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
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,
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 envelopes 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].

Extraskeletal 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.
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 osteosarcomas 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 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 osteoblasts in 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 CDK1 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.

A Pne 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 osteogenesis, 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 LRPS, 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 LRPS tended to have a lower level of event-free survival [109]. Expression of Dickkopf 3 (DKK3), a dominant-negative mutant of LRPS, 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 tumorigene-
sis was discovered by way of a microarray anal-
ysis 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 osteosar-
coma tumors [135, 136, 140, 161, 228, 251].

Ezrin expression provides an early sur-
vival 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 tumorigene-

nosis 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 expres-
sion and metastatic potential were consistently found to vary inversely [78, 326]. One explana-
tion 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 ifos-
famide induce expression of FASL in osteosar-
coma 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 osteosar-
coma 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 gemic-
tabine, 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 tyro-
sine 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 fam-
ily consists of erbB1 (also known as the epider-
mal 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 recep-
tors that trigger more rapid growth in malignant cells [208, 209] and promote cell survival [71].

HER2 is the best known member of the fam-
ily [111, 209, 247, 248, 268]. It has no known lig-
ands [143], but promotes signaling when combi-
ined 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 exonuclease 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,

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...
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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