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REVIEW ARTICLE

Molecular Signaling Pathways as Potential Therapeutic Targets in Osteosarcoma

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Abstract: Among primary bone malignancies, osteosarcoma (OS) is the most common form causing morbidity and mortality in both adults and children. The interesting point about this malignancy is that nearly 10-20% of its newly diagnosed cases have developed metastasis. This adds up to the fact that the survival rate of both metastatic and non-metastatic patients of osteosarcoma has not changed in the past 30 years; therefore, it has been suggested that we need to revise our therapeutic options for OS. In recent years, diverse signaling pathways have drawn the attention of the scientific community since they can be great candidates for treating complicated diseases, such as cancer. In this review, we have tried to explain the pathophysiology of osteosarcoma with the help of different signaling pathways taking part in its initiation/progression and explore how this pathway can be targeted for providing more efficient methods.

Keywords: Osteosarcoma, signaling pathway, STATs, Wnt, MAPK, Akt, PI3K/ERK, Notch.

1. INTRODUCTION

Most of the primary bone tumors arise from the bone. There are multiple types of malignant bone tumors, including osteosarcoma, Ewing's sarcoma, chondrosarcoma [1]. Primary bone malignancies are relatively rare [2]. Malignant mesenchymal cells causing osteoid and immature bone are characteristics of osteosarcoma, which is the most common form of primary bone malignancy in children and young adults [3]. Moreover, there is a slight increase in the incidence of this disease in people older than 60 years [4]. Mainly, the etiology of malignant bone neoplasms is unknown [5]. Apparently, osteosarcoma is a multifactorial disease in which both genetic and environmental factors are involved [6]. Paget disease, benign cartilaginous dysplasia, and radiation injury are some of the identified precancerous conditions [7]. Hereditary retinoblastoma (germline mutation of the *Rb* gene) and Li-Fraumeni syndrome (germline mutation of the *p53* gene) are associated with a higher risk of developing osteosarcoma [8].

Before 1970, the only option for treating patients with osteosarcoma was surgical excision [9]. Within the next two decades after that, neoadjuvant and adjuvant chemotherapy were found as additional options [9]. In spite of great advances in these two decades, no changes are being made in the treatment of patients [3]. Furthermore, the outcome of patients with a localized or metastatic form of the disease has not been changed significantly during the last 30 years. At present, neoadjuvant chemotherapy is the main treatment for osteosarcoma. This treatment is followed by surgical resection and adjuvant chemotherapy. However, radiation therapy is not much effective [10]. When osteosarcoma is diagnosed, approximately 10-20% of cases have evidence of metastasis. In addition to the lungs, which are the most common location of disease dissemination, bone and lymph nodes are other areas where metastases may occur [11, 12]. Same as primary lesions, chemotherapy is being used as the treatment of metastases [13].

In this review, we have explained the connection between the recent research in the relevant biological aspects of osteosarcoma and the translational medicine focusing on the personalized treatment of bone tumors and recent *in vitro, in vivo*, and clinical trials [14, 15].

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2. MOLECULAR SIGNALING PATHWAYS IMPLICATED IN OSTEOSARCOMA PROGRESSION

2.1. PI3K/AKT Signaling Pathway

PI3K/AKT is one of the essential bases of several cellular processes containing cell growth, proliferation, autophagy, *etc.* [16]. This pathway is composed of a kinase family named phosphatidylinositol-4,5-bisphosphate 3-kinase or PI3K and v-akt murine thymoma viral oncogene homologue or Akt [16]. This signaling is involved in the pathogenesis and/or progression of many cancers, including breast, colorectal, and prostate cancer, as well as osteosarcoma [17]. A great body of evidence has shown how this pathway is induced in OS cells either directly or indirectly. While working on the P2X7 receptor, an ATP-gated ion channel, Zhang *et al.* [18] found that oncogenic effects of this receptor are mainly executed through the activation of PI3K/Akt/GSK3β/β-catenin and mTOR/HIF1α/VEGF signaling. HER4 is another protein that works through this pathway in order to improve the metastatic and proliferative features of OS cells [19]. Regarding the accumulative evidence on PI3K/Akt potentials, a line of study has tried to target this signaling in OS.

Piceatannol (PIC) is one of the used agents which is able to target PI3K/Akt directly; thus, it decreases proliferation while inducing apoptosis in OS cells [20]. Honokiol, which can be derived from *Magnolia officinalis*, also seems to be a direct effector on this pathway by altering the expression of PI3K, Akt, and mTOR in OS cells [21]. In the case of RNAs, long non-coding RNA GAS5 was examined by Liu *et al.* [22] and seems to regulate PI3K/Akt signaling by binding to miR-23a-3p, which targets some genes, including PTEN. Long non-coding TDRG1 is also similarly enhancing the proliferation, invasion, migration, and EMT of OS cells; therefore, it can be a great candidate for OS detection [23].

2.2. MAPK/ERK Signaling Pathway

The generic mitogen-activated protein kinases (MAPK) are a family of proteins that trigger four cascades, including the ERK1/2, JNK1/2/3, p38-MAPK, and ERK5. Among these, MAPK/ERK signaling is one of the most important pathways in cancer development [24]. Regarding osteosarcoma, there is not a lot of studies examining this signaling as a therapeutic target. miRNA-497 is one of the RNAs studied in OS, which seems to regulate both proliferation and apoptosis in these cells *via* changing the cellular levels of MAPK, Erk, and P21 [25]. Moreover, Galectin-1 (GAL1) induces the growth and invasion of OS cells by means of decreasing the expression levels of p38MAPK and p-ERK [26].

2.3. Wnt/β-catenin Signaling Pathway

Wnt signaling pathways (canonical and non-canonical) have major roles in development *via* regulating the function of tissue stem cells [27]. This signal transduction pathway is composed of a diversity of ligands, receptors, co-receptors, and intracellular transducers. Nineteen members of the Wnt family are considered as ligands, which have the ability to bind to Frizzled receptors and LRP5/6 co-receptors [28]. Dishevelled (DVL), Axin, and β-catenin are the intracellular components of this pathway, taking part in altering the expression of some target genes as well as regulating chromatin modifications [29, 30]. The Wnt/beta-catenin signalling pathway has a strong association with cancer development, and progression and osteosarcoma is no exception [31]. However, it seems that Wnt signaling is mostly involved in the metastasis of OS tumors. Examinations on LM7 and TCCC-OS84 cells show that this pathway is activated in metastatic tumor cells in a greater amount compared to the primary ones [32].

Zhu *et al.* [33] looked into the activation of Wnt signaling from another point of view. They suggested that increasing the expression of long non-coding RNA SNHG1O is one of the reasons that the activation of this signaling is maintained in OS cells (*in vitro* and *in vivo*) [33]. SNHG10 functions through sponging a microRNA named miR-182-5p, which directly affects FZD3, thereby restraining the whole signaling pathway [33]; thus, increased levels of SNHG10 determine the high levels of FZD3, proliferation, EMT, and invasion in OS cells in comparison to normal cells [33]. LncMAP6-1:3 is another increased long non-coding RNA in OS cells, which affects the expression of beta--catenin. The knockdown of this RNA decreases beta--catenin and its related cellular processes, including proliferation, migration, and invasion in these cells. Hedgehog-GLI increases the levels of β-catenin in OS cells, which is another mechanism for Wnt involvement [34]. In this regard, Xu and colleagues approved that inhibiting the proliferation of OS cells is possible by using siRNAs for the knockdown of GLI-2, which results in β-catenin reduction [34]. Because of the essential part of Wnt signaling in the progression and metastasis of osteosarcoma, a great body of evidence has tried to target it in order to increase the efficacy of common therapies and patients' survival rate. Tegavivint is an inhibitor of this pathway, which is examined *in vitro*, ex-vivo, and *in vivo* on osteosarcoma cell lines and patient-derived xenografts [32]. In this study, No-

mura and colleagues detected that this novel β-catenin/-transducin β-like protein 1 (TBL1) inhibitor is able to inhibit the proliferation of osteosarcoma cells (*in vitro*) after tegavivint treatment for up to 72 hours [32]. They also observed that tegavivint is even more effective than DOX for inhibiting the tumor growth [32].

Chinese traditional medicine seems to be another method for inhibiting this important pathway in OS cells. Piperine is one of these medications which is examined on U2OS and 143B cell lines and is detected to be beneficial for regulating the amounts of beta--catenin. Using non-coding RNAs is also confirmed to be effective for targeting Wnt signaling; for example, long non-coding RNA gastric carcinoma proliferation enhancing transcript 1 (lncGHET1) is examined by Chen and colleagues, who declared that "the inhibition of lncGHET1 attenuated cell proliferation, migration, invasion and epithelial-to-mesenchymal transition, and promoted apoptosis, partly by regulating the Wnt/β-catenin signaling pathway in OS" [35].

2.4. Notch Signaling Pathway

in vitro studies have indicated that the Notch signaling pathway increases the proliferation, migration, and invasion in osteosarcoma cells. Notch signaling also enhances the stem cell-like characteristics and chemo-resistance. Moreover, xenograft models have shown that suppressing Notch signaling leads to a reduction in tumor size and metastasis inhibition [36]. Mechanistically, activated Notch signaling induces the expression of ephrinB1 and enhances the tumor-promoting ephrin reverse signaling. Overall, these findings provide functional evidence for Notch pathway genes as candidate biomarkers to predict prognosis in patients with osteosarcoma and suggest a mechanistic rationale for the use of Notch inhibitors to treat osteosarcoma [36]. Multiple studies have demonstrated that therapeutic methods against osteosarcoma involve a Notch signaling pathway. For instance, cinobufagin is an antitumor agent that inhibits cell growth in osteosarcoma cells by programmed cell death and S phase cell cycle arrest. Treating osteosarcoma cells by cinobufagin results in reduced expression of Hes-1, Hes-5, Hey-1, and Notch-1. Meanwhile, the activation of Notch signaling reduces the apoptosis induced by cinobufagin [37].

In osteosarcoma biopsy specimens, real-time PCR has shown that the expression of some genes is upregulated, including Hey1, Hey2, Jagged1, and Notch2, whereas the expression of DLL1 and Notch1 are reported to be decreased. *in vitro* analysis has indicated that the inhibition of Notch signaling by CBF1 siRNA and inhibitor of *γ*-secretase slows the osteosarcoma growth [38]. Xenograft models have revealed that the suppression of Notch signaling pathway, whether chemically or genetically, leads to a reduction in osteosarcoma growth *in vivo*. Osteosarcoma cell lines and tumor samples of primary human osteosarcoma have shown higher expression levels of Notch and its target genes. Inhibiting the Notch pathway by γ-secretase inhibitors or lentiviral-induced expression of dominant-negative Mastermind-like protein (DN-MAML) results in reduced proliferation of osteosarcoma cells *in vitro*. The upregulation of notch1 target genes, including Hey1 and Hes1, and its ligand, DLL4, has also been confirmed by transcriptional profiling of p53 mutant mice with osteosarcoma [39]. A cohort study on 12 patients has also shown that the expression of notch genes is increased in tumors compared with surrounding normal tissue. Tumor cells are also shown to express high levels of Notch1 intercellular domain (NICD1) and Hes1, notch target gene. The high tumor expression of these genes is reported to be associated with poor responses to chemotherapy [36].

2.5. VEGF Signaling Pathway

VEGF is an essential player in vasculogenesis and angiogenesis. VEGF exerts its biological effects through the vascular homeostasis of different tissues. Meanwhile, it is also involved in the pathogenesis of tumor growth and metastasis [40]. Research has shown that VEGF is associated with different steps of osteosarcoma pathogenesis, such as poor survival and increased risk of metastases [41]. Anlotinib is a multi-targeted tyrosine kinase inhibitor, which is used in the treatment of soft tissue sarcoma and NSLCC. Wang *et al.* has shown that anlotinib inhibits tumor growth, invasion, and migration in osteosarcoma patients while increasing chemo-sensitivity. It is observed that anlotinib inhibits the phosphorylation of VEGFR2 and MET. Moreover, it suppresses the angiogenesis induced by VEGF [42]. Chemokine (C-C motif) ligand 3 (CCL3) is involved in tumor progression and metastasis. A study has reported that CCL3 increases the expression of VEGF-A in osteosarcoma cells. Subsequently, human endothelial progenitor cell (EPC) migration and tube formation is induced. Furthermore, it is observed that the downregulation of miR-347b through p38, JNK, and ERK signaling pathways is involved in the CCL3-promoted expression of VEGF-A [43].

2.6. STAT3

STAT3 is a member of the STAT family, which consists of seven proteins regulating the growth of cells, survival, and differentiation. These proteins play

various biological roles in embryogenesis, hematopoiesis, and immunity [44]. While STAT3 is mainly found inactive in the cytoplasm, growth factors can immediately activate it. Evidence has indicated STAT3 constitutive activation in a number of cancers, such as lymphomas, osteosarcoma, and multiple myeloma [44]. Therefore, STAT3 is a proper candidate to be used for treating different cancers, including osteosarcoma. Cucurbitacin I is an anti-proliferative agent which exerts antitumor effects by inhibiting the activation of STAT3. Administration of cucurbitacin 1 leads to an anti-proliferative effect against different osteosarcoma cell lines, including HOS, 143B, HUO9, MG63, and SAOS-2. Furthermore, it inactivates STAT3 both *in vitro* and *in vivo* [45]. Zou and colleagues have reported that napabucasin is capable of reducing osteosarcoma cell viability and inducing apoptosis. Indeed, napabucasin is revealed to inhibit the phosphorylation and expression of STAT3. Besides, it suppresses the transcription of STAT3 downstream target proteins [46]. Ryu *et al.* [47] has reported that STAT3 is activated in both cultured cell lines and osteosarcoma tissues. They also indicate that pSTAT3 upregulation is correlated with patients' poor prognosis. In addition, CDDO-Me, which is the inhibitor of STAT3, suppresses the growth of tumor cells and induces apoptosis [47]. Polydatin is a natural product that exerts several antitumor effects. It is reported that polydatin suppresses the proliferation of osteosarcoma MG-63 cells and promotes autophagic flux and apoptosis in these cells. Moreover, both phosphorylation and expression of STAT3 have been decreased by polydatin. Meanwhile, co-treating cells with polydatin and colivelin, an activator of STAT3, results in a rescued cytotoxic effect of polydatin. This indicates that STAT3 signaling is involved in the antitumor effects of polydatin against osteosarcoma [48].

Administration of pimozide, an inhibitor of STAT5, has been shown to suppress the colony formation and proliferation in osteosarcoma while inducing apoptosis and cell cycle arrest at G0/G1. Pimozide leads to a reduction in the expression of cyclin D1 and CDK2 as well as the phosphorylation of Rb. Furthermore, it suppresses the phosphorylation of STAT3, STAT5, and ERK in tumor cells [49]. Another study reported that pimozide decreases the expression level of the antioxidant enzyme catalase (CAT) and subsequently induces the generation of ROS in osteosarcoma. Cai *et al.* suggested that pimozide-induced downregulation of CAT may be mediated by the inhibition of cellular STAT3. Thus, pimozide is a potential antitumor agent that can be used in osteosarcoma by involving STAT signaling pathways [50].

3. THE ROLE OF SIGNALING MOLECULES IN DRUG RESISTANCE IN OSTEOSARCOMA

As we mentioned earlier, some osteosarcoma patients develop resistance against the chemotherapeutic drug. Herein, we take a look into the studies investigating the role of signaling molecules in acquired drug resistance and how their inhibitors may be beneficial to overcome this resistance.

3.1. STATs Signaling Pathway

Osteosarcoma cells usually show increased EGFR signaling. While suppressing EGFR signaling to provide a therapeutic option, it consequently activates STAT3 signaling, which, in turn, may lead to drug resistance. Therefore, it is suggested that combined suppression of STAT3 and EGFR could be effective in overcoming the resistance against treatment. Cantharidin is an antitumor agent that inhibits the STAT3 pathway. A study has shown that the co-administration of cantharidin and erlotinib, an EGFR inhibitor, leads to enhanced tumor suppression due to the inhibition of STAT3 feedback activation [51]. As we mentioned earlier, EGFR is a potential target for treating osteosarcoma, and knockdown of this pathway inhibits invasion, migration, and proliferation of osteosarcoma cells both *in vitro* and *in vivo*. Treating osteosarcoma cells with gemcitabine results in an increase in the levels of phosphor-EGFR [52].

3.2. Notch Signaling Pathway

Studies have suggested that Notch signaling plays a role in the regulation of cancer stem cells and tumor resistance against platinum therapy. Dai and colleagues have reported that treating osteosarcoma cells with DAPT, a γ-secretase inhibitor, increases the antitumor effects of cisplatin in resistant osteosarcoma. Furthermore, they implied that the sensitizing effect of DAPT and cisplatin in cisplatin-resistant cells is achieved by Notch signaling downregulation [53]. In another study by Want *et al.*, it is observed that there is a significant association between cisplatin sensitivity and Notch1 expression in osteosarcoma specimens. *in vitro* analysis has indicated that cisplatin sensitivity is significantly lower in the MG63 cell line that has lower levels of Notch1 compared with the SaOS-2 cell line with higher levels of Notch1 [54].

3.3. Wnt/beta-catenin Signaling

From a drug-resistance point of view, circ_001569 is one of the non-coding RNAs, which has helped the understanding of the linkage between Wnt signaling and drug resistance in OS cells [55]. Increased levels

of circ_001569 in OS cells are significantly related to metastasis and advanced stages of OS along with cisplatin-resistance through the activation of Wnt signaling [55]. Tegavivint, which was mentioned before, is also effective in decreasing the resistance of OS cells to chemotherapy [32]. Nomuro *et al.* [32] implanted patient-derived xenograft TCCC-OS63 in four groups of mice (vehicle, tegavivint alone, doxorubicin alone, and a combination of tegavivint and DOX) and treated them *via* ip-injection twice a week for a total of four weeks and detected that using DOX in combination with tegavivint is more effective than using DOX alone [32].

3.4. PI3K/AKT Signaling Pathway

The role of PI3K/AKT signaling in enhancing drug resistance in osteosarcoma is confirmed by the examination of some microRNAs. microRNA-221 is one of these RNAs regulating cell survival, apoptosis, and cisplatin resistance in OS cells [56]. Bioinformatic analysis on the target genes of this miRNA indicates that PTEN expression can be altered by this RNA [56]. miR-22, miR-100, and miR-497 also mediate this effect of PI3K/AKT signaling in OS cells [57-59]. Targeting the ingredients of this signaling pathway has also been considered a therapeutic approach for a while. Wang *et al.* [60] used PHA-665752 for this purpose and revealed that this c-Met inhibitor is able to enhance the efficacy of cisplatin on OS cells through the inhibition of PI3K/AKT signaling. Icariin is another agent utilized for targeting this signaling cascade and has shown some anti-resistance characteristics against doxorubicin in OS cells [61]. Icariin is a Chinese medicine whose inhibitory effects on PI3K/AKT might be taken into consideration in the coming years [61].

CONCLUSION

For the aim of providing more therapeutic options for treating osteosarcoma, we gathered evidence to investigate the efficacy of targeting different signaling pathways in this cancer. According to recent evidence, there are three options for combating these signaling cascades: using their direct inhibitors, Chinese traditional medicine, and non-coding RNAs. Tegavivint, cinobufagin, and Anlotinib are three of the direct inhibitors, which are confirmed to be effective on OS cells, and their mechanisms of action are explained before in detail. In the case of Chinese medications, Icariin and piperine are examined on OS cells and have presented their anti-OS activities as PI3K/AKT and Wnt/beta--catenin inhibitors, respectively (Figs. **1** and **2**). However, we still have a long way till we can completely rely on these results since the mentioned agents are just recently found to be effective as anti-osteosarcoma agents, therefore, more research (both *in vivo* and clinical trials) is needed. Furthermore, it is necessary to precisely investigate the adverse effects of these agents prior for applying them in clinical settings.

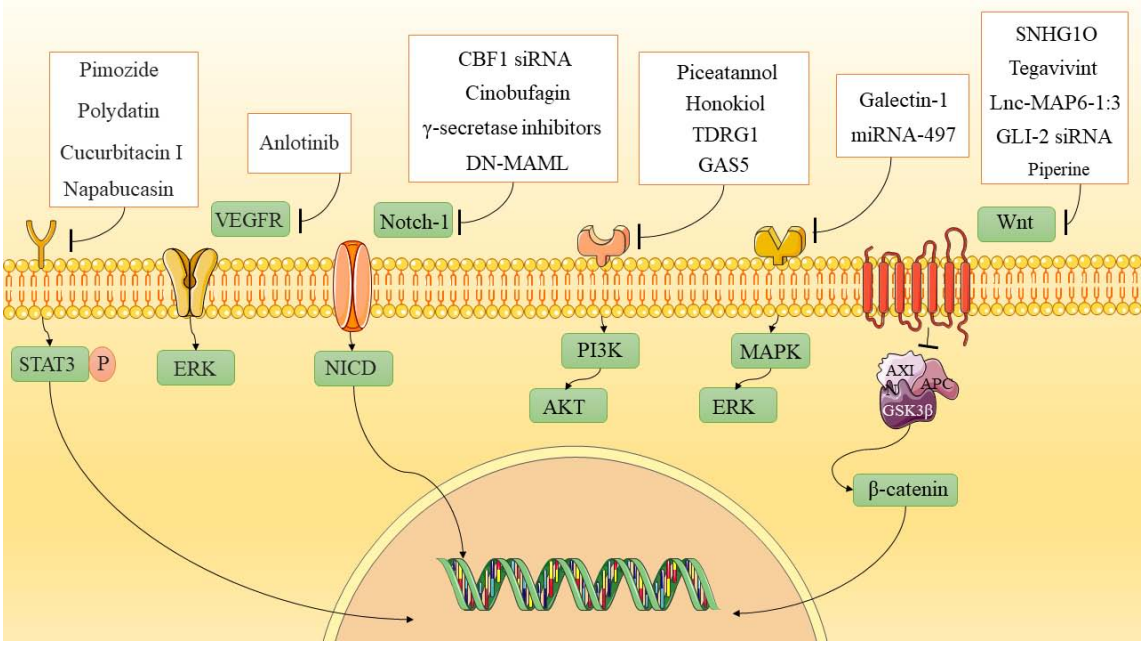

Fig. (1). Schematic representation of molecular signaling pathways in osteosarcoma. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

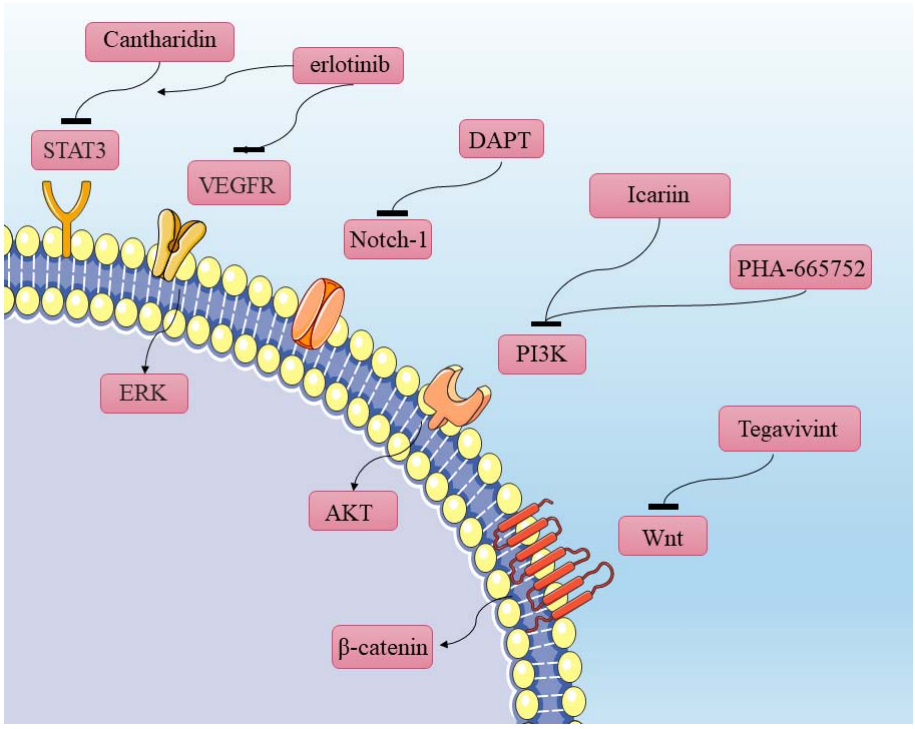

Fig. (2). The role of signaling molecules in drug resistance in osteosarcoma. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

LIST OF ABBREVIATIONS

OS	=	Osteosarcoma
ERK	=	Extracellular Signal-related Kinases
JNK	=	Jun Amino-terminal Kinases

AUTHORS' CONTRIBUTIONS

PM-D, FS, ZA, and BY took part in the creation of the idea, design, and composing of the manuscript.

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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