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Pathogenesis of osteosarcoma and intervention with traditional Chinese medicine

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Abstract

Osteosarcoma (OS) is a malignant bone tumor characterized by rapid progression and a high propensity to metastasis. Elucidating the mechanisms underlying cell proliferation and metastasis is crucial to improving prognosis. Recent advances in OS research span multiple dimensions, such as genetic mutations, epigenetic alterations, and aberrant signaling pathways. Additionally, the roles of the tumor microenvironment and cancer stem cells are increasingly recognized. Furthermore, traditional Chinese medicine (TCM) has gained significant attention due to its ability to regulate OS through multiple targets and pathways. Specifically, TCM formulations combat tumor progression via holistic mechanisms. These include reinforcing healthy Qi, eliminating pathogenic factors, promoting blood circulation, resolving stasis, and clearing heat toxicity. The monomeric components of TCM exert antitumor effects by suppressing tumor growth, inducing apoptosis, modulating the immune microenvironment, and reversing drug resistance. Acupuncture has shown efficacy in alleviating chemotherapy-induced side effects and improving drug sensitivity in tumor cells. This review summarizes the mechanisms of OS development and the progress in TCM-based interventions, emphasizing the need for further integration of modern scientific technologies to elucidate the specific mechanisms of TCM in targeting OS and



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advance its clinical application in OS therapy.

Keywords: OS, pathogenesis, genes, signaling pathways, tumor microenvironment, traditional Chinese medicine, acupuncture, acupoints

INTRODUCTION

Osteosarcoma (OS) is a common primary malignant tumor of the bone tissue, characterized by high malignancy and rapid disease progression, often with lung metastasis^[1]. Its incidence is related to factors such as sex, height, weight, and lifestyle habits^[2,3]. Currently, surgery combined with chemotherapy is the primary treatment for OS. With the development of neoadjuvant chemotherapy, the prognosis of patients with OS has improved significantly. However, the mechanisms underlying OS and related lung metastases have not been fully elucidated, which prevents control of OS prevalence and improvement in patient prognosis^[4]. Traditional Chinese medicine (TCM) has attracted the attention of researchers due to its remarkable ability to inhibit tumor growth^[5], showing great potential for OS treatment. This study reviews recent progress in the multidimensional mechanisms of OS and research on TCM interventions in OS, with the aim of providing constructive insights for the application of TCM in OS diagnosis and treatment.

GENOMIC ALTERATIONS

Inactivation of tumor suppressor genes

TP53

Early prognostic analysis of patients with OS indicated that patients with TP53 mutations have shorter survival times than those without mutations^[6]. Several studies have highlighted a significant role for TP53 mutations in OS^[7-9]. Mirabello *et al.* reported that 74% of adolescent patients with OS had structural variations (SVs) or somatic nucleotide variations in TP53^[10]. Chen *et al.* further confirmed that SVs are common in adolescent OS and are likely to be caused by first intron breakpoint translocation^[11]. Mechanisms by which TP53 mutations contribute to the development of OS are gradually being explored. Lysosomal-associated membrane protein 3 (LAMP3) promotes the survival of OS cells, and TP53 affects OS progression by regulating downstream targets such as LAMP3 and HOXA5: knocking out LAMP3 upregulates TP53 and inhibits OS cell survival^[12], while abnormal expression of HOXA5 promotes OS apoptosis through the p53/p38 α MAPK axis^[13]. Additionally, JAB1 accelerates OS formation through p53 overexpression^[14] and miRNA regulation (e.g., miR-122-5p^[15] inhibits PI3K-Akt-mTOR activation, and miR-181b^[16] forms a negative feedback loop with p53), which further influences proliferation and invasion. Other studies have shown that TP53 mutations can inhibit the osteogenic differentiation of mesenchymal stem cells (MSCs) by silencing transcriptional functions, increasing genomic instability, and promoting the secretion of proinflammatory factors, thus fostering tumor growth^[17].

RB1

RB1 is the second most mutated gene in OS^[18]. The RB1 mutation drives the cell cycle from the G1 to S, leading to genomic instability^[19,20]. In mouse models, the Trp53/Rb1 double knockout upregulates YAP/TAZ expression and promotes glucose metabolic reprogramming, thus accelerating OS progression^[21,22]. Clinical meta-analyses have shown that RB1 mutations are associated with a reduced histological response to chemotherapy and a high risk of metastasis, suggesting that RB1 may serve as a prognostic marker^[23].

CDKN2A

CDKN2A (p16) inhibits tumor cell metastasis by targeting ERK/TGF- β 1. CDKN2A mutations, particularly homozygous deletions at somatic mutation sites, are early events in OS. Escape from CDKN2A/p16 cell

cycle control and deletion of CDKN2A/p14 disrupts p53 and prevents OS apoptosis^[24]. Canine OS models have shown that deletions in the CDKN2A/B locus are associated with susceptibility^[25], and artificial intelligence analysis has identified CDKN2A and PDHA1 as specific biomarkers of OS cell proliferation in OS^[26]. CDKN2A inactivation is closely related to the occurrence and development of OS, and further research on its underlying mechanisms is needed.

Activation of proto-oncogenes

Myc

The overexpression of the *Myc* gene promotes cell proliferation, metabolic reprogramming, and telomerase activity by activating the Myc-associated factor X (MAX) complex^[27]. These transcription factors play a role in driving cell cycle progression in OS^[28,29]. Myc interacts with extracellular signal-regulated kinase (ERK1/2) and diacylglycerol kinase zeta (DGKZ) to promote malignant phenotypes^[30], and the HotTip/c-Myc positive feedback loop accelerates OS progression^[31]. Furthermore, the MYC-miR-193b negative feedback loop leads to sustained MYC activation^[32], and Myc overexpression downregulates CSF1 through miR-17/20a, thus reducing tumor-associated macrophages^[33]. Myc promotes the development of p53-deficient OS by upregulating γ -glutamyl cyclotransferase (GGCT)^[34].

Mesenchymal epithelial transition factor

Mesenchymal epithelial transition factor (MET) overexpression drives OS proliferation, metastasis, and drug resistance via the HGF/c-MET pathway^[35-37]. Neutrophil-derived cytokine lipocalin-2 (LCN2) can inhibit OS cell metastasis by suppressing the mitogen activated extracellular signal regulated kinase-extracellular signalregulated kinase (MEK-ERK) pathway, thus blocking MET^[38]. Crizotinib^[39] and AS1411-SL1 chimeras^[40] have been shown to act as c-MET inhibitors. Collectively, the interplay of tumor suppressor dysregulation (e.g., TP53, RB1, CDKN2A), oncogenic signaling (e.g., Myc, MET), and miRNA-mediated regulatory networks underscores the complexity of OS pathogenesis and chemoresistance. These molecular interactions are shown in [Figure 1](#).

Other gene mutations

Patients with Rothmund-Thomson syndrome with RecQ like helicase (RECQL) mutations are at a higher risk of OS^[41], with mechanisms involving RECQL mutant allele stress^[42]. Miller *et al.* reported that 62% of patients with OS had co-mutations in p53, Rb, and MDM2^[43]. Wang *et al.* confirmed the coexpression of MDM2 and P53 mRNA in OS^[44]. Sodium butyrate (SB) and indole-based MDM2 receptor blockers have been shown to inhibit OS cell proliferation by regulating the MDM2-p53 pathway^[45,46], further confirming the link between MDM2 and P53. Furthermore, RUNX3 was abnormally upregulated in p53-deficient OS, forming a pro-cancer axis^[17], which was corroborated by subsequent studies^[47,48].

EPIGENETIC ALTERATIONS

DNA methylation

Early studies have shown that the incidence of gene methylation in OS is significantly higher than in corresponding normal tissues^[49]. The phosphatase and tensin homolog (PTEN), a key tumor suppressor gene, is closely associated with OS progression through methylation-mediated downregulation^[50,51]. The methylation inhibitor 5-azacytidine (5-Aza-CdR) can reverse PTEN silencing and induce apoptosis in MG-63 cells in a concentration-dependent manner^[52]. Zhang *et al.* reported that hydroxyapatite promoted malignant phenotypes in OS by increasing PTEN methylation levels^[53]. Furthermore, abnormal methylation of SENP3 and FAS may enhance the invasiveness of OS^[54,55].

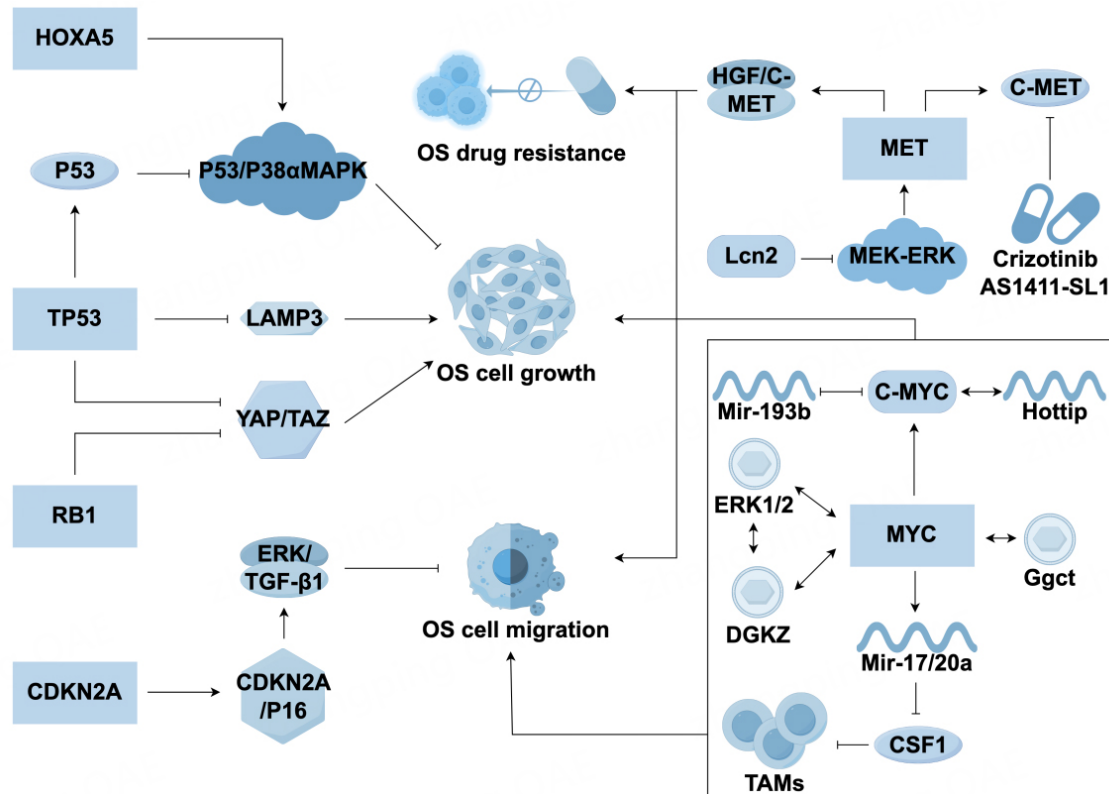


Figure 1. Molecular mechanisms driving OS pathogenesis and drug resistance. OS: Osteosarcoma; TAMs: tumor-associated macrophages;

Histone modifications

Histone modifications can influence the biological behavior of OS by regulating gene activation (H3K4/H3K36/H3K79 acetylation) or silencing (H3K9/H3K27/H4K20 methylation)^[56]. The number of autophagosomes in OS cells is significantly increased, and further studies have shown that PCAF-mediated H3S28 phosphorylation promotes OS progression through ATG5/ATG7-dependent autophagy^[57]. Enrichment of H3K27 acetylation in the COL6A1 promoter region is associated with its high expression, and the histone deacetylase inhibitor SB can inhibit COL6A1-driven invasion and migration^[56]. Histone acetylation or deacetylation can affect the biological behavior of OS and other malignant tumors, and reversing these chemical modifications may benefit patients with OS.

Non-coding RNA

miRNAs regulate the expression of apoptosis-related genes and influence the cell cycle, thus altering proliferation and apoptosis levels. miRNA-296-5p targets Staphylococcal nuclease domain-containing protein 1, exerting tumor-suppressive effects^[58]. Machine learning models suggest that miR-34c-3p and miR-154-3p have the potential to diagnose metastatic OS^[59]. miRNAs, such as hsa-mir-23a-3p^[60], miR-4660^[61], and mRNA-18b-5p^[62], are associated with OS. Liu *et al.*^[63] and Li *et al.*^[64] reported that certain lncRNAs were downregulated in OS tissues while others were upregulated, influencing OS development. lncRNA KIAA0087 inhibits OS through the SOCS1/JAK2/STAT3 pathway^[65], while lncRNAs HCG18 and KCNQ1OT1 enhance aerobic glycolysis to promote OS growth^[66,67]. The lncRNAs RP11-361F15.2^[68] CBR3-AS1^[69], lncRNA-LINC01137^[70], and HOXA-AS3^[71] are associated with OS proliferation and invasion.

ABNORMAL SIGNALING PATHWAYS

Wnt/ β -catenin pathway

Upregulation of the Wnt/ β -catenin pathway is a hallmark of OS^[72,73]. This pathway, often in conjunction with cell cycle and apoptosis regulator 2 (CCAR2), elevates levels of secreted protein acidic and rich in cysteine (SPARC), thereby promoting malignant phenotypes in OS^[74]. Additionally, Mucin 15 (MUC15) drives OS invasion via Wnt/ β -catenin signaling^[75]. Biglycan, a class I small leucine-rich proteoglycan, contributes to chemotherapy resistance by activating this pathway and suppressing autophagy^[76]. Activation of this pathway is related to the presence of tumor stem cell populations and to the phenotypic transformation of OS cells after chemotherapy, suggesting that this mechanism may confer drug resistance to OS cells. Further reinforcing this concept, further studies have shown that CD44 can influence OS cell biological behavior through the Wnt/ β -catenin signaling pathway^[77]. As experimental evidence supporting the pathway's role in therapy resistance and stemness, inhibiting Wnt/ β -catenin in MNNG-HOS cells, while combined with doxorubicin, can prevent the upregulation of factors associated with the transition to OS stem cell states^[78]. Additionally, receptor tyrosine kinase-like orphan receptor 2 (ROR2), a receptor of the atypical Wnt signaling pathway, was shown to be activated by AKT, promoting OS lung metastasis through anoikis resistance^[79].

Notch pathway

The Notch signaling pathway transduces signals only between cells in contact, requiring interaction with other pathways such as PI3K/AKT, NF- κ B, integrins, and miRNAs to regulate OS metastasis^[80]. Notch promotes OS metastasis by phosphorylating ERK (p-Erk)^[81]. Cell migration-inducing protein (CEMIP) and spindle- and kinetochore-associated complex subunit 3 (SKA3) act as downstream effectors driving invasion^[82,83]. Jagged1, a key Notch ligand, significantly inhibited OS proliferation and invasion when knocked out^[84]. Therefore, the Notch signaling pathway promotes tumor proliferation, invasion, and metastasis by activating ERK phosphorylation and upregulating CEMIP/SKA3, depending on the Jagged1 ligand. Interactions between multiple pathways provide new directions for targeted therapies.

PI3K/AKT/mTOR pathway

PI3K/AKT/mTOR dysregulation promotes OS proliferation and regulates apoptosis and cell cycle^[85,86]. The PI3K/Akt/mTOR pathway is involved in resistance to chemotherapy. Notably, lncRNAs are also implicated in stem cell maintenance within this context; specifically, the lncRNA DANCR enhances cancer stem cell (CSC) function by competitively binding miR-33a-5p to upregulate Axl, potentially via the PI3K-Akt pathway^[87].

Studies have shown that rapamycin, an mTOR inhibitor, inhibits OS cell proliferation. However, dual inhibition of PI3K/mTOR was more effective than single-target inhibition in inducing apoptosis in primary mouse and human OS cell cultures^[88]. Meng *et al.* confirmed that cisplatin induces autophagy by inhibiting this pathway^[89]. Qiu *et al.*^[90] and Chen *et al.*^[91] further confirmed this view. Targeting the PI3K/Akt/mTOR pathway may have pro-apoptotic and antiproliferative effects on OS; however, further clinical studies are required to validate these findings. Beyond PI3K/AKT/mTOR, cross-talk with other pathways offers synergistic therapeutic potential. For example, Jing *et al.*^[92] revealed that quercetin can inhibit OS cell proliferation and immune escape by non-covalently binding to the JH2 domain of JAK2, inhibiting the JAK2-STAT3-PD-L1 signaling axis, and improving its water solubility through folate-modified liposome encapsulation, thus providing a new molecular mechanism and drug delivery strategy for quercetin in OS treatment.

Ferroptosis-related pathways

Ferroptosis is a non-apoptotic form of cell death driven by lipid peroxidation^[93]. Several studies have suggested that the promotion of ferroptosis is a promising method to inhibit the development of OS cells. Inhibition of the STAT3/Nrf2/GPX4 signaling pathway can induce ferroptosis, thus improving the sensitivity of OS cells to cisplatin and lipid oxidation^[94]. Chen *et al.*^[95] and Yuan *et al.*^[96] further confirmed that the Nrf2/GPX4 signaling pathway could induce ferroptosis in OS cells. Furthermore, inhibition of the STAT3-MGST2 signaling pathway can achieve similar results^[97], while inhibiting TFEB/FTTH^[98] and activating PI3K/AKT/mTOR^[86] have been observed to inhibit ferroptosis in OS cells.

TUMOR MICROENVIRONMENT

Immune cell regulatory network

Tumor-associated macrophages (TAMs) are primarily polarized into two types: classically activated M1 macrophages and alternatively activated M2 macrophages. M2-type TAMs have weaker antigen-presenting capabilities, promote angiogenesis, and help tumor cells achieve immune escape, which is highly correlated with OS invasiveness^[99,100]. LncRNA RP11-361F15.2 can reduce the polarization of M2-type TAM by inhibiting cytoplasmic polyadenylation element binding protein 4^[69]. After treatment with curcumin and cisplatin, M2-type TAMs are significantly reduced, confirming an association between TAMs and chemotherapy resistance^[101]. In addition, physical stimuli such as ultrasound play an important role in immune activation, promoting the conversion of macrophages to the immune-stimulating M1 phenotype^[102]. Moreover, TAM-secreted exosomes (e.g., C15orf41)^[103] and interleukin-8 (IL-8)^[104] have been found to promote OS metastasis. The PD-1/PD-L1 checkpoint is involved in the immune microenvironment of OS and influences tumor-related events^[105]. Studies have shown that exosomal PD-L1 levels are significantly higher in OS patients than in healthy individuals and that extracellular PD-L1 levels are significantly higher in patients with OS with lung metastasis than in those without metastasis^[106]. PD-L1 immune checkpoint inhibitors are beneficial for the treatment of patients with OS metastases^[107], further confirming the importance of PD-L1 in OS. The detection of extracellular PD-L1 and N-cadherin in serum from patients with OS has been reported to predict the progression of lung metastasis^[106]. The specific mechanisms of PD-L1 are not fully understood but may involve MSTO2P-mediated upregulation of PD-L1^[108], activation of signal transducer and activator of transcription 3 (STAT3) via low-density lipoprotein receptor-related protein 8, leading to PD-L1 expression^[109], and MerTK-mediated phagocytosis enhancing PD-L1-induced immune tolerance^[110].

Angiogenesis

Vascular endothelial growth factor (VEGF) is an important prognostic factor in OS^[111]. Ectopic expression of the RNA-binding protein AUF1 enhances proangiogenic effects in a VEGF-A-dependent manner^[112], while overexpression of microRNA-638 can inhibit VEGF expression^[113]. circ_001621 regulates VEGF expression by sponging miRNA-578^[114]. Another study reported that α -ketoglutarate, a multifunctional intermediate in the Krebs cycle, can enhance the anti-OS effects *in vitro* by inducing apoptosis through JNK and caspase 9-dependent mechanisms and inhibiting VEGF and TGF- β production and metastatic potential^[115].

CSCS

Surface markers

CSC is a promising target for the treatment of OS^[116], and CD133 is a core marker of OS stem cells^[117]. CD133+ cells downregulate P-gp-mediated resistance to CDDP through the Akt/NF- κ B pathway^[118]. Stress-induced phosphoprotein 1 knockdown reduces phosphorylated Akt and ERK1/2 levels, thus inhibiting the invasion of CD133-positive cancer stem cells in the MG63 cell line^[119]. CD133-positive expression is significantly correlated with the invasion stage of patients with OS, local recurrence,

metastasis, and 5-year overall survival rate^[120], confirming the important role of CD133 in OS development. Furthermore, CD133 and CD44 play an important role in OS lung metastasis^[121]. The upregulation of CD44 expression in OS-derived cell-transplanted mice accelerates lung metastasis^[122]. Insulin-like growth factor 1 receptor (IGF1R) expression is highly positively correlated with CD44 expression in patients with OS under 10 years of age^[123], suggesting that CD44 could be a prognostic indicator in combination with IGF1R. Furthermore, CD44 upregulates multidrug resistance protein 1 and improves OS cell resistance to doxorubicin^[124]. Recent research has indicated that DNA polymerase epsilon 2 (POLE2) may enhance CD44 expression by inhibiting MDM2-mediated ubiquitination, thus activating the Rac signaling pathway and affecting OS progression^[125].

Stem cell maintenance mechanisms

Activated MSCs maintain their stemness and stimulate cell migration by secreting IL-6^[126]. B4GALT1-AS1 recruits HuR to enhance YAP and maintain OS stem cell characteristics^[127]. The lncRNA WAC-AS1 can achieve similar effects by sponging miR-5047 to upregulate SOX2^[128]. Another study reported that overexpression of small ubiquitin-like modifier-specific peptidase 1 (SEN1) in OS tissues and cell lines significantly reduced stem maintenance capacity and increased sensitivity to HSVtk/GCV^[129]. Musculoaponeurotic fibrosarcoma oncogene homolog B can upregulate the stem cell regulator Sox9 at the transcriptional level, playing a key role in OS stem cell tumorsphere formation and tumorigenic capacity^[130]. Dual-specificity phosphatase 3 inhibits stem cell differentiation by regulating the EGFR/STAT3/SOX2 axis^[131], which could serve as an effective therapeutic target for OS. Table 1 summarizes the pathogenesis of osteosarcoma.

RESEARCH PROGRESS ON TCM INTERVENTION IN OS

TCM formulations

Patients with OS often experience adverse reactions, such as bone marrow suppression, cancer-related fatigue, and gastrointestinal discomfort during treatment^[132], which severely affect prognosis. TCM formulations based on the principle of holistic regulation have shown increasing influence and unique clinical value for various treatments. A meta-analysis and network pharmacology study suggested that Buzhong Yiqi Tang may alleviate CRF by regulating targets such as AKT1, IL-6, IL-1, PTGS2, CASP3, ESR1, and BCL2, as well as signaling pathways such as TNF, IL-17, TLR, and NF- κ B^[133]. Liu Jun Zi Tang relieved post-chemotherapy nausea and vomiting^[134]. Duan *et al.*^[135] analyzed the main chemical components of Taohong Siwu Tang (THSWD) using UPLC-Q-TOF-MS and confirmed that THSWD exerts antitumor effects by regulating the inflammatory microenvironment and tumor stem cell markers. Huang *et al.*^[136] used network pharmacology to analyze whether Huanglian Jiedu Tang exerts anticancer effects by regulating inflammation-related pathways such as MAPK and TLR. The formulations of TCM are complex, making it difficult to identify the specific active components and elucidate their mechanisms. Current research often adopts an integrated approach involving network pharmacology and experimental validation to reveal the synergistic mechanisms of multiple components and targets in TCM. Future research should explore the synergistic effects of TCM with chemotherapy or targeted therapy and validate their safety and applicability through clinical translation, thus providing new strategies for comprehensive OS treatment.

TCM monomers

Research on the use of TCM monomers in the treatment of OS has gradually gained attention. Quercetin, an important member of the flavonoid family, exhibits antioxidant, anti-inflammatory, and antitumor effects^[137,138]. However, its low solubility and poor bioavailability limit its applications^[139]. Recent mechanistic studies reveal that Saikosaponin A enhances PD-1 inhibitors by downregulating PD-L1 via FASN inhibition. Its delivery in FA-modified thermosensitive nanohydrogel boosts tumor uptake by 40% while preserving > 90% normal cell viability^[140]. Quercetin and saikosaponin A exhibit great clinical translational

Table 1. Comprehensive mechanisms of osteosarcoma pathogenesis

Category	Key elements	Mechanism	Functional impact	References
Genomic alterations	TP53	Somatic mutations/SVs disrupt TP53 function; regulate LAMP3, HOXA5, and miRNA feedback loops	Promotes OS cell survival, inhibits apoptosis, increases genomic instability	[6-17]
	RB1	Loss of function drives cell cycle via YAP/TAZ activation and glucose metabolism reprogramming	Enhances metastasis, reduces chemotherapy response, poor prognosis	[18-23]
	CDKN2A	Homozygous deletions disrupt p53 and cell cycle control	Early event in OS; linked to chemotherapy resistance and tumorigenesis	[24-26]
	Myc	Overexpression activates MAX complex, ERK/DGKZ, and miRNA networks (miR-17/20a)	Promotes OS progression; targeted by crizotinib and AS1411-SL1	[27-34]
	MET	HGF/c-MET pathway activation drives proliferation, metastasis, and drug resistance	Increases OS susceptibility via mutant allele stress	[35-40]
Epigenetic alterations	PTEN methylation	Hypermethylation silences PTEN expression	Promotes OS progression; reversed by 5-Aza-CdR	[50-53]
	SEN3/FAS methylation	Abnormal methylation enhances OS invasiveness	Impairs DNA repair and apoptosis	[54,55]
	H3K27 acetylation	Enriched in the COL6A1 promoter; HDAC inhibitors suppress invasion	Enhances COL6A1 expression and metastasis	[56]
	Non-coding RNAs	- miRNA-296-5p targets SND1, miR-34c-3p/miR-154-3p predict metastasis - lncRNAs (e.g., RP11-361F15.2, HCG18) regulate immune escape and glycolysis	Modulates apoptosis, immune microenvironment, and metabolic reprogramming	[58-71]
Signaling pathway dysregulation	Wnt/ β -catenin	Upregulation of SPARC and MUC15 via β -catenin activation	Promotes stemness, invasion, and chemotherapy resistance	[72-76]
	Notch	Activates ERK phosphorylation and CEMIP/SKA3 to drive metastasis	Enhances invasion; Jagged1 knockout suppresses tumor growth	[80-84]
	PI3K/AKT/mTOR	Dysregulation promotes proliferation, inhibits autophagy, and induces cisplatin resistance	Dual PI3K/mTOR inhibition enhances apoptosis	[85-91]
	Ferroptosis	STAT3/Nrf2/GPX4 axis inhibition induces lipid peroxidation	Enhances cisplatin sensitivity; curcumin and baicalin promote ferroptosis	[93-98]
Tumor microenvironment	Immune cell network	M2-TAMs secrete IL-8 and exosomal C15orf41; PD-L1 exosomes promote immune escape	Facilitates metastasis and chemotherapy resistance	[100-110]
	Angiogenesis	VEGF-A/AUF1/miR-638/circ_001621 axis drives vascularization	Correlates with poor prognosis; AKG inhibits VEGF/TGF- β	[111-115]
	Cancer stem cells (CSCs)	CD133+/CD44+ cells activate PI3K/AKT, Wnt/ β -catenin, and Rac pathways	Mediate lung metastasis, drug resistance, and stemness maintenance	[116-125]
	Stemness maintenance	lncRNAs (DANCR, B4GALT1-AS1) and IL-6/MSCs sustain CSC properties	Promotes tumor recurrence and therapy resistance	[126-131]

OS: Osteosarcoma; SVs: structural variations; LAMP3: lysosomal-associated membrane protein 3; HOXA5: homeobox A5; YAP/TAZ: yes-associated protein/transcriptional coactivator with PDZ-binding motif; 5-Aza-CdR: 5-Aza-2'-deoxycytidine (a DNA methyltransferase inhibitor); HDAC inhibitors: histone deacetylase inhibitors; lncRNAs: long non-coding RNAs; M2-TAMs: M2 tumor-associated macrophages; PD-L1: programmed death ligand-1; VEGF: vascular endothelial growth factor; CSCs: cancer stem cells; PTEN: phosphatase and tensin homolog.

potential for synergistically enhancing the treatment of osteosarcoma. They achieve this by targeting the PD-L1-associated signaling pathway and surmounting their own limitations with the aid of an innovative delivery system.

Curcumin (CUR), a phenolic antioxidant, exerts anticancer effects by regulating signaling pathways^[141]. CUR promotes the nuclear translocation of the transcription factor Nrf2, thereby activating its downstream antioxidant genes (e.g., HMOX1 and NQO1), while simultaneously upregulating the pro-oxidant gene

KLF9. This dual action induces excessive production of reactive oxygen species (ROS), causing oxidative DNA damage and promoting apoptosis in OS cells. The low toxicity of CUR in normal cells^[142] provides a theoretical basis for ROS-targeted combination therapy. CUR and its analogs, demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC), can induce apoptosis in HOS cells. CUR and DMC triggered caspase-dependent apoptosis by activating the Smad2/3 pathway, while BDMC inhibited the Akt pathway. The combination of these three compounds synergistically enhances anticancer effects^[143]. Additionally, CUR induced ferroptosis in OS cells by regulating the Nrf2/GPX4 signaling pathway^[96]. Artemisinin and its derivatives can inhibit tumor cell proliferation, metastasis, angiogenesis, cell cycle arrest, and cell death, regulate the tumor microenvironment, and inhibit glycolysis^[144]. For example, artemisinin inhibits OS angiogenesis by regulating the p38 MAPK/CREB/TSP-1 signaling pathway^[145].

The extract of *H. diffusa*, 2-hydroxy-3-methylantraquinone (HMA), inhibits OS cell activity and induces apoptosis^[146]. Studies have shown that HMA downregulates MYC expression through the PI3K/AKT signaling pathway, thus inhibiting CHK1 and RAD51 activity and ultimately hindering homologous recombination repair. Astragaloside IV, a natural component extracted from astragalus, has antiproliferative and chemotherapy-sensitizing effects in various cancers. Hu *et al.* experimentally verified that astragaloside IV enhanced cisplatin-induced apoptosis and increased sensitivity to chemotherapy by activating the Fas/FasL signaling pathway and improving the caspase cascade reaction^[147]. Baicalin, an extract of *Scutellaria*, promotes ferroptosis by inducing ubiquitin degradation and affecting the stability of the key ferroptosis regulator Nrf2, indirectly inhibiting the expression of Nrf2 downstream targets, GPX4 and xCT, thus achieving anti-OS effects^[148].

Ginseng is known for its many pharmacological effects, including antitumor effects^[149]. Ginsenoside Rg5, an extract of ginseng, was observed through network pharmacology and molecular docking to potentially regulate PI3K/Akt signaling and proteoglycan-related and VEGFA-related pathways, achieving similar effects^[150]. Celastrol caused G2/M phase arrest in OS cells by regulating cell cycle-related proteins. It induces apoptosis through extrinsic and intrinsic apoptotic pathways, mitochondria-mediated apoptosis, and the ROS/JNK signaling pathway, achieving synergistic inhibition of OS cell growth with low toxicity to normal fibroblasts^[151]. Additionally, *in vivo* and *in vitro* pharmacological studies have demonstrated the antitumor activity of Bee venom. The bee toxins in Bee venom have shown potential for the treatment of a wide range of tumors, including OS, while the specific mechanisms remain to be further explored^[152]. The mechanisms involved in the treatment of OS with TCM monomers are summarized in Table 2.

TCM combined with chemotherapy drugs

Enhancing efficacy and reversing drug resistance: Cisplatin resistance poses a significant challenge to OS treatment. To overcome this and enhance chemotherapy effectiveness, diverse strategies leveraging TCM principles and components are being explored. For instance, Groenlandicine, extracted from *Coptis*, increases sensitivity to cisplatin in cisplatin-resistant OS cells through the BAX/Bcl-2/caspase-9/caspase-3 pathway and inhibits tumor growth by downregulating topoisomerase I (TOP1) levels^[153]. Similarly, Polydatin, a precursor of resveratrol, enhances the antitumor effect of paclitaxel by regulating the Akt signaling pathway^[154]. Icariin can reverse doxorubicin resistance in human OS cells by inhibiting the Wnt/ β -Catenin signaling pathway^[155], while Notoginsenoside R1 (NGR1), derived from *Panax notoginseng*, inhibits MSC-induced tumorigenesis and partially reverses resistance to doxorubicin by inhibiting IL-6 secretion and the JAK2/STAT3 pathway^[156].

Innovative delivery and synergistic mechanisms further expand the scope of TCM-chemotherapy combinations. Moving beyond traditional formulations, a copper and *Tremella fuciformis* polysaccharide-

Table 2. Bioactive compounds from natural products demonstrate anti-osteosarcoma potential through elucidated mechanisms

Compound name	Mechanism of action	Key technological breakthrough	References
Quercetin	Inhibits the JAK2-STAT3-PD-L1 axis, blocking tumor immune escape; folate liposomes enhance bioavailability	Development of nanodelivery systems	[92]
CUR	Activation of the Nrf2 and Smad2/3 apoptosis pathways; induction of ferroptosis	Synergistic enhancement mechanisms of analogs	[96,141,142]
Artemisinin	Inhibition of angiogenesis mediated by the p38 MAPK/CREB pathway	Elucidation of multiple anticancer mechanisms	[145]
HMA	Inhibition of CHK1 and RAD51 activity; disruption of homologous recombination repair	Targeting DNA repair pathways	[146]
Astragaloside IV	Activation of the Fas/FasL pathway; enhancement of cisplatin sensitivity	Validation of chemosensitization mechanisms	[147]
Resveratrol	Induction of ubiquitin degradation and ferroptosis	Research on ferroptosis mechanisms	[154]
G-Rg5	PI3K/Akt signaling pathway; proteoglycan-related pathways; VEGFA-related pathways	Network pharmacology prediction combined with experimental validation	[150]
Celastrol	ROS/JNK-mediated apoptosis and autophagy synergy	Research on selective toxicity mechanisms	[151]

CUR: Curcumin; HMA: 2-hydroxy-3-methylantraquinone; G-Rg5: ginsenoside Rg5.

based tumor microenvironment-responsive injectable hydrogel (Cu-TFP-gel) demonstrates promise. Cu-TFP-gel responsively releases copper ions into the tumor microenvironment, inducing copper death in cancer cells and significantly improving the efficacy of chemotherapy drugs through synergy^[157]. Building upon the concept of targeting copper homeostasis and leveraging nanocarriers, celastrol nanoparticles present a sophisticated strategy. These nanoparticles coordinate with copper to potently deplete glutathione and trigger DLAT aggregation, effectively evoking immunogenic cell death. Strikingly, when this dual-action system is combined with anti-PD-L1 therapy, it eradicates 78.1% of lung metastases by enhancing CD8⁺ T cell infiltration and repolarizing macrophages^[158]. Concurrently, advancements in smart drug delivery platforms contribute significantly to precision strategies. For example, cytophilic TPE-PU microcapsules utilize acid-triggered CO₂ generation within tumor lysosomes for instantaneous release of encapsulated chemotherapeutic agents like doxorubicin. Synchronous dual-channel fluorescence imaging enables real-time drug tracking, and complete drug release within 24 h achieves cytotoxicity equivalent to the free drug, overcoming critical limitations of conventional carriers^[159]. This integration of targeted TCM bioactivity, innovative material science, and precision delivery represents a powerful paradigm for improving OS chemotherapy outcomes.

Reduction of side effects: chemotherapy can cause various adverse reactions. Danhong injection contains active components such as danshensu and salvianolic acid A/B, which target apoptosis-related proteins like CASP3 and BCL2, inhibit the mitochondrial apoptosis pathway (Bax/Bcl-2 imbalance and caspase-3 activation), and reverse oxidative stress (reducing MDA and increasing SOD/GSH-Px), effectively reducing doxorubicin-induced cardiotoxicity^[160]. Angelica polysaccharide, a major active component of Angelica, can promote the proliferation and immunosuppressive function of myeloid-derived suppressor cells through STAT1 and STAT3 signaling pathways^[161].

Optimizing OS-targeted therapy with TCM

Therefore, it is important to explore new therapeutic targets and efficient targeting strategies. Ginsenoside Rh2-loaded tumor cell membrane-camouflaged nanoparticles (Rh2@HMnO₂-AM) modified with alendronate and K7M2 cell membranes efficiently targeted bone tumors. By responsively releasing Rh2 into the tumor microenvironment, it enhances the effects of immunotherapy^[162] and can be used for MRI-guided immunochemodynamic therapy for orthotopic OS. Anti-angiogenesis is an effective antitumor strategy but is limited by drug resistance. Dihydroartemisinin (DHA)^[163], a derivative of artemisinin, inhibits VEGFA

secretion by downregulating LOXL2 expression and synergizing with anti-angiogenic drugs. Additionally, DHA can reverse anti-angiogenic drug resistance by interfering with lipid metabolic pathways, particularly fatty acid oxidation. Honokiol (HNK)^[164], a small-molecule polyphenol, has limited applications due to its poor water solubility and lack of targeting. HA-DOPE@Lips/HNK efficiently targeted drug delivery to tumor cells by specifically binding to CD44 receptors and reducing toxicity to normal tissues. The application of nanomaterials in OS chemotherapy shows great potential^[165], providing new strategies for TCM for OS treatment and demonstrating the potential of TCM-modified nanocarriers for targeted drug delivery. To overcome the challenging bone microenvironment in OS therapy, alendronate-based cationic platinum prodrug nanoparticles (Ale NP) employ a cascade-responsive strategy. This system leverages bone targeting and charge reversal for deep tissue penetration, achieves dual drug delivery through glutathione-triggered Pt (II) release, and activates the cGAS-STING pathway to reverse immunosuppression while eliminating core tumors. Such advanced targeting mechanisms exemplify innovative approaches for precision OS therapy^[166]. Concurrently, AI technologies accelerate drug discovery and optimize clinical trial efficiency, as demonstrated in lung cancer research^[167].

TCM in OS immunotherapy

Tumor occurrence is closely related to individual immune functions. As immunotherapy has gained increasing attention, various components of TCM have been shown to inhibit tumors by regulating the immune system^[168]. NGR1 inhibits OS cell proliferation and doxorubicin resistance by inhibiting IL-6 secretion from MSCs and blocking the activation of the JAK2/STAT3 signaling pathway^[156]. Revealing the potential role of TCM in the regulation of the tumor microenvironment provides a theoretical basis for the development of TCM-based OS treatment strategies.

Application of acupuncture and acupoint therapy in tumors

Acupuncture is widely accepted among patients with cancer and can alleviate cancer pain^[169,170], nausea, and vomiting^[171] by stimulating specific acupoints. Acupuncture suppresses systemic inflammation by activating the vagal-adrenal reflex pathway to trigger catecholamine release, while simultaneously inhibiting NF- κ B signaling in macrophages via the cholinergic anti-inflammatory pathway through $\alpha 7$ nicotinic acetylcholine receptors, thereby reducing proinflammatory cytokine levels. Furthermore, activation of the spinal-sympathetic nerve pathway modulates splenic immune cell function through $\beta 2$ -adrenergic receptors, collectively regulating both innate and adaptive immunity, specifically by suppressing proinflammatory macrophage polarization, enhancing natural killer cell cytotoxicity and interferon-gamma secretion, and correcting T-helper cell subset imbalances. This integrated multilevel neuro-immune network ultimately restores immune homeostasis^[172]. Acupuncture (selecting acupoints, such as Shenshu, Baihui, and Zusanli) can significantly delay osteosarcoma progression through three synergistic mechanisms:

Firstly, acupuncture modulates immune homeostasis by suppressing natural killer cell hyperactivation and attenuating cytotoxic T lymphocyte proliferation. This immunoregulation mitigates systemic stress responses. Secondly, it inhibits critical cytokine storms by significantly blunting pathological elevations in serum TNF- α and IL-6 while normalizing splenic IFN- γ levels toward physiological baselines. Finally, acupuncture restructures gut microbiota composition by reversing the pathological Firmicutes/Bacteroidetes ratio elevation, suppressing proinflammatory genus expansion, and modulating systemic immunity via the gut-immune axis. This multi-targeted regulation constitutes an integrated framework for delaying tumorigenesis through systemic network modulation^[173]. Acupuncture can lower serum levels of proinflammatory factors (IL-6, TNF- α , and IFN- γ), partially reverse abnormal CD8⁺ elevation and CD4⁺ reduction, and exert systemic antitumor effects by improving gut microbiota imbalance through the “immune-microbiota axis”. Studies have shown that acupuncture can enhance the body’s immune response

by regulating the activity of natural killer cells (NK cells), macrophages, mast cells, and microglia and can modulate T and B cell immune responses, enhancing antitumor immunity^[174]. Acupuncture is widely accepted among patients with cancer worldwide due to its significant and rapid efficacy. Although physiological and pathological regulatory mechanisms are not fully understood, ongoing research will help to resolve these issues.

Mechanism research on TCM treatment of OS

The PI3K/AKT pathway plays a key role in OS^[175]. Important monomers, such as HMA^[143] and ginsenosides^[149], can inhibit tumors by PI3K/AKT. BDMC, an extract of turmeric rhizomes^[176], can significantly downregulate PI3K, p-Akt, NF- κ B, and GSK3 β expression, inhibiting OS cell migration. Cardamonin^[177] inhibits OS through PI3K/AKT and ERK, but its effect is weaker than that of the P38-JNK pathway.

Casticin^[178] can upregulate the expression of heme oxygenase 1 (HMOX1), microtubule-associated protein 1 light chain 3 (LC3), and nuclear receptor coactivator 4, thus activating the MAPK signaling pathway. MAPK amplifies ferroptosis by regulating oxidative stress and cell death signals, thus inhibiting tumor growth. Cryptotanshinone^[179] and matrine^[180] inhibit MAPK pathway activity and alter the expression of cell proliferation-related proteins, thus inhibiting the proliferation of OS cells. Berberine (BBR)^[181] activates the p38 MAPK and JNK signaling pathways, inducing apoptosis in MG-63 and U2OS cells. BBR downregulates MMP-2 and MMP-9, inhibits cell migration and invasion, downregulates Bcl-2, upregulates Bax, and activates caspase-3 to promote apoptosis. Furthermore, BBR^[182] combined with cisplatin improved the inhibitory effect in MG-63 cells by inhibiting the MAPK signaling pathway. In addition, BDMC and other TCM-active substances can inhibit MAPK signaling^[176], affecting cell proliferation and invasion.

Tanshinone I, an extract from *Salvia miltiorrhiza*, inhibits IL-6-induced JAK1, 2, and STAT3 phosphorylation, blocks JAK/STAT3 signaling pathway activation, inhibits STAT3 binding to target genes (e.g., Bcl-2), reduces anti-apoptotic protein Bcl-2 expression, lowers matrix metalloproteinase MMP2 and MMP9 levels, and indirectly downregulates JAK/STAT3 to inhibit OS^[183]. Andrographolide can inhibit the PI3K/AKT pathway, upregulate pro-apoptotic proteins (Bax and caspase-3), downregulate anti-apoptotic proteins (Bcl-2), and arrest the cell cycle (G0/G1 phase) to induce tumor cell apoptosis^[184]. β -caryophyllene induces mitochondrial apoptosis by reducing the mitochondrial membrane potential and upregulates JAK1 and STAT3 protein expression^[185]. Furthermore, curcumin^[186], quercetin^[92], and cryptotanshinone^[179] can inhibit OS cell proliferation and invasion by inhibiting the JAK2/STAT3 signaling pathway. These findings provide strong evidence for the potential use of TCM monomers as OS therapeutic drugs and offer a theoretical basis for future clinical applications.

SUMMARY AND OUTLOOK

The pathogenesis of OS involves multiple dimensions, including genomics, epigenetics, aberrant signaling pathways, and the tumor microenvironment. Elucidating these mechanisms will lay the groundwork for developing novel therapeutic targets. TCM has demonstrated advantages in multi-target and multi-pathway intervention for OS treatment, particularly showing significant promise in combination with modern medical therapies. However, the clinical translation of current TCM research faces substantial challenges. Foremost is the lack of standardization, encompassing variations in the specific composition and dosage of different herbal formulations, as well as individualized differences in acupuncture point selection and manipulation techniques. These factors significantly compromise the reproducibility and reliability of TCM therapeutic strategies. Additionally, existing studies predominantly rely on *in vitro* models, which possess inherent limitations in replicating the complex human microenvironment. Furthermore, inconsistent

nomenclature for TCM components or therapies across different studies impedes the comparison and integration of findings. Most critically, despite numerous preclinical studies revealing the mechanisms of TCM action, its clinical efficacy urgently requires validation through rigorously designed, large-scale randomized controlled trials.

Looking ahead, advancing the application of TCM in OS therapy necessitates addressing several key issues: First, establishing standardized protocols for TCM formulations and acupuncture practices is imperative to enhance research reproducibility and result comparability. Second, the low bioavailability of active constituents must be resolved, necessitating optimization through novel delivery systems such as nanocarriers. Third, accelerating clinical translation requires designing robust clinical trials, especially investigating synergistic effects when TCM is combined with chemotherapy, targeted therapy, or immunotherapy. Fourth, deepening mechanistic exploration involves actively integrating multi-omics technologies (genomics, proteomics, metabolomics) and incorporating artificial intelligence analysis to systematically dissect the multidimensional network mechanisms by which TCM modulates OS. The ultimate objective is to deeply integrate modern technology into TCM research - not only to clarify its specific targets and pathways in OS treatment but, crucially, to propel its clinical practice through high-quality evidence, thereby establishing evidence-based clinical guidelines to provide more effective, comprehensive treatment strategies for osteosarcoma patients.

DECLARATIONS

Authors' contributions

Conceptualization, investigation, visualization, writing - original draft, writing - review and editing: Liang M, Ni X

Investigation, data curation, writing - original draft: Dong Z

Investigation, validation, writing - original draft: Xue Q

Investigation, Formal Analysis, Writing - Original Draft: Li Z

Supervision, writing - review and editing, project administration: Xia P

Conceptualization, supervision, writing - review and editing, project administration, funding acquisition: Pu F

Availability of data and materials

Not applicable.

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Conflict of interest

Pu F is a Junior Editorial Board member of *Journal of Cancer Metastasis and Treatment*. Pu F was not involved in any steps of editorial processing, notably including reviewers' selection, manuscript handling and decision making. The other authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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