Chapter

Bone Tumors: Types and Treatments

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Abstract

The tumors associated with bone are mostly of mesenchymal origin and contribute to approximately 1% of all the known tumors. These could be primary/benign tumors (that originate in the bone), secondary tumors (that originate in some other tissue/organ and metastasize to the bone), or malignant primary bone tumors (that originate in bone and metastasize to distant tissue). These tumors are majorly due to defects in the regulation of tumor suppressor genes and oncogenes and/or misregulation of signal transduction pathways. Chemotherapy and radiotherapy used for the treatment have several side effects. During the recent years, therapeutic strategies involving hormone deprivation (estrogen, androgen), hormone replacements (estrogen analogs), hormone receptor modulators (SERMs), growth factors and cytokines, small-molecule inhibitors, and gene therapy have emerged as a promising alternative to chemo- and radiotherapy. In the present chapter, we have provided an extensive account of tumors associated with the bone and various therapeutic options related to hormone deprivation, hormone replacements, hormone receptor modulators, and hormone inhibition.

Keywords: osteosarcoma, chondrosarcoma, androgen, estrogen, metastasis, hormone

1. Introduction

Primary bone tumors are rare in occurrence accounting for approximately 0.2% of all the tumors. Generally, primary tumors are localized, intra-compartmental, or extended, extra-compartmental [1]. Most benign tumors that have not spread to lymph nodes or other organs remain asymptomatic until their presence is indicated by a trivial insult [1, 2]. Therefore, it becomes important to categorize bone lesions in the early stages of their development for prognosis and diagnosis [2]. On the other hand, skeletal metastases are frequent in patients with breast, prostate, and lung cancer and also occur in other tumors such as myeloma, thyroid and renal cancer, lymphoma, and Ewing's sarcoma [3]. The bone becomes the most common site of metastases in humans due to its highly vascular nature, and this results in pain, pathologic fracture, and decreased quality of life [3].

Bone metastases are either osteolytic or osteoblastic, depending on the dominance of osteoclastic activity or osteoblasts, respectively [3]. As a result the radiological appearance of bone metastasis is lytic, sclerotic, or mixed [4]. General pathogenesis of bone tumors sequentially involves proliferation of primary

neoplasm, local tissue invasion, intravasation into blood vessels, extravasation into bone marrow, tumor cell dormancy, proliferation in bone, and modification of bone microenvironment [3]. The site of metastases is governed by the "seed and soil" hypothesis by Paget which states that neoplastic cells grow or proliferate only in a suitable environment [3]. Tumor cells therefore migrate to the heavily vascularized areas of the skeleton, particularly the red bone marrow of the axial skeleton and the proximal ends of the long bones, the ribs, and the vertebral column [5]. Chemotactic factors such as CXCL10 (CXC motif ligand [CXCL]), CXCL12, and osteopontin have been reported to play a major role in tumor migration [6].

Tumors can be classified into low-grade (Grade I), intermediate-grade (Grade II), and high-grade (Grade III) tumors [1]. Grading of bone tumors is roughly based on the cellularity of the lesions compared to the extracellular matrix, nuclear features, the presence of mitotic figures, and necrosis [7]. Grade II tumors are more cellular, with a greater degree of nuclear atypia, hyperchromasia, and nuclear size. Grade III tumors have significant areas of marked pleomorphism, large cells with more hyperchromatic nuclei than Grade II tumors, occasional giant cells, and abundant necrosis [1]. High-grade bone tumors are the fastest growing and most aggressive group of classic osteoblastic subtype. On the basis of histological appearance, bone tumors are classified as parosteal and periosteal. Parosteal tumors belong to low-grade subtype of osteosarcoma, whereas periosteal belongs to the intermediate-grade subtype. Parosteal tumors have fibroblast appearance and are limited to bone surface, but periosteal tumors appear chondroblastic upon histology.

2. Primary malignant tumors

2.1 Giant cell tumor of bone (GCT)

This tumor accounts for 5% of all primary and 20% of the benign skeletal tumors [8]. GCT is an aggressive osteolytic benign bone tumor developing in the long bones, which is characterized by giant, multinucleated osteoclast-like cells, recruited by stromal cells such as osteoblasts [8, 9]. The stromal cells of GCT highly express parathyroid hormone-related peptide (PTHrP) and thus increase the bone tumor cell local invasiveness and migration [9].

Distal femur, proximal tibia, and distal radius are the most common locations for the occurrence of GCT, but due to its association with Paget's disease, it may also occur in the skull, pelvis, facial bones, and spine. It has been reported to rarely affect greater trochanter of the femur. Though some of the reports claim an equal rate of occurrence in both the genders, some reports show an increased prevalence in females [8].

These tumors have been associated with reduction in length of telomeres [7]. Radiological examination in patients with closed physes displays lytic lesions with well-defined, non-sclerotic margins that are eccentric in location and extend near the articular surface. Thus GCT involves cortical expansion or destruction [8].

2.2 Chondrosarcoma

Chondrosarcomas majorly involve tumorous growth of chondrocytes, which are mostly found in bones that elongate due to endochondral ossification and involve differentiation in the epiphyseal growth plates of long bones. For example, hypertrophic chondrocytes are found in clear cell chondrosarcoma. The most common sites include proximal femur, proximal humerus, distal femur, and ribs. Sex hormones such as estrogen play a major role in governing the nuclear signaling pathways involved in this process of ossification [1].

There are different histologic variants of chondrosarcoma.

1. Mesenchymal chondrosarcomas are highly aggressive tumors, composed of resting chondrocytes, and are radiographically and histologically similar to Ewing's sarcoma [1, 10]. Such sarcomas frequently occur in the pelvic bones, femur, and humerus and less commonly in the head, spine, breast, and prostate [1]. These have high risk of local recurrence and distant metastasis [10].

2. Dedifferentiated chondrosarcomas are aggressive neoplasms and have poor prognosis [10].

3. Clear cell chondrosarcomas are low-grade tumors and involve the epiphyseal end of the long bone. The radiographs show a lytic defect at the epiphyseal end of long bones, sharply demarcated with sclerotic margins [10].

4. Extraskeletal myxoid chondrosarcomas are slow-growing tumors, characterized histologically by prominent myxoid degeneration, and are considered as differentiated tumors [10].

5. Juxtacortical chondrosarcoma arises on the surface of the bone and is histologically identical to conventional intramedullary chondrosarcoma [1].

2.3 Ewing's sarcoma

Ewing's sarcoma is an exception to all the bone tumors, because bone tumors are mostly mesenchymal in origin, but Ewing's sarcoma is reported to have neuroectodermal precursor cells [7]. It has been found to be associated with neuroblastomas in patients younger than 5 years, whereas in patients above 30 years, it is associated with small round cell tumors (e.g., small-cell carcinoma) and large-cell lymphoma [1]. Ewing's sarcoma is most likely to occur in younger individuals and most commonly in males [11].

Ewing's sarcoma is characterized by small round cell bone tumor and involves pain at the site of tumor and soft tissue swelling around it [1]. Unlike other primary tumors of the bone, Ewing's sarcoma is associated with a characteristic translocation in the 11th and 22nd chromosomes. This translocation results in production of an aberrant transcription factor EWS/FLI1 that forms a complex with RNA helicase A and drives the pathogenesis of Ewing's sarcoma [7, 11]. Its metastasis involves certain non-specific signs of inflammation, anorexia, fever, malaise, fatigue, and weight loss [1].

2.4 Osteosarcoma

Osteosarcoma is an osteoid-producing malignancy of mesenchymal origin [12]. It is the third most frequent type of cancer in adolescence and represents more than 56% of all bone tumors [13]. It is the most common bone sarcoma and affects 60% of the patients below 25 years of age and 30% of the patients above 40 years [14, 15]. It is associated with extensive genomic disruptions and propensity of metastatic spread. Seventy-six percent of the osteosarcomas have been found to be associated with reduced expression of *HACE1* gene localized to human chromosome 6q21, thus resulting in poor survival [14]. Approximately, 30–40% children with pediatric osteosarcoma die due to metastasis to lungs [1, 16].

Environmental factors such as exposure to radiation, teriparatide (parathyroid hormone 1–34) usage, and consumption of fluorinated drinking water during

childhood increase the risk of osteosarcoma [12]. Its localization in regions that are largely cut off from the vasculature reduces the effectiveness of systemically administered chemotherapeutics [17]. The subtypes of osteosarcoma include osteoblastic, chondroblastic, fibroblastic, small cell, telangiectatic, high-grade surface, extraskeletal, and other lower-grade forms of tumors including parosteal and periosteal [12].

Osteosarcomas are one of the widely studied sarcomas of the skeletal system. Several investigations have reported the association of germline mutation disorders such as hereditary retinoblastoma, Rothmund-Thomson syndrome, Li-Fraumeni syndrome, and Bloom syndrome, with increased risk of osteosarcoma [12]. Various other pathways discussed below have been reported to be responsible for supporting growth and metastasis of osteosarcoma.

1. Constitutively active signal transducers and activators of transcription 3 (STAT3) signaling have been found to be necessary for osteosarcoma survival and migration in vitro and tumor growth in vivo. STAT3 is a proto-oncogene that stimulates self-proliferation (due to expression of cyclin D1), mediates immune evasion, promotes angiogenesis, and confers apoptosis resistance (induced by conventional therapies, due to expression of BCL2) [18]. Similarly an upregulation in the expression of survivin (an oncogenic protein) has also been observed in osteosarcoma (**Figure 1**) [19].

2. Attenuation of cell cycle arrest by p53 has been found to affect the upstream p53 signaling pathways [20]. Approximately 26.5% of non-hereditary osteosarcomas have been reported to be associated with somatic loss of p53, out of which 60% are high-grade osteosarcomas, whereas 1% are low-grade osteosarcomas [14]. Tumor suppressor genes such as p15 and p27 have been commonly found to be silenced due to methylation of promoter by DNA methyl transferase that adds methyl group to the fifth carbon position of cytosine ring in CpG islands, leading to heterochromatin and inhibition of gene expression (**Figure 1**) [21].

3. Sex steroid hormones have been found to play an important role in development and progression of bone tumors. Previous investigations have revealed the role of aromatase and sulfatase pathways in the in situ formation of active estrogen. Aromatase pathway involves aromatization of androgens to produce estrogen. In sulfatase pathway, estrone 3-sulfate is taken up by the cells and is activated by the removal of sulfate by steroid sulfatase, thus converting inactive estrogen to unconjugated and bioactive estrogen. Both these pathways allow generation of estrogen and androgen by the bone cells in the bone microenvironment (**Figure 2**) [24].

4. Growth factors such as transforming growth factor (TGF)-β, insulin-like growth factors (IGFs), bone morphogenetic proteins (BMPs), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF) released from degraded bone matrices also promote tumor cell proliferation by production of PTHrP that interacts with parathyroid hormone (PTH)/PTHrP receptors in the bone and kidney to cause hypercalcemia, osteoclast-mediated bone resorption, increased nephrogenous cyclic AMP, and phosphate excretion [4–6, 25]. PTH is known to regulate osteosarcoma cells, by inducing transcription of c-Fos that in turn targets calcium/cAMP-response element (CRE) through activation of protein kinase A (PKA) [26]. c-Fos is a member of activator protein-1 (AP-1) family of transcription factors containing c-Fos (FosB, Fra-1, Fra-2) (**Figure 1**) [27].

Additionally, FGF also regulates multiple signaling cascades in both autocrine and paracrine manner [4]. FGF receptor 1 was identified as a c-Fos-regulated gene playing an important role in lung metastases of osteosarcoma. The FGF receptors 1–4 belong to a family of receptor tyrosine kinase (RTK) that triggers intracellular signaling cascades through mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT), STAT pathways, and signal transducers phospholipase Cγ (PLCγ) and casitas B-lineage lymphoma (CBL) (**Figure 1**) [27].

5. Majority of the osteosarcomas develop as a result of metastases of breast, prostate, or lung tumors into the bone. Breast cancer cells metastasize to the bone and secrete various factors in the bone microenvironment that enhance osteoclastogenesis and inhibit osteoblastogenesis, thus developing several skeletal-related events (SREs) such as pathological fracture, spinal cord compression, bone pain, and hypercalcemia [28]. Similarly, androgen signaling components such as androgen receptor, ARV7, v-ets avian erythroblastosis virus E26 oncogene homolog (ERG), cytochrome P450, and family 17 subfamily A polypeptide 1 (CYP17) and molecules such as phospho-Met, phospho-Src, glucocorticoid receptor, and Ki67 have been implicated to play a major role in the metastasis of castration-resistant prostate cancer (CRPC) [29].

Some of the reports associate migration and invasion of breast cancer cells to the upregulation of micro RNAs, miR-10b, miR-373, and miR-520c. Out of these, miR-373 and miR-520c have been found to silence *CD44* gene that codes for hyaluronan receptor (plays an important in cellular adhesion). Similarly, miR-218 that is involved in osteoblast differentiation promotes breast cancer cell osteomimicry (ability to acquire bone cell phenotype for immune escape). miR-154 and miR-379 overexpression in bone metastatic cells was associated with mesenchymal properties and enhanced invasive potential [30].

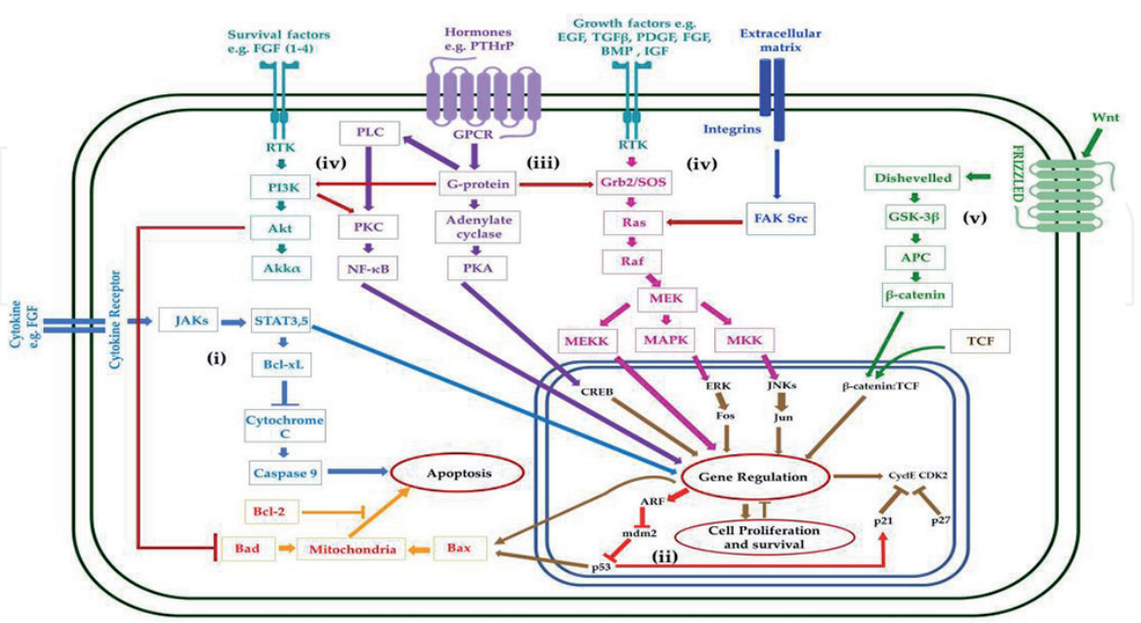

Figure 1.
Cross talk of various signaling pathways in osteosarcoma. (i) JAK–STAT pathway confers apoptosis resistance, thus enhancing tumor cell survival. (ii) Inactivation of p53 favors cell cycle progression in cells with DNA damage and promotes tumorigenesis. (iii) Cross talk between GPCR-regulated PTHrP signaling and RTK-mediated survival/growth factors. (iv) Role of RTK in supporting tumor cell survival and proliferation as a response to various growth factors. (v) Wnt signaling mechanism plays a major role in tumors associated with bone tissue.

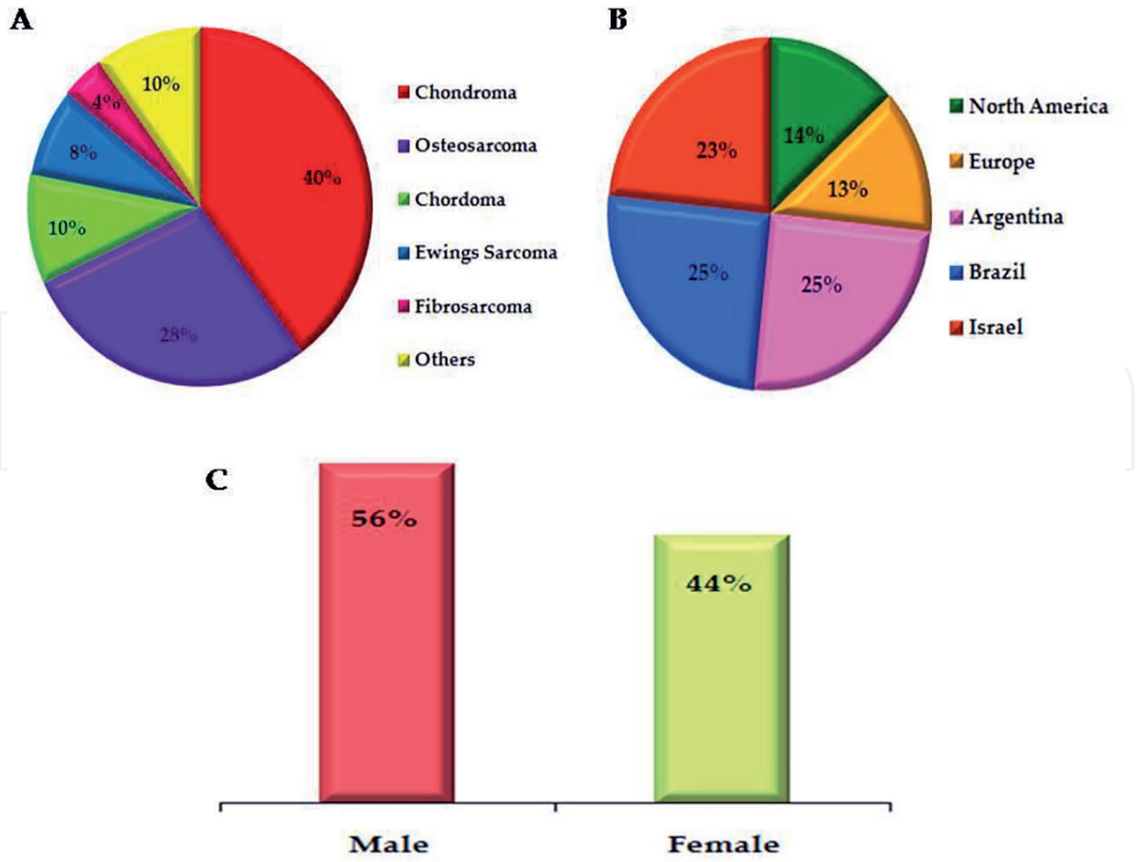

Figure 2.
(A) Percentage of primary bone cancers in adult human population [22]. (B) Osteosarcoma cases per 100,000 population per year [23]. (C) Percentage of males and females diagnosed with primary bone tumor in the United States in the year 2017 [22].

3. Pathophysiology of bone tumors

General pathogenesis of bone tumors sequentially involves proliferation of primary neoplasm, local tissue invasion, intravasation into blood vessels, extravasation into bone marrow, tumor cell dormancy, proliferation in the bone, and modification of bone microenvironment. The site of metastases is governed by the "seed and soil" hypothesis by Paget which states that neoplastic cells grow or proliferate only in a suitable environment [3].

The initial step in metastasis involves escape of cancer cells from primary tumor into the systemic circulation, through epithelial–mesenchymal transition (EMT). EMT involves loss of cell surface intercellular adhesion proteins and epithelial polarization. EMT is followed by dissolution of the extracellular matrix by secreting certain proteolytic enzymes and migration into surrounding tissue to enter systemic circulation through intravasation. Such circulating tumor cells (CTCs) escape anoikis (cell apoptosis due to loss of cell-matrix or cell-cell interactions, preferably through overexpression of tyrosine kinase receptor, TrkB) resulting in activation of PI3K-AKT pro-survival pathways. These tumor cells also upregulate certain proteins on their surface (e.g., CD47) to escape destruction by macrophages [4]. The two major factors that govern the localization of CTCs are blood flow and molecular signaling. For example, the metastasis of breast cancer to thoracic spine is due to the venous drainage of the breast to thoracic region, whereas lung cancer shows general skeletal distribution due to the drainage of pulmonary veins into the left side of the heart, followed by eventual entry into systemic circulation. Alternatively, prostate cancer majorly

displays metastasis to the axial skeletal in the lumbar spine, sacrum, and pelvis due to their drainage through pelvic plexus. As far as the signaling pathways are concerned, CXCL12-CXCR4 (CXC motif ligand [CXCL], CXC chemokine receptor [CXCR]) axis has been found to regulate CTC homing to the bone. CXCL12, also called as stromal cell-derived factor 1 (SDF1), is a chemokine factor secreted by bone marrow MSCs, endothelial cells, and osteoblast and primarily binds to G protein-coupled receptor, CXCR4, thus activating cell survival, chemotaxis, and expression of integrin αvβ3 on the surface of CTCs [4]. Another receptor involved in osteotropism is the calcium-sensing receptor (CaSR), expressed by advanced primary breast tumors that causes enhanced calcium-induced migration to the bone [30]. In addition to CXCL12-CXCR4 axis, non-receptor cytoplasmic tyrosine kinase, Src, has also been shown to mediate improved survival of breast cancer cells in the bone marrow by increasing the resistance to tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) and activation of AKT signaling [4].

After tumor cells colonize the bone, they induce the expression of receptor activator of nuclear factor-κB ligand (RANKL) via production of PTHrP, prostaglandin E2 (PGE2), interleukin 6 (IL-6), IL-1β, TNF, and epidermal growth factor (EGF), which promote osteoclast differentiation and activation. RANKL induces osteoclastic bone resorption resulting in growth of osteolytic tumor that causes hypercalcemia, and it also acts as a chemoattractant to the bone for tumor cells [6]. In relation to this, hypoxia-inducible factor (HIF)-1α has been reported to act as an upstream master switch to many of the osteolytic factors such as IL-11 and IL-8 and angiogenic factors such as PDGF and vascular endothelial growth factor (VEGF) [4, 31]. TGF-β plays a central role in the pathogenesis of osteolytic bone metastasis from breast carcinoma via potentiation of estrogen receptor (ER)-α-mediated transcription induced by constitutively active ERα [25]. A higher expression of ERα in the cortical bone suggests its role in bone formation, whereas trabecular bone cells show a higher expression of ERβ [32].

Osteolytic bone metastases are most often caused by breast cancer and multiple myeloma [6, 33], whereas osteoblastic metastases are mostly observed in bone metastasis of prostate cancer, which is due to osteoblast stimulation by cancer cells [6, 34]. Factors that are locally produced by cancer cells, such as bone BMP, IGF, FGF, TGF-β, and endothelin-1, also promote osteoblast proliferation and bone formation [6].

Another important cellular component of bone microenvironment that is involved in tumor metastases is osteocytes. Osteocytes present in the bone regulate osteoclast development through expression of RANKL, macrophage colony-stimulating factor (M-CSF), and osteoprotegerin (OPG) and inhibit osteoblast differentiation by the expression of sclerostin. Osteocytes have an interesting ability to respond to mechanical stress and pressure. An increase in pressure due to prostate cancer metastasis thus results in upregulation of matrix metalloproteinases (MMPs) and chemokine (C-C motif) ligand 5 (CCL-5). Additionally, apoptotic osteocytes have been shown to release IL-11 that enhances osteoclast differentiation. Endothelial cells are yet another important component of the bone marrow that contributes to the bone metastatic process. Endothelial cells in the metaphysis of the long bone aid CTC adhesion due to constitutive expression of P-selectin, E-selectin, vascular cell adhesion molecule 1 (VCAM1), and intercellular adhesion molecule A (ICAM-1). Decrease in shear forces due to reduced blood flow velocity in the large volume of sinusoids also favors CTC attachment [4].

The bone marrow is also a major reservoir for dendritic cells, macrophages, myeloid-derived cells, and different subsets of T cells. T and B cells are known to produce RANKL and impact osteoclastogenesis, whereas production of IL-6, IL-23, and IL-1 by dendritic cells in the bone microenvironment of multiple

myeloma patients causes an increase in Th17 cells that in turn increase IL-17. IL-17 is an important cytokine that promotes osteoclast and myeloma proliferation and also mediates interactions between T cells and bone metastatic environment. Additionally, myeloid-derived suppressor cells from the bone marrow suppress innate and adaptive immune responses by impairing T-cell antigen recognition and promotion of regulatory T cells [4].

However, as an individual ages, the hematopoietic red bone marrow gets converted to adipose tissue-rich yellow bone marrow that has a significant impact on the development of bone metastasis. These bone marrow adipocytes not only serve as an energy source but also secrete several pro-inflammatory mediators such as IL-1β, IL-6, leptin, adiponectin, VCAM-1, TNF-α, and CXCL12 that increase cancer cell survival and proliferation [4].

4. Tumor biomarkers

Histological examination of tissue biopsy has been the most commonly used procedure for the diagnosis of bone tumors. Clinical and radiological observations also aid in diagnosis and provide a complete staging of bone cancers. But molecular and genetic markers increase the accuracy of diagnosis, assist in subtyping bone tumors, and also provide an overview of target molecules for designing therapeutic approaches. The biomarkers can be specific or non-specific; diagnostic, prognostic, or therapeutic; and serological, genetic, or histological. The clinical presentation of bone tumors is non-specific, and the most common symptoms include pain and swelling. The clinical features involve limited movement, skin hyperthermia, weight loss, and the presence of a visible mass in the anatomical profile [10].

The serological markers are generally a reflection of osteoblastic and osteoclastic activities in the bone [10]. As mentioned in the earlier sections, breast cancer metastases are mostly osteolytic, whereas metastases of prostate cancer are generally osteoblastic. Therefore, elevated levels of urinary N-terminal cross-linked telopeptide (NTx of type I collagen) and serum carboxyterminal cross-linked telopeptide (ICTP of type I collagen) in solid tumor patients and serum tartrate-resistant acid phosphatase type 5b (TRAcP-5b) in patients with breast tumor metastasis can be used for diagnosis. On the other hand, serum levels of bone-specific alkaline phosphatase (BSAP), procollagen type I N-terminal propeptide (PINP), and OPG serve as the biomarkers for prostate cancer metastasis [10, 30].

With reference to the genetic changes, sarcomas can be divided into three categories: sarcomas with specific translocations (e.g., Ewing's sarcoma, aneurysmal bone cyst), tumors with gene mutations or amplifications (e.g., chondrosarcomas, fibrous dysplasia), and sarcomas with genetic instability. These cytogenetic changes can be detected using banding and multicolor fluorescence in situ hybridization (FISH), array comparative genomic hybridization (array CGH), targeted detection techniques such as qPCR, and techniques to detect mutation [10].

There are several markers that are used for prognosis or diagnosis of different types of tumors that are discussed below:

4.1 Osteosarcoma

4.1.1 Serum markers

1. Degradation of collagen and the ground substance in the bone (due to prolonged exposure to fluoride) results in increased concentration of serum sialic acid that can be used as a serum biomarker for osteosarcoma [10].

2. Expression of heat shock protein (HSP gp96), in the cytoplasm of osteoblastic sarcoma, has been found to be associated with pathogenesis of bone tumors. But it does not provide any idea regarding the degree of malignancy [10].

3. The osteosarcoma patients displayed increased levels of endostatin, placental growth factor (PlGF), and FGF-1 and FGF-2 in serum [10].

4. Gas chromatography–mass spectrometry profiles of small-molecule metabolites in urine and serum samples of osteosarcoma patients displayed a disrupted energy metabolism, downregulated amino acid metabolism, and increase in glutathione metabolism and polyamine metabolism [10].

5. qPCR and western blot analysis for detection of IGF-1 receptor showed its increased expression in osteosarcoma tissues, suggesting it as a prognostic marker. Western blotting and enzyme-linked immunosorbent assay (ELISA) confirmed a decrease in serum levels of gelsolin in the osteosarcoma samples [10].

6. The presence of FGF-2 or leukemia inhibitory factor (Lif) serves as a biomarker, suggesting reduction of osteogenesis on osteosarcoma cells. Elevated serum levels of CXCL4 and CXCL in osteosarcoma patients affirmed the role of these markers in clinical manifestation. In addition to this, biomarker Snail2 is also useful in prognosis of bone tumors [10].

7. Metastatic prostate cancers have been found to express the well-known markers of aggressiveness, namely, prostatic-specific antigen (PSA) [30].

8. Increase in erythrocyte sedimentation rate (ESR), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) are indicators of osteosarcoma [1, 12].

9. Immunohistochemistry of primary osteosarcomas showed expression of mitotic arrest defective protein 2 (MAD2). Immunohistochemical analysis of osteosarcoma biopsies indicated reduced expression of cysteine-rich protein with Kazal motifs (RECK). Immunohistochemistry analysis also showed expression of WNT-5a and ROR2 in patients with advanced stages of osteosarcoma [10].

4.1.2 Genetic markers

Assessment of *CCN3* expression levels at diagnosis may represent a useful molecular tool for early identification of patients with osteosarcoma. Gene alteration of c-kit protein also serves as a prognostic marker for osteosarcoma. The transcriptional regulator, Oct-4, has been found to play a marked role in proliferation and spread of cancer. A reduced expression and inactivation of miR-34 gene have been reported to be associated with osteosarcomas. The action of miR-34 is p53 dependent. A dominant polymorphic variant of TGFβ receptor 1 (TGFBR1), TGFBR1*6A, is found to be associated with increased susceptibility of osteosarcoma for metastasis. Bcl-xL, a member of Bcl-2 (B-cell lymphoma (BCL)) protein family, has been investigated to function as a dominant regulator of apoptotic cell death and plays an important role in malignant transformation. Cytotoxic T-lymphocyte antigen-4 (CTLA-4), a molecule that decreases immune response mediated by T cells, promotes development of osteosarcoma. Overexpressions of *Cortactin* (*CTTN*) gene, present in 11q13 amplicon, serve as a valid biomarker for osteosarcoma [10].

4.2 Ewing's sarcoma

Ewing's sarcomas are associated with rearrangement of the *EWS* gene on chromosome 22q12 with an erythroblast transformation-specific (*ETS*) gene family member, resulting in formation of EWS-ETS fusion protein (EWS-FLI). FLI1 has been suggested as a useful marker particularly when hematolymphoid markers are negative. This translocation defines Ewing's sarcoma family of tumors (ESFT) and provides a major tool for their accurate diagnosis. The translocation results in different types of genetic abnormalities, e.g., five forms of EWSR1-FLI1, three forms of EWSR1-ERG, and one form of EWSR1-FEV. A high expression of BMI-1 in ESFT cells was found to significantly affect survival and proliferation. Expression of CXCR4 has been reported to increase the risk of tumor metastases, whereas CXCR7 expression is associated with shorter survival [10].

Ewing's sarcoma has been reported to be associated with modulation of RANKL by VEGF-165, thus resulting in activation of osteoclast-mediated bone destruction [10].

4.3 Chondrosarcoma

On the basis of histological observations, chondrosarcomas are classified into three categories:

Grade I (low grade)—cytology similar to enchondroma and hyperchromatic plump nuclei of uniform size [10].

Grade II (intermediate grade)—increased cellularity, hyperchromasia, distinct nucleoli, and foci of myxoid alteration [10].

Grade III (high grade)—increased cellularity and nuclear atypia, occasional giant cells, abundant necrosis, and presence of mitosis [10].

4.3.1 Genetic markers

Deletions in the loci of *CDKN2A*, *EXT1*, and *EXT2* genes, *p53* mutation as late event in tumor progression, and amplification of 12q13 and loss of 9p21 are genetic aberrations found in conventional chondrosarcomas [10].

Higher expression of PTHR1 and Bcl-2 was found to be associated with increasing histological grade in chondrosarcoma, suggesting its involvement in tumor progression. A higher expression of Aurora kinases A and B was relevant as prognosis marker for chondrosarcoma. Somatic heterozygous isocitrate dehydrogenase 1 (IDH1) hot spots (R132C and R132H) or IDH2 (R172S) mutations are specifically found in cartilaginous tumors [10].

4.3.2 Biological and molecular markers

qPCR analysis showed a high expression of COX-2 protein in solitary peripheral chondrosarcoma. Some of the studies reported a significant role of nitrotyrosine, COX-2, CD34, and lymphatic marker podoplanin with histological grades of chondrosarcoma. Molecules such as integrin-linked kinase α and β-parvin and Mig-2 allow attachment of cells to matrix and govern cell motility and growth, thus playing an important role in progression and prognosis of chondrosarcomas [10].

Significantly high serum levels of receptor activator of nuclear factor-κB (RANK), OPG, IL-8, IL-6, and OPG/soluble RANKL ratio have been used to detect bone tumors. Osteosarcoma patients display a higher serum concentration of IL-16 as compared to chondrosarcoma patients [35].

5. Diagnosis

1. **Radiographic diagnosis**: Plain radiographs, computed tomography (CT) scans, and magnetic resonance imaging (MRI) are used to investigate the extent of tumors and to study the surrounding structure such as blood vessels, nerves, and soft tissues [1, 12].

2. **Positron emission tomography (PET)-CT**: [F-18]-Fluorodeoxy-D-glucose (FDG)-PET is a noninvasive imaging tool used for accurate discrimination between responding and nonresponding osseous tumors [1, 12].

3. **Bone scintigraphy**: This method involves total body scan and identifies axial and appendicular skeletal metastasis. It helps in determining intraosseous extension of tumors and sites of metastasis [1, 12].

4. **Thallium scintigraphy**: This method is used for determining tumor response to neoadjuvant (preoperative) chemotherapy when MRI is not helpful and also for detection of local recurrence [1].

5. **Incisional or core needle biopsy** is the final step in the diagnostic process. The tumor is staged using the Musculoskeletal Tumor Society staging scheme or the American Joint Commission on Cancer (AJCC) system [12].

6. **Increased uptake of technetium diphosphonate** in the clinical bone scans of osteolytic lesions in cancer patients also provides a diagnostic tool to identify increased cellular activity and metastasis [36].

Bone scans, X-rays, and histologic evaluation of autopsy specimens are commonly used for radiologic and histologic assessment of tumor sites [5].

6. Therapy

Osteosarcoma is typically treated with surgery and adjuvant chemotherapy that usually includes a combination of methotrexate, doxorubicin, and cisplatin [37]. Once the cancer has spread to the bones, it can rarely be cured, but often it can be treated to slow down its growth [38]. The therapeutic strategies for bone tumors should involve the following:

1. Treatment of cancer cells: This involves inhibition of tumor cell proliferation or killing of cancer cells to extend the patient's survival time. This could be achieved by usage of cytotoxic drugs, hormonal deprivation, or inhibition of specific signaling pathways by targeted agents [4].

2. Disruption of the vicious cycle created due to complex biological signaling between cancer cells and bone resident cells [4].

3. Palliative therapies to reduce the extremely debilitating and painful symptom of bone metastasis and improve the quality of life for cancer patients [4].

As discussed in the previous sections, various signaling pathway are involved in the proliferation and migration of tumor cells. Targeting these signaling pathways by use of different inhibitors could hamper the survival of tumor cells. However, it has

already been known that the sex hormones play a major role in tumor cell survival and metastases. Thus, hormone therapy for curing tumors would basically involve hormone deprivation approaches. These strategies might inhibit the action of hormones responsible to bone metastases, resulting in osteolytic or osteoblastic tumors.

6.1 Androgen deprivation therapy (ADT)

Ubiquitous and extensive expression of androgen receptor in the bone marrow of both males and females of all ages provides a direct evidence of action of androgen on the bone marrow and offers clues to clinicopathological correlates [39]. Most prostate cancers and their stages depend upon androgen and androgen receptor (AR) for their growth and survival. Androgen receptor is a transcription factor that regulates the expression of several genes in response to binding of androgen (such as testosterone and dihydrotestosterone) and thus regulates the process of proliferation and survival. As mentioned earlier, bone metastasis of prostate cancers majorly results in osteoblastic bone tumors [40]. Therefore, systemic treatment for advanced prostate cancer involves androgen deprivation therapy (ADT) that includes the following approaches:

a. To reduce the levels of circulating androgen by surgical or chemical castration. Surgical castration results in reduction of circulating androgen levels by >90% within 24 hours, whereas chemical castration is achieved by application of analogs of luteinizing hormone-releasing hormone (LH-RH) and results in reduction of circulating levels of testosterone [40].

LH-RH is a neurohormone, secreted by the hypothalamus, and regulates the secretion of gonadotropin, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) from the pituitary. LH-RH acts via binding to its receptor, LH-RHR. These receptors have also been found in the cytoplasm of many tumor cells that involve both reproductive and nonreproductive tissues. LH-RH agonists and antagonists have been found to downregulate these receptors and thus inhibit tumor growth [41]. Leuprolide acetate (Lupron, Eligard), goserelin acetate (Zoladex), triptorelin (Trelstar), and histrelin (Vantas) are some of the LH-RH agonists, whereas degarelix is an antagonist [4, 40]. LH-RHR can also be targeted specifically by peptides conjugated to anticancer drugs, thus developing cytotoxic analogs [41]. AN-152, commercially designated as AEZS-108, has been developed by conjugating 14-OH group of doxorubicin (DOX) to epsilon-amino group of D-Lys side chain of carrier peptide, through a glutaric acid spacer. The drug is endocytosed by cells through receptor-mediated endocytosis and thus selectively acts on cells that express its receptor. After internalization the drug is cleaved from the LH-RH moiety and accumulates in the nucleus. Because of receptor-mediated entry, the drug shows lesser side effects and also overcomes the resistance [41].

Administration of LH-RH agonist or antagonist for ADT not only results in suppression of testosterone to castration levels but also depletes estradiol, because it is derived by aromatization of testosterone [42]. Estradiol deficiency negatively impacts the bone health resulting in decline of bone mineral density (BMD) and increased risk of fractures [42]. This decrease in bone density also results in development of renal stones leading to risk of urinary calculi [43]. Recently parenteral (e.g., intravenous, intramuscular, or transdermal) administration of estradiol has been investigated to suppress androgen production through negative feedback loop involving hypothalamic–pituitary axis and avoids fall in endogenous estradiol levels. This also eliminates the risk of embolic cardiovascular toxicity that was caused due to oral administration of estradiol [42].

b. To prevent binding of androgen to AR, by competitive inhibition using antiandrogens. These molecules compete with androgen for the ligand-binding domain of AR [40]. The antiandrogens can be of two categories, steroidal and nonsteroidal. Cyproterone acetate, a derivative of hydroxyprogesterone, is a steroidal antiandrogen and an antigonadotropin, which has a binding affinity for AR. But it has been found that it is not a pure antagonist but rather a partial agonist that adversely affects the survival of prostate cancer patients when combined with castration [40]. Among the nonsteroidal antiandrogens are the first-generation flutamide, nilutamide, and bicalutamide and second-generation enzalutamide and the cytochrome P450 c17 (CYP17, a critical enzyme in testosterone synthesis) inhibitor, abiraterone acetate, which prevents synthesis of androgens. Abiraterone inhibits 17-α-hydroxylase/17,20 lyase, a testosterone synthesis enzyme found in the adrenals, testis, and tumor [40, 44]. All these nonsteroidal antiandrogens are similar in terms of the chemical structure of their moiety that binds to the ligand-binding pocket [40]. It has been found that treatment with abiraterone acetate plus prednisone prolongs survival among patients with metastatic castration-resistant prostate cancer [44], though back pain, nausea, constipation, bone pain, arthralgia, urinary tract infection, edema, cardiac events, and elevation in levels of aminotransferase are some of the side effects associated with administration of abiraterone [44, 45].

Flutamide was the first nonsteroidal antiandrogen drug approved by the US Food and Drug Administration (FDA) for prostate cancer and forms the basis for all other nonsteroidal antiandrogens. The recommended dose of flutamide is 250 mg three times per day, so as to achieve a Cmax and Cmin of approximately 1.7 and 0.8 μg/ml, respectively. It acts via blocking the binding of androgen to the ligand-binding pocket of AR, resulting in inhibition of nuclear translocation of androgen-bound AR. But improvement in disease upon cessation of flutamide treatment has been observed in patients, due to gain-of-function mutation in the ligand-binding domain of AR, T877A. Flutamide gets eliminated through the kidney, and liver toxicity is one of the common adverse effects [40].

Enzalutamide (previously called MDV3100) also acts via inhibiting the binding of androgen to AR, thus blocking its nuclear translocation and interaction with co-activators [4, 29, 40, 46]. Its recommended dose is 160 mg/day [40, 47]. However, clinical resistance due to gain-of-function mutation in AR ligand-binding domain (F876 L) and constitutive expression of active spice variants of AR that lack ligand-binding domain results in poor survival rates. Apalutamide and darolutamide also belong to the second generation of nonsteroidal antiandrogen that blocks the androgen binding to AR [40].

A novel first class of drug, ralaniten, is currently under clinical trials for patients who have previously received abiraterone, enzalutamide, or both. This class of drug binds to the unique region in the N-terminal domain of both full-length and truncated constitutively active splice variant of AR [40].

c. ADT that effectively reduces the serum testosterone levels has been a core tool for treating metastatic and advanced prostate cancer [48]. However, neoadjuvant ADT has been suggested to have several advantages in prostate cancer patients undergoing transperineal prostate brachytherapy. The agents that are mainly used as adjuvant ADT include estrogens, antiandrogen monotherapy, and combined androgen blockade (CAB) using antiandrogen plus a gonadotropin-releasing hormone receptor (GnRH) agonist. It has been reported that in comparison to GnRH agonist, degarelix, a GnRH receptor antagonist, is more efficient in achieving castration levels of testosterone and PSA, without risk of testosterone flare [48, 49].

ADT is the mainstay of treatment for advanced prostate cancer, but eventual development of castration-resistant prostate cancer (CRPC) reduces the survival rates. One of the main reasons for development of CRPC is the sustained levels of androgen within the tumor due to suboptimal androgen suppression by primary ADT. Moreover, apart from the hormone-independent subsets, the other subsets of CRPC cells adapt themselves to the low testosterone environment induced by ADT and become hypersensitive to even lower concentrations of testosterone and other androgen precursors. Therefore, secondary hormone therapies are proving to be more efficient to achieve maximum suppression of testosterone. GTx-758 (3-fluoro-N-(4-fluorophenyl)-4-hydroxy-N-(4-hydroxyphenyl) benzamide) is an oral nonsteroidal selective estrogen receptor (ERα) agonist that lowers the free testosterone and PSA levels by increasing sex hormone-binding globulin (SHBG). This also helps to avoid side effects related to estrogen deficiency [50].

Finasteride and dutasteride (5α-reductase enzyme inhibitors) are found to inhibit 5α-reductase-mediated conversion of testosterone to the high affinity androgen receptor ligand, 5α-dihydrotestosterone [51].

6.2 Selective estrogen receptor modulators (SERMs)

Estrogen is chemically related compounds derived from androgen precursors but contain a defining aromatic and hydroxyl group at the 17th position. Estrogens comprise the natural ligands for estrogen receptors (ERs), with 17β-estradiol being a potent agonist. 17β-estradiol has been reported to inhibit metastasis-associated lung adenocarcinoma transcript 1 (MALAT-1)-mediated osteosarcoma migration, invasion, metastasis, and induction of cell apoptosis, in an estrogen receptor α (ERα)-independent manner [15]. Earlier it was thought that binding of ER agonists induces a conformational change in the receptors, conferring the ability for co-activators to bind, whereas ER antagonists were thought to compete for binding [52]. But later studies with tamoxifen revealed that the same molecule can behave as an agonist (tamoxifen acts as an estrogen agonist in the uterus, promoting hypertrophy) as well as an antagonist (tamoxifen exhibited estrogenic activity in the bone, thus protecting against bone loss), depending on the tissue context. Recently it has been found that oxysterols such as 27-hydroxycholesterol (27 HC) also modulate the activity of estrogen receptors (ERs) and are therefore classified as endogenous SERMs. 27 HC is derived from cholesterol in the presence of enzyme CYP27A1 (cytochrome P450 enzyme). Breast cancer is the most common cancer in women, and its metastasis is majorly hormone (estrogen receptor) dependent. Some of the reports emphasize on the role of 27 HC in cancer progression and drug resistance, but several other reports also highlight its beneficial role in inhibiting proliferation and invasion of prostate cancer cells by blocking sterol-regulatory element-binding protein 2 (SREBP2). However, their different affinities for the different subtypes of ERs (α and β) and different relative expressions of these subtypes in tissues may explain some the of SERMs' pharmacology. Recent evidence also suggests that binding of the receptor even by structurally related compounds could result in unique conformational changes, thus allowing recruitment of distinct sets of co-activators and/or corepressors to the receptor [52].

SERMs such as genistein, daidzein, and 4-hydroxytamoxifen have been reported to downregulate the expression of epidermal growth factor (EGFR) in vitro in osteosarcoma cells in an ER-dependent manner. The reduction in EGFR expression resulted in upregulation of markers for osteoblast differentiation, thus resulting in suppression of tumor cell proliferation [53].

Isoflavones such as genistein and daidzein are abundantly found in soybeans and soy-based food products. Isoflavones, coumestans, and lignans belong to a

class of phytoestrogens. Phytoestrogens are plant-derived substances that resemble 17β-estradiol and can bind to activate intracellular estrogen receptors. These dietary phytoestrogens have been reported to exhibit bone-protecting effect without the risk of breast cancer [54].

Genistein has also been demonstrated to elicit different cell responses through different signaling mechanisms. A combination of genistein and 17β-estradiol has been shown to significantly increase apoptosis of breast cancer cells by increasing the BAX/BCL-2 (BCL-2-associated X protein (BAX)) ratio and reducing phosphorylation of extracellular signal-regulated kinase (ERK) ½ and AKT [55].

6.3 Activation of ERα

Osteosarcoma is a malignant tumor in the bone that originates from osteoblasts or osteoblast precursors. The reports clarify that normal osteoblasts express ERα, whereas osteosarcomas do not (due to promoter DNA methylation). Thus a treatment strategy that involves induction of ERα expression in osteosarcoma cells in combination with estrogen administration would reduce proliferation of osteosarcoma and increase cell differentiation. In vitro treatment of osteosarcoma cells with decitabine (DAC, 5-Aza-2′-deoxycytidine) has been found to induce the expression of ERα but reduce the expression of metastasis-associated markers such as vimentin, slug, zeb1, and MMP9, with simultaneous decrease in stem cell markers such as *SOX2*, *OCT4*, and *NANOG*. Subsequent treatment with 17β-estradiol synergized with DAC in reducing cell proliferation and inducing differentiation markers such as alkaline phosphatase, osterix, and bone sialoproteins [21].

6.4 Estrogen inhibitors

The bone is the frequent site for metastasis of breast cancer. Estrogen plays a critical role in development and progression of breast cancer by interacting with ERα and ERβ. In postmenopausal women, estrogens (estrone and estradiol) are synthesized from androgens (androstenedione and testosterone) at extragonadal sites, including the breast. Thus the third generation of therapy involves inhibition of these aromatase enzymes, catalyzing the conversion of androgens to estrogens [56]. The aromatase inhibitors fall into two categories: steroidal and nonsteroidal. Letrozole and anastrozole are the third-generation nonsteroidal aromatase inhibitors that block the extragonadal conversion of androgens to estrogens and give rise to an estrogen-depleted environment [51, 56, 57]. This lowers the estrogen in breast tissues and reduces their metastasis to the bone [56]. But in patients with hormone receptor-positive breast cancer, both the disease and its therapeutic treatment with antiestrogenic agents negatively impact the bone and result in decrease in bone mineral density. Therefore anti-hormonal therapy is considered only in cases where cancer cells express the ERα [58]. However, unlike nonsteroidal aromatase inhibitors, a steroidal aromatase inhibitor, e.g., exemestane (probably due to its steroid structure), has been reported to exert beneficial effects on the bone through its primary metabolite 17-hydroexemestane [51, 57].

Fulvestrant, an alkylosulfonian derivative of estradiol, is another category of estrogen inhibitors (estrogen receptor antagonist), which competitively binds to ER with high affinity and downregulates expression of ERβ by functional blockade [59, 60]. Fulvestrant has been reported to induce mitochondrial depolarization at high concentrations that results in release of apoptogenic factors, loss of oxidative phosphorylation, and eventually cell death due to apoptosis [60].

2-Methoxyestradiol (2-ME) belongs to another class of anticancer drugs, which act via induction of neuronal nitric oxide synthase and generation of nitric oxide

in the nuclei of cancer cells. However, recently 2-ME has been found to activate epigenetically silenced ERβ, resulting in apoptosis of malignant cancer cells [60].

Furthermore, some of the recent reports emphasize the role of mutant ERα gene (*ESR1*) in cancer progression and drug resistance. These mutations have been observed to get accumulated in circulating DNA of bone metastasis patients [32].

6.5 Estrogen replacement

Zoledronic acid is a known anti-resorptive agent and exhibits antitumor effects in ER-ve breast cancers. Some of the recent studies emphasize that it's the menopausal status (and not the hormone receptor status) that determines its anticancer efficiency [61]. This differential effect of zoledronic acid in pre- and postmenopausal bone metastasis patients has been suggested to be regulated by bone turnover effect of estrogen. Estrogen inhibits osteoclastogenesis via its direct effect on osteoclast and their precursors. Similarly, zoledronic acid also exhibits pro-apoptotic effects on osteoclasts by inhibiting mevalonate pathway and thus prevents release of growth factors that stimulate tumor growth. But in contrast to estrogen, zoledronic acid also reduces the number and activity of osteoblasts. Therefore, replacement of estrogen with zoledronic acid could be a more effective antitumor therapy in a low-estrogen bone microenvironment. Though administration of zoledronic acid does not alter growth of ER+ve cells at the primary site of tumor, it hampers their dissemination in the bone. As the cells evade the bone microenvironment, zoledronic acid-mediated bone turnover inhibits their proliferation and prevents overt metastases. Thus zoledronic acid could inhibit bone metastases of both ER-ve and ER+ve breast cancer cells [61].

One of the study reports dealing with in vitro microarray data analysis revealed that glucocorticoid was more efficient in controlling osteosarcoma cell growth than 17β estradiol. Glucocorticoid upregulated the expression of tumor suppressor genes resulting in apoptosis and downregulated the oncogenes associated with cell cycle and mitosis, whereas estradiol had an opposite action [62].

7. Conclusion

Primary bone tumors are a rare occurrence, and most of the bone tumors arise due to metastases of breast, prostate, or lung cancers. The bone is the preferred site for metastases because of its highly vascular nature and extensive molecular signaling. A large number of bone tumor cases have been observed in an adolescent population experiencing a growth spurt and hormonal changes. Therefore, the treatment methodologies for bone tumors rarely involve the use of hormones as drugs but rather deal with hormone deprivation or inhibition. Though therapeutic approaches involving deprivation or replacement of hormones negatively affect bone health, the hormonal therapy alone or in combination with chemotherapeutic drugs offers a promising strategy for inhibition of bone tumors and improving the survival rates.

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Conflict of interest

The authors declare no conflict of interest with relation to this study.

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References

[1] Dai X, Ma W, He X, Jha RK. Review of therapeutic strategies for osteosarcoma, chondrosarcoma, and Ewing's sarcoma. Medical Science Monitor. 2013;**17**:RA177-RA190. DOI: 10.12659/msm.881893

[2] Ibrahim T, Mercatali L, Amadori D. Bone and cancer: The osteoncology. Clinical Cases in Mineral and Bone Metabolism. 2013;**10**:121-123. DOI: 10.11138/ccmbm/2013.10.2.121

[3] Simmons JK, Hildreth BE, Supsavhad W, Elshafae SM, Hassan BB, Dirksen WP, et al. Animal models of bone metastasis. Veterinary Pathology. 2015;**52**:827-841. DOI: 10.1177/0300985815586223

[4] Gdowski AS, Ranjan A, Vishwanatha JK. Current concepts in bone metastasis, contemporary therapeutic strategies and ongoing clinical trials. Journal of Experimental & Clinical Cancer Research. 2017;**36**:1-13. DOI: 10.1186/s13046-017-0578-1

[5] Kakonen S-M, Mundy GR. Mechanisms of osteolytic bone metastases in breast carcinoma. Cancer. 2003;**97**:834-839. DOI: 10.1002/cncr.11132

[6] Nakai Y, Okamoto K, Terashima A, Ehata S, Nishida J, Imamura T, et al. Efficacy of an orally active small-molecule inhibitor of RANKL in bone metastasis. Bone Research. 2019;**7**:1-10. DOI: 10.1038/s41413-018-0036-5

[7] Schrage Y, Bovée J. Bone tumors: An overview. The Atlas of Genetics and Cytogenetics in Oncology and Haematology. 2011;**9**:166-170. DOI: 10.4267/2042/38197

[8] Chakarun CJ, Forrester DM, Gottsegen CJ, Patel DB, White EA, Matcuk GR. Giant cell tumor of bone: Review, mimics, and new developments in treatment. Radiographics. 2013;**33**:197-211. DOI: 10.1148/rg.331125089

[9] Mak IWY, Turcotte RE, Ghert M. Parathyroid hormone-related protein (PTHrP) modulates adhesion, migration and invasion in bone tumor cells. Bone. 2013;**55**:198-207. DOI: 10.1016/j.bone.2013.02.020

[10] Evola FR, Costarella L, Pavone V, Caff G, Cannavò L, Sessa A, et al. Biomarkers of osteosarcoma, chondrosarcoma, and ewing sarcoma. Frontiers in Pharmacology. 2017;**8**:1-14

[11] Schwab JH, Springfield DS, Raskin KA, Mankin HJ, Hornicek FJ. What's new in primary bone tumors. Journal of Bone and Joint Surgery. 2012;**94**:1913-1919. DOI: 10.2106/JBJS.L.00955

[12] Lindsey BA, Markel JE, Kleinerman ES. Osteosarcoma Overview. Rheumatology and Therapy. 2016;**4**:25-43. DOI: 10.1007/s40744-016-0050-2

[13] Li X, Yan ML, Yu Q. Identification of candidate drugs for the treatment of metastatic osteosarcoma through a subpathway analysis method. Oncology Letters. 2017;**13**:4378-4384. DOI: 10.3892/ol.2017.5953

[14] El-Naggar AM, Clarkson PW, Negri GL, Turgu B, Zhang F, Anglesio MS, et al. HACE1 is a potential tumor suppressor in osteosarcoma. Cell Death & Disease. 2019;**10**. DOI: 10.1038/s41419-018-1276-4

[15] Fang D, Yang H, Lin J, Teng Y, Jiang Y, Chen J, et al. 17β-Estradiol regulates cell proliferation, colony formation, migration, invasion and promotes apoptosis by upregulating miR-9 and thus degrades MALAT-1 in osteosarcoma cell MG-63 in an estrogen receptor-independent manner. Biochemical and Biophysical Research

Communications. 2015;**457**:500-506. DOI: 10.1016/j.bbrc.2014.12.114

[16] Yu PY, Gardner HL, Roberts R, Cam H, Hariharan S, Ren L, et al. Target specificity, in vivo pharmacokinetics, and efficacy of the putative STAT3 inhibitor LY5 in osteosarcoma, Ewing's sarcoma, and rhabdomyosarcoma. PLoS One. 2017;**12**:1-18. DOI: 10.1371/journal.pone.0181885

[17] Wu VM, Mickens J, Uskoković V. Bisphosphonate-functionalized hydroxyapatite nanoparticles for the delivery of the bromodomain inhibitor JQ1 in the treatment of osteosarcoma. ACS Applied Materials & Interfaces. 2017;**9**:25887-25904. DOI: 10.1021/acsami.7b08108

[18] Onimoe GI, Liu A, Lin L, Wei CC, Schwartz EB, Bhasin D, et al. Small molecules, LLL12 and FLLL32, inhibit STAT3 and exhibit potent growth suppressive activity in osteosarcoma cells and tumor growth in mice. Investigational New Drugs. 2012;**30**:916-926. DOI: 10.1007/s10637-011-9645-1

[19] Zhang S, Wang X, Gu Z, Wang L. Small molecule survivin inhibitor YM155 displays potent activity against human osteosarcoma cells. Cancer Investigation. 2016;**34**:401-407. DOI: 10.1080/07357907.2016.1212205

[20] Tovar C, Rosinski J, Filipovic Z, Higgins B, Kolinsky K, Hilton H, et al. Small-molecule MDM2 antagonists reveal aberrant p53 signaling in cancer: Implications for therapy. Proceedings of the National Academy of Sciences of the United States of America. 2006;**103**:1888-1893. DOI: 10.1073/pnas.0507493103

[21] Lillo Osuna MA, Garcia-Lopez J, El Ayachi I, Fatima I, Khalid AB, Kumpati J, et al. Activation of estrogen receptor alpha by decitabine inhibits osteosarcoma growth and metastasis. Cancer Research. 2018. DOI: 10.1158/0008-5472.can-18-1255

[22] Bone Cancer: Statistics [Internet]. Nov. 2018. Available from: https://www.cancer.net/cancer-types/bone-cancer/statistics

[23] Dorfman HD, Vanel D, Czerniak B, Park YK, Kotz R, Unni KK. WHO classification of tumours of bone: Introduction. World Health Organization. 2013:226-232

[24] Svoboda M, Hamilton G, Thalhammer T. Steroid hormone metabolizing enzymes in benign and malignant human bone tumors. Expert Opinion on Drug Metabolism & Toxicology. 2010;**6**:427-437. DOI: 10.1517/17425251003592129

[25] Guise TA. Molecular mechanisms of osteolytic bone metastases. Cancer. 1999;**88**:15-16. DOI: 10.1002/1097-0142(20000615)88:12+<2892::AID-CNCR2>3.0.CO;2-Y

[26] Nikitovic D, Kavasi RM, Berdiaki A, Papachristou DJ, Tsiaoussis J, Spandidos DA, et al. Parathyroid hormone/parathyroid hormone-related peptide regulate osteosarcoma cell functions: Focus on the extracellular matrix (review). Oncology Reports. 2016;**36**:1787-1792. DOI: 10.3892/or.2016.4986

[27] Weekes D, Kashima TG, Zandueta C, Perurena N, Thomas DP, Sunters A, et al. Regulation of osteosarcoma cell lung metastasis by the c-Fos/AP-1 target FGFR1. Oncogene. 2016;**35**:2852-2861. DOI: 10.1038/onc.2015.344

[28] Jiang M, Yan Y, Yang K, Liu Z, Qi J, Zhou H, et al. Small molecule nAS-E targeting cAMP response element binding protein (CREB) and CREB-binding protein interaction inhibits breast cancer bone metastasis. Journal of Cellular and Molecular Medicine. 2019;**23**:1224-1234. DOI: 10.1111/jcmm.14024

[29] Efstathiou E, Titus M, Wen S, Hoang A, Karlou M, Ashe R, et al. Molecular characterization of enzalutamide-treated bone metastatic castration-resistant prostate cancer. European Urology. 2015;**67**:53-60. DOI: 10.1016/j.eururo.2014.05.005

[30] D'Oronzo S, Brown J, Coleman R. The value of biomarkers in bone metastasis. European Journal of Cancer Care. 2017;**26**:1-10. DOI: 10.1111/ecc.12725

[31] Chirgwin JM, Wong DH, Corey E, Higgins LS, Stebbins EG, Guise TA, et al. Basic mechanisms responsible for osteolytic and osteoblastic bone metastases. Clinical Cancer Research. 2006;**12**:6213s-6216s. DOI: 10.1158/1078-0432.ccr-06-1007

[32] Bado I, Gugala Z, Fuqua SAW, Zhang XH-F. Estrogen receptors in breast and bone: From virtue of remodeling to vileness of metastasis. Oncogene. 2017;**36**:4527-4537. DOI: 10.1038/onc.2017.94

[33] Bone Cancer: Introduction [Internet]. 2018. Available from: https://www.cancer.net/cancer-types/bone-cancer/introduction

[34] Logothetis CJ, Lin SH. Osteoblasts in prostate cancer metastasis to bone. Nature Reviews. Cancer. 2005;**5**:21-28. DOI: 10.1038/nrc1528

[35] Kushlinskii NE, Timofeev YS, Solov'ev YN, Gerstein ES, Lyubimova NV, Bulycheva IV. Components of the RANK/RANKL/OPG system, IL-6, IL-8, IL-16, MMP-2, and calcitonin in the sera of patients with bone tumors. Bulletin of Experimental Biology and Medicine. 2014;**157**:520-523. DOI: 10.1007/s10517-014-2605-y

[36] Suva LJ, Washam C, Nicholas RW, Griffin RJ. Bone metastasis: Mechanisms and therapeutic opportunities. Nature Reviews Endocrinology. 2011;**7**:208-218. DOI: 10.1038/nrendo.2010.227

[37] Duan Z, Zhang J, Ye S, Shen J, Choy E, Cote G, et al. A-770041 reverses paclitaxel and doxorubicin resistance in osteosarcoma cells. BMC Cancer. 2014;**14**:1-11. DOI: 10.1186/1471-2407-14-681

[38] Macedo F, Ladeira K, Pinho F, Saraiva N, Bonito N, Pinto L, et al. Bone metastases: An overview. Oncology Reviews. 2017;**11**:43-49. DOI: 10.4081/oncol.2017.321

[39] Mantalaris A, Panoskaltsis N, Sakai Y, Bourne P, Chang C, Messing EM, et al. Localization of androgen receptor expression in human bone marrow. The Journal of Pathology. 2001;**193**:361-366. DOI: 10.1002/1096-9896(0000)9999:9999<::AID-PATH803>3.0.CO;2-W

[40] Ito Y, Sadar MD. Enzalutamide and blocking androgen receptor in advanced prostate cancer: Lessons learnt from the history of drug development of antiandrogens. Research and Reports in Urology. 2018;**10**:23-32. DOI: 10.2147/RRU.S157116

[41] Deivaraju C, Temple HT, Block N, Robinson P, Schally AV. LHRH receptor expression in sarcomas of bone and soft tissue. Hormone Molecular Biology and Clinical Investigation. 2016;**28**:105-111. DOI: 10.1515/hmbci-2016-0001

[42] Langley RE, Kynaston HG, Alhasso AA, Duong T, Paez EM, Jovic G, et al. A randomised comparison evaluating changes in bone mineral density in advanced prostate cancer: Luteinising hormone-releasing hormone agonists versus transdermal oestradiol. European Urology: European Association of Urology. 2016;**69**:1016-1025. DOI: 10.1016/j.eururo.2015.11.030

[43] Diaz-Convalia E, Arrabal-Polo MA. Cano-Garcia M del C, Dominguez-Amillo A, Canales-Casco N, Arrabal-Martin M. Risk of renal stone formation in patients treated with luteinising hormone-releasing hormone analogues for prostate

cancer: Importance of bone metabolism and urine calcium. International Urology and Nephrology. 2018;**50**:419-425. DOI: 10.1007/s11255-018-1793-1

[44] de Bono JS, Logothetis CJ, Molina A, Fizazi K, North S, Chu L, et al. Abiraterone and increased survival in metastatic prostate cancer. The New England Journal of Medicine. 2011;**364**:1995-2005. DOI: 10.1056/NEJMoa1014618

[45] Gravanis I, Lopez AS, Hemmings RJ, Jimenez JC, Garcia-Carbonero R, Gallego IG, et al. The European medicines agency review of abiraterone for the treatment of metastatic castration-resistant prostate cancer in adult men after docetaxel chemotherapy and in chemotherapy-naive disease: Summary of the scientific assessment of the committee for medicinal products for human use. The Oncologist. 2013;**18**:1032-1042. DOI: 10.1634/theoncologist.2013-0092

[46] Body J-J, Casimiro S, Costa L. Targeting bone metastases in prostate cancer: improving clinical outcome. Nature Reviews Urology. 2015;**12**: 340-356. DOI: 10.1038/nrurol.2015.90

[47] El-Amm J, Patel N, Freeman A, Aragon-Ching JB. Metastatic castration-resistant prostate cancer: Critical review of enzalutamide. Clinical Medicine Insights: Oncology. 2013;**7**:235-245. DOI: 10.4137/CMO.S11670

[48] Miki K, Sasaki H, Kido M, Takahashi H, Aoki M, Egawa S. A comparative study on the efficacies of gonadotropin-releasing hormone (GnRH) agonist and GnRH antagonist in neoadjuvant androgen deprivation therapy combined with transperineal prostate brachytherapy for localized prostate cancer. BMC Cancer. 2016;**16**: 1-7. DOI: 10.1186/s12885-016-2737-8

[49] Kashiwabara T, Suda S. Usefulness of combined androgen blockade therapy with gonadotropin-releasing hormone antagonist for bone metastatic prostate cancer with pretreatment prostate-specific antigen level ≥ 50 ng/mL. BMC Cancer. 2018;**18**:1-11. DOI: 10.1186/s12885-018-4541-0

[50] Yu EY, Getzenberg RH, Coss CC, Gittelman MM, Keane T, Tutrone R, et al. Selective estrogen receptor alpha agonist GTx-758 decreases testosterone with reduced side effects of androgen deprivation therapy in men with advanced prostate cancer. European Urology: European Association of Urology. 2015;**67**:334-341. DOI: 10.1016/j.eururo.2014.06.011

[51] Hofbauer LC, Rachner TD, Coleman RE, Jakob F. Endocrine aspects of bone metastases. The Lancet Diabetes and Endocrinology. 2014;**2**:500-512. DOI: 10.1016/S2213-8587(13)70203-1

[52] He S, Nelson ER. 27-Hydroxycholesterol, an endogenous selective estrogen receptor modulator. Maturitas. 2017;**104**:29-35. DOI: 10.1016/j.maturitas.2017.07.014

[53] Salvatori L, Caporuscio F, Coroniti G, Starace G, Frati L, Russo MA, et al. Down-regulation of epidermal growth factor receptor induced by estrogens and phytoestrogens promotes the differentiation of U2OS human osteosarcoma cells. Journal of Cellular Physiology. 2009;**220**:35-44. DOI: 10.1002/jcp.21724

[54] Chrzan BG, Bradford PG. Phytoestrogens activate estrogen receptor β1 and estrogenic responses in human breast and bone cancer cell lines. Molecular Nutrition & Food Research. 2007;**51**:171-177. DOI: 10.1002/mnfr.200600091

[55] Rajah TT, Peine KJ, Du N, Serret CA, Drews NR. Physiological concentrations of genistein and 17β-estradiol inhibit MDA-MB-231 breast cancer cell growth by increasing

BAX/BCL-2 and reducing pERK1/2. Anticancer Research. 2012;**32**:1181-1191

[56] Wang W, Belosay A, Yang X, Hartman JA, Song H, Iwaniec UT, et al. Effects of letrozole on breast cancer micro-metastatic tumor growth in bone and lung in mice inoculated with murine 4T1 cells. Clinical & Experimental Metastasis. 2016;**33**:475-485. DOI: 10.1007/s10585-016-9792-z

[57] Ariazi EA, Leitao A, Oprea TI, Chen B, Louis T, Bertucci AM, et al. Exemestane's 17-hydroxylated metabolite exerts biological effects as an androgen. Molecular Cancer Therapeutics. 2007;**6**:2817-2827. DOI: 10.1158/1535-7163.mct-07-0312

[58] Bartels S, Christgen M, Luft A, Persing S, Jödecke K, Lehmann U, et al. Estrogen receptor (ESR1) mutation in bone metastases from breast cancer. Modern Pathology. 2018;**31**:56-61. DOI: 10.1038/modpathol.2017.95

[59] Kang E, Cho JH, Choi J-H, Yoo H-W. Etiology and therapeutic outcomes of children with gonadotropin-independent precocious puberty. Annals of Pediatric Endocrinology & Metabolism. 2016;**21**:136. DOI: 10.6065/apem.2016.21.3.136

[60] Gorska M, Wyszkowska RM, Kuban-Jankowska A, Wozniak M. Impact of apparent antagonism of estrogen receptor β by fulvestrant on anticancer activity of 2-methoxyestradiol. Anticancer Research [Internet]. 2016;**36**:2217-2226. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27127126

[61] Holen I, Walker M, Nutter F, Fowles A, Evans CA, Eaton CL, et al. Oestrogen receptor positive breast cancer metastasis to bone: inhibition by targeting the bone microenvironment in vivo. Clinical & Experimental Metastasis. 2016;**33**:211-224. DOI: 10.1007/s10585-015-9770-x

[62] Zhou Q, Shen L, Liu C, Liu C, Chen H, Liu J. The effects of estradiol and glucocorticoid on human osteosarcoma cells: Similarities and differences. Anticancer Research. 2016;**36**:1683-1691