

# Molecular Characterization of Bone Tumors and Implications for Treatment and Prognosis

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## Abstract

Neoplastic transformation is a consequence of maladaptive alterations in the cellular processes normally involved in cell growth, proliferation, differentiation, and survival. Despite the relative infrequent nature of skeletal neoplasms, current understanding of the pathobiology underlying these conditions is becoming increasingly characterized. This article highlights some of the established molecular abnormalities identified in various benign and malignant skeletal neoplasms and how they pertain to tumor biology, diagnosis, and prognosis. Most of the commonly accepted cellular aberrancies in skeletal neoplasms pertain to mutations, copy number changes, and/or chromosomal rearrangements. However, it is becoming increasingly understood that the complexity of tumorigenic pathways necessary for neoplastic growth are manipulated by numerous overlapping alterations in the genetic code and are further influenced by higher-order molecular programs, such as pretranscriptional and posttranscriptional regulation and chromatin reorganization. Over time, identification and quantification of these increasingly recognized neoplastic processes will gradually translate into valuable clinical applications, enhancing the current diagnostic and prognostic capabilities. (*J Natl Compr Canc Netw* 2014;12:214–220)

**B**oth benign and malignant bone neoplasms are the product of myriad molecular aberrations. At some fundamental level, the molecular perturbations present in any neoplasm disrupt or enhance the normal pathways that define a cell's character, including tissue traits, speed of growth, and responsiveness to signals from neighboring cells or circulating factors.

Despite the increasing complexity of cellular pathways involved in cell growth, proliferation, and differentiation, the central tenet of oncogenic transformation involves the collective dysregulation of 7 key regulatory cell pathways: self-sufficiency in growth signals, insensitivity to antigrowth signals, evasion of apoptosis, limitless replicative potential, angiogenesis, metastasis and tissue invasion, and immune evasion.<sup>1,2</sup> Subtle differences in how these essential pathways are disrupted dictates the various biological attributes of different benign and malignant neoplasms, and greatly influences clinical variation among different patients with an identical diagnosis.

Perhaps because of limits in current understanding, neoplasia is considered to be a genetic disease process. Neoplastic cells will ultimately display different tissue traits, speed of growth, or responsiveness to outside signals because they express different levels of certain proteins or express uniquely altered proteins that gain non-native functions. These proteins all derive from genes, leading to an understanding of neoplasia and oncogenic transformation primarily through genes.

Much investigational effort in the past decade has elucidated a variety of mechanisms to impact gene expression. Certainly some neoplastic genetic aberrations derive from deleterious alterations in the genetic code itself, such as gene mutations, amplifications, deletions, or higher-order events that alter chromosomes, but many more are likely dysregulated through nongenetic or epigenetic mechanisms, such as micro-RNAs or chromatin remodeling. Historically, as much was learned about the genes lost specifically in cancer cells by studying the germline genetics of heritable cancer predisposition syndromes 20 years ago, most of the nongenetic molecular mechanisms at work in neoplasia is understood by translating each mechanism into the more tractable language of which genes or pathways each upregulates or downregulates.

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Currently, none of these not-strictly-genetic molecular mechanisms that are likely at work in neoplasia have even approached clinical importance in the field of bone tumors; although they probably will in the future, this review focuses on genes instead. Even the application of genetic knowledge to bone tumors strays deeply into the research realm and away from clinical standards and practices. For each specific genetic association discussed, it will be made clear which have clinical implications currently or in the foreseeable future. Study of the other lesser-understood genetic associations focuses on the hope that understanding the genetic mechanisms of disease will highlight improved targets for therapy or diagnostic testing.

## Benign Bone Neoplasms

### Osteochondromas

Osteochondromas are one of the most common benign skeletal neoplasms, characterized by a cartilage-capped bony outgrowth, which is contiguous with the underlying medullary canal. The cell of origin is a proliferating chondrocyte derived from the growing physis.<sup>3</sup> The hallmark genetic abnormality in these cartilage growths is biallelic inactivation of the *EXT1* or *EXT2* gene in some portion of the chondrocytes. These gene inactivations are either acquired sporadically or through loss of heterozygosity after inheriting one inactive allele. The latter, hereditary form is called *multiple osteochondromas* (MO) or *multiple hereditary exostosis* (MHE). Both *EXT* gene products are fundamentally involved in heparan sulfate (HS) synthesis, and HS deficiency in physal chondrocytes is thought to promote proliferation and loss of polarity.<sup>4</sup> In nearly 80% of sporadic osteochondromas, biallelic loss of *EXT1* is observed in the cartilaginous cap.<sup>5</sup> MHE is an autosomal dominant condition, characterized by a heterozygous mutation in either *EXT1* or *EXT2* in 65% and 35% of cases, respectively.<sup>6</sup> The risk of malignant transformation to secondary peripheral chondrosarcoma is rare: less than 1% of solitary lesions and 1% to 5% in patients with MHE.<sup>7,8</sup>

### Enchondromas

Enchondromas are also benign cartilage neoplasms, which originate in the medullary cavity of bone. Most lesions are solitary; however, multiple lesions

(enchondromatosis) are observed in rare skeletal dysplasias, such as Ollier disease and Maffucci syndrome. Mutations of the isocitrate dehydrogenase genes, *IDH1* and *IDH2*, are observed in roughly 50% of solitary lesions and 90% of patients with Ollier disease and Maffucci syndrome.<sup>9,10</sup> Ollier disease and Maffucci syndrome are not hereditary conditions; mutations of the *IDH1* and *IDH2* genes are acquired, likely in the mesoderm, causing a somatic mosaic distribution. Consequently, these mutations are infrequently observed in normal tissues and normal chondrocytes within the cartilage cap.<sup>9,11</sup> *IDH1* mutations are most common, which lead to gain of function, resulting in accumulation of a metabolite, D-2-hydroxyglutarate, and transcriptional repression of various genes.<sup>12</sup> *IDH1* mutations are also observed in other benign and malignant cartilaginous neoplasms, such as periosteal chondroma and central chondrosarcoma, while absent from others, such as synovial chondromatosis, chondroblastoma, chondromyxoid fibroma, and clear cell chondrosarcoma.<sup>10,11</sup> The risk of malignant transformation in solitary enchondromas is rare, whereas malignant degeneration into secondary central chondrosarcomas occurs in 25% to 50% and greater than 50% of patients with Ollier disease and Maffucci syndrome, respectively.<sup>13,14</sup> Lesions involving the pelvic bones in these patients are particularly high risk.<sup>13</sup>

### Fibrous Dysplasia

Fibrous dysplasia is a benign monostotic or polyostotic fibro-osseous metaplasia involving the medullary bone cavity and cortex. Lesions often develop during adolescence or early adulthood, but can remain undetected for years. Similar to enchondromatosis, fibrous dysplasia results from a postzygotic mutation, in this case an activating mutation in the *GNAS* gene, which encodes the  $\alpha$ -subunit of the stimulatory protein, GS- $\alpha$ .<sup>15</sup> Replacement of Arg201 with a Cys or His residue results in constitutive activation of GS- $\alpha$ , which in turn constitutively activates adenylyl cyclase, resulting in the accumulation of cyclic adenosine monophosphate and stimulation of various downstream cellular processes.<sup>16</sup> It has been demonstrated that *GNAS* mutations in marrow stromal cells impair differentiation potential into adipocyte lineages and promotes osteoclastogenic signaling, mediated partly by receptor activator of nuclear factor- $\kappa$ B ligand and interleukin 6.<sup>15</sup> These alterations impair the normal cellular and mechani-

cal architecture of the local marrow cavity, giving rise to the classic skeletal lesions observed in fibrous dysplasia. Generalized osteomalacia can also be associated with fibrous dysplasia attributable to elevated serum levels of fibroblast growth factor-23 (FGF-23). Posttranslational modification of inactive FGF-23 (iFGF-23) by fibrous dysplasia cells is thought to result in increased circulating levels of active FGF-23 (cFGF-23), which acts on the kidney to promote a net phosphate loss.<sup>17</sup> It has been suggested that quantification of serum FGF-23 levels can provide an indirect measure of disease burden in fibrous dysplasia, although this is not yet used widely.<sup>18</sup>

### Aneurysmal Bone Cyst

Aneurysmal bone cysts (ABCs) are locally aggressive, benign, expansile bone lesions characterized by multiloculated blood-filled cystic cavities. ABCs can present as primary lesions or secondary lesions within other benign and malignant neoplasms, such as giant cell tumor of bone, osteoblastoma, fibrous dysplasia, chondrosarcoma, and osteosarcoma. Although decidedly lacking any metastatic potential, ABCs are highly destructive, promote mechanical insufficiency in the host bone, and frequently recur after excision. Approximately 70% to 80% of primary ABCs harbor a somatic translocation involving rearrangement of the ubiquitin specific peptidase 6 (*USP6*) gene on chromosome 17, most commonly to the cadherin 11 gene (*CDH11*) on chromosome 16 (t16:17).<sup>19</sup> Other fusion partners include *TRAP150*, *ZNF9*, *OMD*, and *COL1A1*, although they account for less than 30% of translocation-positive ABCs. Rather than generating fusion protein products, these translocations bring the *USP6* gene expression under the control of the promoters of the more highly expressed partner loci. These rearrangements occur in a low-prevalence spindle cell population within the ABC. The increased transcriptional level of *USP6* is thought to facilitate tumorigenesis via nuclear factor- $\kappa$ B (NF- $\kappa$ B) induction of matrix metalloproteinase expression and recruitment of many reactive cells that constitute most of the tumor tissue.<sup>20</sup> The diagnostic utility of detecting *USP6* rearrangements is questionable given the absence of these abnormalities in 30% of primary lesions and all secondary lesions, and lack of any treatment or prognostic implication of molecular testing.

## Malignant Bone Tumors

### Conventional Osteosarcoma

Conventional osteosarcomas are high-grade lesions characterized by a plethora of complex numerical and structural chromosomal abnormalities. This high degree of chromosomal instability in osteosarcoma predisposes to significant intra- and intertumoral heterogeneity.<sup>21</sup> Regardless, high-resolution microarray and in situ hybridization studies have identified consistent genomic regions of copy number amplifications and deletions in conventional osteosarcoma.

Deletions or copy number losses of the retinoblastoma gene *RB1* (13q14), *TP53* (17p13), *CDKN2A* (9p21), and *LSAMP* (3q13) are common observations in conventional osteosarcoma. Osteosarcomas are the second most common malignancy in patients with hereditary retinoblastoma, attributable to mutations within or epigenetic inactivation of the *RB1* locus. A total of 60% of retinoblastoma cases are the result of sporadic mutations/inactivation, whereas 40% are an inherited autosomal dominant germline mutation with 100% penetrance.<sup>22</sup> The RB protein is one of the master regulators of cell cycle progression, involved in G1 checkpoint control, regulation of apoptosis, maintenance of senescence, chromosome stability, and chromatin organization.<sup>22</sup> The RB protein also functions as a transcriptional coactivator of osteoblast differentiation, which may explain why inactivation of the *RB1* gene predisposes to osteosarcomagenesis.<sup>23</sup> Roughly 35% of sporadic osteosarcomas are associated with direct inactivation of the *RB1* locus (DNA mutations, loss of heterozygosity, and deletion), although RB inactivation does not seem to correlate with prognosis.<sup>24</sup>

Mutations or losses at the *TP53* locus are also common in sporadic osteosarcomas, present in 40% of cases. Similar to the RB protein, TP53 is an essential tumor suppressor protein that functions as a transcriptional activator for a variety of vital cellular processes, such as initiation of DNA repair, cell cycle arrest, senescence, and apoptosis.<sup>25</sup> In 10% of cases, amplifications of the *MDM2* locus (12q15) are observed, which functions as a TP53 antagonist. In parosteal osteosarcomas (low grade, surface variant), *MDM2* amplifications are observed in 80% to 90% of cases, often associated with a supernumerary ring chromosome.<sup>26</sup> Again, similar to RB inactivation, *TP53* loss does not

prognosticate histologic response to chemotherapy or overall survival.<sup>27</sup>

The *CDKN2A* locus encodes for 2 important tumor suppressor genes: *INK4A* (also called p16) and *ARF* (also called p14). Both are translated from the same transcript, the second from an alternate reading frame (the source of its name). *INK4A* loss essentially silences the RB1 cell cycle checkpoint, and *ARF* loss concomitantly silences the TP53 cell cycle checkpoint. *CDKN2A* is deleted in 15% of conventional osteosarcomas and has been associated with inferior clinical outcomes.<sup>28</sup>

*LSAMP* is a membrane-associated protein originally associated with development of the central nervous system, but recent reports have suggested that it also functions as a tumor suppressor in osteosarcoma.<sup>29</sup> *LSAMP* loss also seems to be associated with advanced disease and poor survival.<sup>29,30</sup>

Alternatively, the 2 most common amplified genomic regions observed in conventional osteosarcoma house the *RUNX2* gene and the *MYC* oncogene. Both of these targets are amplified in 40% to 50% of cases. *RUNX2* expression is associated with osteoblast differentiation, and overexpression in osteosarcomas is associated with an inferior histologic response to chemotherapy.<sup>31</sup>

### Chondrosarcomas

Primary central chondrosarcoma is classified primarily based on histologic grade. Presently, histologic grade also remains the single most important prognostic variable. *IDH1* and *IDH2* mutations are observed in 50% to 60% of primary central chondrosarcoma cases, in contrast to a mutation frequency of 50% and 90% in solitary enchondromas and multiple enchondromas associated with Ollier disease and Maffucci syndrome, respectively. *IDH1* and *IDH2* mutations are not observed in osteosarcomas, and therefore the detection of *IDH1* mutations has been suggested as a means to differentiate chondroblastic osteosarcomas from chondrosarcomas in ambiguous cases.<sup>10,11</sup> *IDH1* and *IDH2* mutations are also observed in periosteal chondrosarcomas and secondary central chondrosarcomas, but not osteochondromas or secondary peripheral chondrosarcomas.<sup>11</sup> Interestingly, in secondary peripheral chondrosarcomas, homozygous *EXT1* or *EXT2* mutations are not common, despite their prevalence in osteochondromas.<sup>32</sup> These results suggest that malignant transformation of osteochondromas to form peripheral chondrosar-

comas often occurs in the wild-type chondrocyte passenger cells in the mosaic cartilaginous cap.

Complex copy number alterations and polyploidization are associated with increasing histologic grades in chondrosarcomas.<sup>33</sup> Dysregulation of the *RB1* pathway is present in more than 80% of high-grade lesions.<sup>34</sup> In dedifferentiated chondrosarcomas, which are associated with dismal 5-year survival, *IDH1/IDH2* mutations are also common (50%), as are *TP53* mutations and *MYC* amplifications. Many of these mutations and amplifications are shared between both differentiated and dedifferentiated components, suggesting a common cellular origin.<sup>35,36</sup>

### Ewing Sarcoma

Ewing sarcoma is a quintessential translocation-associated malignancy; virtually all tumors harbor a balanced chromosomal translocation fusing the *EWSR1* gene on chromosome 22 with 1 of 5 members of the ETS family of transcription factors. The *EWS/FLI* fusion, t(11;22), is most common, constituting 85% of cases, followed by *EWS/ERG* (5%–10%). Fusions involving *ETV1*, *ETV4*, and *FEV* are infrequent, representing fewer than 5% of cases together.<sup>37</sup> Other Ewing-like sarcomas have been observed, characterized by *EWS* fusions to other non-ETS proteins, such as *NFATc2*, *SMARCA5*, *PATZ1*, and *SP3*. Ewing sarcomas are frequently recognized as a “small round blue cell” malignancy, characterized by monolayered, homogenous, undifferentiated malignant cells. Histologic preparations of Ewing sarcoma usually stain with immunohistochemistry against the membrane-associated protein CD99, although lymphoblastic leukemias, synovial sarcomas, and myeloid sarcomas may also stain for CD99, rendering the test anything but pathognomonic.<sup>38,39</sup> Consequently, molecular studies directed at detecting *EWSR1* rearrangements via reverse transcriptase PCR or fluorescence in situ hybridization with an *EWSR1* break-apart probe are now used in concert with histologic evaluation to render a definitive diagnosis. These tests do not negate the need for appropriate clinical history and diagnostic imaging, because other musculoskeletal sarcomas harbor *EWSR1* rearrangements, such as desmoplastic round cell tumor (*EWS/WTI*), extraskeletal myxoid chondrosarcoma (*EWS/NRYA3*), myxoid liposarcoma (*EWS/DDIT3*), and clear cell sarcoma (*EWS/ATF1*).

EWS/FLI and related EWS/ETS chimeras are potent oncogenic transcription factors, known to up- and downregulate numerous direct and indirect target genes important for oncogenesis.<sup>40</sup> One of these direct targets, *NKX2.2*, is a transcription factor responsible for a significant portion of the EWS/FLI-repressed transcriptional signature,<sup>40</sup> and positive immunostaining for this protein has been shown to differentiate Ewing sarcoma from other small round blue cell malignancies, with a sensitivity and specificity of 93% and 89%, respectively.<sup>41</sup> *TP53* mutations and *CDKN2A* deletions are infrequent in Ewing sarcoma (10%–15% of cases). Loss of either has been associated with inferior clinical outcomes, although this remains controversial.<sup>42</sup> The type of EWS/ETS fusion or the varied *EWSR1* breakpoints are not considered prognostic.<sup>43,44</sup> Like other malignant bone tumors, the clinical detec-

tion of metastatic disease is the most important prognostic indicator in Ewing sarcoma.

## Conclusions

Table 1 summarizes the identified molecular abnormalities associated with various benign and malignant bone neoplasms. Importantly, despite the increasing discovery of genetic mutations, copy number variations, and molecular dysregulation essential for tumorigenesis in skeletal neoplasms, most cases remain diagnosed through integrating clinical information with detailed radiographic studies and meticulous histologic evaluations. Except for Ewing sarcoma, most molecular diagnostic tests for malignant bone neoplasms are not pathognomonic of a specific tissue diagnosis. As each is also expensive and technically challenging, the use of most molecu-

**Table 1 Molecular Abnormalities Associated With Bone Neoplasms**

	Inactivated Tumor Suppressors	Activated Oncogenes	Chromosomal Abnormalities and Translocations	Associated Syndromes	Diagnostic Aberrations	Prognostic Aberrations
<b>Benign bone lesions</b>						
Osteochondroma		<i>EXT1/EXT2</i>		MHE		
Enchondroma		<i>IDH1/IDH2</i> <i>PTH1R</i>		Ollier disease Maffucci syndrome		
Fibrous dysplasia		<i>GNAS</i> (constitutive activation of adenylyl cyclase)		McCune-Albright and Mazabraud syndrome		
Aneurysmal bone cyst			<i>t(16;17) – CDH11/USP6</i> <i>t(17;17) – COL1A1/USP6</i>		<i>USP6</i> break-apart FISH	
<b>Malignant bone lesions</b>						
Conventional osteosarcoma	<i>RB</i> , <i>TP53</i> , <i>INK4A</i> , <i>INK2A</i> , <i>LSAMP</i>	<i>MDM2</i> , <i>CDK4</i> , <i>cMYC</i> , <i>CCND1</i> , <i>ERBB2</i>		Retinoblastoma Li-Fraumeni Rothmund-Thompson Werner syndrome		<i>LSMAP</i> loss <i>INK4A/ARF</i> loss
Parosteal osteosarcoma		<i>MDM2</i> , <i>CDK4</i> , <i>SAS</i>	Supernumerary ring chromosomes			
Chondrosarcoma		<i>IDH1/IDH2</i>		Maffucci syndrome Ollier disease MHE	<i>IDH1</i> mutations	
Dedifferentiated chondrosarcoma		<i>IDH1/IDH2</i> <i>cMYC</i>				
Ewing sarcoma	<i>TP53</i> (10%–15%) <i>INK4A</i> (10%–15%)		<i>t(11;22) – EWS/FLI</i> <i>t(21;22) – EWS/ERG</i> <i>t(7;22) – EWS/ETV1</i> <i>t(17;22) – EWS/ETV4</i> <i>t(2;22) – EWS/FEV</i>		EWS/ETS fusion RT-PCR <i>EWSR1</i> break-apart FISH IHC – CD99, NKX2.2	<i>TP53</i> mutation? <i>INK4A</i> (p16) loss?

Abbreviations: FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; MHE, multiple hereditary exostoses.

lar characterization of bone neoplasia is currently reserved for research purposes or the deciphering of ambiguous cases. Currently, histologic grade and detectable disseminated disease remain the most important prognostic variables for malignant bone sarcomas. However, the ongoing elucidation of key, measureable molecular pathways associated with important variations in tumor biology may soon provide the opportunity to more effectively stratify individual disease behaviors and select more efficacious, targeted therapies.

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