Elucidation of the Molecular Mechanisms of Malignant Bone Tumors: Refining Diagnosis and Identifying Novel Targets for Treatment

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Abstract
Primary bone tumors are rare, accounting for less than 0.2% of all cancers diagnosed yearly in the United States. Study of the molecular mechanisms of these diseases has given insight into their pathobiology. It has also identified molecular alterations that, if present, may be used in conjunction with histologic evaluation to further refine diagnosis, allowing cases to be stratified into prognostic groups more or less likely to show response to current cytotoxic protocols. Recent findings have lead to the identification of molecular pathways that may serve as targets of novel therapies, especially in the case of Ewing sarcoma. Telomere maintenance mechanisms are also emerging as potential targets for anticancer therapy. As the molecular mechanisms underlying malignant bone tumors are better understood, new anticancer agents targeting specific pathways are likely to emerge. This may make it possible to tailor treatment for each individual patient, using a combination of cytotoxic and targeted therapies based on the histologic and molecular profile of the patient's tumor. (JNCCN 2005;3:142–146)

Molecular Pathogenesis of Bone Tumors

Osteogenic Sarcoma
Osteogenic sarcoma (OS), the most common primary cancer of bone, is a malignancy in which the neoplastic cells directly produce osteoid. It is characterized by complex genetic changes, including loss and amplification of chromosomal regions, and frequent alterations in tumor suppressor gene pathways. Mutation of p53 is commonly associated with OS, and correlates with high levels of genetic instability. The MDM2 gene, a regulator of p53 protein function localized to chromosome 12q13, is frequently amplified in OS. In a study of OS patient samples, Ladanyi et al. found that MDM2 gene amplification was detected more frequently in metastatic or recurrent OS than in primary OS, implicating MDM2 in tumor progression and metastasis. Other tumor suppressor genes, such as INK4A, are also known to be altered in OS. INK4A inhibits CDK4, a cyclin-dependent kinase...
encoded by a gene on chromosome 12q13 that may arrest the cell cycle via Rb (Figure 1). Such genetic alterations dysregulating the restriction checkpoint of the cell cycle are common in OS.

Man et al.10 characterized the genetic changes in OS using array-based comparative genomic hybridization to screen OS patient samples. They found that gains were more common than losses, with the most frequently amplified clones mapping to six chromosomal regions. Further investigations into the molecular mechanisms of OS have led to examination of the Wnt family of proteins and its coreceptor, LRP5. They play an important role in human skeletal development by modulating cell proliferation during embryogenesis.11 In a series of experiments using patient tumor samples, Hoang et al.12 showed that LRP5 is commonly expressed in OS. In vitro, blockade of the LRP5 receptor reduced invasion and motility of human OS cell lines. Furthermore, it induced changes in beta-catenin localization consistent with increased cell to cell adhesion.13 These findings suggest a role for Wnt signaling in the pathobiology of OS.

Because of the frequency of genetic alterations characteristic of OS, several studies have examined the relation of these alterations to various clinical parameters. Morris et al.14 evaluated HER/erbB-2 expression in OS patient samples. They found HER/erbB-2 overexpression in approximately 43% of patient samples assayed. Interestingly, they noted higher levels of expression in samples obtained from patients with relapse or who presented with metastatic disease. Furthermore, HER/erbB-2 expression was associated with inferior event-free survival and significantly less tumor necrosis after preoperative chemotherapy. The significance of these findings is unclear, however, because other groups have not corroborated HER/erbB-2 overexpression in OS.15 A separate in vivo study of canine OS revealed that expression of the membrane-cytoskeleton linker, ezrin, was associated with early metastasis.16 Accordingly, high ezrin expression correlated with poor outcome in pediatric OS patients.

Ewing Sarcoma

Ewing sarcoma is the second most common malignant bone tumor of late childhood and early adulthood. It is a malignant, small, blue, round cell tumor belonging to the family of primitive neuroectodermal tumors (PNET).5 Although the cause of Ewing sarcoma is unknown, it is characterized by the reciprocal translocation t(11;22)(q24;12), which is present in its classical or variant form in approximately 90% and 10% of cases, respectively.17

The classic, or type 1, translocation results in the chimeric transcript EWS-FLI1, whereas alternate translocations result in the EWS-ERG fusion transcript, among others. Because these chimeric transcripts are so common, their detection is useful for diagnosing Ewing sarcoma, and outcome is strongly correlated with which translocation breakpoint (type 1 vs. non-type 1) occurs.18 Comparison of the EWS-FLI1 transcript with the EWS-ERG transcript reveals a lower proliferative rate in former,19 although no significant phenotypic difference was seen between the two.20

Although the reciprocal translocation involving the EWS gene is the primary genetic alteration in Ewing sarcoma, less commonly, secondary genetic changes may also influence tumor progression. The most frequent secondary genetic alteration in Ewing sarcoma is deletion of the cell cycle regulator gene INK4A (Figure 1). INK4A deletion has been found to be a strong negative factor for disease-specific survival21.
and may help identify a subset of patients with Ewing sarcoma who have a poor prognosis. Alterations of p53 have also been identified in Ewing sarcoma patients, and they appear to define a subset of patients with markedly poor outcome.32

Telomere Maintenance Mechanisms
Recent interest has focused on telomere maintenance mechanisms in sarcomas. Telomeres are the repetitive sequences at the ends of chromosomes that shorten with each cell division. Critical telomere shortening leads to cell senescence. Cancer cells avoid senescence by maintaining telomere length by one of two mechanisms: telomerase activity and alternative lengthening of telomeres. Examination of patient tumor samples has revealed that telomerase activation predominates in sarcomas with specific chromosomal translocations, such as Ewing sarcoma. Alternative lengthening of telomeres, conversely, is more common in sarcomas with non-specific, complex karyotypes, such as osteogenic sarcoma.33 Furthermore, an absence of any detectable telomere maintenance mechanism may indicate a subset of OS patients with a more favorable prognosis.24

Treatment of Malignant Bone Tumors
Treatment of osteogenic sarcoma and Ewing sarcoma consists of cytotoxic chemotherapy, usually combined with surgery, radiation, or both. Current research is focused on refining the molecular mechanisms underlying bone sarcomas to try to identify earlier which patients are unlikely to benefit from traditional cytotoxic therapies. Development of novel therapies for these patients is a priority.

Osteogenic Sarcoma
Experts now well understand that osteogenic sarcoma is a systemic disease, with the majority of patients presenting with micrometastatic disease at diagnosis. The current treatment paradigm for OS consists of induction chemotherapy and surgical resection. Most chemotherapy regimens consist of doxorubicin, cisplatin, and high-dose methotrexate, with or without other agents. Five-year event-free survival is approximately 70%. New cytotoxic agents have shown modest activity in OS,25,26 and current efforts are focused on identifying targets for molecular-based therapies. The complex karyotypes that characterize OS make these targets elusive, although dihydrofolate reductase27 is a potential target.

Ewing Sarcoma
Ewing sarcoma, like OS, is a systemic disease, with 90% of patients having micrometastatic disease at presentation. Five-year event-free survival is 70% for patients with localized disease at presentation, and 30% for patients presenting with clinically detectable metastasis.4 Clinical trials have identified two groups of patients with Ewing sarcoma.28 The first group, consisting primarily of patients with localized disease, can experience event-free and overall survival with traditional cytotoxic therapies. The second group consists of patients with metastatic disease at presentation. These patients typically do not benefit from traditional therapies, and novel therapies are needed for them.

Because the EWS-FLI1 chimeric transcript is so common and the different translocation breakpoints impart different transforming ability in vitro and in vivo, it is an attractive target for molecular-based therapy. Another potential target is the beta-platelet-derived growth factor receptor (PDGFR) signaling pathway, which has been found to mediate motility and proliferation of Ewing sarcoma cell lines in vitro.29

Chordoma
Chordoma is a rare malignant bone tumor arising from developmental remnants of notochordal tissue. It is typically a slowly growing, low-grade tumor, although occasionally it may exhibit high-grade areas with a spindle cell component. Its pathobiology is poorly understood, although a locus for familial chordoma has been mapped to chromosome 7q33.30,31 Furthermore, chordoma has shown microsatellite instability, an indirect marker of globally defective DNA mismatch repair.32 In a study of chordoma patient samples, Riva et al.33 found a common molecular lesion at chromosome 1p36.13. Interestingly, telomerase activity has been seen in chordoma,34 suggesting that tumor behavior parallels that which is seen in sarcomas with specific chromosomal translocations, such as Ewing sarcoma. Cell cycle analysis of conventional chordoma has revealed no association between diploid or aneuploid DNA pattern and local recurrence or overall survival.35 However, aneuploidy has been found to be more common in chordomas with spindle cell components than in conventional chordoma.36 Complete surgical
resection is the principal treatment modality for conventional chordoma, because chordoma is largely refractory to chemotherapy. Recently, however, imatinib mesylate was shown to exhibit antitumor activity in chordoma patients whose tumors were positive for beta-PDGFR. As the molecular mechanisms of chordoma are further characterized, novel treatments for this malignancy may be developed.

Conclusions
Elucidation of the molecular mechanisms of bone tumors has given significant insight into their pathobiology. Diagnosis is no longer limited to histologic findings, but rather incorporates molecular alterations that characterize these tumors. Hopefully, this will allow for substratification to identify patients who will not experience a response to traditional cytotoxic therapies early in the course of treatment. As the molecular alterations underlying malignant bone tumors are better characterized, therapies targeting specific pathways will probably emerge. In the future, this may make it possible for each patient to receive a combination of cytotoxic and targeted therapies based on the histologic and molecular profile of his or her tumor.

References


