Chapter 8

Osteosarcomagenesis: Biology, Development, Metastasis, and Mechanisms of Pain

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Additional information is available at the end of the chapter

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Abstract

Osteosarcoma is the most common primary cancer of the bone and third most common cancer in children and adolescents with approximately 900 new cases annually in the United States. A major facet of osteosarcoma is its high level of genomic instability, in particular chromosomal instability, which is the result of increased or decreased chromosome number in a cell. Furthermore, pain is the most common symptomatic feature of osteosarcoma that lacks effective therapy. Pain in osteosarcoma is relatively more complicated than many other painful conditions requiring a more thorough understanding of its etiology, pathobiology, and neurobiology to allow the development of better therapies for reducing pain in osteosarcoma patients. Studies are underway to define the diverse modalities of presentation, growth, development, metastases, and nociception in osteosarcoma. New data from human studies in combination with data from studies incorporating transgenic mouse models of osteosarcoma are providing valuable insights into the mechanisms underlying the development of both the tumor and the tumor-induced pain. These new data will undoubtedly lead to improved prognoses, as well as the development of novel therapeutics that will significantly decrease bone cancer pain.

Keywords: osteosarcoma, genetics, pain, bone tumors, pathobiology

1. Introduction

Osteosarcoma is the most common malignant bone tumor found in children and adolescents and is associated with many complications including metastases and intractable cancer pain [1, 2]. Typically, the prevalence of osteosarcoma shows a strong relationship with skeletal growth. The main incidence peak occurs in the second decade of life and generally

is associated with a highly defined phenotype. Osteosarcoma also occurs in elderly adults in the sixth and seventh decades of life and is often preceded by certain genetic predispositions [3]. Osteosarcomas predominately form in the metaphyses of the long bones in the major growth centers such as the distal femur, proximal tibia, and proximal humerus. Osteosarcomas are quite aggressive locally but often produce early, lethal systemic metastases [4]. According to the National Cancer Institute, as many as 20% of patients will have radiographically detectable metastases at diagnosis, and ultimately nearly 90% of patients have radiographically undetectable metastatic lesions, particularly to the lungs [5, 6]. However, chest CTs have been estimated to miss nearly 25% of metastatic nodules found during thoracotomy, and up to 14% of metastases are not nodular in shape, which complicates the metastatic picture in many patients [7]. With no known precursor to osteosarcoma, treatment options are extremely limited. Adjuvant chemotherapy and surgical resection are standard therapies, but treatment efficacy still remains poor for over one-third of osteosarcoma patients [5, 8]. Although our understanding of the mechanisms underlying tumor development, tumor progression, and metastasis is improving [6, 9–12], the complex nature of the bone tumor microenvironment presents unique challenges to identifying novel drug targets and treatment strategies.

The most common presenting symptom of osteosarcoma is pain, particularly with activity. Osteosarcoma pain can start in adolescence, leading to hospitalization, reduced survival, and poor quality of life. Pain in osteosarcoma is unique because of unpredictable and recurrent episodes of acute pain due to vaso-occlusive crises (VOC), in addition to chronic pain experienced by a majority of adult patients on a daily basis [13]. As detection and survival among cancer patients have improved, pain has become an increasing challenge, because traditional therapies are often only partially effective [14]. In this regard, the treatment of osteosarcoma pain is complicated because long-term treatment choices remain limited and generally involve opioids, which impose liabilities of their own including constipation, mast cell activation, addiction, and respiratory depression [15]. Moreover, significantly larger doses of opioids are required to treat pain in osteosarcoma as compared to other acute and chronic pain conditions. Pain can be lifelong in osteosarcoma and may therefore influence cognitive function and lead to depression and anxiety, which can in turn promote the perception of pain [13]. In general, the treatment of chronic pain remains unsatisfactory, perhaps due to the diverse pathobiology in different diseases. Therefore, it is critical to understand the mechanisms specific to the genesis of osteosarcoma-related pain to develop targeted therapies.

2. Normal bone development

The bone is a readily adaptive, mineralized tissue that performs diverse functions including enabling locomotion, storing nutrients such as phosphate and calcium, and protecting soft tissues among many others. Despite its static appearance, osteoclasts and osteoblasts, two major cell types abundant throughout bone tissue are constantly remodeling bone.

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Osteoblasts are the resident bone-producing cells of the body. Osteoblasts are derived from a lineage of cells arising from a mesenchymal origin [16], while osteoclasts arise from a hematopoietic lineage [17]. The remodeling of the bone is a tightly coupled process. From a physiological perspective, distinct differentiation and maturation pathways of these two cell types allows for uninterrupted maintenance of bone homeostasis [18]. The differentiation process of osteoblasts is often divided in specific stages: mesenchymal progenitors, preosteoblasts, and osteoblasts. Similarly, this process also occurs in osteoclastogenesis where cells of myeloid origin differentiate into one of four cell types, one being osteoclasts [17]. While differentiation stages are useful for cellular identification, the maturation process is not well understood. Often, the identities of cells during each stage are characterized by expression of various molecular markers highly associated with osteoblast development. Markers of cells from mesenchymal origin are not well defined and are still a matter of intense debate. Similarly, preosteoblast markers are also difficult to identify. These cells are highly heterogeneous in their expression patterns, as this stage can encompass various cell types during maturation into osteoblasts, bone-lining cells, or osteocytes. However, two common transcription factors, Runt-Related Transcription Factor 2 (*RUNX2*) and later OSTERIX (also known as *SP7*), are expressed during maturation and are highly associated with maturation of the preosteoblast lineage [19], while hematopoietic transcription factor (*PU.1*), microphthalmia-associated transcription factor (*MITF*), and *c-FOS* are associated with osteoclast precursors [17].

3. Tumorigenesis

There is currently no known origin to osteosarcoma; however, much research has pointed to osteoblasts as the progenitor cell type. The single most shared feature of all osteosarcomas histologically is the presence of osteoid matrix, secreted by malignant cells in the growing tumor. In addition, the presence and quantity of this matrix do not define the disease, as osteosarcomas may be composed of many tissue types including chondroblastic, fibroblastic, and osteoblastic [20]. Osteosarcoma has been shown to be associated with loss of key tumor suppressor genes such as tumor protein P53 (*TP53*) and retinoblastoma 1 (*RB1*) [21], and alterations in *p53* are associated with reduced event-free survival [22]. Moreover, sporadic osteosarcomas are highly associated with mutations in the *RB* gene. Additionally, germ line mutations in the *p53* gene predispose patients with Li-Fraumeni syndrome (LFS) to osteosarcoma [23] with an incidence of up to 12% [24].

4. Chromosomal abnormalities

Despite its well-defined phenotypic characteristics, genetically speaking, osteosarcoma is chaotic and disordered. It is often associated with massive chromosomal rearrangements, cytogenetic aberrations, and numerous mutations [25]. Numerous cytogenetic and molecu-

lar studies of osteosarcoma have been conducted in recent years yielding interesting results but often with conflicting findings [26]. Many of these studies have limited prognostic and diagnostic value and fail to understand the driving events necessary for osteosarcoma development. However, the overall infrequency of this disease makes elucidating these factors all the more challenging. It has been apparent since early studies that osteosarcomas have a significant propensity toward aneuploidy with over 96% of high-grade cases being hyperploid [26]. Furthermore, cytogenetic analyses have provided evidence for enormous variation in karyotypic alterations. A major facet of osteosarcoma is its high level of genomic instability, in particular chromosomal instability (CIN). CIN can contribute to tumor initiation and progression through altering the expression of proto-oncogenes or tumor suppressor genes. The rate of gain or loss of entire chromosomes or sections is significant in the pathogenesis of osteosarcoma resulting in numerous aberrations and wide variability between cells and tumors [27].

5. Oncogene/tumor suppressor gene dysfunction

The search for specific drivers of osteosarcoma development has stemmed from the early cytogenetic and molecular findings. Initial genetic studies sought to identify important genes involved in cancers although not necessarily specific to osteosarcoma. These early studies have implicated a number of important tumor suppressors, oncogenes, and growth factors that are implicated in other sarcomas and carcinomas as well [26]. As stated earlier, perhaps the best two characterized examples of this are the tumor suppressor gene *TP53* and the retinoblastoma *RB1* gene. The location of *TP53* on chromosome 17p13 is an area frequently altered in osteosarcomas and is readily apparent in cytogenetic analyses. Alterations in the *TP53* gene have significant effects on the downstream signaling targets, many of which are normally involved in cell cycle control and apoptosis. Gene rearrangements, point mutations, epigenetic modification [28, 29], and/or allelic loss can presumably lead to inactivation of normal *TP53* function, and these aberrations have been associated with the development of osteosarcoma. In a recent study that characterized the genomic landscape of osteosarcoma via whole genome sequencing (WGS), the majority of *TP53* inactivation in osteosarcoma was found to be due to translocations [30]. Furthermore, this study highlights the fact that *TP53* mutation is highly prevalent in osteosarcoma, with >90% of all tumors containing a mutation in at least one allele, and upward of 80% containing mutations in both alleles. In addition to the *TP53* gene, associations between osteosarcoma and *RB1* are well recognized as well, especially in patients with hereditary retinoblastoma, in which osteosarcoma incidence is 1000 times higher than in the general population. The loss of heterozygosity and/or sporadic alterations in the *RB1* gene is apparent in >60% of osteosarcoma cases, and these genetic changes have significant prognostic value [31]. As only these prototypical cancer genes and a few others have been definitively implicated in osteosarcoma, there is a pressing need to identify more genes and pathways governing its development and metastasis to better treat osteosarcoma patients and their associated pain.

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6. Axonal guidance genes in osteosarcoma

Recently, a new pathway has been identified using the Sleeping Beauty mutagenesis system in mice implicating axon guidance genes such as semaphorin-4D (*SEMA4D*) in osteosarcoma [25]. During normal bone homeostasis, osteoclasts express high levels of *Sema4d*, whereas osteoblasts do not. Instead, osteoblasts express its cognate receptor and co-receptor, Plexin B1 (*Plxnb1*) and Erb-B2 receptor tyrosine kinase 2 (*Erbb2*), respectively. Thus, it is possible that misexpression of *SEMA4D* and MET proto-oncogene, receptor tyrosine kinase (*MET*) in osteoblasts might give rise to a subset of osteosarcomas. Similarly, the tumorigenic properties induced by overexpression of *SEMA4D* in human osteosarcoma cells are dependent on MET and ERBB2 levels, which has been reported [32]. Previous studies in osteosarcoma showed that high levels of *ERBB2* are associated with a good outcome and that overexpression of *MET* can directly transform osteoblasts into osteosarcomas [33, 34]. The fact that *SEMA4D* is a cell surface receptor makes it an attractive candidate for novel therapies. This will be addressed later in the chapter.

7. Metastasis

Approximately 20–30% of osteosarcoma patients have overt metastases at diagnosis, and about 40% of patients will develop lung metastases during the course of treatment [35–37]. Analysis of clinical outcomes of patients without overt metastasis at diagnosis prior to the advent of chemotherapy demonstrated >90% of patients developed lung metastasis 6–36 months after surgical resection, indicating the majority of seemingly nonmetastatic patients actually have micrometastatic disease at diagnosis [30]. While it is largely believed that the implementation of chemotherapy eradicates these developing micrometastases in many cases, these data highlight the fact that metastasis is the most important factor associated with poor outcome in osteosarcoma [38]. Recent work from Moriarity and colleagues identified many genes that promote osteosarcoma development and metastasis through a forward genetic screen in mice using the *SB* transposon-based mutagenesis system. Both classes of genes (oncogenes and tumor suppressors) may be critical for development of detectable metastases present at diagnosis and/or the ability of latent micrometastases to develop to a detectable level. Subsets of genes identified via the SB screen were only present in the metastatic lesions, while others were found both in primary tumors and metastases. Among those genes were phosphatase and tensin homolog (*Pten*), glycogen synthase kinase 3 beta (*Gsk3b*), synaptosome-associated protein 23 kDa (*Snap23*), mitogen-activated protein kinase kinase kinase kinase 3 (*Map4k3*), Rho GTPase-activating protein 35 (*Arhgap35* (*Grlf1*)), Rho/Rac guanine nucleotide exchange factor 18 (*Arhgef18*), Axin 1 (*Axin1*), Raf-1 proto-oncogene, serine/threonine kinase (*Raf1*), and ubiquitin-associated protein 2 like (*Ubap2l*). Interestingly, all of these genes have been previously implicated in metastasis of other cancers, which supports the conclusion that these genes are likely involved in osteosarcoma metastasis [25]. Additionally, bone metastases are also highly painful. The ability of osteosarcoma to successfully metastasize relies in part on its ability to exploit many mechanisms of normal bone remodeling [39]. Two such examples of this are the Wnt family of proteins and bone morphogenetic proteins (BMPs), both of which are

critical in bone development and are implicated in cancer pain [40, 41]. The evolving physiologic and pathological roles of the Wnt/β-catenin signaling pathway may offer attractive therapeutic targets for novel antagonists and inhibitors for patients with primary and metastatic osteosarcoma. Likewise, BMPs are responsible for numerous osteoinductive cellular processes including bone growth, differentiation, and matrix maintenance [40]. In vitro examination in numerous osteosarcoma cell lines revealed highly abundant expression of BMPs in virtually all lines tested [42–44]. Moreover, BMP expression has been found to correlate with metastasis in osteosarcoma [45], while overexpression of BMP-9 reduces invasion and migration properties of osteosarcoma cells [46]. Conversely, analysis of 47 human osteosarcomas found no correlations between BMP expression and prognostic outcomes [47]. Furthermore, BMPs have also been shown to induce bone formation in human osteosarcoma cells [48]. As mentioned earlier, osteosarcomas can present with mixed cell lineages and differentiation patterns [20]. These studies and others suggest that perhaps BMPs may be expressed at differing levels depending on the cellular state, and their presence may offer an attractive therapeutic option for the treatment of osteosarcoma. While the significance of BMP signaling in osteosarcomagenesis is not yet fully understood, current research suggests BMPs may play an important role.

8. Characteristics of pain in osteosarcoma

Cancer-induced bone pain is a complex pain state involving a combination of background, spontaneous, and incident (movement-evoked) pain [14, 49]. Regional pain alone or in conjunction with a palpable mass are the two main reasons that osteosarcoma patients consult a doctor. Patients with osteosarcoma of the jaws typically present with pain, swelling, ulceration, or neurological deficit [50], but again pain is a major symptom causing these patients to seek medical attention. Currently only about half of patients with cancer-induced bone pain experience temporary relief from conventional therapies [51], which stresses the need for the development of more effective treatments. **Table 1** summarizes some of the types of pain experienced by bone cancer patients and animal models of bone cancer pain.

Characteristics of pain	**Pain phenotyping method**	
	Subjects with OSA	**Mice with bone tumor**
Mechanical allodynia	Patients with cancer pain were evaluated for mechanical allodynia [52]	Paw withdrawal responses to von Frey monofilaments in bone cancer mice or rats [1, 53–55]
Heat hyperalgesia	Patients with cancer pain were evaluated for heat hyperalgesia [52]	Paw withdrawal latency and frequency in response to static heat stimuli in mice or rats [53, 54, 56]
Movement-evoked pain	The use of a pain verbal rating scale during movement [57]	Count the number of spontaneous flinches of the hind limb in bone tumor mice [55]

Note: most human cancer pain studies have employed visual analogue scales (VAS), numerical rating scales, or verbal reporting to quantify pain, and thus it is often difficult to compare nociceptive behavioral testing results from cancer pain studies using animal models with human cancer pain studies because of the differences in pain assessment.

Table 1. Characteristics of pain in osteosarcoma.

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9. Peripheral and central mechanisms of pain in osteosarcoma

While our knowledge of the mechanisms of bone cancer pain is ever expanding, part of our failure to adequately manage osteosarcoma and other forms of bone cancer pain is an inadequate understanding of the etiology and mechanisms involved. Cancer-induced bone pain is a mixed-mechanism pain state exhibiting elements of inflammatory, neuropathic, and ischemic pain, but with distinctive effects on the tissues and nerves in the periphery, as well as unique biochemical changes within the spinal cord [14, 58].

9.1. Peripheral mechanisms

As summarized by Falk and colleagues, the biology of cancer-induced bone pain involves a complex interplay among the tumor cells, peripheral nerves, and cells of the bone [14]. The tumor cells trigger a number of nociceptive and immune responses that ultimately recruit inflammatory cells including macrophages, neutrophils, and T cells to the bone resulting in release of a plethora of endogenous chemicals acting on bone cells, cancer cells, and importantly on primary afferent nerve fibers [59, 60]. Thus, tumor and tumor-associated cells in the cancer microenvironment may release various peripheral mediators. These include adenosine triphosphate (ATP), formaldehyde, protons, proteases, endothelin, bradykinin, tumor necrosis factor (TNF), and nerve growth factor (NGF) that result in the activation and/or sensitization of peripheral and central neurons [61, 62]. The complexity of this neuroimmune and inflammatory effect on cancer pain has been recently reviewed [63]. Ultimately this cascade of events leads to the activation and sensitization of nociceptors, the degradation of the bone, and subsequent increased tumor growth [14]. Furthermore, studies have shown that cancer cells in the bone induce a highly disorganized sprouting of sensory and sympathetic fibers, leading to the formation of small neuromas. These disorganized bundles of nerve fibers are thought to contribute to episodes of breakthrough pain or even movement-induced pain [64, 65]. In addition to changes in nerve fibers, bone tumors in mice have also been shown to be associated with changes in both blood vessels and lymphatics, which may facilitate metastasis and which, interestingly, can be altered by acupuncture treatment [66].

9.2. Central mechanisms

Both the spinal cord dorsal horn and dorsal root ganglia undergo unique changes induced by bone tumors suggesting that the peripheral alterations drive central alterations. Some of the bone tumor-induced changes observed in the spinal glial and neurons are distinct, but many are reflective of changes seen with other chronic pain states. Thus, tumors are associated with increased expression of dynorphin with accompanying spinal astrocyte hypertrophy and upregulation of galanin and AF3 [14, 66]. Clearly, the recent identification of a vast number of mediators and receptors that contribute to bone cancer-related pain, as well as more detailed knowledge of the peripheral and central mechanisms underlying the development of bone tumor nociception, will provide novel therapeutic targets for treating patients with osteosarcoma pain. Subsets of these targets are discussed in more detail below.

10. Treatment of osteosarcoma

From the discussion above, it is clear that due to the complex nature of osteosarcoma pathobiology and the neurobiological mechanisms that underlie the development of bone cancer pain, it may be necessary to target multiple receptors, mediators, and genes to adequately treat osteosarcomas and osteosarcoma-associated pain. Below we review some of the established and novel targets for the treatment of osteosarcoma and its associated pain.

10.1. Immunomodulation

10.1.1. Semaphorins

VX15/2503 (Vaccinex, Inc.) is a highly novel, immunomodulatory monoclonal antibody that specifically targets *SEMA4D* (CD100), a receptor and soluble protein from the semaphorin family known to be involved in immune modulation [67] and regulation of normal bone formation [68]. Initial interest in this monoclonal antibody was rooted in results indicating that immune cell-dependent interactions were in fact responsible for its antitumor activity [67]. Early preclinical studies have determined that high concentrations of *SEMA4D* are expressed at the invasive border of many human cancers and that this border restricts the antitumor cell infiltrate from effectively combating the growing tumor. Treatment with anti-*SEMA4D* restores the inhibited immune response leading to reduced tumor burden and delayed growth in animal models. Furthermore, anti-*SEMA4D* blockade results in phase I clinical trials of 42 adult patients with advanced solid tumors have been well tolerated with many exhibiting stable disease over various treatment regimens. Antibody therapy targeting *SEMA4D* has also been shown to reduce tumor growth in a xenograft model of soft tissue sarcoma (STS) when combined with antibody therapy for vascular endothelial growth factor (VEGF) [69].

10.2. Intracellular signaling pathway inhibitors

10.2.1. Hedgehog (Hh)

While encompassing diverse functions such as tissue homeostasis and embryonic development, Hedgehog (Hh) signaling is highly complex and not completely understood [70]. Signaling through its receptor Patched-1 (PTCH), Smoothened is activated and promotes subsequent downstream signaling pathway of the Hedgehog (Hh) pathway [71]. This activation has been implicated in many cancers including osteosarcoma where aberrant activation increases cell proliferation but can be reduced through inhibition of the signaling [72]. Hedgehog inhibitors have been successfully tested in clinical trials of other cancers such as chondrosarcoma [73, 74], carcinoma [75], and medulloblastoma [76] providing solid evidence for consideration as a novel therapeutic in osteosarcoma.

10.2.2. Mammalian target of rapamycin (mTOR)

The mammalian target of rapamycin (mTOR) is a protein kinase that regulates cell survival and proliferation [77]. Due to its diverse functions, mTOR is implicated in many cancers making it an attractive target in treating tumors, including osteosarcoma. In one recent study,

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activated mTOR was visualized in osteosarcoma and the staining positively correlated to metastasis and necrosis [78]. Targeted inhibition of the signaling pathway of mTOR has been shown to reduce metastatic behavior in a mouse model of osteosarcoma [79] as well as human xenograft models [80]. Current and future clinical trials using mTOR inhibitors may prove therapeutically fruitful in the treatment of osteosarcoma [81].

10.3. Tyrosine kinase receptors

10.3.1. Human epidermal growth factor receptor 2 (HER2)

HER2 is a member of the human epidermal growth factor receptor family. Located on chromosome 17, activation and overexpression have been implicated in a number of cancers including osteosarcoma. *HER2* is overexpressed in ~40% of osteosarcomas and has been found to occur more frequently in metastatic patients [82]. Expression was also found to correlate with decreased tumor necrosis and event-free survival [83]. Chimeric antigen receptor (CAR)-modified T cells have been shown to kill *HER2*-positive osteosarcoma cells in xenograft and metastatic mouse models [84].

10.3.2. Vascular endothelial growth factor (VEGF)

VEGF expression has been shown to involve in osteosarcoma [85], correlated with overall survival [86] and implicated in metastatic development [82]. A recent paper by Zhou and colleagues found anti-VEGF strategies to be antiangiogenic in osteosarcoma [69]. Interestingly, *SEMA4D* blockade enhanced the anticancer activity of anti-VEGF treatment that provides a viable adjunct to *VEGF* therapy alone. While discussed above, development of a highly novel monoclonal antibody to *SEMA4D* is underway and may provide further insight into targeting *VEGF*-resistant tumors as well as associated malignancies.

11. Treatment of osteosarcoma pain

With a growing population of patients receiving inadequate treatment for intractable bone cancer pain, new targets need to be considered to better address this largely unmet clinical need for improving their quality of life. In general, while there are a variety of methods that are used to treat bone cancer pain, including bisphosphonates, radiation therapy, chemotherapy, hormone therapy, and surgery, the clinical treatment of bone cancer pain still focuses on the three-step program. This program was established by the World Health Organization and includes NSAIDs and narcotics as therapeutic treatment options. However, as we learn more about the mechanisms responsible for cancer pain and the genetic basis for the development of osteosarcomas, there are several areas that offer hope for the development of novel treatments for bone cancer pain. It is important to point out that bone cancer pain can be treated by both systemic and local administration of drugs as well as alternative medical approaches. The obvious advantage of peripheral targets is the reduced potential for CNS side effects, such as the sedation and nausea that often accompany opiate analgesics.

11.1. Cytokines

Pain is a complex trait, and thus, the influence of genetics on pain sensitivity and the efficacy of analgesics are an ongoing challenge. A recent study found that polymorphisms in the Interleukin 1 Beta (IL-1β) family have a significant influence on cytokine serum levels and maximum pain intensity in cancer patients, as well as affecting cancer proliferation [87]. IL-1β has been shown to be expressed in astrocytes and microglia and in nociceptive dorsal root ganglion neurons [88] and thus may represent a target for the treatment of cancer pain.

11.2. TRPV1 receptors

TRP channels were first identified in Drosophila [89], and TRPV1 denotes the transient receptor potential channel family number 1 and was the first mammalian TRP channel to be cloned [90]. Capsaicin and other TRPV1 agonists selectively stimulate nociceptive neurons, and thus while it induces pain, it is possible to treat pain by boosting analgesic pathways [91]. In this regard the use of the TRPV1 agonist, resiniferatoxin (RTX), to block cancer pain has recently been reviewed [92]. In human cancer patients, RTX was given by intrathecal injection into the lumbar cistern, and all patients experienced substantial analgesia without significant side effects. In addition, a recent study has shown that cancer cells undergo numerous metabolic changes that include increased glutamine catabolism and overexpression of the enzyme glutaminase, which mediates glutaminolysis. This produces large pools of intracellular glutamate [93]. This is coupled to an upregulation of the plasma membrane antiporter, system x_c^-. System x_c^- is an amino acid antiporter that typically mediates the exchange of extracellular l-cystine and intracellular L-glutamate across the cellular plasma membrane. The exchange-mediated export of L-glutamate is particularly important within the nervous system, since it represents a non-vesicular route of release through which glutamate can participate in either neuronal signaling or in excitotoxic pathology. With respect to osteosarcomas, the excess glutamate is released directly from the cancer cells and can act on peripheral glutamate receptors located on nerve fibers. It is known that glutamate receptors can modulate peripheral TRPV1 receptors [78]. Thus, the released glutamate converges on peripheral afferent nerve terminals to transmit nociceptive signals through TRPV1. Activation of TRPV1 receptors can ultimately initiate central sensitization in response to tumor-released glutamate [93]. Thus, using RTX to block peripheral TRPV1 channels would block this excess glutamate effect on TRPV1 and reduce both tumor-induced peripheral and central sensitization.

11.3. Opioid receptors

Three members of the opioid receptor family were cloned in the early 1990s, including the delta-opioid receptor (DOR1), the mu-opioid receptor (MOR1), and the kappa-opioid receptor (KOR1) [94]. These three receptors and their corresponding peptide systems are significantly implicated in antinociceptive processes. Opiates have long been the mainstay of treatment for chronic bone cancer pain. However, there is increasing pressure to ensure that prescribing opioid analgesics is minimized to reduce not only the risk of dependence and illicit diversion but also the potential harms associated with tolerance, side effects, and complications,

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since opioid doses required for bone cancer patients are associated with adverse side effects further diminishing their quality of life [95]. This has often led to opiate underdosing [96]. It is important to note that while opioids are routinely used to treat tumor-induced bone pain, Parreca's lab has shown that sustained morphine use increases pain, osteolysis, bone loss, and spontaneous fracture, as well as markers of neuronal damage in DRG cells and the expression of pro-inflammatory cytokines in a rodent model of bone cancer [97]. More recent studies indicate that morphine contributes to chemoresistance via expanding the population of cancer stem cells, promotes tumor angiogenesis, and promotes tumor growth, thereby revealing a novel role of morphine and providing some new guidelines for the clinical use of morphine [98, 99]. It is also worth noting that treatment guidelines tend to consider morphine and morphine-like opioids comparable and interchangeable in the treatment of chronic cancer pain, but individual responses can vary. A recent clinical trial found that while there were no significant analgesic differences among morphine, oxycodone, transdermal fentanyl, or buprenorphine, the dose escalation was greater with fentanyl and switches, and discontinuations were more frequent with morphine [100]. Interestingly, this study identified groups of patients that were nonresponders to opiate treatment ranging from 11.5% (morphine) to 14.4% (buprenorphine). Thus, subsets of patients that do not respond to opiates are found in the general population, and like nonresponders for acupuncture analgesia (discussed below), these patients should be considered for alternative treatments. Finally, cannabis may be used both to treat chronic cancer pain and importantly to significantly reduce opiate usage. Thus, a 2011 clinical trial that examined the administration of vaporized plant cannabis in chronic cancer pain patients on a daily regimen of morphine or oxycodone reported that inhaled cannabis augments the analgesic effect of opioids [101].

11.4. Complementary/alternative therapies

Controlling cancer pain is an important part of the palliative care of cancer patients. Although conventional medicine has well-established guidelines to systemically control cancer-related pain, over half of cancer patients still suffer from pain as indicated above. Pharmacological therapeutic approaches are not always sufficient and may produce serious side effects. Thus these limitations have led to the use of complementary and alternative medicine approaches. While acupuncture has been around for thousands of years, it is only recently that it has been evolving as a promising approach to relieve chronic cancer pain [102, 103]. A recent study has shown that acupuncture and related therapies are effective in reducing pain and fatigue and in improving quality of life when compared with conventional intervention alone among cancer patients [104]. On the other hand, a subgroup analysis of five randomized controlled trials (RCTs) that evaluated acupuncture's effect on cancer pain did not include cancer-induced bone pain, because none of the studies made any reference to bone pain [105]. At this point in time, there is not convincing evidence that acupuncture significantly reduces cancer pain in the human literature. That being said there are several studies using animal models of cancer bone pain that suggest that acupuncture can reduce bone tumor-induced pain including osteosarcoma-induced pain [1, 106, 107]. The animal data suggest that electroacupuncture can alleviate bone cancer pain, at least in part by suppressing IL-1β expression and by altering nerve innervation and the vasculature of osteosarcoma. However, the acupuncture treat-

ment schedule can effect tumor growth, and therefore the sequence of acupuncture treatment must be determined carefully [66]. Clearly, more clinical research is required to address whether acupuncture can reproducibly reduce pain in human osteosarcoma patients without unwanted tumor-related consequences.

12. Conclusion

Despite advances in our knowledge of osteosarcoma biology, development, metastasis, and its associated pain, the current treatment options have not changed over the last four decades and continue to rely on tumor resection and nonspecific combination chemotherapy, which results in a dismal 5-year survival rate of 0–29% for patients with clinically detectable metastases [38, 108]. Additionally, severe lack of knowledge regarding osteosarcoma metastasis hinders advancement of clinical treatment in pediatric patients. With limited human samples available, animal models hold promise for further understanding of the biology, pathways, and treatment options for osteosarcoma patients. While each model has its specific limitations, the collective insight into the genetics has proven extremely fruitful. With the advent of novel genetic engineering approaches, future studies will be instrumental in better modeling of the disease and uncovering new and valuable information. While conventional chemotherapy and surgical resection remain the mainstays of osteosarcoma treatment, when these approaches are used in combination with the above novel therapies, this will lead to prolonged if not remissive prognoses as well as significantly decreased pain for osteosarcoma patients in the future.

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