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Emerging Prognostic Factors for Clinical Care

Molecular Abnormalities

Current investigations are searching for molecular abnormalities that provide information related to prognosis and treatment; however, the field is still in the early stage of development. The following summarizes the critical molecular abnormalities of the most important sarcomas of bone.

Critical Molecular Abnormalities of Primary Sarcomas of Bone

Osteosarcoma

High-grade conventional osteosarcoma. Osteosarcomas are characterized by complex DNA copy number alterations, with few recurrent abnormalities and a high level of genomic instability. To date, the search for common molecular therapeutic targets in osteosarcoma has been disappointing. However, the genomic chaos characteristic of osteosarcoma is shedding light on new mutation patterns through recent whole-genome sequencing studies. Approximately 33% of primary osteosarcomas show evidence of chromothripsis, defined as a single catastrophic event resulting in massive genomic rearrangements and remodeling of a chromosome, compared with 2–3% of cancers overall. Furthermore, half of all osteosarcomas exhibit kataegis, a pattern of localized hypermutation colocalized with regions of somatic genome rearrangements. The regions affected by kataegis are not recurrent, and most mutated genes are not located in these regions. Up to 80% of primary osteosarcoma samples harbor RB1 gene aberrations, whereas 20% of osteosarcomas have either a deletion of CDKN2A (encoding p16-INK4A) or an amplification of CDK4. These findings, along with the reciprocal relationship between RB1 and CDKN2A alterations, suggest that G1/S deregulation by RB1 loss, CDK4 amplification, or CDKN2A loss is nearly universal in osteosarcoma. Another gene significantly associated with osteosarcoma is *TP53*. The frequency of somatic TP53 mutations in osteosarcomas ranges from 19–38%, and TP53 mutations are associated with high levels of genomic instability. An additional 5% of conventional osteosarcomas harbor gene amplification of MDM2 . A high copy number gain of the MYC oncogene at 8q24 was found in 43% of osteosarcomas.

Low-grade osteosarcoma. The presence of ring chromosomes resulting from 12q13-15 gains/amplifications is the cytogenetic hallmark of parosteal osteosarcoma. This abnormality may be investigated in clinical practice either by fluorescence *in situ* hybridization (FISH) for MDM2/CDK4 gene

amplifications or by IHC showing overexpression for MDM2 and/or CDK4 in most cases. Thus, MDM2/CDK4 gene amplification and/or protein overexpression represents a useful adjunct test in challenging diagnoses, reliably distinguishing low-grade osteosarcoma from benign histologic mimics. Although a small subset of conventional high-grade osteosarcomas have been documented to harbor MDM2 gene amplifications, it is likely that high-grade osteosarcomas showing coexpression of MDM2 and CDK4 represent dedifferentiated examples of low-grade osteosarcomas; careful examination in these cases to identify the low-grade component, corroborated with detailed radiographic review, might facilitate accurate subclassification.

Although GNAS mutations initially were detected exclusively in fibrous dysplasia, a more recent study showed GNAS mutations in five of nine cases (55%) of parosteal osteosarcoma, regardless of the presence of a dedifferentiation. These results have not been confirmed in more recent, larger studies, and their validity remains questionable.

Chondrosarcoma

The most prevalent genetic alteration detected in cartilaginous tumors is the somatic mutation of isocitrate dehydrogenase (IDH) genes. Mutations in *IDH1* and *IDH2* are present in 56–61% of chondrosarcomas. Among cartilaginous tumors, IDH mutations appear to be limited to enchondromas, periosteal chondrosarcomas, and central (intramedullary) chondrosarcomas of conventional or dedifferentiated histology. IDH mutations have not been found in secondary peripheral chondrosarcomas, which instead share some of the molecular characteristics of osteochondromas. *IDH1* and *IDH2* mutations also appear to be absent in osteochondromas and osteosarcomas, including chondroblastic osteosarcomas; thus, this molecular test has been proposed as useful in challenging diagnoses. However, a recent report identified the presence of IDH mutations in a subset of conventional osteosarcomas (25%); further studies are needed to validate this finding.

The common IDH mutations in chondrosarcoma affect *IDH1* R132 (~90% of IDH-mutant cases) and *IDH2* at the homologous position, R172 (~10%). These mutations also are common in glioma and acute myeloid leukemia. The mutations block the ability of the enzymes to convert isocitrate to α-ketoglutarate, which in turn increases levels of HIF1A, a subunit of a transcription factor that facilitates tumor growth in hypoxic environments and also interferes with enzymes responsible for demethylation of histones and DNA.

Additionally, a recent whole-exome sequencing study showed that 37% of chondrosarcomas have insertions, deletions, or rearrangements of *COL2A1*, which encodes the α-chain of type II collagen fibers, the major collagen constituent of articular cartilage. These mutations may interfere with the production

of mature collagen fibrils. This study also showed mutations in *IDH1/2* (59%), *TP53* (20%), and genes of the RB1 pathway (33%) and the Hedgehog pathway (18%).

Ewing Sarcoma/PNET

Ewing sarcoma/PNET is characterized by translocations that fuse EWSR1, located at chromosome 22q12, and a gene of the ETS family of transcription factors. In 90–95% of cases, there is a recurrent t(11;22)(q24;q12), resulting in an *EWSR1–FLI1* gene fusion, which contains the N-terminal portion of *EWSR1* and the C-terminal portion of *FLI1*. In the EWSR1–FLI fusion protein, the EWSR1 portion functions as a strong transcriptional activation domain, whereas the FLI portion contributes an ETS-type DNA-binding domain. An *EWSR1–ERG* fusion, resulting from a t(21;22)(q22;q12), is found in 5–10% of cases. Less frequently, *EWSR1* is fused to *ETV1* (7p22), *E1A-F* (17q21), or *FEV* (2q35–36).

EWSR1–FLI1 is structurally heterogeneous, with at least 18 possible types of in-frame EWSR1–FLI1 chimeric transcripts. The two main types, fusion of EWSR1 exon 7 to FLI1 exon 6 (type 1) and fusion of EWSR1 exon 7 to FLI1 exon 5 (type 2), account for about 85–90% of EWSR1–FLI1 fusions. The molecular methods used in clinical practice for detecting this recurrent translocation include reverse transcription polymerase chain reaction (RT-PCR) and FISH. Because of the multiple variants of EWSR1–FLI1 fusion transcripts, several RT-PCR assays with different primer pair designs typically are needed to reliably exclude the presence of an EWSR1–FLI1 fusion. Furthermore, depending on fixation methods, the RNA quality extracted from archival material is suboptimal in up to 30–50% of cases. For these technical reasons, FISH testing for the presence of *EWSR1* gene rearrangements has been applied widely and has increasingly replaced the RT-PCR method in most cases. One important caveat regarding FISH assay is that it interrogates abnormalities in only one fusion gene (i.e., *EWSR1*) and does not provide information on the status of its fusion partner; thus, a positive result may be insufficient to rule out other EWSR1-rearranged positive mesenchymal neoplasms.

Un/Poorly Differentiated Small Round/Spindle Cell Sarcoma with Alternative Gene Fusions (SRC/SCT)

EWSR1-positive small blue round/spindle cell tumors (SRC/SCTs) with alternative non-ETS partners. Rare cases of small blue round cell tumors (SRC/SCTs) recently were reported to carry a fusion between *EWSR1* and genes outside the ETS gene family members. Among this group, a few cases showed *EWSR1* fusions with genes encoding a member of the zinc-finger family of proteins, including EWSR1–PATZ1 or EWSR1–SP3; the *NFATc2* gene, encoding for a member of the nuclear factor of activated T cells (NFAT) transcription factor family; or *SMARCA5*, a chromatin-reorganizing gene.

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FUS-rearranged SRC/SCTs. In a handful of cases, FUS (fused in sarcoma) has been found to substitute for the *EWSR1* gene, being fused to members of the ETS transcription factor family, with FUS–ERG fusion in five cases and FUS–FEV in one case.

CIC–DUX4–positive SRC/SCTs. CIC–DUX4 fusions are emerging as the most prevalent genetic event in EWSR1-negative SRC/SCTs, accounting for two thirds of cases in this group. CIC–DUX4 fusion results from two possible translocation events, either a t(4;19)(q35;q13) or t(10;19)(q26.3;q13). In contrast to classic Ewing sarcoma, these tumors occur preferentially in the soft tissue (90%) and in an older age group (young adults). Microscopically, CIC–DUX4–positive SRC/SCTs are associated with a higher degree of heterogeneity in nuclear shape and size compared with the consistent appearance seen with classic Ewing sarcoma. In some tumors, the neoplastic cells may be spindled. Furthermore, the CD99 immunostain is less diffuse, ranging from patchy to occasionally negative. Preliminary data regarding neoadjuvant therapy and response in metastatic disease indicate that this sarcoma subtype is less sensitive than Ewing sarcoma to standard chemotherapy agents (doxorubicin/ifosfamide or combination five-drug therapy with vincristine/doxorubicin/cyclophosphamide with ifosfamide/etoposide). However, the finding of a CIC rearrangement in nonpediatric patients with an EWSR1-negative SRC/SCT may support adapting the therapeutic plan according to the Ewing sarcoma family of tumors rather than following the adult-type soft tissue sarcoma guidelines.

BCOR–CCNB3–positive SRC/SCTs. A novel X-chromosomal paracentric inversion, resulting in a BCOR–CCNB3 fusion, was described recently in a subset of SRC/SCTs occurring preferentially in bone and often in young male patients. Despite remarkable clinical and pathological (some tumors may contain spindle cells) similarities with the Ewing sarcoma group, gene profiling and single-nucleotide polymorphism array analyses indicate that this group of tumors is biologically distinct from Ewing sarcoma and does not share the EWSR1–ETS expression signature. The latter subset may be identified by detecting CCNB3 overexpression using IHC.

Chordoma

T gene, encoding the protein brachyury, has been implicated in the pathogenesis of chordoma. Brachyury is a tissue-specific transcription factor expressed in the nucleus of notochord cells and is essential for proper notochord development and maintenance. Copy number gain of *T* gene (amplifications, polysomy, minor allelic gain) has been reported in sporadic chordomas, with similar percentages in sacrococcygeal, mobile spine, and skull base tumors.

Recently, a common genetic variant in *T* (rs2305089) was significantly associated with the risk of sporadic chordoma. The susceptibility related to *T*, however, appears to be complex, involving multiple mechanisms, including *T* duplication (essentially seen only in families), and multiple common and rare

variants. Among sporadic cases, another common variant (rs3816300) was significantly associated with risk, and the association was significantly stronger in cases with early-age onset, including cases of skull base chordoma.

Update on Molecular Aberrations in Relation to Prognosis

Ewing Sarcoma/PNET

Based on retrospective studies, the survival of patients whose tumors contain the type 1 EWSR1–FLI1 fusion appears to be better than that of patients with other EWSR1–FLI1 fusion types. However, in contrast, prospective evaluation did not confirm a prognostic benefit for type 1 EWSR1–FLI1 fusions. The EWSR1–ERG fusion is associated with clinical phenotypes indistinguishable from those of EWSR1–FLI1–positive Ewing sarcoma. Whole-genome sequencing has shown that Ewing sarcomas harboring mutations in *STAG2* and *TP53* have a poor outcome.

Risk Assessment Models

The AJCC recently established guidelines that will be used to evaluate published statistical prediction models for the purpose of granting endorsement for clinical use. Although this is a monumental step toward the goal of precision medicine, this work was published only very recently. Therefore, the existing models that have been published or may be in clinical use have not yet been evaluated for this cancer site by the Precision Medicine Core of the AJCC. In the future, the statistical prediction models for this cancer site will be evaluated, and those that meet all AJCC criteria will be endorsed.

Recommendations for Clinical Trial Stratification

The new staging strategies for appendicular skeleton, trunk, skull, and facial bones; spine; and pelvis should be incorporated in clinical trial design. Additionally, stratification based on surgical margin reporting (R0, R1, and R2) is strongly recommended. As newly modified, reporting of pathological grade should use a three-grade system.

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