

2.

Genetics of Osteosarcoma

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Primary malignant tumors of bone are rare and constitute one of the more uncommon types of neoplasms. Only about 1,500 new bone sarcomas are reported in the United States each year. Yet, because of the effects of radical surgery and chemotherapy, the very existence of these tumors leads to a significant reduction in the quality of life in children and adolescents. Notwithstanding their rarity, primary tumors are important for understanding cancer and its treatment.

Osteosarcoma is the most common primary tumor of bone and accounts for approximately 19% of all malignant tumors in bone and 40–60% of all primary malignant tumors of bone [59, 128, 186, 236, 297]. It is the most common solid tumor in teenagers and the third most common malignancy in children, accounting for 7% of all adolescent cancers [321]. Twenty years ago, the advent of a multidisciplinary approach that combined multi-agent chemotherapy and limb-sparing surgery greatly improved the survival rate of patients with osteosarcoma. Sadly, since then the 5-year survival has plateaued at approximately 70% and outcome has not improved significantly; indeed, long-term complications of osteosarcoma survivors treated with intensive chemotherapy have increased [90]. Furthermore, the prognosis for patients with metastatic disease or those with local relapse is much worse; if patients develop extrapulmonary metastatic disease, they almost never survive [12, 128].

2.1 Histopathology of Osteosarcoma

The defining characteristic of osteosarcoma is the production of osteoid [297]. Beyond this, osteosarcoma can be divided into several subtypes based on histopathological and clinical features. Most broadly, the tumors can be divided into those that arise within the bone (intramedullary) and those that arise on the surface of the bone [199]. Most intramedullary osteosarcomas are highly malignant and most frequently occur during adolescence [297]. In contrast, most osteosarcomas that occur on the surface of the bone tend to be less aggressive and contain cells that are either well or moderately well differentiated.

Intramedullary osteosarcoma tumors are typically localized to the metaphyseal portion of the long bones, with the majority of tumors occurring in the distal femur and proximal tibia. These tumors can have predominant elements of osteoblastic, chondroblastic, or fibroblastic differentiation (Fig. 2.1). Other histopathological features include a small round cell variation [10, 11, 202] and a variation with giant osteoclast-like cells [19, 201, 253]. The molecular and/or genetic bases of these histologic variations have yet to be systematically explored.

Osteosarcoma is characterized by osteoblast-like tumor cells that produce a disorganized field

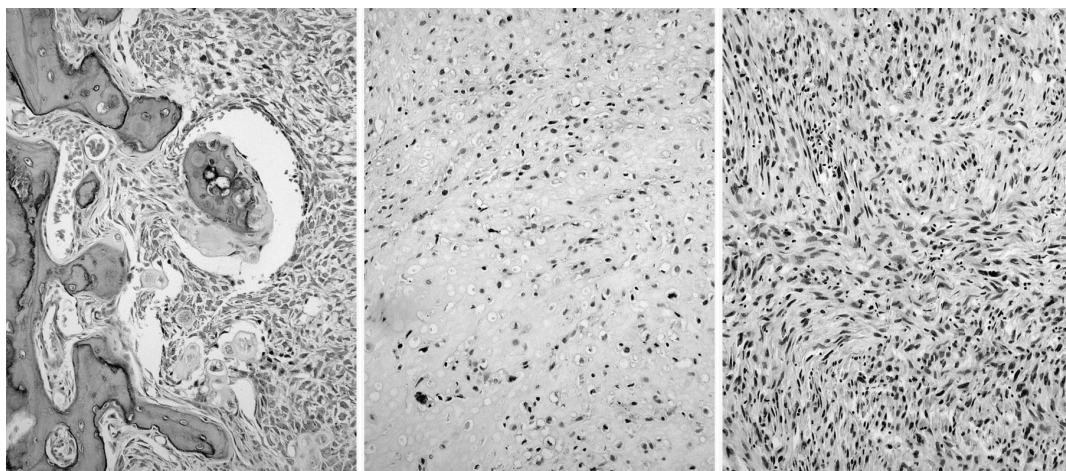


Figure 2.1. Histopathology of conventional intramedullary osteosarcoma. Shown left to right are examples of osteoblastic, chondroblastic, and fibroblastic subtypes of conventional intramedullary osteosarcoma. In each example, obviously anaplastic cells and osteoid production can be detected. In the osteoblastic example, reactive bone is found retained within the neoplasm and serves as a scaffold upon which osteoid is deposited by the neoplastic cells. In the chondroblastic subtype, osteoid production by neoplastic cells requires careful search to be detected in the cartilaginous material. In the fibroblastic subtype, there is an admixture of pleomorphic spindled cells and enlarged polygonal cells and only focal presence of osteoid.

of calcified tissue, including osteoid and bone. Osteosarcoma tumors can be highly cellular, with little osteoid production, or sparsely cellular, with abundant calcified osteoid matrix. Unusual or undifferentiated tumor cells occur frequently in osteosarcoma tumors, as are foci of neoplastic cartilage or fibrous tissue. This can result in misdiagnosis of chondrosarcoma or fibrosarcoma in poorly sampled pathological specimens.

Osteoid production is associated with well-vascularized tumors, whereas malignant cartilage is more commonly associated with poorly vascularized tumors. This may be one reason that chondroblastic differentiation is associated with a slightly worse response to chemotherapy than other types of intramedullary osteosarcomas, owing to poor delivery of the drug. Predominantly osteoblastic tumors are typically sparsely cellular, with unusual mineralized matrix, and are in juxtaposition to native trabecular and cortical bone. Sheets of tumor cells are pushed against malignant bone with no osteoblasts lining the surface.

Although cell types vary, osteosarcomas have in common cytological characteristics such as pleomorphism, hyperchromatism, and abundant atypical mitoses. Epithelial-like cells have been found in some osteosarcomas. This finding suggests that some osteosarcomas

arise from primitive pluripotent mesenchymal stem cells [151]. Other osteosarcomas appear to arise from mesenchymal stem cells with rhabdomyosarcomatous-like or lipomatous-like features [154, 177, 196, 243], or from more committed osteoprogenitor lineage cells [30].

Unfortunately, the histopathological classification has little or no prognostic significance. Osteosarcomas also can be divided into sclerotic and lytic subgroups, but this too has no value in clinical prognosis. At present, more than 80% of patients with appendicular osteosarcoma with no distant metastases will become long-term survivors [152].

2.2 Unconventional Osteosarcoma Subtypes

One unusual subtype of intramedullary osteosarcoma is telangiectatic osteosarcoma [185, 200] (Fig. 2.2). The tumor is almost completely lytic in appearance, resembling an aneurysmal bone cyst with large hemorrhagic cystic cavities that contain blood clots, tumor fragments, and tissue debris. Curiously, these tumors appear to arise in the metaphysis and then to extend into the diaphysis. Histologically,

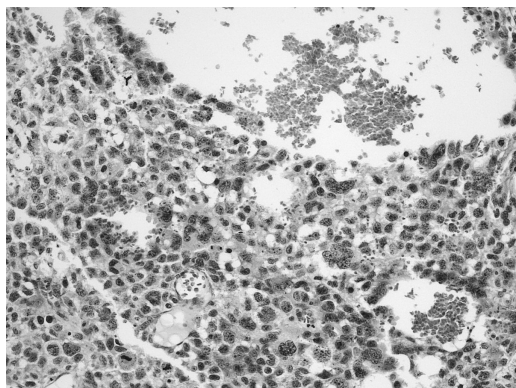


Figure 2.2. Histopathology of telangiectatic osteosarcoma. Note the characteristic cystic vasculature spaces at the top and right of the example. Osteoid production is minimal. These tumors can be confused with aneurysmal bone cysts.

the hemorrhagic cysts contain tumor cells and giant cells that line the septa of the cysts. Osteoid is produced by the tumor cells within these cysts. Response of these tumors to chemotherapy appears to be similar to that of other intramedullary osteosarcomas [13, 317].

Surface osteosarcomas arise and are confined to the surface of the bone and do not involve the medullary canal. They are divided into three categories: periosteal, parosteal, and high-grade surface osteosarcomas [132, 241, 254]. Periosteal osteosarcoma typically occurs as a diaphyseal lesion on the tibia or femur and can be mistaken for periosteal chondrosarcoma [94, 227, 245, 246]. Histologically, periosteal chondrosarcoma is composed of lobules of atypical proliferation, with the center of the tumor displaying mineralization, whereas the peripheral portions of the tumor tend to be composed of proliferative spindle-shaped cells. Whether chemotherapy affects the long-term outcome of periosteal osteosarcoma is controversial [85, 132].

Parosteal osteosarcoma is a slow-growing, relatively indolent tumor [166, 218, 262, 299] that is densely mineralized and envelops the shaft of the bone. It is characterized by low-grade fibroblast-like spindle cells with minimal cellular atypia that line the long axis of the bone with embedded sheets of fibrous stroma. The osteoid that lines the tumor merges with the underlying fibrous tissue. Parosteal osteosarcoma rarely metastasizes, with recurrence locally the major

risk. Occasionally, indolent tumors become anaplastic. The resulting condition is designated dedifferentiated parosteal osteosarcoma [1, 262, 280, 322]. The dedifferentiated component is characterized by a pleomorphic spindle cell phenotype. Although surgical resection appears sufficient for the more indolent tumor, adjuvant chemotherapy is recommended for the dedifferentiated form of the disease [262].

High-grade surface osteosarcomas are rare variants of the surface osteosarcoma. The tumors appear similar on histological examination to conventional intramedullary osteosarcomas, except that they are confined to the surface of the bone. Osteoid and bone production also are similar to intramedullary tumors. Outcome is generally similar to intramedullary osteosarcoma [113, 219].

Extraskelatal osteosarcomas provide an interesting insight into the disease. They arise within the muscle or soft tissues, usually of the thigh and buttock regions, and do not involve bone [2, 14, 35, 53, 157, 164] (Fig. 2.3). Mean age of onset is later than the bony osteosarcomas. Histologically, the tumors present with any of the differentiation patterns of intramedullary osteosarcoma: chondroblastic, fibroblastic, osteoblastic, small cell, giant cell-rich tumors, or even telangiectatic phenotypes [53]. It is tempting to think these tumors arise from mesenchymal stem cells located within the soft tissues and undergo osteoprogenitor differentiation as part of their tumorigenic process.

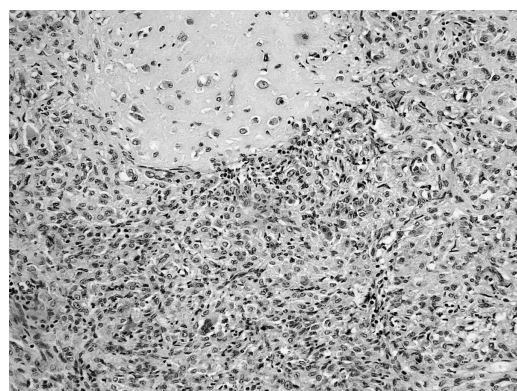


Figure 2.3. Histopathology of extraskelatal osteosarcoma. Note the chaotic mixture of cell types that compose the tumor.

2.3 Head and Neck Osteosarcoma

About 6–13% of osteosarcomas occur in the head and neck, with the most common site being the mandible, followed by the maxilla and the other bones of the skull [37, 60, 123, 178]. Craniofacial osteosarcoma can be either primary, i.e., arise in the absence of known predisposing factors, or secondary, i.e., arising in response to and arising within radiation fields (as in radiation-treated bilateral retinoblastoma patients) or in response to other disease conditions, such as Paget's disease [183]. Secondary osteosarcomas of the head and neck are aggressive lesions that are clinically similar to osteosarcomas of the long bones.

Appendicular osteosarcomas occur between the ages of 10 and 18, coinciding with the major post-pubescent growth spurt [230]. Primary craniofacial osteosarcomas have a median onset in the fourth decade of life [18, 24, 60, 123, 178, 216, 269, 284, 304]. Appendicular and secondary craniofacial osteosarcomas metastasize widely within a year of the initial diagnosis. It is the distant metastases that are the most common cause of death. In contrast, primary craniofacial osteosarcomas do not metastasize aggressively and spread more slowly, with the mean interval between initial treatment and discovery of a metastatic lesion some 20+ months [24]. Local recurrence is the major complication and the leading cause of death in primary craniofacial osteosarcomas. Appendicular osteosarcoma and secondary craniofacial osteosarcoma are both characterized by pronounced cellular atypia. Histologically, craniofacial osteosarcomas are most frequently chondroblastic in appearance [60, 127], show little cellular atypia, and are frequently confused with benign or reactive bony lesions (Fig. 2.4). Neither pathologic staging of primary craniofacial osteosarcomas nor extension of the osteosarcoma into the surrounding soft tissues correlates well with survival. In many cases, only the completeness of the surgical resection as determined by margin status has correlated well with outcome, whereas incomplete resection correlates with local relapse and poor survival. In osteosarcomas of the head and neck, tumors of the mandible excepted, it

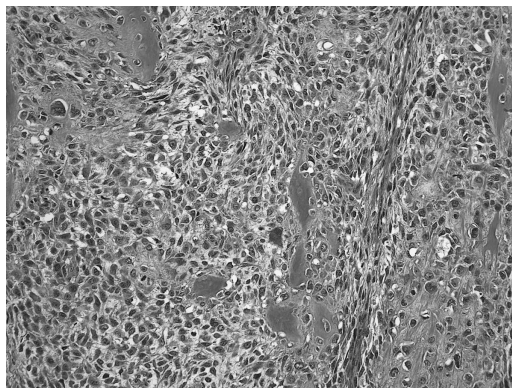


Figure 2.4. Histopathology of primary osteosarcoma of the mandible.

is difficult to achieve complete surgical resection. Osteosarcoma of the mandible, therefore, has a better prognosis than other types of craniofacial osteosarcoma. However, even in patients with mandibular osteosarcoma, complete surgical resection is achieved in only one-third of the cases [15]. The reason for the low rate of complete resection is extension of the tumor into adjacent structures, which occurs in 50% of patients with mandibular osteosarcoma.

One of the possible reasons for the difference in phenotype between primary craniofacial osteosarcomas and appendicular osteosarcomas is that the bones of the head and neck undergo a different program of development from those of the long bones of the skeleton [36, 39, 55]. In the precursors of the craniofacial bones (the calvaria of the skull, the maxilla, and the mandible), neural crest-derived cells differentiate into osteoblasts in a process known as intramembranous ossification. In the appendicular portions of the skeleton, mesenchymal cells differentiate into bone through a process called endochondral ossification. This difference in origin may be reflected in the distinct clinical and biological behavior of the two tumor types.

2.4 Osteosarcoma and Bilateral Retinoblastoma

Predisposition to osteosarcoma has been associated with several inherited syndromes.

Kitchin and Ellsworth [142] had observed that patients with bilateral retinoblastoma were at an increased risk for secondary tumors, notably osteosarcoma, whether or not the patient had been treated with radiation for the first tumor. They concluded that the increased risk was due to the pleiotropic effect of the susceptibility for retinoblastoma. This hypothesis was strengthened by the discovery that osteosarcoma tumors from patients with bilateral retinoblastoma lost constitutional heterozygosity (LoH) in the same region of chromosome 13 as in retinoblastoma tumors [50, 96, 256].

Cloning the gene for retinoblastoma susceptibility (RB1) demonstrated that the association between retinoblastoma and osteosarcoma was due to mutations in a common gene called RB1 [65, 66, 121, 160], consistent with its role as a tumor suppressor [7, 257, 307, 316, 327]. Furthermore, reintroduction of the RB1 gene into osteosarcoma tumor cells resulted in reduced tumorigenicity, both in vivo and in vitro [114].

2.5 Osteosarcoma and Li–Fraumeni Syndrome

The second association between osteosarcoma and an inherited predisposition was detected in the cancer syndrome first described by Li and Fraumeni [162]. These investigators and others [102, 163, 234] identified osteosarcoma as one of the more common tumors associated with rhabdomyosarcoma, breast cancers, and other neoplasms. The link between these disparate tumors was first suggested by the discovery of mutations in the TP53 gene in sporadic osteosarcoma tumors [181]. This was followed by the discovery of inherited mutations in the TP53 gene in several familial Li–Fraumeni syndromes [172]. As with RB1, TP53 is frequently mutated in sporadic osteosarcomas [189, 190, 291] and insertion of TP53 into osteosarcoma tumor cells has led to a loss of tumorigenicity in vivo and in vitro [48].

Li–Fraumeni syndrome, a heterogeneous disease, is associated with inherited mutations in the CHK2 gene in some families [159]. Activated CHK2 stabilizes TP53, as well as acting on other

genes in the TP53 pathway. Inherited mutations in the CHK2 gene have been identified in sporadic osteosarcomas and in osteosarcomas in families with Li–Fraumeni syndrome [191].

2.6 Osteosarcoma and Rothmund–Thomson Syndrome

Osteosarcoma is also associated with a rare autosomal recessive syndrome termed Rothmund–Thomson syndrome [249, 290], characterized by progressive poikilodermatous skin changes, juvenile cataracts, and skeletal abnormalities [305]. Individuals with this syndrome have an increased incidence of malignancies, including osteosarcoma. The predisposing mutation involves mutations in a helicase gene RECQL4 [141] and other mutations in RECQL4 [16, 167]. Also, osteosarcomas in Rothmund–Thomson patients were found associated with truncation of the RECQL4 gene [311]. Curiously, in contrast with the osteosarcomas associated with RB1 and TP53, sporadic osteosarcomas were not associated with mutations in RECQL4 [213].

An increased risk of osteosarcoma has also been associated with Werner's syndrome, caused by mutations in the related helicase, WRN/RECQL2 gene [80, 198]. Osteosarcoma may therefore be sensitive to changes in DNA repair that result in chromosomal instability.

2.7 Osteosarcoma and Paget's Disease of Bone

Osteosarcoma also has been associated with Paget's disease. Paget's disease is the second most common metabolic bone disease that affects up to 4% of the U.S. population by age 60 [63, 265, 266]. Rapid bone turnover in this condition alters the strength and shape of the newly formed bone [63, 226, 242, 265, 266]. The familial form of the disease is inherited in an autosomal dominant fashion with variable penetrance [261, 267]. Predisposition to familial Paget's disease has been linked to a number of loci [43], with osteosarcoma associated in 84% of cases [92, 118, 193, 235, 270]. Pagetic sarcoma

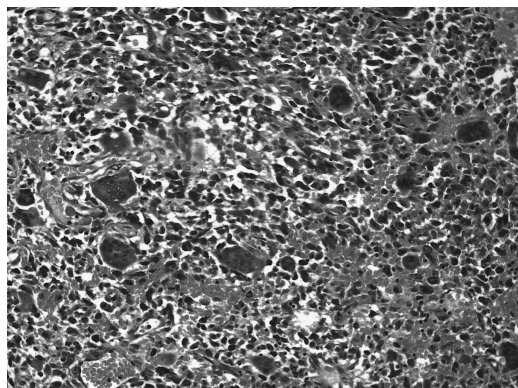


Figure 2.5. Histopathology of pagetic osteosarcoma. Note the presence of large osteoclast-like giant cells within the tumor.

occurs in 0.7–5% of patients with Paget's disease [64, 82, 91, 93, 319]. Osteosarcomas related to Paget's disease account for approximately 3% of all osteosarcomas [298], but account for 20% of all osteosarcomas in patients over 40 years of age [319] and for 50% of osteosarcomas in patients over 60 years of age [117].

Most osteosarcomas that develop in the Pagetic bone are conventional high-grade intramedullary tumors characterized by a highly pleomorphic, often spindle-cell sarcoma [82] (Fig. 2.5). The tumors are marked by the presence of many osteoclastic giant cells and atypical osteoblasts that seem responsible for the high rate of bone remodeling typical of Paget's disease.

The molecular basis for the increased risk for osteosarcoma in Paget's disease is unclear [97]. Analysis of LoH patterns identified a putative tumor suppressor locus in the same region of chromosome 18q that has been implicated as predisposing to some forms of familial Paget's disease [126, 184, 207], but no common mutations have been identified.

2.8 Genetics of Osteosarcoma

Osteosarcoma, despite its relative rarity, has played a significant role in the discovery of tumor suppressor genes such as *RB1* [50, 65, 66, 96, 160] and *TP53* [48, 189], as well as in the discovery of proto-oncogenes such as *FOS* [41,

42, 76, 84, 187, 250, 303, 313, 314] and *MDM2* [190, 222]. Indeed, many more cancer genes have been identified in leukemias, lymphomas, and sarcomas than in any other type of cancer, even though they account for only 10% of human cancers [67].

2.9 RB1 and Osteosarcoma

RB1 was the first human tumor suppressor gene to be cloned, but its mechanistic role in tumorigenesis remains incompletely understood. *RB1* plays a role in many cell processes, including cell cycle regulation [99, 281], DNA damage response and repair [69, 144, 145, 310], DNA replication [210], apoptosis [32, 99, 107], and differentiation [38, 45, 148, 168].

Mutations in *RB1* in osteosarcoma were some of the earliest mutations detected in *RB1* [65, 66, 160]. Subsequent analysis has shown that inactivation of *RB1* is the most common mutational event in osteosarcoma [45, 307, 316, 327].

RB1's role in regulating cell cycle progression may be through its repression of gene expression mediated by E2F1 and other members of the E2F family of transcription factors [54]. However, *RB1* also regulates gene expression by recruiting chromatin remodeling complexes to promoter regions that mediate chromatin condensation and inhibit transcription [22, 23, 98, 100].

RB1 is regulated by a group of cyclin-dependent kinases (CDKs) in response to mitogenic stimulation during cell cycle progression, allowing the cell to pass through the G1/S boundary [46]. *RB1* phosphorylation leads to disruption of the *RB1*/E2F1 association and depression of a variety of E2F1-regulated genes. This, in turn, leads to a proliferative response. CDKs are regulated by a group of CDK inhibitors, which prevent CDKs from phosphorylating *RB1*. Mutations in the CDKI proteins [188, 212], as well as amplification of CDK genes [68, 130, 137, 238, 315, 328], have been found in some osteosarcomas; this suggests alternative mechanisms to inactivate the *RB1* pathway.

An alternative regulatory mechanism has been identified. During apoptosis, *RB1* is degraded by caspases in response to TNF-alpha-

mediated apoptotic signals [282, 283]. This leads to derepression of the E2F1-regulated gene APAF1 [87, 195]. APAF1 is a key component of the mitochondria-dependent apoptotic machinery [27, 312, 337]. However, thus far no mutations in APAF1 have been identified in human osteosarcoma.

2.10 TP53 and Osteosarcoma

TP53 is one of the most commonly mutated genes in human cancer [276, 292, 306]. Mutations leading to inactivation of TP53 are common in osteosarcoma tumorigenesis [120, 134, 181, 189, 190, 224, 229, 231, 237, 257, 308]. As described previously, germline mutations in TP53 can predispose to osteosarcoma.

TP53 plays a crucial role in a number of pathways related to cellular stress, DNA repair, and apoptosis [276]. TP53 induces cell cycle arrest, senescence, differentiation, and apoptosis depending on the genetic environment of the cell. In response to genotoxic damage, TP53 can contribute to DNA repair. However, most often induction of TP53 by genotoxic damage leads to irreversible activation of apoptosis. TP53 function can be lost through mutation of the TP53 gene, or through mutations of genes within the TP53 signaling pathway [101]. TP53 is regulated by MDM2, a protein that blocks the activity of the TP53 protein by directing it to the ubiquitin-mediated degradation pathway [25, 122, 292]. MDM2 is negatively regulated by p14ARF [333], whereas CHK2-mediated phosphorylation of TP53 prevents MDM2 inactivation of TP53 [233]. Overexpression of MDM2, which results in functional loss of TP53 activity, occurs in osteosarcoma [134, 137, 190, 206, 229, 231, 238, 315, 328] as do mutations in the p14ARF and CHK2 genes that lead to functional inactivation of these genes [17, 170, 191].

2.11 Wnt Signaling Pathway

Signaling through the canonical Wnt pathway is critical for the differentiation of progenitor cells into osteoblasts [73, 74]. During osteogen-

esis, stimulation by bone morphogenic protein 2, a bone differentiation factor, is sustained by Wnt signaling. When Wnt signaling is inhibited, mesenchymal stem cells enter the cell cycle and osteogenesis is breached. Dickkopf 1 (DKK1) disrupts the Wnt signaling cascade [211], resulting in the inhibition of osteogenesis [83].

Serum levels of DKK1 are significantly elevated in pediatric osteosarcoma patients [158], with DKK1 expression at a maximum in the osteosarcoma cells located at the periphery of the tumor. When human mesenchymal cells are cultured in conditioned media from osteosarcoma tumor cells, osteogenesis is reduced in the same fashion as when DKK1 is added. Immunodepletion of DKK1 or addition of an inhibitor blocks the inhibitory effect on osteogenesis [158].

The level of expression of LRP5, a co-receptor in the Wnt signaling pathway, has been found to correlate positively and significantly with a rise in tumor metastasis. Patients whose tumors were positive for LRP5 tend to have a lower level of event-free survival [109]. Expression of Dickkopf 3 (DKK3), a dominant-negative mutant of LRP5, reduced invasion and motility in an osteosarcoma tumor cell line by modulating the Wnt-beta-catenin pathway [110]. Specifically DKK3 upregulated E-cadherin and downregulated Slug and Twist, transcription factors associated with regulation of metastasis. DKK3 expression also led to reduced expression of matrix metalloproteinases MMP2 and MMP14, as well as of Met and hepatocyte growth factor (HGF), enzymes that are involved in invasion and cell motility [86].

Wnt signaling therefore may play an important role in osteosarcoma tumorigenesis by inhibiting repair of the surrounding bone and by increasing the motility and invasiveness of the tumor cells.

2.12 Ezrin and Metastasis

Ezrin is a gene associated with motility, invasion, and adherence. Together with radixin and moesin, it is a component of the ERM proteins, which act as links between the plasma membrane and the actin cytoskeleton [116]. The ERM

proteins are involved in cell adhesion, migration, and the organization of cell surface structures. The role of Ezrin in osteosarcoma tumorigenesis was discovered by way of a microarray analysis of a mouse model of osteosarcoma [135]. Subsequent analyses have shown that Ezrin is overexpressed in aggressive mouse and canine tumors, as well as in metastatic human osteosarcoma tumors [135, 136, 140, 161, 228, 251].

Ezrin expression provides an early survival advantage for metastatic osteosarcoma tumor cells that reach the lungs in that AKT and MAPK phosphorylation and activity were reduced when Ezrin protein was suppressed [136]. Khanna and colleagues [136] also found that Ezrin-mediated early metastatic survival was partially dependent on activation of MAPK, but not of AKT.

Another member of the ERM protein family, Merlin, the product of the NF2 gene, is linked to highly metastatic osteosarcomas in mice [182]. This is surprising, as mutations in the NF2 gene in humans do not show increased predisposition to osteosarcoma. Moreover, analysis of NF2 in human osteosarcoma has not detected any mutations [274]. Possibly, another member of the ERM protein family compensates for loss of Merlin function in human osteoblasts.

2.13 FAS and FASL Signaling

The FAS receptor and its ligand (FASL) belong to the tumor necrosis factor death receptor superfamily and participate in regulating tumorigenesis in several types of primary malignancies and metastases [309]. Low expression of FAS in different tumors, including osteosarcoma, correlates with poor prognosis. Osteosarcoma lung metastases express low levels of FAS, whereas the primary tumors from the same patients often express high FAS levels [77, 149, 153]. In mouse models of osteosarcoma, FAS expression and metastatic potential were consistently found to vary inversely [78, 326]. One explanation is that FASL is constitutively expressed in lung tissue and that FAS-positive osteosarcoma tumor cells that enter the lungs bind to the FASL and induce apoptosis [78, 149]. This explana-

tion is consistent with the earlier observation that cyclophosphamide and its derivative ifosfamide induce expression of FASL in osteosarcoma cells [52]. Induction of FASL mediates apoptosis in osteosarcoma tumor cells via an autocrine–paracrine loop by cross-linking with cell surface FAS. Duan et al. [51,52] also showed that IL-12 enhanced the sensitivity of osteosarcoma cells to cyclophosphamide by upregulating FAS. This is consistent with FAS/FASL regulation in osteosarcoma, inasmuch as cells with high FAS expression are likely to be more sensitive to agents that upregulate FASL.

Chemotherapy agents that upregulate FAS would be expected to inhibit lung metastases. Gemcitabine, a pyrimidine antimetabolite and an analog of cytosine arabinoside, caused growth inhibition and cell death in human osteosarcoma tumor cell lines [124]. When mice were treated with an aerosol form of gemcitabine, FAS expression increased and the tumor regressed [5, 78, 124, 150].

2.14 erbB2/HER2 and Its Role in Osteosarcoma

The erbB family of type I protein receptor tyrosine kinases may be one group of genes which, when their mechanism of action is better understood, may lead to the identification of new targets for osteosarcoma therapy. This erbB family consists of erbB1 (also known as the epidermal growth factor receptor EGFR), erbB2 (also known as HER2 or neu), erbB3 (also known as HER3, and erbB4 (also known as HER4) [26, 106, 111, 119, 221]. These cell surface receptors form homodimers and heterodimers [40, 330, 331] to create functional growth factor receptors that trigger more rapid growth in malignant cells [208, 209] and promote cell survival [71].

HER2 is the best known member of the family [111, 209, 247, 248, 268]. It has no known ligands [143], but promotes signaling when combined as a heterodimer with any other family members that have ligands [220, 221, 278, 279]. Other erbB family members will preferentially partner with HER2 when co-expressed [81, 295]. Immunohistochemical examination of HER2 in

breast cancer cells has revealed strong antigen staining along the edges of tumor cells. This is consistent with membrane staining [268]. Overexpression of HER2 is correlated with genomic amplification to the point where identification of *HER2* amplification by fluorescent in situ hybridization (FISH) has been approved by the US Food and Drug Administration as a procedure to identify patients at high risk for recurrence and death due to node-negative invasive breast cancer [129, 332].

HER2 expression and gene amplification in osteosarcoma have been examined in many published reports [3, 4, 6, 58, 79, 115, 139, 171, 223, 271, 289, 294, 300, 320, 334]. The results of these studies appear to be contradictory: several studies report that HER2 plays a prognostic role, whereas other reports show no significance. The difference may be due to the definition of HER2 overexpression. In breast cancer, HER2 cytoplasmic immunostaining is considered to be an artifact [288, 289] and only complete membrane staining is considered to be clinically relevant [20]. Moreover, overexpression must be accompanied by genomic amplification; this has not been routinely observed in osteosarcoma [171], except in a single study that utilized FISH analysis [334]. In general, when HER2 expression in osteosarcoma was examined by immunohistochemistry, the pattern of staining was faint and diffuse; this suggests localization in the cytoplasm rather than in the plasma membrane [115]. Therefore, if and how HER2 expression affects osteosarcoma biology if the receptor is not expressed on the cell surface is unresolved.

2.15 RECQL4 and Genomic Stability

RECQL helicases represent a highly conserved protein family that is needed to maintain genome integrity [95, 108, 205, 301]. Three of the RECQL family members predispose to cancer predisposition syndromes: Bloom's Syndrome, Werner's syndrome, and Rothmund-Thomson syndrome. All three syndromes share a common phenotype of genomic instability [108, 301]. An important function of the RECQL helicases

appears to be the unwinding of intermediates of recombination, thereby preventing uncontrolled recombination [205].

Loss of function of the RECQL family of helicases gives rise to an increase in the levels of recombination. This in turn results in chromosomal aberrations that include LOH, a common chromosomal change associated with malignancies [108, 205]. RECQL4 may play a role in initiating DNA replication and in sister-chromatid cohesion [155, 176]. In normal human fibroblasts, RECQL4 is predominantly localized in the cytoplasm; relocation from nucleus to nucleolus or other nuclear foci occurs in response to UV or oxidative cell stress [232, 318, 325]. RECQL4 also associates with RAD51; this suggests that RECQL4 has a role in repairing double-strand breaks of DNA by homologous recombination [232].

One difference between RECQL4 and other mutated genes that predispose to osteosarcoma (RB1, TP53) is that no somatic mutations of RECQL4 have been identified in sporadic cases of osteosarcoma [213]. This may reflect the fact that mutations in RECQL4 would only have an indirect effect on tumorigenesis, whereas RB1 and TP53 have more direct effects.

2.16 Role of Chromosomal Instability and Telomere Maintenance in Osteosarcoma

One of the striking features of osteosarcoma is the high frequency of genomic amplification, rearrangement, deletion, and loss of heterozygosity across the genome [8, 112, 156, 174, 197, 258, 259, 273, 277, 286, 324, 329, 336]. This chromosomal instability is rare in childhood tumors.

Chromosomal instability is common in cancer cells. Mechanisms that lead to numerical and structural chromosomal instability in cancer cells include defects in chromosomal segregation, defects in cellular checkpoints that guard against reproduction of abnormal cells, defects in telomere stability, and defects in the DNA damage response. A long-standing debate in cancer genetics is whether genomic instability is an

early or late event in tumorigenesis [169, 179, 180, 214, 263, 264, 293]. Chromosomal instability has been studied primarily in epithelial tumors, notably colorectal carcinoma. Approximately 15% of colorectal cancers show a form of genetic instability that is characterized by mismatch repair deficiency. The remaining 85% of colorectal cancers, and an even larger proportion of other solid tumor types, show an abnormal chromosomal content that reflects chromosomal instability [240]. Unlike microsatellite instability, which is caused by genes in the DNA mismatch repair pathways, chromosomal instability is due to errors in chromosomal segregation, telomere stability, and in the repair of damage to double-stranded DNA [33, 75, 146, 147, 239, 302].

Alterations in over 100 genes have been shown to give rise to chromosomal instability in *Saccharomyces cerevisiae* [146]. Many of these have one or more homologs in humans. These include those involved in cell cycle regulation, chromosome condensation, sister-chromatid cohesion, spindle assembly, kinetochore structure and function, microtubule formation and dynamics, as well as cell cycle checkpoints.

Alterations in telomeres have been associated with increased genomic instability [44, 180]. Terminal deletions induced by telomere shortening in the absence of telomerase may be initiated by end-to-end chromosome fusion and breakage or by exonucleolytic end resection. In telomerase-deficient mice, end-to-end chromosome fusion is the most prominent chromosomal abnormality [21], with fusions a primary consequence of telomere shortening [104]. In human tumors with telomere dysfunction, deletions in the terminal regions of chromosomes precede an increase in global instability [44, 57, 72].

Decreased telomerase activity leads to chromosomal end lesions, which promote either genomic instability and carcinogenesis or apoptotic cell death [34]. Telomerase may therefore have a dual role in promoting tumorigenesis and protecting the cell from genomic instability [31, 47, 88, 89]. Studies using a model for Li-Fraumeni syndrome have suggested that telomere shortening is the primary driving force for the genomic instability characteristic of Li-Fraumeni syndrome cells [56].

Telomeres are maintained in human tumors by activation and by alternative lengthening of telomeres (ALT) [29, 33, 105, 125, 275]. Most human tumors maintain telomeres by activating telomerase. However, in appendicular osteosarcoma ALT occurs at a higher frequency than in other types of tumors [9, 204, 255, 272, 296]. Absence of telomerase activation or presence of ALT correlates with a favorable prognosis in osteosarcoma [138, 252, 272, 296]. The ALT and telomerase-dependent mechanisms serve the same end, but they are not equivalent. Telomerase-dependent osteosarcoma cell lines have short telomeres with a minimum range of length, whereas ALT-dependent osteosarcoma cell lines have telomeres that are long, but vary in length. ALT-positive cell lines also have greater genetic instability and more translocations than the telomerase-positive cell lines [255].

One function of telomere maintenance is in stem cells. A controversial hypothesis proposed that cancers have stem cell-like subpopulations and that it is these self-renewing cells that drive tumor proliferation [244]. Stem cell-like cells have been identified in osteosarcoma tumors [70]. These cells express activated STAT3, OCT3/4, and NANOG, all of which are marker genes for pluripotent embryonic stem cells [28].

2.17 Comparative Genomic Hybridization

Since the completion of the sequencing of the human genome, efforts have accelerated to examine chromosomal abnormalities including large-scale amplifications, deletions, and variations in the number of copies in various types of cancer. This work has been catapulted by the availability of high-throughput array-based technologies that can scan the entire genome with high resolution. Comparative genome hybridization has utilized arrayed BAC or oligonucleotide probes to detect genotype variation [61]. By means of comparative genome hybridization analysis, the chromosomal instability phenotype of osteosarcoma tumors has been confirmed, with many chromosomal alterations in each tumor [8, 15, 49, 62, 103, 133, 156,

165, 174, 217, 225, 258, 260, 273, 277, 285–287, 335, 336].

Notwithstanding much variation in these analyses, some common chromosomal gains were observed for 1p, 5p, 6p, 8q, and 17p. Common chromosomal losses were observed for 2q, 10p, 14q, 15q, and 16p. Common amplification regions were observed for 1q21-q22, 1p34-p36, 5p13-p15, 6p12-21, 12q12-q14, and Xp11.2. The most common amplifications detected involved two chromosomal regions: 8q23-q24 and 17p11.2-p12. The only common deletion observed was 18q21-q22. In all cases, amplifications outnumbered deletions.

The 8q23–q24 region of amplification includes the MYC gene, as well as the TNFRSF11B, COL14A1, COL22A1, and RECQL4 genes (Fig. 2.6). The 17p11.2–p12 region contains the TNFRSF13B, MAP2K4, MAPK7 genes and TOP3A genes (Fig. 2.7). The latter forms a

complex with the BLM gene, which regulates recombination in somatic cells.

2.18 Microarray Analysis of Osteosarcoma

Even though the identification of genetic alterations in osteosarcoma has progressed steadily, no single molecular marker has greater prognostic significance in osteosarcoma treatment than the current clinical markers. Clearly more comprehensive analytical technologies are needed to develop more informative classification systems and to identify new therapeutic targets.

Gene expression analysis by oligonucleotide microarray has been increasingly utilized to analyze tumors including osteosarcoma. These arrays permit a nearly comprehensive survey of

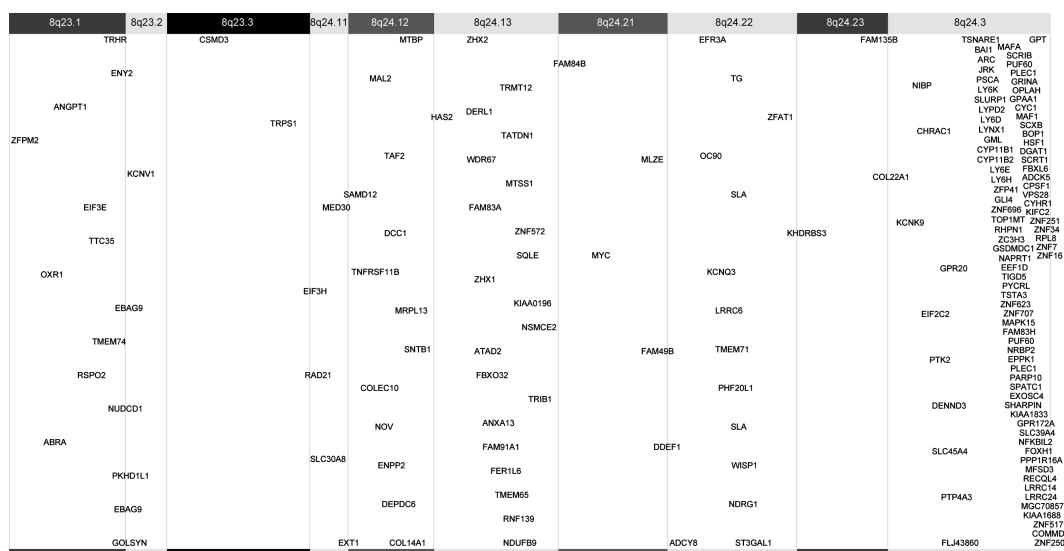


Figure 2.6. Map of the 8q23–q24 region of frequent chromosomal amplification in osteosarcoma. Map adapted from the UCSC Human Genome Browser Project [131] (<http://genome.ucsc.edu/>).

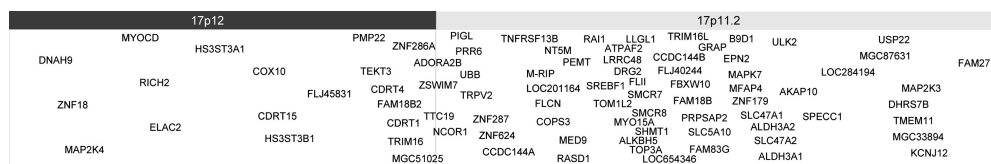


Figure 2.7. Map of the 17p11.2–p12 region of frequent chromosomal amplification in osteosarcoma. Map adapted from the UCSC Human Genome Browser Project [131] (<http://genome.ucsc.edu/>).

the expression patterns in the tumors, which in turn can be used to identify molecular pathways and targets for diagnosis and treatment. Microarray analysis alone can be used to develop genomic expression signatures that distinguish between outcome and therapeutic response. The method also helps divide tumors into molecularly defined categories that are associated with specific genetic pathways that can suggest novel therapeutic approaches. The use of microarrays for clinical purposes remains a challenge because of difficulties with specimen collection and their heterogeneity. In order for microarray results to be interpreted within the clinical context, they need to be validated by complimentary techniques and supported by strong bioinformatics. To reduce complexity, some microarray analyses have focused on osteosarcoma tumor cell lines [175, 194, 203, 323, 338] and mouse models [135] to identify specific known target pathways and their perturbations.

Other analyses have focused on the clinical question of identifying patients that will or will not respond to chemotherapy [173, 192, 215], thereby identifying chemotherapy-resistant pediatric osteosarcomas. Ochi et al. [215] identified a signature of 60 genes whose expression correlated with response to chemotherapy. Mintz et al. [192] identified a signature of 104 genes that correlated with response to chemotherapy. Mann et al. [176] identified a signature of 45 genes that also correlated with response to chemotherapy. Curiously, there is almost no overlap in the three gene groups. However, most genes in the three signatures groups were at high expression when there was a poor response to chemotherapy. The full significance of these findings remains uncertain. Clearly there is a need to identify a robust signature group of genes that predict response to therapy.

2.19 Summary

Osteosarcoma is a fascinating disease. Its variability in presentation, association with a number of inherited syndromes, the lack of benign precursors or other morphological determinants all make it necessary to develop molecular classification schemes for screening and

identifying tumors and their likely outcome. Much progress notwithstanding, understanding of osteosarcoma remains elusive. New discoveries are therefore likely to have a profound impact on understanding the disease.

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References

1. Abdelwahab IF, Kenan S, Hermann G, and Klein MJ, (1997) Dedifferentiated parosteal osteosarcoma of the radius. *Skeletal Radiol* 26: 242–245.
2. Abramovici LC, Hytirogrou P, Klein RM, Karkavelas G, Drevelegas A, Panousi E, and Steiner GC, (2005) Well-differentiated extraskeletal osteosarcoma: report of 2 cases, 1 with dedifferentiation. *Hum Pathol* 36: 439–443.
3. Akatsuka T, Wada T, Kokai Y, Kawaguchi S, Isu K, Yamashiro K, Yamashita T, Sawada N, Yamawaki S, and Ishii S, (2002) ErbB2 expression is correlated with increased survival of patients with osteosarcoma. *Cancer* 94: 1397–1404.
4. Akatsuka T, Wada T, Kokai Y, Sawada N, Yamawaki S, and Ishii S, (2001) Loss of ErbB2 expression in pulmonary metastatic lesions in osteosarcoma. *Oncology* 60: 361–366.
5. Ando T, Ichikawa J, Okamoto A, Tasaka K, Nakao A, and Hamada Y, (2005) Gemcitabine inhibits viability, growth, and metastasis of osteosarcoma cell lines. *J Orthop Res* 23: 964–969.
6. Anninga JK, van de Vijver MJ, Cleton-Jansen AM, Kristel PM, Taminiau AH, Nooij M, Egeler RM, and Hogendoorn PC, (2004) Overexpression of the HER-2 oncogene does not play a role in high-grade osteosarcomas. *Eur J Cancer* 40: 963–970.
7. Araki N, Uchida A, Kimura T, Yoshikawa H, Aoki Y, Ueda T, Takai S, Miki T, and Ono K, (1991) Involvement of the retinoblastoma gene in primary osteosarcomas and other bone and soft-tissue tumors. *Clin Orthop Relat Res* 271–277.

8. Atiye J, Wolf M, Kaur S, Monni O, Bohling T, Kivioja A, Tas E, Serra M, Tarkkanen M, and Knuutila S, (2005) Gene amplifications in osteosarcoma – CGH microarray analysis. *Genes Chromosomes Cancer* 42: 158–163.
9. Aue G, Muralidhar B, Schwartz HS, and Butler MG, (1998) Telomerase activity in skeletal sarcomas. *Ann Surg Oncol* 5: 627–634.
10. Ayala AG, Ro JY, Papadopoulos NK, Raymond AK, and Edeiken J, (1993) Small cell osteosarcoma. *Cancer Treat Res* 62: 139–149.
11. Ayala AG, Ro JY, Raymond AK, Jaffe N, Chawla S, Carrasco H, Link M, Jimenez J, Edeiken J, Wallace S et al., (1989) Small cell osteosarcoma. A clinicopathologic study of 27 cases. *Cancer* 64: 2162–2173.
12. Bacci G, Briccoli A, Rocca M, Ferrari S, Donati D, Longhi A, Bertoni F, Bacchini P, Giacomini S, Fornì C, Manfrini M, and Galletti S, (2003) Neoadjuvant chemotherapy for osteosarcoma of the extremities with metastases at presentation: recent experience at the Rizzoli Institute in 57 patients treated with cisplatin, doxorubicin, and a high dose of methotrexate and ifosfamide. *Ann Oncol* 14: 1126–1134.
13. Bacci G, Ferrari S, Ruggieri P, Biagini R, Fabbri N, Campanacci L, Bacchini P, Longhi A, Fornì C, and Bertoni F, (2001) Telangiectatic osteosarcoma of the extremity: neoadjuvant chemotherapy in 24 cases. *Acta Orthop Scand* 72: 167–172.
14. Bane BL, Evans HL, Ro JY, Carrasco CH, Grignon DJ, Benjamin RS, and Ayala AG, (1990) Extraskelatal osteosarcoma. A clinicopathologic review of 26 cases. *Cancer* 65: 2762–2770.
15. Batanian JR, Cavalli LR, Aldosari NM, Ma E, Sotelo-Avila C, Ramos MB, Rone JD, Thorpe CM, and Haddad BR, (2002) Evaluation of paediatric osteosarcomas by classic cytogenetic and CGH analyses. *Mol Pathol* 55: 389–393.
16. Beghini A, Castorina P, Roversi G, Modiano P, and Larizza L, (2003) RNA processing defects of the helicase gene RECQL4 in a compound heterozygous Rothmund–Thomson patient. *Am J Med Genet A* 120: 395–399.
17. Benassi MS, Molendini L, Gamberi G, Magagnoli G, Ragazzini P, Gobbi GA, Sangiorgi L, Pazzaglia L, Asp J, Brantsing C, and Picci P, (2001) Involvement of INK4A gene products in the pathogenesis and development of human osteosarcoma. *Cancer* 92: 3062–3067.
18. Bennett JH, Thomas G, Evans AW, and Speight PM, (2000) Osteosarcoma of the jaws: a 30-year retrospective review. *Oral Surg, Oral Med, Oral Pathol, Oral Radiol, Endod* 90: 323–332.
19. Bertoni F, Bacchini P, and Staals EL, (2003) Giant cell-rich osteosarcoma. *Orthopedics* 26: 179–181.
20. Bilous M, Dowsett M, Hanna W, Isola J, Lebeau A, Moreno A, Penault-Llorca F, Ruschoff J, Tomasic G, and van de Vijver M, (2003) Current perspectives on HER2 testing: a review of national testing guidelines. *Mod Pathol* 16: 173–182.
21. Blasco MA, Lee HW, Hanke MP, Samper E, Lansdorp PM, DePinho RA, and Greider CW, (1997) Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. *Cell* 91: 25–34.
22. Brehm A and Kouzarides T, (1999) Retinoblastoma protein meets chromatin. *Trends Biochem Sci* 24: 142–145.
23. Brehm A, Miska EA, McCance DJ, Reid JL, Bannister AJ, and Kouzarides T, (1998) Retinoblastoma protein recruits histone deacetylase to repress transcription. *Nature* 391: 597–601.
24. Briant TD and Bird R, (1981) Osteogenic sarcoma of the mandible. *J Otolaryngol* 10: 149–161.
25. Brooks CL and Gu W, (2006) p53 ubiquitination: Mdm2 and beyond. *Mol Cell* 21: 307–315.
26. Casalini P, Iorio MV, Galmozzi E, and Menard S, (2004) Role of HER receptors family in development and differentiation. *J Cell Physiol* 200: 343–350.
27. Cecconi F, Alvarez-Bolado G, Meyer BI, Roth KA, and Gruss P, (1998) Apaf1 (CED-4 homolog) regulates programmed cell death in mammalian development. *Cell* 94: 727–737.
28. Chambers I, (2004) The molecular basis of pluripotency in mouse embryonic stem cells. *Cloning Stem Cells* 6: 386–391.
29. Chang S, Khoo CM, Naylor ML, Maser RS, and DePinho RA, (2003) Telomere-based crisis: functional differences between telomerase activation and ALT in tumor progression. *Genes Dev* 17: 88–100.
30. Chano T, Matsumoto K, Ishizawa M, Morimoto S, Hukuda S, Okabe H, Kato H, and Fujino S, (1996) Analysis of the presence of osteocalcin, S-100 protein, and proliferating cell nuclear antigen in cells of various types of osteosarcomas. *Eur J Histochem* 40: 189–198.
31. Charames GS and Bapat B, (2003) Genomic instability and cancer. *Curr Mol Med* 3: 589–596.
32. Chau BN and Wang JY, (2003) Coordinated regulation of life and death by RB. *Nat Rev Cancer* 3: 130–138.
33. Cheung AL and Deng W, (2008) Telomere dysfunction, genome instability and cancer. *Front Biosci* 13: 2075–2090.
34. Chou WC, Hawkins AL, Barrett JF, Griffin CA, and Dang CV, (2001) Arsenic inhibition of telomerase transcription leads to genetic instability. *J Clin Invest* 108: 1541–1547.
35. Chung EB and Enzinger FM, (1987) Extraskelatal osteosarcoma. *Cancer* 60: 1132–1142.
36. Chung UI, Kawaguchi H, Takato T, and Nakamura K, (2004) Distinct osteogenic mechanisms of bones of distinct origins. *J Orthop Sci* 9: 410–414.
37. Clark JL, Unni KK, Dahlin DC, and Devine KD, (1983) Osteosarcoma of the jaw. *Cancer* 51: 2311–2316.
38. Classon M and Harlow E, (2002) The retinoblastoma tumour suppressor in development and cancer. *Nat Rev Cancer* 2: 910–917.
39. Cohen MM, Jr., (2000) Merging the old skeletal biology with the new. I. Intramembranous ossification, endochondral ossification, ectopic bone, secondary cartilage, and pathologic considerations. *J Craniofac Genet Dev Biol* 20: 84–93.

40. Cohen S, Ushiro H, Stoscheck C, and Chinkers M, (1982) A native 170,000 epidermal growth factor receptor-kinase complex from shed plasma membrane vesicles. *J Biol Chem* 257: 1523–1531.
41. Curran T, MacConnell WP, van Straaten F, and Verma IM, (1983) Structure of the FBJ murine osteosarcoma virus genome: molecular cloning of its associated helper virus and the cellular homolog of the v-fos gene from mouse and human cells. *Mol Cell Biol* 3: 914–921.
42. Curran T, Peters G, Van Beveren C, Teich NM, and Verma IM, (1982) FBJ murine osteosarcoma virus: identification and molecular cloning of biologically active proviral DNA. *J Virol* 44: 674–682.
43. Daroszewska A and Ralston SH, (2005) Genetics of Paget's disease of bone. *Clin Sci (Lond)* 109: 257–263.
44. De Lange T, (2005) Telomere-related genome instability in cancer. *Cold Spring Harb Symp Quant Biol* 70: 197–204.
45. Deshpande A and Hinds PW, (2006) The retinoblastoma protein in osteoblast differentiation and osteosarcoma. *Curr Mol Med* 6: 809–817.
46. Deshpande A, Sicinski P, and Hinds PW, (2005) Cyclins and cdk in development and cancer: a perspective. *Oncogene* 24: 2909–2915.
47. Desmaze C, Soria JC, Freulet-Marriere MA, Mathieu N, and Sabatier L, (2003) Telomere-driven genomic instability in cancer cells. *Cancer Lett* 194: 173–182.
48. Diller L, Kassel J, Nelson CE, Gryka MA, Litwak G, Gebhardt M, Bressac B, Ozturk M, Baker SJ, Vogelstein B, et al., (1990) p53 functions as a cell cycle control protein in osteosarcomas. *Mol Cell Biol* 10: 5772–5781.
49. dos Santos Aguiar S, de Jesus Giroto Zambaldi L, dos Santos AM, Pinto W, Jr., and Brandalise SR, (2007) Comparative genomic hybridization analysis of abnormalities in chromosome 21 in childhood osteosarcoma. *Cancer Genet Cytogenet* 175: 35–40.
50. Dryja TP, Rapaport JM, Epstein J, Goorin AM, Weichselbaum R, Koufos A, and Cavenee WK, (1986) Chromosome 13 homozygosity in osteosarcoma without retinoblastoma. *Am J Hum Genet* 38: 59–66.
51. Duan X, Jia SF, Koshkina N, and Kleinerman ES, (2006) Intranasal interleukin-12 gene therapy enhanced the activity of ifosfamide against osteosarcoma lung metastases. *Cancer* 106: 1382–1388.
52. Duan X, Zhou Z, Jia SF, Colvin M, Lafleur EA, and Kleinerman ES, (2004) Interleukin-12 enhances the sensitivity of human osteosarcoma cells to 4-hydroperoxycyclophosphamide by a mechanism involving the Fas/Fas-ligand pathway. *Clin Cancer Res* 10: 777–783.
53. Dubec JJ, Munk PL, O'Connell JX, Lee MJ, Janzen D, Connell D, Masri B, and Logan PM, (1997) Soft tissue osteosarcoma with telangiectatic features: MR imaging findings in two cases. *Skeletal Radiol* 26: 732–736.
54. Dyson N, (1998) The regulation of E2F by pRB-family proteins. *Genes Dev* 12: 2245–2262.
55. Eames BF, de la Fuente L, and Helms JA, (2003) Molecular ontogeny of the skeleton. *Birth Defects Res C Embryo Today* 69: 93–101.
56. Elmore LW, Turner KC, Gollahon LS, Landon MR, Jackson-Cook CK, and Holt SE, (2002) Telomerase protects cancer-prone human cells from chromosomal instability and spontaneous immortalization. *Cancer Biol Ther* 1: 391–397.
57. Feldser DM, Hackett JA, and Greider CW, (2003) Telomere dysfunction and the initiation of genome instability. *Nat Rev Cancer* 3: 623–627.
58. Fellenberg J, Krauthoff A, Pollandt K, Delling G, and Parsch D, (2004) Evaluation of the predictive value of Her-2/neu gene expression on osteosarcoma therapy in laser-microdissected paraffin-embedded tissue. *Lab Invest* 84: 113–121.
59. Ferguson WS and Goorin AM, (2001) Current treatment of osteosarcoma. *Cancer Invest* 19: 292–315.
60. Fernandes R, Nikitakis NG, Pazoki A, and Ord RA, (2007) Osteogenic sarcoma of the jaw: a 10-year experience. *J Oral Maxillofac Surg* 65: 1286–1291.
61. Feuk L, Carson AR, and Scherer SW, (2006) Structural variation in the human genome. *Nat Rev Genet* 7: 85–97.
62. Forus A, Weghuis DO, Smeets D, Fodstad O, Myklebost O, and Geurts van Kessel A, (1995) Comparative genomic hybridization analysis of human sarcomas: II. Identification of novel amplicons at 6p and 17p in osteosarcomas. *Genes Chromosomes Cancer* 14: 15–21.
63. Fraser WD, (1997) Paget's disease of bone. *Curr Opin Rheumatol* 9: 347–354.
64. Freydinge JE, Duhig JT, and Mc DL, (1963) Sarcoma complicating Paget's disease of bone. A study of seven cases with report of one long survival after surgery. *Arch Pathol* 75: 496–500.
65. Friend SH, Bernards R, Rogelj S, Weinberg RA, Rapaport JM, Albert DM, and Dryja TP, (1986) A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. *Nature* 323: 643–646.
66. Fung YK, Murphree AL, T'Ang A, Qian J, Hinrichs SH, and Benedict WF, (1987) Structural evidence for the authenticity of the human retinoblastoma gene. *Science* 236: 1657–1661.
67. Futreal PA, Coin L, Marshall M, Down T, Hubbard T, Wooster R, Rahman N, and Stratton MR, (2004) A census of human cancer genes. *Nat Rev Cancer* 4: 177–183.
68. Gamberi G, Ragazzini P, Benassi MS, Ferrari C, Sollazzo MR, Molendini L, Merli M, Magagnoli G, Ruggieri P, Balladelli A, Orlando C, Bacchini P, Pazzagli M, and Picci P, (2000) Analysis of 12q13–15 genes in parosteal osteosarcoma. *Clin Orthop Relat Res* 195–204.
69. Genovese C, Trani D, Caputi M, and Claudio PP, (2006) Cell cycle control and beyond: emerging roles for the retinoblastoma gene family. *Oncogene* 25: 5201–5209.
70. Gibbs CP, Kukekov VG, Reith JD, Tchigrinova O, Suslov ON, Scott EW, Ghivizzani SC, Ignatova TN, and Steindler DA, (2005) Stem-like cells in bone sarcomas: implications for tumorigenesis. *Neoplasia* 7: 967–976.

71. Gilmore AP, Valentijn AJ, Wang P, Ranger AM, Bundred N, O'Hare MJ, Wakeling A, Korsmeyer SJ, and Streuli CH, (2002) Activation of BAD by therapeutic inhibition of epidermal growth factor receptor and transactivation by insulin-like growth factor receptor. *J Biol Chem* 277: 27643–27650.
72. Gisselsson D, Jonson T, Petersen A, Strombeck B, Dal Cin P, Hoglund M, Mitelman F, Mertens F, and Mandahl N, (2001) Telomere dysfunction triggers extensive DNA fragmentation and evolution of complex chromosome abnormalities in human malignant tumors. *Proc Natl Acad Sci USA* 98: 12683–12688.
73. Glass DA, 2nd and Karsenty G, (2006) Molecular bases of the regulation of bone remodeling by the canonical Wnt signaling pathway. *Curr Top Dev Biol* 73: 43–84.
74. Glass DA, 2nd and Karsenty G, (2007) In vivo analysis of Wnt signaling in bone. *Endocrinology* 148: 2630–2634.
75. Gollin SM, (2004) Chromosomal instability. *Curr Opin Oncol* 16: 25–31.
76. Goralczyk R, Closs EI, Ruther U, Wagner EF, Strauss PG, Erfle V, and Schmidt J, (1990) Characterization of fos-induced osteogenic tumours and tumour-derived murine cell lines. *Differentiation* 44: 122–131.
77. Gordon N, Arndt CA, Hawkins DS, Doherty DK, Inwards CY, Munsell MF, Stewart J, Koshkina NV, and Kleinerman ES, (2005) Fas expression in lung metastasis from osteosarcoma patients. *J Pediatr Hematol Oncol* 27: 611–615.
78. Gordon N, Koshkina NV, Jia SF, Khanna C, Mendoza A, Worth LL, and Kleinerman ES, (2007) Corruption of the Fas pathway delays the pulmonary clearance of murine osteosarcoma cells, enhances their metastatic potential, and reduces the effect of aerosol gemcitabine. *Clin Cancer Res* 13: 4503–4510.
79. Gorlick R, Huvo AG, Heller G, Aledo A, Beardsley GP, Healey JH, and Meyers PA, (1999) Expression of HER2/erbB-2 correlates with survival in osteosarcoma. *J Clin Oncol* 17: 2781–2788.
80. Goto M, Miller RW, Ishikawa Y, and Sugano H, (1996) Excess of rare cancers in Werner syndrome (adult progeria). *Cancer Epidemiol Biomarkers Prev* 5: 239–246.
81. Graus-Porta D, Beerli RR, Daly JM, and Hynes NE, (1997) ErbB-2, the preferred heterodimerization partner of all ErbB receptors, is a mediator of lateral signaling. *Embo J* 16: 1647–1655.
82. Greditzer HG, 3rd, McLeod RA, Unni KK, and Beabout JW, (1983) Bone sarcomas in Paget's disease. *Radiology* 146: 327–333.
83. Gregory CA, Singh H, Perry AS, and Prockop DJ, (2003) The Wnt signaling inhibitor dickkopf-1 is required for reentry into the cell cycle of human adult stem cells from bone marrow. *J Biol Chem* 278: 28067–28078.
84. Grigoriadis AE, Schellander K, Wang ZQ, and Wagner EF, (1993) Osteoblasts are target cells for transformation in c-fos transgenic mice. *J Cell Biol* 122: 685–701.
85. Grimer RJ, Bielack S, Flege S, Cannon SR, Folaras G, Andreeff I, Sokolov T, Taminiau A, Dominkus M, San-Julian M, Kollender Y, and Gosheger G, (2005) Periosteal osteosarcoma—a European review of outcome. *Eur J Cancer* 41: 2806–2811.
86. Guo Y, Zi X, Koontz Z, Kim A, Xie J, Gorlick R, Holcombe RF, and Hoang BH, (2007) Blocking Wnt/LRP5 signaling by a soluble receptor modulates the epithelial to mesenchymal transition and suppresses metastasis and metalloproteinases in osteosarcoma Saos-2 cells. *J Orthop Res* 25: 964–971.
87. Guo Z, Yikang S, Yoshida H, Mak TW, and Zacksteinhaus E, (2001) Inactivation of the retinoblastoma tumor suppressor induces apoptosis protease-activating factor-1 dependent and independent apoptotic pathways during embryogenesis. *Cancer Res* 61: 8395–8400.
88. Hackett JA, Feldser DM, and Greider CW, (2001) Telomere dysfunction increases mutation rate and genomic instability. *Cell* 106: 275–286.
89. Hackett JA and Greider CW, (2002) Balancing instability: dual roles for telomerase and telomere dysfunction in tumorigenesis. *Oncogene* 21: 619–626.
90. Haddy TB, Mosher RB, Dinndorf PA, and Reaman GH, (2004) Second neoplasms in survivors of childhood and adolescent cancer are often treatable. *J Adolesc Health* 34: 324–329.
91. Hadjipavlou A, Lander P, Srolovitz H, and Enker IP, (1992) Malignant transformation in Paget's disease of bone. *Cancer* 70: 2802–2808.
92. Haibach H, Farrell C, and Dittrich FJ, (1985) Neoplasms arising in Paget's disease of bone: a study of 82 cases. *Am J Clin Pathol* 83: 594–600.
93. Hall FM, (1983) Incidence of bone sarcoma in Paget's disease. *Radiology* 148: 865.
94. Hall RB, Robinson LH, Malawar MM, and Dunham WK, (1985) Periosteal osteosarcoma. *Cancer* 55: 165–171.
95. Hanada K and Hickson ID, (2007) Molecular genetics of RecQ helicase disorders. *Cell Mol Life Sci* 64: 2306–2322.
96. Hansen MF, Koufos A, Gallie BL, Phillips RA, Fodstad O, Brogger A, Gedde-Dahl T, and Cavenee WK, (1985) Osteosarcoma and retinoblastoma: a shared chromosomal mechanism revealing recessive predisposition. *Proc Natl Acad Sci USA* 82: 6216–6220.
97. Hansen MF, Nellissery MJ, and Bhatia P, (1999) Common mechanisms of osteosarcoma and Paget's disease. *J Bone Miner Res* 14 Suppl 2: 39–44.
98. Harbour JW and Dean DC, (2000) Chromatin remodeling and Rb activity. *Curr Opin Cell Biol* 12: 685–689.
99. Harbour JW and Dean DC, (2000) Rb function in cell-cycle regulation and apoptosis. *Nat Cell Biol* 2: E65–67.
100. Harbour JW and Dean DC, (2001) Corepressors and retinoblastoma protein function. *Curr Top Microbiol Immunol* 254: 137–144.
101. Harris SL and Levine AJ, (2005) The p53 pathway: positive and negative feedback loops. *Oncogene* 24: 2899–2908.

102. Hartley AL, Birch JM, Marsden HB, and Harris M, (1986) Breast cancer risk in mothers of children with osteosarcoma and chondrosarcoma. *Br J Cancer* 54: 819–823.
103. Hattinger CM, Reverter-Branchat G, Remondini D, Castellani GC, Benini S, Pasello M, Manara MC, Scotlandi K, Picci P, and Serra M, (2003) Genomic imbalances associated with methotrexate resistance in human osteosarcoma cell lines detected by comparative genomic hybridization-based techniques. *Eur J Cell Biol* 82: 483–493.
104. Hemann MT, Strong MA, Hao LY, and Greider CW, (2001) The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. *Cell* 107: 67–77.
105. Henson JD, Neumann AA, Yeager TR, and Reddel RR, (2002) Alternative lengthening of telomeres in mammalian cells. *Oncogene* 21: 598–610.
106. Herbst RS, (2004) Review of epidermal growth factor receptor biology. *Int J Radiat Oncol Biol Phys* 59: 21–26.
107. Hickman ES, Moroni MC, and Helin K, (2002) The role of p53 and pRB in apoptosis and cancer. *Curr Opin Genet Dev* 12: 60–66.
108. Hickson ID, (2003) RecQ helicases: caretakers of the genome. *Nat Rev Cancer* 3: 169–178.
109. Hoang BH, Kubo T, Healey JH, Sowers R, Mazza B, Yang R, Huvos AG, Meyers PA, and Gorlick R, (2004) Expression of LDL receptor-related protein 5 (LRP5) as a novel marker for disease progression in high-grade osteosarcoma. *Int J Cancer* 109: 106–111.
110. Hoang BH, Kubo T, Healey JH, Yang R, Nathan SS, Kolb EA, Mazza B, Meyers PA, and Gorlick R, (2004) Dickkopf 3 inhibits invasion and motility of Saos-2 osteosarcoma cells by modulating the Wnt-beta-catenin pathway. *Cancer Res* 64: 2734–2739.
111. Holbro T, Civenni G, and Hynes NE, (2003) The ErbB receptors and their role in cancer progression. *Exp Cell Res* 284: 99–110.
112. Hoogerwerf WA, Hawkins AL, Perlman EJ, and Griffin CA, (1994) Chromosome analysis of nine osteosarcomas. *Genes Chromosomes Cancer* 9: 88–92.
113. Hoshi M, Matsumoto S, Manabe J, Tanizawa T, Shigemitsu T, Takeuchi K, and Kawaguchi N, (2006) Report of four cases with high-grade surface osteosarcoma. *Jpn J Clin Oncol* 36: 180–184.
114. Huang HJ, Yee JK, Shew JY, Chen PL, Bookstein R, Friedmann T, Lee EY, and Lee WH, (1988) Suppression of the neoplastic phenotype by replacement of the RB gene in human cancer cells. *Science* 242: 1563–1566.
115. Hughes DP, Thomas DG, Giordano TJ, Baker LH, and McDonagh KT, (2004) Cell surface expression of epidermal growth factor receptor and Her-2 with nuclear expression of Her-4 in primary osteosarcoma. *Cancer Res* 64: 2047–2053.
116. Hughes SC and Fehon RG, (2007) Understanding ERM proteins—the awesome power of genetics finally brought to bear. *Curr Opin Cell Biol* 19: 51–56.
117. Huvos AG, (1986) Osteogenic sarcoma of bones and soft tissues in older persons. A clinicopathologic analysis of 117 patients older than 60 years. *Cancer* 57: 1442–1449.
118. Huvos AG, Butler A, and Bretsky SS, (1983) Osteogenic sarcoma associated with Paget's disease of bone. A clinicopathologic study of 65 patients. *Cancer* 52: 1489–1495.
119. Hynes NE, Horsch K, Olayioye MA, and Badache A, (2001) The ErbB receptor tyrosine family as signal integrators. *Endocr Relat Cancer* 8: 151–159.
120. Isfort RJ, Cody DB, Lovell G, and Doersen CJ, (1995) Analysis of oncogenes, tumor suppressor genes, autocrine growth-factor production, and differentiation state of human osteosarcoma cell lines. *Mol Carcinog* 14: 170–178.
121. Issing WJ, Wustrow TP, Oeckler R, Mezger J, and Nerlich A, (1993) An association of the RB gene with osteosarcoma: molecular genetic evaluation of a case of hereditary retinoblastoma. *Eur Arch Otorhinolaryngol* 250: 277–280.
122. Iwakuma T and Lozano G, (2003) MDM2, an introduction. *Mol Cancer Res* 1: 993–1000.
123. Jasnaus S, Meyer U, Potratz J, Jundt G, Kevric M, Joos UK, Jurgens H, and Bielack SS, (2008) Craniofacial osteosarcoma experience of the cooperative German-Austrian-Swiss osteosarcoma study group. *Oral Oncol* 44: 286–294.
124. Jia SF, Worth LL, Turan M, Duan Xp XP, and Kleinerman ES, (2002) Eradication of osteosarcoma lung metastasis using intranasal gemcitabine. *Anticancer Drugs* 13: 155–161.
125. Johnson JE and Broccoli D, (2007) Telomere maintenance in sarcomas. *Curr Opin Oncol* 19: 377–382.
126. Johnson-Pais TL, Nellissery MJ, Ammerman DG, Pathmanathan D, Bhatia P, Buller CL, Leach RJ, and Hansen MF, (2003) Determination of a minimal region of loss of heterozygosity on chromosome 18q21.33 in osteosarcoma. *Int J Cancer* 105: 285–288.
127. Junior AT, de Abreu Alves F, Pinto CA, Carvalho AL, Kowalski LP, and Lopes MA, (2003) Clinicopathological and immunohistochemical analysis of twenty-five head and neck osteosarcomas. *Oral Oncol* 39: 521–530.
128. Kager L, Zoubek A, Potschger U, Kastner U, Flege S, Kempf-Bielack B, Branschke D, Kotz R, Salzer-Kuntschik M, Winkelmann W, Jundt G, Kabisch H, Reichardt P, Jurgens H, Gadner H, and Bielack SS, (2003) Primary metastatic osteosarcoma: presentation and outcome of patients treated on neoadjuvant Cooperative Osteosarcoma Study Group protocols. *J Clin Oncol* 21: 2011–2018.
129. Kallioniemi OP, Kallioniemi A, Kurisu W, Thor A, Chen LC, Smith HS, Waldman FM, Pinkel D, and Gray JW, (1992) ERBB2 amplification in breast cancer analyzed by fluorescence in situ hybridization. *Proc Natl Acad Sci USA* 89: 5321–5325.
130. Kanoe H, Nakayama T, Murakami H, Hosaka T, Yamamoto H, Nakashima Y, Tsuboyama T, Nakamura T, Sasaki MS, and Toguchida J, (1998) Amplification

- of the CDK4 gene in sarcomas: tumor specificity and relationship with the RB gene mutation. *Anticancer Res* 18: 2317–2321.
131. Karolchik D, Baertsch R, Diekhans M, Furey TS, Hinrichs A, Lu YT, Roskin KM, Schwartz M, Sugnet CW, Thomas DJ, Weber RJ, Haussler D, and Kent WJ, (2003) The UCSC genome browser database. *Nucleic Acids Res* 31: 51–54.
132. Kaste SC, Fuller CE, Saharia A, Neel MD, Rao BN, and Daw NC, (2006) Pediatric surface osteosarcoma: clinical, pathologic, and radiologic features. *Pediatr Blood Cancer* 47: 152–162.
133. Kaur S, Larramendy ML, Vauhkonen H, Bohling T, and Knuutila S, (2007) Loss of TP53 in sarcomas with 17p12 to approximately p11 gain. A fine-resolution oligonucleotide array comparative genomic hybridization study. *Cytogenet Genome Res* 116: 153–157.
134. Kawaguchi K, Oda Y, Sakamoto A, Saito T, Tamiya S, Iwamoto Y, and Tsuneyoshi M, (2002) Molecular analysis of p53, MDM2, and H-ras genes in osteosarcoma and malignant fibrous histiocytoma of bone in patients older than 40 years. *Mod Pathol* 15: 878–888.
135. Khanna C, Khan J, Nguyen P, Prehn J, Caylor J, Yeung C, Trepel J, Meltzer P, and Helman L, (2001) Metastasis-associated differences in gene expression in a murine model of osteosarcoma. *Cancer Res* 61: 3750–3759.
136. Khanna C, Wan X, Bose S, Cassaday R, Olomu O, Mendoza A, Yeung C, Gorlick R, Hewitt SM, and Helman LJ, (2004) The membrane–cytoskeleton linker ezrin is necessary for osteosarcoma metastasis. *Nat Med* 10: 182–186.
137. Khatib ZA, Matsushime H, Valentine M, Shapiro DN, Sherr CJ, and Look AT, (1993) Coamplification of the CDK4 gene with MDM2 and GLI in human sarcomas. *Cancer Res* 53: 5535–5541.
138. Kido A, Schneider-Stock R, Hauptmann K, and Roessner A, (2003) Telomerase activity in juxtacortical and conventional high-grade osteosarcomas: correlation with grade, proliferative activity and clinical response to chemotherapy. *Cancer Lett* 196: 109–115.
139. Kilpatrick SE, Geisinger KR, King TS, Sciarrotta J, Ward WG, Gold SH, and Bos GD, (2001) Clinicopathologic analysis of HER-2/neu immunorexpression among various histologic subtypes and grades of osteosarcoma. *Mod Pathol* 14: 1277–1283.
140. Kim MS, Song WS, Cho WH, Lee SY, and Jeon DG, (2007) Ezrin expression predicts survival in stage IIB osteosarcomas. *Clin Orthop Relat Res* 459: 229–236.
141. Kitao S, Shimamoto A, Goto M, Miller RW, Smithson WA, Lindor NM, and Furuichi Y, (1999) Mutations in RECQL4 cause a subset of cases of Rothmund–Thomson syndrome. *Nat Genet* 22: 82–84.
142. Kitchin FD and Ellsworth RM, (1974) Pleiotropic effects of the gene for retinoblastoma. *J Med Genet* 11: 244–246.
143. Klapper LN, Glathe S, Vaisman N, Hynes NE, Andrews GC, Sela M, and Yarden Y, (1999) The ErbB-2/HER2 oncoprotein of human carcinomas may function solely as a shared coreceptor for multiple stroma-derived growth factors. *Proc Natl Acad Sci USA* 96: 4995–5000.
144. Knudsen ES, Sexton CR, and Mayhew CN, (2006) Role of the retinoblastoma tumor suppressor in the maintenance of genome integrity. *Curr Mol Med* 6: 749–757.
145. Knudsen KE, Booth D, Naderi S, Sever-Chroneos Z, Fribourg AF, Hunton IC, Feramisco JR, Wang JY, and Knudsen ES, (2000) RB-dependent S-phase response to DNA damage. *Mol Cell Biol* 20: 7751–7763.
146. Kolodner RD, Putnam CD, and Myung K, (2002) Maintenance of genome stability in *Saccharomyces cerevisiae*. *Science* 297: 552–557.
147. Kops GJ, Weaver BA, and Cleveland DW, (2005) On the road to cancer: aneuploidy and the mitotic checkpoint. *Nat Rev Cancer* 5: 773–785.
148. Korenjak M and Brehm A, (2005) E2F–Rb complexes regulating transcription of genes important for differentiation and development. *Curr Opin Genet Dev* 15: 520–527.
149. Koshkina NV, Khanna C, Mendoza A, Guan H, DeLauter L, and Kleiner ES, (2007) Fas-negative osteosarcoma tumor cells are selected during metastasis to the lungs: the role of the Fas pathway in the metastatic process of osteosarcoma. *Mol Cancer Res* 5: 991–999.
150. Koshkina NV and Kleiner ES, (2005) Aerosol gemcitabine inhibits the growth of primary osteosarcoma and osteosarcoma lung metastases. *Int J Cancer* 116: 458–463.
151. Kramer K, Hicks DG, Palis J, Rosier RN, Oppenheimer J, Fallon MD, and Cohen HJ, (1993) Epithelioid osteosarcoma of bone. Immunocytochemical evidence suggesting divergent epithelial and mesenchymal differentiation in a primary osseous neoplasm. *Cancer* 71: 2977–2982.
152. La Quaglia MP, (1998) Osteosarcoma. Specific tumor management and results. *Chest Surg Clin N Am* 8: 77–95.
153. Lafleur EA, Koshkina NV, Stewart J, Jia SF, Worth LL, Duan X, and Kleiner ES, (2004) Increased Fas expression reduces the metastatic potential of human osteosarcoma cells. *Clin Cancer Res* 10: 8114–8119.
154. Lamovec J, Zidar A, Bracko M, and Golouh R, (1994) Primary bone sarcoma with rhabdomyosarcomatous component. *Pathol Res Pract* 190: 51–60.
155. Larizza L, Magnani I, and Roversi G, (2006) Rothmund–Thomson syndrome and RECQL4 defect: splitting and lumping. *Cancer Lett* 232: 107–120.
156. Lau CC, Harris CP, Lu XY, Perlaky L, Gogineni S, Chintagumpala M, Hicks J, Johnson ME, Davino NA, Huvos AG, Meyers PA, Healy JH, Gorlick R, and Rao PH, (2004) Frequent amplification and rearrangement of chromosomal bands 6p12–p21 and 17p11.2 in osteosarcoma. *Genes Chromosomes Cancer* 39: 11–21.
157. Lee JS, Fetsch JF, Wasdhal DA, Lee BP, Pritchard DJ, and Nascimento AG, (1995) A review of 40 patients with extraskeletal osteosarcoma. *Cancer* 76: 2253–2259.

158. Lee N, Smolarz AJ, Olson S, David O, Reiser J, Kutner R, Daw NC, Prockop DJ, Horwitz EM, and Gregory CA, (2007) A potential role for Dkk-1 in the pathogenesis of osteosarcoma predicts novel diagnostic and treatment strategies. *Br J Cancer* 97: 1552–1559.
159. Lee SB, Kim SH, Bell DW, Wahrer DC, Schiripo TA, Jorczak MM, Sgroi DC, Garber JE, Li FP, Nichols KE, Varley JM, Godwin AK, Shannon KM, Harlow E, and Haber DA, (2001) Destabilization of CHK2 by a missense mutation associated with Li–Fraumeni Syndrome. *Cancer Res* 61: 8062–8067.
160. Lee WH, Bookstein R, Hong F, Young LJ, Shew JY, and Lee EY, (1987) Human retinoblastoma susceptibility gene: cloning, identification, and sequence. *Science* 235: 1394–1399.
161. Leonard P, Sharp T, Henderson S, Hewitt D, Pringle J, Sandison A, Goodship A, Whelan J, and Boshoff C, (2003) Gene expression array profile of human osteosarcoma. *Br J Cancer* 89: 2284–2288.
162. Li FP and Fraumeni JF, Jr., (1969) Soft-tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome? *Ann Intern Med* 71: 747–752.
163. Li FP, Fraumeni JF, Jr., Mulvihill JJ, Blattner WA, Dreyfus MG, Tucker MA, and Miller RW, (1988) A cancer family syndrome in twenty-four kindreds. *Cancer Res* 48: 5358–5362.
164. Lidang JM, Schumacher B, Myhre JO, Steen NO, and Keller J, (1998) Extraskeletal osteosarcomas: a clinicopathologic study of 25 cases. *Am J Surg Pathol* 22: 588–594.
165. Lim G, Karaskova J, Vukovic B, Bayani J, Beheshti B, Bernardini M, Squire JA, and Zielenska M, (2004) Combined spectral karyotyping, multicolor banding, and microarray comparative genomic hybridization analysis provides a detailed characterization of complex structural chromosomal rearrangements associated with gene amplification in the osteosarcoma cell line MG-63. *Cancer Genet Cytogenet* 153: 158–164.
166. Lindell MM, Jr., Shirkhoda A, Raymond AK, Murray JA, and Harle TS, (1987) Parosteal osteosarcoma: radiologic–pathologic correlation with emphasis on CT. *AJR Am J Roentgenol* 148: 323–328.
167. Lindor NM, Furuichi Y, Kitao S, Shimamoto A, Arndt C, and Jalal S, (2000) Rothmund–Thomson syndrome due to RECQ4 helicase mutations: report and clinical and molecular comparisons with Bloom syndrome and Werner syndrome. *Am J Med Genet* 90: 223–228.
168. Lipinski MM and Jacks T, (1999) The retinoblastoma gene family in differentiation and development. *Oncogene* 18: 7873–7882.
169. Loeb LA, Springgate CF, and Battula N, (1974) Errors in DNA replication as a basis of malignant changes. *Cancer Res* 34: 2311–2321.
170. Lopez-Guerrero JA, Lopez-Gines C, Pellin A, Carda C, and Llombart-Bosch A, (2004) Deregulation of the G1 to S-phase cell cycle checkpoint is involved in the pathogenesis of human osteosarcoma. *Diagn Mol Pathol* 13: 81–91.
171. Maitra A, Wanzer D, Weinberg AG, and Ashfaq R, (2001) Amplification of the HER-2/neu oncogene is uncommon in pediatric osteosarcomas. *Cancer* 92: 677–683.
172. Malkin D, Li FP, Strong LC, Fraumeni JF, Jr., Nelson CE, Kim DH, Kassel J, Gryka MA, Bischoff FZ, Tainsky MA et al., (1990) Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science* 250: 1233–1238.
173. Man TK, Chintagumpala M, Visvanathan J, Shen J, Perlaky L, Hicks J, Johnson M, Davino N, Murray J, Helman L, Meyer W, Triche T, Wong KK, and Lau CC, (2005) Expression profiles of osteosarcoma that can predict response to chemotherapy. *Cancer Res* 65: 8142–8150.
174. Man TK, Lu XY, Jaeweon K, Perlaky L, Harris CP, Shah S, Ladanyi M, Gorlick R, Lau CC, and Rao PH, (2004) Genome-wide array comparative genomic hybridization analysis reveals distinct amplifications in osteosarcoma. *BMC Cancer* 4: 45.
175. Mandal D, Srivastava A, Mahlum E, Desai D, Maran A, Yaszemski M, Jalal SM, Gitelis S, Bertoni F, Damron T, Irwin R, O'Connor M, Schwartz H, Bolander ME, and Sarkar G, (2007) Severe suppression of Frzb/sFRP3 transcription in osteogenic sarcoma. *Gene* 386: 131–138.
176. Mann MB, Hodges CA, Barnes E, Vogel H, Hassold TJ, and Luo G, (2005) Defective sister-chromatid cohesion, aneuploidy and cancer predisposition in a mouse model of type II Rothmund–Thomson syndrome. *Hum Mol Genet* 14: 813–825.
177. Marcial-Seoane RA, Marcial-Seoane MA, Davila-Toro FJ, and Marcial-Rojas RA, (1990) Bone tumors of mixed origin: osteo-liposarcoma and osteo-rhabdomyosarcoma. *Bol Asoc Med P R* 82: 378–393.
178. Mardinger O, Givol N, Talmi YP, and Taicher S, (2001) Osteosarcoma of the jaw: The Chaim Sheba Medical Center experience. *Oral Surg, Oral Med, Oral Pathol, Oral Radiol, Endod* 91: 445–451.
179. Marx J, (2002) Debate surges over the origins of genomic defects in cancer. *Science* 297: 544–546.
180. Maser RS and DePinho RA, (2002) Connecting chromosomes, crisis, and cancer. *Science* 297: 565–569.
181. Masuda H, Miller C, Koeffler HP, Battifora H, and Cline MJ, (1987) Rearrangement of the p53 gene in human osteogenic sarcomas. *Proc Natl Acad Sci USA* 84: 7716–7719.
182. McClatchey AI, Saotome I, Mercer K, Crowley D, Gusella JF, Bronson RT, and Jacks T, (1998) Mice heterozygous for a mutation at the Nf2 tumor suppressor locus develop a range of highly metastatic tumors. *Genes Dev* 12: 1121–1133.
183. McHugh JB, Thomas DG, Herman JM, Ray ME, Baker LH, Adsay NV, Rabah R, and Lucas DR, (2006) Primary versus radiation-associated craniofacial osteosarcoma: Biologic and clinicopathologic comparisons. *Cancer* 107: 554–562.
184. McNairn JD, Damron TA, Landas SK, Ambrose JL, and Shrimpton AE, (2001) Inheritance of osteosarcoma

- and Paget's disease of bone: a familial loss of heterozygosity study. *J Mol Diagn* 3: 171-177.
185. Mervak TR, Unni KK, Pritchard DJ, and McLeod RA, (1991) Telangiectatic osteosarcoma. *Clin Orthop Relat Res* 135-139.
 186. Meyers PA and Gorlick R, (1997) Osteosarcoma. *Pediatr Clin North Am* 44: 973-989.
 187. Miller AD, Curran T, and Verma IM, (1984) c-fos protein can induce cellular transformation: a novel mechanism of activation of a cellular oncogene. *Cell* 36: 51-60.
 188. Miller CW, Aslo A, Campbell MJ, Kawamata N, Lampkin BC, and Koeffler HP, (1996) Alterations of the p15, p16, and p18 genes in osteosarcoma. *Cancer Genet Cytogenet* 86: 136-142.
 189. Miller CW, Aslo A, Tsay C, Slamon D, Ishizaki K, Toguchida J, Yamamuro T, Lampkin B, and Koeffler HP, (1990) Frequency and structure of p53 rearrangements in human osteosarcoma. *Cancer Res* 50: 7950-7954.
 190. Miller CW, Aslo A, Won A, Tan M, Lampkin B, and Koeffler HP, (1996) Alterations of the p53, Rb and MDM2 genes in osteosarcoma. *J Cancer Res Clin Oncol* 122: 559-565.
 191. Miller CW, Ikezoe T, Krug U, Hofmann WK, Tavor S, Vegesna V, Tsukasaki K, Takeuchi S, and Koeffler HP, (2002) Mutations of the CHK2 gene are found in some osteosarcomas, but are rare in breast, lung, and ovarian tumors. *Genes Chromosomes Cancer* 33: 17-21.
 192. Mintz MB, Sowers R, Brown KM, Hilmer SC, Mazza B, Huvos AG, Meyers PA, Lafleur B, McDonough WS, Henry MM, Ramsey KE, Antonescu CR, Chen W, Healey JH, Daluski A, Berens ME, Macdonald TJ, Gorlick R, and Stephan DA, (2005) An expression signature classifies chemotherapy-resistant pediatric osteosarcoma. *Cancer Res* 65: 1748-1754.
 193. Moore TE, King AR, Kathol MH, el-Khoury GY, Palmer R, and Downey PR, (1991) Sarcoma in Paget's disease of bone: clinical, radiologic, and pathologic features in 22 cases. *AJR Am J Roentgenol* 156: 1199-1203.
 194. Mori K, Berreur M, Blanchard F, Chevalier C, Guislemarsollier I, Masson M, Redini F, and Heymann D, (2007) Receptor activator of nuclear factor-kappaB ligand (RANKL) directly modulates the gene expression profile of RANK-positive Saos-2 human osteosarcoma cells. *Oncol Rep* 18: 1365-1371.
 195. Moroni MC, Hickman ES, Lazzerini Denchi E, Caprara G, Colli E, Cecconi F, Muller H, and Helin K, (2001) Apaf-1 is a transcriptional target for E2F and p53. *Nat Cell Biol* 3: 552-558.
 196. Mrad K, Sassi S, Smida M, Oubiche F, Mekni A, and Romdhane KB, (2004) Osteosarcoma with rhabdomyosarcomatous component or so-called malignant mesenchymoma of bone. *Pathologica* 96: 475-478.
 197. Murata H, Kusuzaki K, Hirasawa Y, Ashihara T, Abe T, and Inazawa J, (1999) Relationship between chromosomal aberrations by fluorescence in situ hybridization and DNA ploidy by cytofluorometry in osteosarcoma. *Cancer Lett* 139: 221-226.
 198. Murata K, Hatamochi A, Shinkai H, Ishikawa Y, Kawaguchi N, and Goto M, (1999) A case of Werner's syndrome associated with osteosarcoma. *J Dermatol* 26: 682-686.
 199. Murphey MD, Robbin MR, McRae GA, Flemming DJ, Temple HT, and Kransdorf MJ, (1997) The many faces of osteosarcoma. *Radiographics* 17: 1205-1231.
 200. Murphey MD, wan Jaovisidha S, Temple HT, Gannon FH, Jelinek JS, and Malawer MM, (2003) Telangiectatic osteosarcoma: radiologic-pathologic comparison. *Radiology* 229: 545-553.
 201. Nagata S, Nishimura H, Uchida M, Hayabuchi N, Zenmyou M, and Harada H, (2006) Giant cell-rich osteosarcoma of the distal femur: radiographic and magnetic resonance imaging findings. *Radiat Med* 24: 228-232.
 202. Nakajima H, Sim FH, Bond JR, and Unni KK, (1997) Small cell osteosarcoma of bone. Review of 72 cases. *Cancer* 79: 2095-2106.
 203. Nakano T, Tani M, Ishibashi Y, Kimura K, Park YB, Imaizumi N, Tsuda H, Aoyagi K, Sasaki H, Ohwada S, and Yokota J, (2003) Biological properties and gene expression associated with metastatic potential of human osteosarcoma. *Clin Exp Metastasis* 20: 665-674.
 204. Nakashima H, Nishida Y, Sugiura H, Katagiri H, Yonekawa M, Yamada Y, Iwata H, Nagasaka T, and Ishiguro N, (2003) Telomerase, p53 and PCNA activity in osteosarcoma. *Eur J Surg Oncol* 29: 564-567.
 205. Nakayama H, (2002) RecQ family helicases: roles as tumor suppressor proteins. *Oncogene* 21: 9008-9021.
 206. Nakayama T, Toguchida J, Wadayama B, Kanoe H, Kotoura Y, and Sasaki MS, (1995) MDM2 gene amplification in bone and soft-tissue tumors: association with tumor progression in differentiated adipose-tissue tumors. *Int J Cancer* 64: 342-346.
 207. Nellisery MJ, Padalecki SS, Brkanac Z, Singer FR, Roodman GD, Unni KK, Leach RJ, and Hansen MF, (1998) Evidence for a novel osteosarcoma tumor-suppressor gene in the chromosome 18 region genetically linked with Paget's disease of bone. *Am J Hum Genet* 63: 817-824.
 208. Neve RM, Holbro T, and Hynes NE, (2002) Distinct roles for phosphoinositide 3-kinase, mitogen-activated protein kinase and p38 MAPK in mediating cell cycle progression of breast cancer cells. *Oncogene* 21: 4567-4576.
 209. Neve RM, Lane HA, and Hynes NE, (2001) The role of overexpressed HER2 in transformation. *Ann Oncol* 12 Suppl 1: S9-13.
 210. Nevins JR, (2001) The Rb/E2F pathway and cancer. *Hum Mol Genet* 10: 699-703.
 211. Niehrs C, (2006) Function and biological roles of the Dickkopf family of Wnt modulators. *Oncogene* 25: 7469-7481.
 212. Nielsen GP, Burns KL, Rosenberg AE, and Louis DN, (1998) CDKN2A gene deletions and loss of p16 expres-

- sion occur in osteosarcomas that lack RB alterations. *Am J Pathol* 153: 159–163.
213. Nishijo K, Nakayama T, Aoyama T, Okamoto T, Ishibe T, Yasura K, Shima Y, Shibata KR, Tsuboyama T, Nakamura T, and Toguchida J, (2004) Mutation analysis of the RECQL4 gene in sporadic osteosarcomas. *Int J Cancer* 111: 367–372.
 214. Nowak MA, Komarova NL, Sengupta A, Jallepalli PV, Shih Ie M, Vogelstein B, and Lengauer C, (2002) The role of chromosomal instability in tumor initiation. *Proc Natl Acad Sci USA* 99: 16226–16231.
 215. Ochi K, Daigo Y, Katagiri T, Nagayama S, Tsunoda T, Myoui A, Naka N, Araki N, Kudawara I, Ieguchi M, Toyama Y, Toguchida J, Yoshikawa H, and Nakamura Y, (2004) Prediction of response to neoadjuvant chemotherapy for osteosarcoma by gene-expression profiles. *Int J Oncol* 24: 647–655.
 216. Oda D, Bavisotto LM, Schmidt RA, McNutt M, Bruckner JD, Conrad EU, 3rd, and Weymuller EA, Jr., (1997) Head and neck osteosarcoma at the University of Washington. *Head Neck* 19: 513–523.
 217. Ohata N, Ito S, Yoshida A, Kunisada T, Numoto K, Jitsumori Y, Kanzaki H, Ozaki T, Shimizu K, and Ouchida M, (2006) Highly frequent allelic loss of chromosome 6q16–23 in osteosarcoma: involvement of cyclin C in osteosarcoma. *Int J Mol Med* 18: 1153–1158.
 218. Okada K, Frassica FJ, Sim FH, Beabout JW, Bond JR, and Unni KK, (1994) Parosteal osteosarcoma. A clinicopathological study. *J Bone Joint Surg Am* 76: 366–378.
 219. Okada K, Unni KK, Swee RG, and Sim FH, (1999) High grade surface osteosarcoma: a clinicopathologic study of 46 cases. *Cancer* 85: 1044–1054.
 220. Olayioye MA, Graus-Porta D, Beerli RR, Rohrer J, Gay B, and Hynes NE, (1998) ErbB-1 and ErbB-2 acquire distinct signaling properties dependent upon their dimerization partner. *Mol Cell Biol* 18: 5042–5051.
 221. Olayioye MA, Neve RM, Lane HA, and Hynes NE, (2000) The ErbB signaling network: receptor heterodimerization in development and cancer. *Embo J* 19: 3159–3167.
 222. Oliner JD, Kinzler KW, Meltzer PS, George DL, and Vogelstein B, (1992) Amplification of a gene encoding a p53-associated protein in human sarcomas. *Nature* 358: 80–83.
 223. Onda M, Matsuda S, Higaki S, Iijima T, Fukushima J, Yokokura A, Kojima T, Horiuchi H, Kurokawa T, and Yamamoto T, (1996) ErbB-2 expression is correlated with poor prognosis for patients with osteosarcoma. *Cancer* 77: 71–78.
 224. Overholtzer M, Rao PH, Favis R, Lu XY, Elowitz MB, Barany F, Ladanyi M, Gorlick R, and Levine AJ, (2003) The presence of p53 mutations in human osteosarcomas correlates with high levels of genomic instability. *Proc Natl Acad Sci USA* 100: 11547–11552.
 225. Ozaki T, Neumann T, Wai D, Schafer KL, van Valen F, Lindner N, Scheel C, Bocker W, Winkelmann W, Dockhorn-Dworniczak B, Horst J, and Poremba C, (2003) Chromosomal alterations in osteosarcoma cell lines revealed by comparative genomic hybridization and multicolor karyotyping. *Cancer Genet Cytogenet* 140: 145–152.
 226. Paget J, (1877) On a form of chronic inflammation of bones (osteitis deformans). *Med Chir Trans* 60: 37–63.
 227. Papagelopoulos PJ, Galanis E, Sim FH, and Unni KK, (1999) Periosteal osteosarcoma. *Orthopedics* 22: 971–974.
 228. Park HR, Jung WW, Bacchini P, Bertoni F, Kim YW, and Park YK, (2006) Ezrin in osteosarcoma: comparison between conventional high-grade and central low-grade osteosarcoma. *Pathol Res Pract* 202: 509–515.
 229. Park HR, Jung WW, Bertoni F, Bacchini P, Park JH, Kim YW, and Park YK, (2004) Molecular analysis of p53, MDM2 and H-ras genes in low-grade central osteosarcoma. *Pathol Res Pract* 200: 439–445.
 230. Parkin DM, Stiller CA, and Nectoux J, (1993) International variations in the incidence of childhood bone tumors. *Int J Cancer* 53: 371–376.
 231. Pellin A, Boix-Ferrero J, Carpio D, Lopez-Terrada D, Carda C, Navarro S, Peydro-Olaya A, Triche TJ, and Llombart-Bosch A, (1997) Molecular alterations of the RB1, TP53, and MDM2 genes in primary and xenografted human osteosarcomas. *Diagn Mol Pathol* 6: 333–341.
 232. Petkovic M, Dietschy T, Freire R, Jiao R, and Staglar I, (2005) The human Rothmund–Thomson syndrome gene product, RECQL4, localizes to distinct nuclear foci that coincide with proteins involved in the maintenance of genome stability. *J Cell Sci* 118: 4261–4269.
 233. Pommier Y, Weinstein JN, Aladjem MI, and Kohn KW, (2006) Chk2 molecular interaction map and rationale for Chk2 inhibitors. *Clin Cancer Res* 12: 2657–2661.
 234. Porter DE, Holden ST, Steel CM, Cohen BB, Wallace MR, and Reid R, (1992) A significant proportion of patients with osteosarcoma may belong to Li–Fraumeni cancer families. *J Bone Joint Surg Br* 74: 883–886.
 235. Price CH and Goldie W, (1969) Paget's sarcoma of bone. A study of eighty cases from the Bristol and the Leeds bone tumour registries. *J Bone Joint Surg Br* 51: 205–224.
 236. Provisor AJ, Ettinger LJ, Nachman JB, Krailo MD, Makley JT, Yunis EJ, Huvo AG, Betcher DL, Baum ES, Kisker CT, and Miser JS, (1997) Treatment of non-metastatic osteosarcoma of the extremity with pre-operative and postoperative chemotherapy: a report from the Children's Cancer Group. *J Clin Oncol* 15: 76–84.
 237. Radig K, Schneider-Stock R, Oda Y, Neumann W, Mittler U, and Roessner A, (1996) Mutation spectrum of p53 gene in highly malignant human osteosarcomas. *Gen Diagn Pathol* 142: 25–32.
 238. Ragazzini P, Gamberi G, Benassi MS, Orlando C, Sestini R, Ferrari C, Molendini L, Sollazzo MR, Merli M, Magagnoli G, Bertoni F, Bohling T, Pazzagli M, and Picci P, (1999) Analysis of SAS gene and CDK4 and

- MDM2 proteins in low-grade osteosarcoma. *Cancer Detect Prev* 23: 129–136.
239. Rajagopalan H and Lengauer C, (2004) Aneuploidy and cancer. *Nature* 432: 338–341.
 240. Rajagopalan H, Nowak MA, Vogelstein B, and Lengauer C, (2003) The significance of unstable chromosomes in colorectal cancer. *Nat Rev Cancer* 3: 695–701.
 241. Raymond AK, (1991) Surface osteosarcoma. *Clin Orthop Relat Res* 140–148.
 242. Reddy SV, (2004) Etiology of Paget's disease and osteoclast abnormalities. *J Cell Biochem* 93: 688–696.
 243. Reith JD, Donahue FI, and Hornicek FJ, (1999) Dedifferentiated parosteal osteosarcoma with rhabdomyosarcomatous differentiation. *Skeletal Radiol* 28: 527–531.
 244. Reya T, Morrison SJ, Clarke MF, and Weissman IL, (2001) Stem cells, cancer, and cancer stem cells. *Nature* 414: 105–111.
 245. Ritts GD, Pritchard DJ, Unni KK, Beabout JW, and Eckardt JJ, (1987) Periosteal osteosarcoma. *Clin Orthop Relat Res* 299–307.
 246. Rose PS, Dickey ID, Wenger DE, Unni KK, and Sim FH, (2006) Periosteal osteosarcoma: long-term outcome and risk of late recurrence. *Clin Orthop Relat Res* 453: 314–317.
 247. Roskoski R, Jr., (2004) The ErbB/HER receptor protein-tyrosine kinases and cancer. *Biochem Biophys Res Commun* 319: 1–11.
 248. Ross JS and Fletcher JA, (1998) The HER-2/neu oncogene in breast cancer: prognostic factor, predictive factor, and target for therapy. *Stem Cells* 16: 413–428.
 249. Rothmund A, (1868) Ueber Cataracte in Verbindung mit einer eigenthuemlichen Hautdegeneration. *Albrecht von Graefes Arch Klin Exp Ophthal* 14: 159–182.
 250. Ruther U, Komitowski D, Schubert FR, and Wagner EF, (1989) c-fos expression induces bone tumors in transgenic mice. *Oncogene* 4: 861–865.
 251. Salas S, Bartoli C, Deville JL, Gaudart J, Fina F, Calisti A, Bollini G, Curvale G, Gentet JC, Duffaud F, Figarella-Branger D, and Bouvier C, (2007) Ezrin and alpha-smooth muscle actin are immunohistochemical prognostic markers in conventional osteosarcomas. *Virchows Arch* 451: 999–1007.
 252. Sangiorgi L, Gobbi GA, Lucarelli E, Sartorio SM, Mordenti M, Ghedini I, Maini V, Scrimieri F, Reggiani M, Bertoja AZ, Benassi MS, and Picci P, (2001) Presence of telomerase activity in different musculoskeletal tumor histotypes and correlation with aggressiveness. *Int J Cancer* 95: 156–161.
 253. Sato K, Yamamura S, Iwata H, Sugiura H, Nakashima N, and Nagasaka T, (1996) Giant cell-rich osteosarcoma: a case report. *Nagoya J Med Sci* 59: 151–157.
 254. Schajowicz F, McGuire MH, Santini Araujo E, Muscolo DL, and Gitelis S, (1988) Osteosarcomas arising on the surfaces of long bones. *J Bone Joint Surg Am* 70: 555–564.
 255. Scheel C, Schaefer KL, Jauch A, Keller M, Wai D, Brinkschmidt C, van Valen F, Boecker W, Dockhorn-Dworniczak B, and Poremba C, (2001) Alternative lengthening of telomeres is associated with chromosomal instability in osteosarcomas. *Oncogene* 20: 3835–3844.
 256. Scheffer H, Kruize YC, Osinga J, Kuiken G, Oosterhuis JW, Leeuw JA, Schraffordt Koops H, and Buys CH, (1991) Complete association of loss of heterozygosity of chromosomes 13 and 17 in osteosarcoma. *Cancer Genet Cytogenet* 53: 45–55.
 257. Scholz RB, Kabisch H, Weber B, Roser K, Delling G, and Winkler K, (1992) Studies of the RB1 gene and the p53 gene in human osteosarcomas. *Pediatr Hematol Oncol* 9: 125–137.
 258. Selvarajah S, Yoshimoto M, Maire G, Paderova J, Bayani J, Squire JA, and Zielenska M, (2007) Identification of cryptic microaberrations in osteosarcoma by high-definition oligonucleotide array comparative genomic hybridization. *Cancer Genet Cytogenet* 179: 52–61.
 259. Selvarajah S, Yoshimoto M, Park PC, Maire G, Paderova J, Bayani J, Lim G, Al-Romaih K, Squire JA, and Zielenska M, (2006) The breakage-fusion-bridge (BFB) cycle as a mechanism for generating genetic heterogeneity in osteosarcoma. *Chromosoma* 115: 459–467.
 260. Serra M, Tarkkanen M, Baldini N, Scotlandi K, Sarti M, Maurici D, Manara MC, Benini S, Bacchini P, Knuutila S, and Picci P, (2001) Simultaneous paired analysis of numerical chromosomal aberrations and DNA content in osteosarcoma. *Mod Pathol* 14: 710–716.
 261. Seton M, Choi HK, Hansen MF, Seibaldt RJ, and Cooper C, (2003) Analysis of environmental factors in familial versus sporadic Paget's disease of bone – the New England Registry for Paget's disease of bone. *J Bone Miner Res* 18: 1519–1524.
 262. Sheth DS, Yasko AW, Raymond AK, Ayala AG, Carrasco CH, Benjamin RS, Jaffe N, and Murray JA, (1996) Conventional and dedifferentiated parosteal osteosarcoma. Diagnosis, treatment, and outcome. *Cancer* 78: 2136–2145.
 263. Shih IM, Zhou W, Goodman SN, Lengauer C, Kinzler KW, and Vogelstein B, (2001) Evidence that genetic instability occurs at an early stage of colorectal tumorigenesis. *Cancer Res* 61: 818–822.
 264. Sieber OM, Heinimann K, and Tomlinson IP, (2003) Genomic instability – the engine of tumorigenesis? *Nat Rev Cancer* 3: 701–708.
 265. Siris ES, (1998) Paget's disease of bone. *J Bone Miner Res* 13: 1061–1065.
 266. Siris ES, Jacobs TP, and Canfield RE, (1980) Paget's disease of bone. *Bull N Y Acad Med* 56: 285–304.
 267. Siris ES, Ottman R, Flaster E, and Kelsey JL, (1991) Familial aggregation of Paget's disease of bone. *J Bone Miner Res* 6: 495–500.
 268. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, and McGuire WL, (1987) Human breast cancer: corre-

- lation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 235: 177–182.
269. Slootweg PJ and Muller H, (1985) Osteosarcoma of the jaw bones. Analysis of 18 cases. *J Maxillofac Surg* 13: 158–166.
 270. Smith J, Botet JF, and Yeh SD, (1984) Bone sarcomas in Paget's Disease: a study of 85 patients. *Radiology* 152: 583–590.
 271. Somers GR, Ho M, Zielenska M, Squire JA, and Thorner PS, (2005) HER2 amplification and overexpression is not present in pediatric osteosarcoma: a tissue microarray study. *Pediatr Dev Pathol* 8: 525–532.
 272. Sotillo-Pineiro E, Sierrasesumaga L, and Patino-Garcia A, (2004) Telomerase activity and telomere length in primary and metastatic tumors from pediatric bone cancer patients. *Pediatr Res* 55: 231–235.
 273. Squire JA, Pei J, Marrano P, Beheshti B, Bayani J, Lim G, Moldovan L, and Zielenska M, (2003) High-resolution mapping of amplifications and deletions in pediatric osteosarcoma by use of CGH analysis of cDNA microarrays. *Genes Chromosomes Cancer* 38: 215–225.
 274. Stemmer-Rachamimov AO, Nielsen GP, Rosenberg AE, Louis DN, Jones D, Ramesh V, Gusella JF, and Jacoby LB, (1998) The NF2 gene and merlin protein in human osteosarcomas. *Neurogenetics* 2: 73–74.
 275. Stewart SA, (2005) Telomere maintenance and tumorigenesis: an "ALT"ernative road. *Curr Mol Med* 5: 253–257.
 276. Stiewe T, (2007) The p53 family in differentiation and tumorigenesis. *Nat Rev Cancer* 7: 165–168.
 277. Stock C, Kager L, Fink FM, Gadner H, and Ambros PF, (2000) Chromosomal regions involved in the pathogenesis of osteosarcomas. *Genes Chromosomes Cancer* 28: 329–336.
 278. Sweeney C and Carraway KL, 3rd, (2000) Ligand discrimination by ErbB receptors: differential signaling through differential phosphorylation site usage. *Oncogene* 19: 5568–5573.
 279. Sweeney C, Fambrough D, Huard C, Diamonti AJ, Lander ES, Cantley LC, and Carraway KL, 3rd, (2001) Growth factor-specific signaling pathway stimulation and gene expression mediated by ErbB receptors. *J Biol Chem* 276: 22685–22698.
 280. Takeuchi K, Morii T, Yabe H, Morioka H, Mukai M, and Toyama Y, (2006) Dedifferentiated parosteal osteosarcoma with well-differentiated metastases. *Skeletal Radiol* 35: 778–782.
 281. Tamrakar S, Rubin E, and Ludlow JW, (2000) Role of pRB dephosphorylation in cell cycle regulation. *Front Biosci* 5: D121–137.
 282. Tan X, Martin SJ, Green DR, and Wang JY, (1997) Degradation of retinoblastoma protein in tumor necrosis factor- and CD95-induced cell death. *J Biol Chem* 272: 9613–9616.
 283. Tan X and Wang JY, (1998) The caspase-RB connection in cell death. *Trends Cell Biol* 8: 116–120.
 284. Tanzawa H, Uchiyama S, and Sato K, (1991) Statistical observation of osteosarcoma of the maxillofacial region in Japan. Analysis of 114 Japanese cases reported between 1930 and 1989. *Oral Surg Oral Med Oral Pathol* 72: 444–448.
 285. Tarkkanen M, Bohling T, Gamberi G, Ragazzini P, Benassi MS, Kivioja A, Kallio P, Elomaa I, Picci P, and Knuutila S, (1998) Comparative genomic hybridization of low-grade central osteosarcoma. *Mod Pathol* 11: 421–426.
 286. Tarkkanen M, Elomaa I, Blomqvist C, Kivioja AH, Kellokumpu-Lehtinen P, Bohling T, Valle J, and Knuutila S, (1999) DNA sequence copy number increase at 8q: a potential new prognostic marker in high-grade osteosarcoma. *Int J Cancer* 84: 114–121.
 287. Tarkkanen M, Karhu R, Kallioniemi A, Elomaa I, Kivioja AH, Nevalainen J, Bohling T, Karaharju E, Hyytinen E, Knuutila S, et al., (1995) Gains and losses of DNA sequences in osteosarcomas by comparative genomic hybridization. *Cancer Res* 55: 1334–1338.
 288. Taylor SL, Rudland PS, and Barraclough R, (1999) C-erbB-2 mRNA in breast cancer specimens that exhibit membrane or cytoplasmic immunoreactivity for c-erbB-2. *Oncol Res* 11: 311–317.
 289. Thomas DG, Giordano TJ, Sanders D, Biermann JS, and Baker L, (2002) Absence of HER2/neu gene expression in osteosarcoma and skeletal Ewing's sarcoma. *Clin Cancer Res* 8: 788–793.
 290. Thomson MS, (1936) Poikiloderma congenitale. *Brit J Derm* 48: 221–234.
 291. Toguchida J, Yamaguchi T, Dayton SH, Beauchamp RL, Herrera GE, Ishizaki K, Yamamoto T, Meyers PA, Little JB, Sasaki MS, et al., (1992) Prevalence and spectrum of germline mutations of the p53 gene among patients with sarcoma. *N Engl J Med* 326: 1301–1308.
 292. Toledo F and Wahl GM, (2006) Regulating the p53 pathway: in vitro hypotheses, in vivo veritas. *Nat Rev Cancer* 6: 909–923.
 293. Tomlinson I and Bodmer W, (1999) Selection, the mutation rate and cancer: ensuring that the tail does not wag the dog. *Nat Med* 5: 11–12.
 294. Tsai JY, Aviv H, Benevenia J, Chang VT, Patterson F, Aisner S, and Hameed M, (2004) HER-2/neu and p53 in osteosarcoma: an immunohistochemical and fluorescence in situ hybridization analysis. *Cancer Invest* 22: 16–24.
 295. Tzahar E, Waterman H, Chen X, Levkowitz G, Karunagaran D, Lavi S, Ratzkin BJ, and Yarden Y, (1996) A hierarchical network of interreceptor interactions determines signal transduction by Neu differentiation factor/neuregulin and epidermal growth factor. *Mol Cell Biol* 16: 5276–5287.
 296. Ulaner GA, Huang HY, Otero J, Zhao Z, Ben-Porat L, Satagopan JM, Gorlick R, Meyers P, Healey JH, Huvos AG, Hoffman AR, and Ladanyi M, (2003) Absence of a telomere maintenance mechanism as a favorable prognostic factor in patients with osteosarcoma. *Cancer Res* 63: 1759–1763.

297. Unni KK, (1998) Osteosarcoma of bone. *J Orthop Sci* 3: 287–294.
298. Unni KK and Dahlin DC, (1979) Premalignant tumors and conditions of bone. *Am J Surg Pathol* 3: 47–60.
299. Unni KK, Dahlin DC, Beabout JW, and Ivins JC, (1976) Parosteal osteogenic sarcoma. *Cancer* 37: 2466–2475.
300. Valabrega G, Fagioli F, Corso S, Madon E, Brach del Prever A, Biasin E, Linari A, Aglietta M, and Giordano S, (2003) ErbB2 and bone sialoprotein as markers for metastatic osteosarcoma cells. *Br J Cancer* 88: 396–400.
301. van Brabant AJ, Stan R, and Ellis NA, (2000) DNA helicases, genomic instability, and human genetic disease. *Annu Rev Genomics Hum Genet* 1: 409–459.
302. van Gent DC, Hoeijmakers JH, and Kanaar R, (2001) Chromosomal stability and the DNA double-stranded break connection. *Nat Rev Genet* 2: 196–206.
303. van Straaten F, Muller R, Curran T, Van Beveren C, and Verma IM, (1983) Complete nucleotide sequence of a human c-onc gene: deduced amino acid sequence of the human c-fos protein. *Proc Natl Acad Sci USA* 80: 3183–3187.
304. Vener J, Rice DH, and Newman AN, (1984) Osteosarcoma and chondrosarcoma of the head and neck. *Laryngoscope* 94: 240–242.
305. Vennos EM and James WD, (1995) Rothmund–Thomson syndrome. *Dermatol Clin* 13: 143–150.
306. Vousden KH and Lu X, (2002) Live or let die: the cell's response to p53. *Nat Rev Cancer* 2: 594–604.
307. Wadayama B, Toguchida J, Shimizu T, Ishizaki K, Sasaki MS, Kotoura Y, and Yamamuro T, (1994) Mutation spectrum of the retinoblastoma gene in osteosarcomas. *Cancer Res* 54: 3042–3048.
308. Wadayama B, Toguchida J, Yamaguchi T, Sasaki MS, and Yamamuro T, (1993) p53 expression and its relationship to DNA alterations in bone and soft tissue sarcomas. *Br J Cancer* 68: 1134–1139.
309. Wajant H, (2002) The Fas signaling pathway: more than a paradigm. *Science* 296: 1635–1636.
310. Wang JY, Naderi S, and Chen TT, (2001) Role of retinoblastoma tumor suppressor protein in DNA damage response. *Acta Oncol* 40: 689–695.
311. Wang LL, Gannavarapu A, Kozinetz CA, Levy ML, Lewis RA, Chintagumpala MM, Ruiz-Maldonado R, Contreras-Ruiz J, Cunniff C, Erickson RP, Lev D, Rogers M, Zackai EH, and Plon SE, (2003) Association between osteosarcoma and deleterious mutations in the RECQL4 gene in Rothmund–Thomson syndrome. *J Natl Cancer Inst* 95: 669–674.
312. Wang X, (2001) The expanding role of mitochondria in apoptosis. *Genes Dev* 15: 2922–2933.
313. Wang ZQ, Liang J, Schellander K, Wagner EF, and Grigoriadis AE, (1995) c-fos-induced osteosarcoma formation in transgenic mice: cooperativity with c-jun and the role of endogenous c-fos. *Cancer Res* 55: 6244–6251.
314. Wang ZQ, Ovitt C, Grigoriadis AE, Mohle-Steinlein U, Ruther U, and Wagner EF, (1992) Bone and haematopoietic defects in mice lacking c-fos. *Nature* 360: 741–745.
315. Wei G, Lonardo F, Ueda T, Kim T, Huvos AG, Healey JH, and Ladanyi M, (1999) CDK4 gene amplification in osteosarcoma: reciprocal relationship with INK4A gene alterations and mapping of 12q13 amplicons. *Int J Cancer* 80: 199–204.
316. Weichselbaum RR, Beckett M, and Diamond A, (1988) Some retinoblastomas, osteosarcomas, and soft tissue sarcomas may share a common etiology. *Proc Natl Acad Sci USA* 85: 2106–2109.
317. Weiss A, Khoury JD, Hoffer FA, Wu J, Billups CA, Heck RK, Quintana J, Poe D, Rao BN, and Daw NC, (2007) Telangiectatic osteosarcoma: the St. Jude Children's Research Hospital's experience. *Cancer* 109: 1627–1637.
318. Werner SR, Prahalad AK, Yang J, and Hock JM, (2006) RECQL4-deficient cells are hypersensitive to oxidative stress/damage: Insights for osteosarcoma prevalence and heterogeneity in Rothmund–Thomson syndrome. *Biochem Biophys Res Commun* 345: 403–409.
319. Wick MR, Siegal GP, Unni KK, McLeod RA, and Greditzer HG, 3rd, (1981) Sarcomas of bone complicating osteitis deformans (Paget's disease): fifty years' experience. *Am J Surg Pathol* 5: 47–59.
320. Willmore-Payne C, Holden JA, Zhou H, Gupta D, Hirschowitz S, Wittwer CT, and Layfield LJ, (2006) Evaluation of Her-2/neu gene status in osteosarcoma by fluorescence in situ hybridization and multiplex and monoplex polymerase chain reactions. *Arch Pathol Lab Med* 130: 691–698.
321. Wittig JC, Bickels J, Priebat D, Jelinek J, Kellar-Graney K, Shmookler B, and Malawer MM, (2002) Osteosarcoma: a multidisciplinary approach to diagnosis and treatment. *Am Fam Physician* 65: 1123–1132.
322. Wold LE, Unni KK, Beabout JW, Sim FH, and Dahlin DC, (1984) Dedifferentiated parosteal osteosarcoma. *J Bone Joint Surg Am* 66: 53–59.
323. Wolf M, El-Rifai W, Tarkkanen M, Kononen J, Serra M, Eriksen EF, Elomaa I, Kallioniemi A, Kallioniemi OP, and Knuutila S, (2000) Novel findings in gene expression detected in human osteosarcoma by cDNA microarray. *Cancer Genet Cytogenet* 123: 128–132.
324. Wong KK, Tsang YT, Shen J, Cheng RS, Chang YM, Man TK, and Lau CC, (2004) Allelic imbalance analysis by high-density single-nucleotide polymorphic allele (SNP) array with whole genome amplified DNA. *Nucleic Acids Res* 32: e69.
325. Woo LL, Futami K, Shimamoto A, Furuichi Y, and Frank KM, (2006) The Rothmund–Thomson gene product RECQL4 localizes to the nucleolus in response to oxidative stress. *Exp Cell Res* 312: 3443–3457.
326. Worth LL, Lafleur EA, Jia SF, and Kleinerman ES, (2002) Fas expression inversely correlates with metastatic potential in osteosarcoma cells. *Oncol Rep* 9: 823–827.

327. Wunder JS, Czitrom AA, Kandel R, and Andrulis IL, (1991) Analysis of alterations in the retinoblastoma gene and tumor grade in bone and soft-tissue sarcomas. *J Natl Cancer Inst* 83: 194–200.
328. Wunder JS, Eppert K, Burrow SR, Gokgoz N, Bell RS, and Andrulis IL, (1999) Co-amplification and overexpression of CDK4, SAS and MDM2 occurs frequently in human parosteal osteosarcomas. *Oncogene* 18: 783–788.
329. Yamaguchi T, Toguchida J, Yamamuro T, Kotoura Y, Takada N, Kawaguchi N, Kaneko Y, Nakamura Y, Sasaki MS, and Ishizaki K, (1992) Allelotype analysis in osteosarcomas: frequent allele loss on 3q, 13q, 17p, and 18q. *Cancer Res* 52: 2419–2423.
330. Yarden Y and Schlessinger J, (1987) Epidermal growth factor induces rapid, reversible aggregation of the purified epidermal growth factor receptor. *Biochemistry* 26: 1443–1451.
331. Yarden Y and Schlessinger J, (1987) Self-phosphorylation of epidermal growth factor receptor: evidence for a model of intermolecular allosteric activation. *Biochemistry* 26: 1434–1442.
332. Yeh IT, (2002) Measuring HER-2 in breast cancer. Immunohistochemistry, FISH, or ELISA? *Am J Clin Pathol* 117 Suppl: S26–35.
333. Zhang Y and Xiong Y, (2001) Control of p53 ubiquitination and nuclear export by MDM2 and ARF. *Cell Growth Differ* 12: 175–186.
334. Zhou H, Randall RL, Brothman AR, Maxwell T, Coffin CM, and Goldsby RE, (2003) Her-2/neu expression in osteosarcoma increases risk of lung metastasis and can be associated with gene amplification. *J Pediatr Hematol Oncol* 25: 27–32.
335. Zielenska M, Bayani J, Pandita A, Toledo S, Marrano P, Andrade J, Petrilli A, Thorner P, Sorensen P, and Squire JA, (2001) Comparative genomic hybridization analysis identifies gains of 1p35 approximately p36 and chromosome 19 in osteosarcoma. *Cancer Genet Cytogenet* 130: 14–21.
336. Zielenska M, Marrano P, Thorner P, Pei J, Beheshti B, Ho M, Bayani J, Liu Y, Sun BC, Squire JA, and Hao XS, (2004) High-resolution cDNA microarray CGH mapping of genomic imbalances in osteosarcoma using formalin-fixed paraffin-embedded tissue. *Cytogenet Genome Res* 107: 77–82.
337. Zou H, Henzel WJ, Liu X, Lutschg A, and Wang X, (1997) Apaf-1, a human protein homologous to C. elegans CED-4, participates in cytochrome c-dependent activation of caspase-3. *Cell* 90: 405–413.
338. Zucchini C, Bianchini M, Valvassori L, Perdichizzi S, Benini S, Manara MC, Solmi R, Strippoli P, Picci P, Carinci P, and Scotlandi K, (2004) Identification of candidate genes involved in the reversal of malignant phenotype of osteosarcoma cells transfected with the liver/bone/kidney alkaline phosphatase gene. *Bone* 34: 672–679.

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