Focus on sarcomas

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Introduction and epidemiology

Sarcomas in general are rare tumors, accounting for less than 5% of adult neoplasms and less than 20% of pediatric malignancies. They represent a much larger proportion of pediatric malignancies compared to their proportional representation in adults, although their absolute numbers are much higher in adults. Several of these tumors including, osteogenic sarcoma, Ewing's sarcoma, and synovial sarcoma, have peak incidences in adolescents and young adults, while other tumors such as chondrosarcomas have a peak incidence in patients over the age of 65. These tumors are derived from mesodermal or ectodermal germ layers, in contradistinction to the more common carcinomas, which are typically derived from endoderm. Sarcomas are histologically diverse, and although the histogenesis of some sarcomas is unknown, others exhibit features resembling normal connective tissue elements, such as bone, cartilage, fat, and muscle, from which they appear to rise (see Figure 1). The relative rarity and diversity of histologies have hindered large randomized treatment studies. In general, the etiology of these tumors is obscure, and the overwhelming majority of sarcomas appear to occur through sporadic mutations. However, there are currently four known familiar cancer syndromes associated with sarcomas. Patients with germline mutations of the retinoblastoma (RB) gene have a much higher frequency of osteosarcomas (Abramson et al., 1984). Patients with Li-Fraumeni syndrome and germline mutations of the p53 gene (Li and Fraumeni, 1969; Malkin et al., 1990) have an increased incidence of a variety of sarcomas typically diagnosed before the age of 45. Another sarcoma, malignant peripheral nerve sheath tumor, or MPNST, occurs in up to 50% of cases in the setting of neurofibromatosis 1 (NF-1) that is associated with germline loss of the NF1 gene (King et al., 2000). Finally, a recently described familial gastrointestinal stromal tumor (GIST) syndrome was described in a kindred with germline mutations of c-kit (Nishida et al., 1998). Known environmental risk factors for sarcoma development include ionizing irradiation, which is associated with the development of a variety of sarcomas, and vinyl chloride, which is an established cause of hepatic angiosarcoma.

Genetics

A major recent advance in our understanding of sarcomas has been the identification of specific genetic abnormalities corresponding to specific histologic subtypes of sarcomas. This understanding has led to new specific diagnostic entities, as well as new approaches to the treatment of these tumors. Rather than treating all sarcomas in a uniform fashion, each specific genetic/histologic subtype should be considered a unique therapeutic target. Of particular interest to the molecular biologist, a subset of sarcomas is cytogenetically relatively simple and exhibits reciprocal chromosome translocations resulting in the formation of fusion genes (Table 1). Not only have these translocations provided new highly specific diagnostic markers, but they also hold the key to new insights into the biology of these tumors. It appears clear that these disease-specific translocations are necessary for tumorigenesis, but they may not be sufficient. Other "second hits" and/or a uniquely permissive environment present in an early mesenchymal stem and/or progenitor cell may be necessary for malignant transformation (Deneen and Denny, 2001). One model holds that a primary effect of the sarcoma-specific translocations is to dictate lineage commitment and/or impair differentiation, as exemplified by the myogenic commitment of NIH-3T3 cells following expression of the *PAX3-FKHR* translocation which is found in alveolar rhabdomyosarcoma (Khan et al., 1999), thus expanding the pool of cells that may be susceptible to the "second hit." Similar models are emerging in leukemia biology wherein specific translocations induce differentiation arrest at distinct stages of hematopoiesis but second genetic events are required for the full induction of the leukemic state (Dash et al., 2002; Kelly et al., 2002).

A second group of sarcomas (exemplified by osteosarcoma and leiomyosarcoma) is characterized by a chaotic karyotype and the lack of distinct fusion genes. Mutations in the tumor suppressor genes Rb and p53 are found with varying frequency in both groups, but it appears that disturbances in mechanisms that regulate genomic integrity are a common feature of the cytogenetically complex sarcomas. Aside from the translocations, few initiating events in sarcomas have been described. Amplification of the cell-cycle-related genes *CDK4* and *MDM2* occurs sporadically in various sarcomas, but otherwise little is known of cooperating genes that contribute to tumor progression. Obtaining a more comprehensive description of the genes involved in the initiation and progression of sarcomas remains a major goal for future research. It is of interest to note that two common benign mesenchymal tumors, leiomyomas and lipomas, have a high incidence of recurrent rearrangements of chromosome 12q involving the high mobility protein group gene *HMGIC* (Schoenmakers et al., 1995), but these translocations are not seen in the corresponding leiomyosarcomas or liposarcomas, indicating that the benign tumors are not precursor lesions in these cases. In contrast, plexiform neurofibromas, benign tumors associated with NF-1 syndrome, have loss of neurofibromin expression, and this does appear to be the precursor lesion in MPNST occurring in the setting of NF-1. In addition, sarcoma genetics may yield important insights into the stem cell biology of connective tissue, which could potentially impact novel areas of biomedical research such as tissue engineering.

Conventional diagnostics and therapeutics

Until recently, the diagnosis of a sarcoma depended solely upon

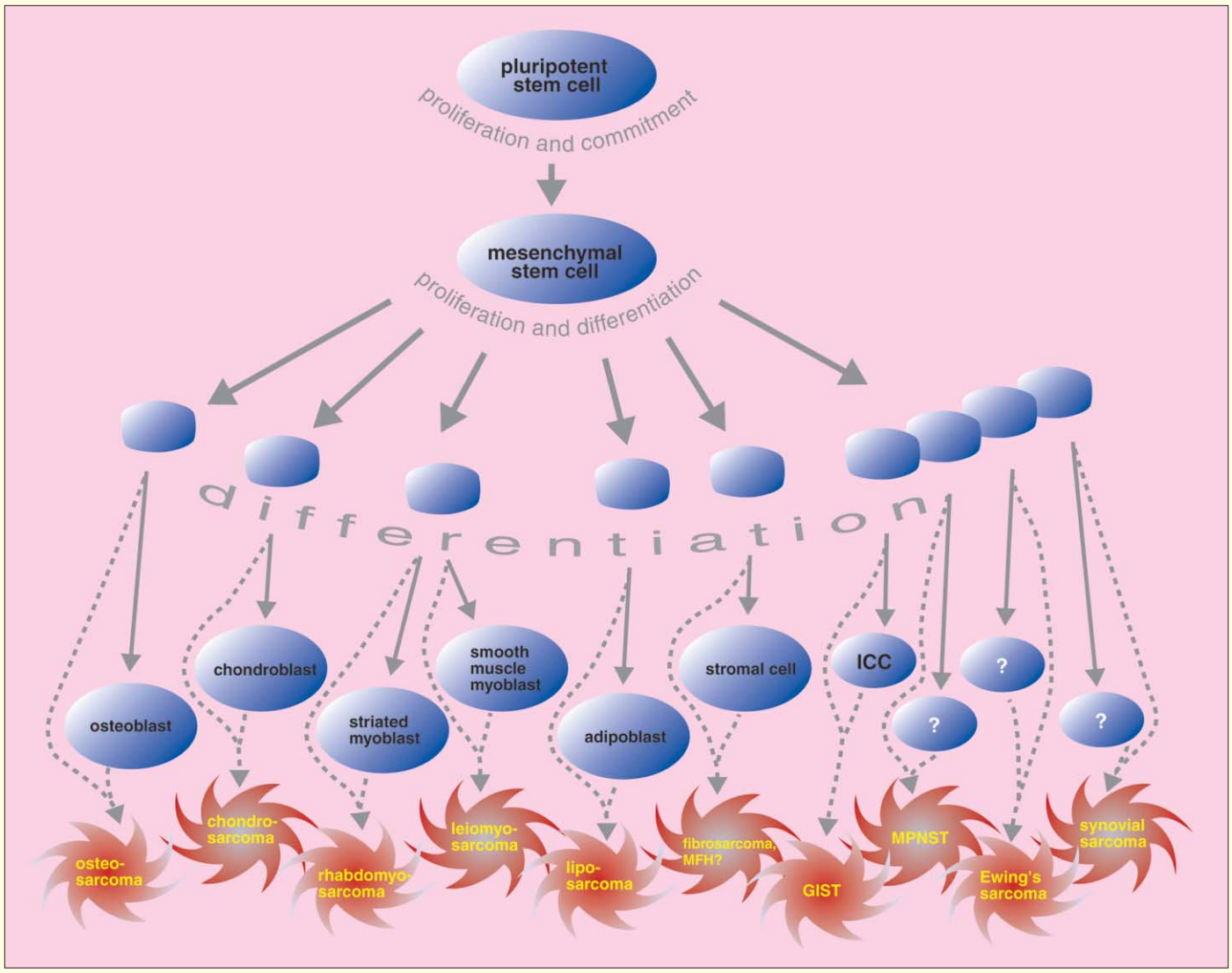

Figure 1. Schematic model for differentiation of mesenchymal tissues and sarcoma tumorigenesis

Boxes represent progenitor cells for each lineage shown. Solid lines designate normal differentiation and dotted lines represent potential pathways for sarcoma tumorigenesis. For some sarcomas (e.g., MPNST, SS, and Ewing's), the exact lineage remains unknown. ICC = interstitial cells of Cajal.

histopathologic review of a biopsy sample, and this remains an important part of the overall diagnostic evaluation. The tumor is deemed to be malignant based on standard criteria such as anaplasia and invasion, among others, and tumors are given specific diagnoses based upon assignment to a particular differentiation lineage (fat, cartilage, or bone, for example). Differentiation lineage is generally determined based upon histologic appearance using both H&E staining and immunohistochemical stains using lineage-specific antibodies. But, even with the use of these techniques, the specific diagnosis of a sarcoma can often be difficult, and the addition of cytogenetic and molecular cytogenetic techniques has proven to be particularly useful in the final classification of these difficult cases. In those tumors with reciprocal translocations generating tumor-specific fusion proteins, numerous studies have confirmed the utility of applying RT-PCR techniques to aid in diagnosis (Bennicelli and Barr, 2002).

Staging of sarcomas is determined by the extent of disease and the tumor grade. Extent of disease is related to tumor size, depth of invasion, and the presence or absence of metastases. Unlike carcinomas, which typically spread via lymphatics, sarcomas spread hematogenously, with the lungs and bone being the most common sites of metastasis. High mitotic activity and necrosis within a tumor leads to the assignment of a high grade. While low-stage tumors are generally treated with surgical resection alone and high-stage tumors are treated with adjuvant therapy (e.g., radiation therapy and/or chemotherapy), it is important to note that histologic subtype plays a primary role in determining the effectiveness of adjuvant therapy and overall outcome. For example, adjuvant therapy has shown dramatic success in pediatric sarcomas (e.g., Ewing's sarcoma, osteosarcoma, and rhabdomyosarcoma), where survival has increased from <20% to approximately 70% by the addition of cytotoxic chemotherapy to local therapies (Arndt and Crist,

1999). In other sarcomas, such as synovial sarcoma, MPNST, and myxoid liposarcoma, clinical responses to chemotherapy are frequently seen, but a clear survival advantage has not been demonstrated (Bramwell et al., 1994). Still other sarcomas, such as nonuterine leiomyosarcomas, alveolar soft-part sarcomas, and clear-cell sarcomas, only rarely respond to cytotoxic chemotherapy, and thus new therapeutic targets are critically needed (Singer et al., 2000).

New targets and novel therapies

Because causal-unique molecular genetic alterations have been identified in a number of these tumors, it is predicted that effective targeted therapies could be developed for individual histologies. Gastrointestinal stromal tumors (GISTs) provide a specific example of how molecular characterization of a tumor entity led to both a new diagnostic category of tumor and a new and effective targeted therapy in a tumor that previously had no effective therapy. GISTs had previously been referred to as gastrointestinal leiomyosarcomas, emphasizing a presumed smooth muscle origin based upon histopathology. But unlike uterine leiomyosarcomas that may respond to cytotoxic chemotherapy, "leiomyosarcomas" of the GI-tract are almost uniformly unresponsive to chemotherapy. In 1998, activating mutations in the c-kit oncogene were identified as a distinguishing feature of these tumors (Hirota et al., 1998). Within two years, it was determined that these tumors likely arise from the interstitial cells of Cajal, cells that coordinate peristaltic activity throughout the GI tract and that appear to depend on c-kit signaling for normal differentiation. These tumors were renamed GISTs, and over 90% have now been shown to harbor somatic c-kit mutations (Berman and O'Leary, 2001; Rubin et al., 2001). Of note, true leiomyosarcomas of the GI tract are much rarer than GISTs and do not have any evidence of c-kit mutations. The observation that somatic gain-of-function mutations in the c-kit tyrosine kinase characterized GIST suggested that constitutive activation of c-kit was the critical event in the malignant transformation of this tumor. This was confirmed by evidence that the tyrosine kinase inhibitor STI571 induced apoptosis of GIST cell lines in vitro (Tuveson et al., 2001) and induced dramatic clinical responses as well (van Oosterom et al., 2001). The c-kit specificity of this activity is extrapolated from the evidence that rare GISTs without activating c-kit mutations show much less sensitivity to STI571. Thus, identification and targeting of critical genetic alterations in GIST have dramatically changed both the classification and clinical outcome of this disease (Dematteo et al., 2002).

The identification of similar critical targets in other sarcomas remains the challenge of the future. Another sarcoma, dermatofibrosarcoma protuberans, may also respond to STI571, since the tumor-specific translocation in this tumor leads to overexpression of PDGFβ, whose receptor tyrosine kinase is also inhibited by STI571 (See Table 1). As noted in the introduction, MPNST tumors are associated with homozygous loss of the *NF1* gene, often occurring in the setting of *NF1* with germline loss of one *NF1* allele. The *NF1* gene encodes for a protein known as neurofibromin, which shares homology with the Ras-GAP protein. Ras-GAP inhibits Ras signaling, and the presumption is that loss of NF1 may enhance Ras activity in MPNST tumors. Thus, the use of agents targeting the Ras pathway has been of interest in these tumors, and there is an ongoing study evaluating a farnesyl transferase inhibitor (targeting Ras) in plexiform neurofibromas, a precursor lesion for MPNST.

Importantly, however, even in cases where a causal genetic alteration has been described, the critical downstream targets responsible for initiating and/or maintaining the malignant phenotype often remain elusive. For example, in Ewing's sarcoma, the t(11;22) is generally accepted as the necessary and essential genetic event that initiates and maintains this sarcoma, but pertinent downstream targets that could be inhibited therapeutically have remained elusive (Arvand and Denny, 2001). In the absence of unique causal targets, current research is aimed at targeting growth or death pathways that have some impact on tumor growth, either alone or in concert with other therapies. For example, despite an absence of constitutive c-kit activation, Ewing's sarcomas often express c-kit, and SCF-mediated signaling has been implicated as one of many growth loops involved in survival of this tumor (Landuzzi et al., 2000). STI571 inhibits growth of this tumor, but at higher concentrations than those required by GIST. Whether inhibition of the SCF-mediated growth factor pathway or inhibition of another, less sensitive kinase is responsible for STI571's effects in Ewing's sarcoma is currently under study. In either case, this example illustrates the concept that even secondary growth factor-mediated pathways may render tumors sensitive to the effects of targeted therapeutics (Heinrich et al., 2002).

Another potentially promising approach is the activation of death-receptor pathways in sarcomas. Proof-of-principle that such agents can induce selective tumor kill comes from the efficacy of limb perfusion using TNFα with cytotoxic agents to convert unresectable sarcomas to resectable (Lejeune et al., 2000). Indeed, these trials have led to the approval of TNFα in Europe as a therapeutic agent for unresectable limb sarcoma. While toxicity limits systemic use of TNFα, another member of the TNF family, TRAIL/Apo2L, shows a higher level of tumor specificity (Ashkenazi et al., 1999). TRAIL/Apo2L induces cell death in a variety of sarcomas—including Ewing's sarcoma (Kontny et al., 2001), osteosarcoma (Evdokiou et al., 2002), and rhabdomyosarcoma (Petak et al., 2000)—either alone or in con-

Table 1. Sarcoma translocations resulting in gene fusions

Tumor	Translocation	Genes
Ewing's sarcoma	t(11:22)(q24;q12) t(21;22)(q22;q12) t(7;22)(p22;q12) t(17;22)(q21;q12) t(2;22)(q33;q12)	*EWSR1-FLI1* *EWSR1-ERG* *EWSR1-ETV1* *EWSR1-ETV4* *EWSR1-FEV*
clear cell sarcoma	t(12;22)(q13;q12)	*EWSR1-ATF1*
desmoplastic small round cell tumor	t(11;22)(p13:q12)	*EWSR1-WT1*
myxoid chondrosarcoma	t(9;22)(q22-31;q11-12)	*EWSR1-NR4A3*
myxoid liposarcoma	t(12:16)(q13;p11) t(12;22)(q13;q12)	*FUS-DDIT3* *EWSR1-DDIT3*
Alveolar rhabdomyosarcoma	t(2:13)(q35:q14) t(1:13)(p36:q14)	*PAX3-FOXO1A* *PAX7-FOXO1A*
Synovial sarcoma	t(X;18)(p11:q11)	*SYT-SSX*
Dermatofibrosarcoma protuberans	t(17:22)(q22:q13)	*COL1A1-PDGFB*
Congenital fibrosarcoma	t(12;15)(p13;q25)	*ETV6-NTRK3*
Inflammatory myofibroblastic tumor	2p23 rearrangements	*TMP3-ALK;* *TMP4-ALK*
Alveolar soft part sarcoma	t(X;17)(p11.2;q25)	*ASPL-TFE3*

cert with immunomodulating or cytotoxic therapies. Clinical agents that can ligate the death domain containing TRAIL receptors currently are being developed.

Future challenges

In summary, sarcomas represent a heterogeneous group of diseases, each with a unique and complex pathway to carcinogenesis. The challenge of the next decade will be to define sarcomas according to their unique molecular alterations and to treat them accordingly. The implementation of genomic technologies such as microarray analysis will facilitate this biologically based tumor classification and accelerate drug-target discovery (Allander et al., 2001; Khan et al., 2001). The intriguing possibility that identification of critical genetic events will lead to specific, nontoxic therapies for individual tumors has led to enthusiasm within the scientific community for dissecting the genomic and proteomic pathways that fuel these tumors. The challenge is finding not one, but many "magic bullets" that can effectively target the numerous and varied sarcomas.

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