblastic nephromas, confirming a relationship with congenital fibrosarcoma.
[b]This translocation is seen also in giant cell fibroblastoma, confirming a relationship with dermatofibrosarcoma protuberans.
[c]Rearrangement also frequently seen as a ring chromosome.