REVIEW

Ubiquitination in osteosarcoma: unveiling the impact on cell biology and therapeutic strategies

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ABSTRACT   Ubiquitination, a multifaceted post-translational modification, regulates protein function, degradation, and gene expression. The pivotal role of ubiquitination in the pathogenesis and progression of cancer, including colorectal, breast, and liver cancer, is well-established. Osteosarcoma, an aggressive bone tumor predominantly affecting adolescents, also exhibits dysregulation of the ubiquitination system, encompassing both ubiquitination and deubiquitination processes. This dysregulation is now recognized as a key driver of osteosarcoma development, progression, and chemoresistance. This review highlights recent progress in elucidating how ubiquitination modulates tumor behavior across signaling pathways. We then focus on the mechanisms by which ubiquitination influences osteosarcoma cell function. Finally, we discuss the potential for targeting the ubiquitin-proteasome system in osteosarcoma therapy. By unraveling the impact of ubiquitination on osteosarcoma cell physiology, we aim to facilitate the development of novel strategies for prognosis, staging, treatment, and overcoming chemoresistance.

KEYWORDS   Ubiquitination; osteosarcoma; cancer development; therapeutic target

Introduction

Osteosarcoma (OS), a highly aggressive bone tumor, is characterized by the presence of malignant mesenchymal cells that form osteoid or immature bone tissue and exhibits a bimodal incidence that primarily affects children and adolescents in addition to individuals > 60 years of age[1]. Approximately 25% of patients have metastatic spread, most commonly to the lungs[2]. Despite advancements in treatment strategies, including extensive surgical resection and neoadjuvant/adjuvant chemotherapy, improving patient survival remains a significant challenge[3,4]. While targeted therapy offers promise due to selectivity and reduced toxicity, efficacy is hampered by rapid development of drug resistance in patients undergoing prolonged chemotherapy with relapse salvage rates hovering at approximately 30%[5]. Furthermore, the prognosis for patients with metastatic or recurrent osteosarcoma has had little improvement in recent decades[6]. This stagnation, coupled with limitations in early detection methods, presents a major obstacle to improving survival rates[7].

Ubiquitination, a ubiquitous post-translational modification, has a central role in orchestrating cellular processes[8]. Recent research has unveiled an intricate interplay between ubiquitination and deubiquitination in cancer development and progression[9-12]. This interplay holds promising clinical applications, including overcoming drug resistance in OS and providing novel targets for targeted therapy[13-15]. Therefore, understanding the role of ubiquitination in OS holds immense potential for improving both disease prognosis and therapeutic efficacy. This article examines the

role of ubiquitination in regulating osteosarcoma proliferation, invasion, migration, and other malignant behaviors. Furthermore, the potential of ubiquitin-based research to create novel therapeutic strategies for OS is discussed. We posit that ubiquitination dysregulation is a primary driver of OS development and drug resistance. Consequently, targeting ubiquitination holds significant promise for future OS treatment. By comprehensively reviewing the involvement of ubiquitination in OS pathogenesis and the current state of research, this work identifies knowledge gaps and outlines potential avenues for future investigation. The findings provide a foundation for developing novel therapeutic strategies targeting the ubiquitination pathway in OS while deepening our understanding of the disease through a focused examination of the critical role of ubiquitination (**Figure 1**).

Ubiquitination and osteosarcoma

Burden of OS

OS, the most common primary bone tumor, primarily affects adolescents and older individuals with pre-existing bone deformities[16]. OS typically arises in the ends of long bones, particularly the limbs[17]. Characteristically, this cancer involves the abnormal production of immature bone and osteoid by transformed osteoblasts, resulting in remarkable intratumoral heterogeneity in which different tissue types
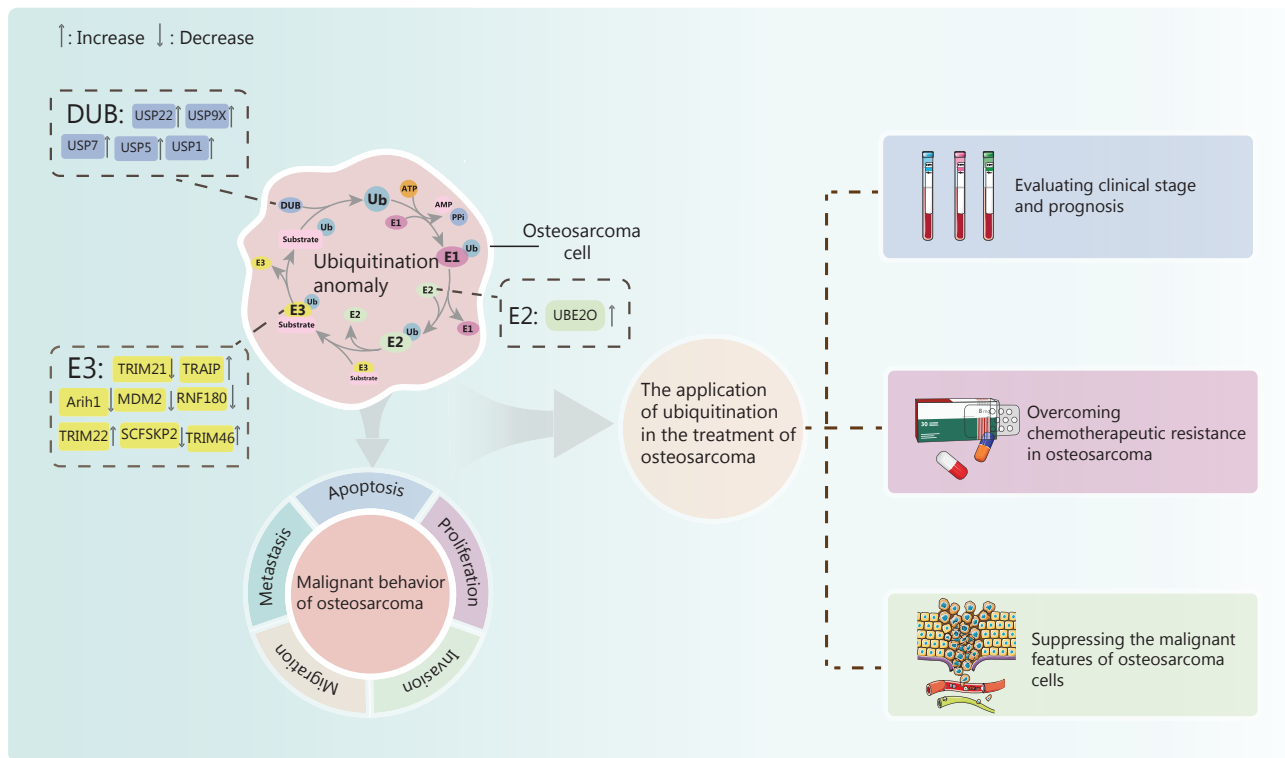

Figure 1 Effect of ubiquitination imbalance on osteosarcoma cells and its clinical application. Aberrant expression of E1, E2, and E3 enzymes within the ubiquitin-proteasome system, as well as deubiquitinases, can disrupt ubiquitination and deubiquitination pathways, contributing to the malignant phenotypes of osteosarcoma cells, including proliferation, invasion, migration, and metastasis. By investigating the impact of these ubiquitin modification dysregulations on osteosarcoma, researchers have made significant strides in evaluating tumor prognosis, overcoming drug resistance, and inhibiting tumor progression. Together, these findings promote the development of osteosarcoma treatment, such as judging the prognosis of osteosarcoma, overcoming drug resistance, and inhibiting tumor occurrence. As illustrated in the figure, corresponding solid and dashed lines at the upstream and downstream of the signaling pathway indicate consistent activation or inhibition, respectively. The red line signifies a distinct process. Upon activation of the signaling pathway, expression or interaction of a downstream ubiquitin ligase is induced, subsequently influencing tumorigenesis. This effect is independent of the illustrated upstream signaling events.

and behaviors co-exist[18]. This complexity underscores the challenge of managing this disease. Both environmental and genetic factors contribute to OS development, including age, exposure to ionizing radiation, and pre-existing bone conditions, such as osteoarthritis[19]. Patients typically present with localized swelling, pain, and limited joint movement. In approximately 15% of advanced cases there is radiographic evidence of metastasis, mainly to the lungs[5]. Imaging has a crucial role in diagnosis, revealing different patterns within the tumor, including osteogenic, osteolytic, or mixed forms. Notably, the triangular periosteal calcification at the tumor-bone interface, known as the Codman triangle, is a highly characteristic feature[20,21].

The mainstay of OS treatment involves surgery, radiotherapy, and pre- or postoperative chemotherapy[3]. However, effective options remain limited for patients with advanced lung metastasis, resistance to chemotherapy, or drug intolerance. Furthermore, chemoresistance significantly hinders improvements in survival rates[22]. This grim reality highlights the urgent need for intensified research and clinical trials to overcome these limitations[23,24].

Ubiquitin-proteasome system

Ubiquitination and deubiquitination dynamically regulate intracellular protein modification and gene expression. Dysregulation of these processes frequently drives tumorigenesis and progression by altering the expression of both modified and unmodified genes, leading to aberrant signaling and malignant behavior. The resulting altered gene expression profile offers potential as a cancer biomarker for prognostication and therapeutic targeting[12,25].

Ubiquitination, a pivotal post-translational modification orchestrated by a coordinated enzymatic cascade, utilizes a 76-amino acid ubiquitin molecule[13]. This key modifier harbors a C-terminal tail with a conserved diglycine (GG) motif and seven lysine (Lys) residues, serving as attachment sites for additional ubiquitin moieties[26]. The cascade involves the following complex interplay of enzymes: E1 family members activate ubiquitin; E2 enzymes categorized by function accept ubiquitin; and E3 ligases (particularly HECT, RING, and RBR types) recognize the target protein and facilitate ubiquitin attachment to specific Lys residues[27-29]. This intricate collaboration orchestrates a vast array of cellular functions, highlighting the potential of ubiquitination as a therapeutic target. Notably, the hundreds of E3 ligases in eukaryotes exemplify

the remarkable power and versatility of ubiquitination in regulating life[30-32].

Deubiquitination, the counterpoint to ubiquitination, relies on deubiquitinating enzymes (DUBs) to release the attached ubiquitin molecule from its target protein, effectively reversing the functional impact[33]. DUBs are currently classified into seven main groups, each with unique structural and functional features: ubiquitin-specific protease (USP); ubiquitin C-terminal hydrolase (UCH); ovarian tumor protease (OTU); machado-josephin domain protease (MJD); Jab1/MPN+/Mov34 (JAMM) domain protease; monocyte chemoattractant protein inducible protein (MCPIP); and motifs interacting with ubiquitin-containing novel DUB family (MINDY)[34].

Link between OS and ubiquitination

A growing body of research has highlighted dysregulation of the ubiquitin-proteasome system (UPS) in OS. Components of the UPS, including ubiquitin ligases and DUBs, are frequently aberrantly expressed in OS cells, which contributes to tumorigenesis, progression, and metastasis[35,36]. For example, several ubiquitin ligases have been shown to promote osteosarcoma cell growth and survival by targeting tumor suppressors for degradation. Conversely, DUBs stabilize oncoproteins, thereby driving tumor progression. Understanding the precise mechanisms by which ubiquitination contributes to OS pathogenesis is essential for developing novel therapeutic strategies. By elucidating the specific roles of ubiquitin-related enzymes in this cancer type, researchers can identify potential drug targets and biomarkers for patient stratification.

Subsequently, we will examine the current state of ubiquitination research across diverse tumor types. This will be followed by an in-depth exploration of the influence of ubiquitination on the malignant phenotype of OS. Finally, we will discuss the clinical implications and potential applications of targeting ubiquitination in OS treatment.

Ubiquitin modification of proteins in key signaling pathways in cancer

The intricate interplay between ubiquitination and deubiquitination tightly regulates diverse signaling pathways, demonstrably influencing tumorigenesis and cancer progression (**Figure 2**). This dynamic duo acts as a cellular
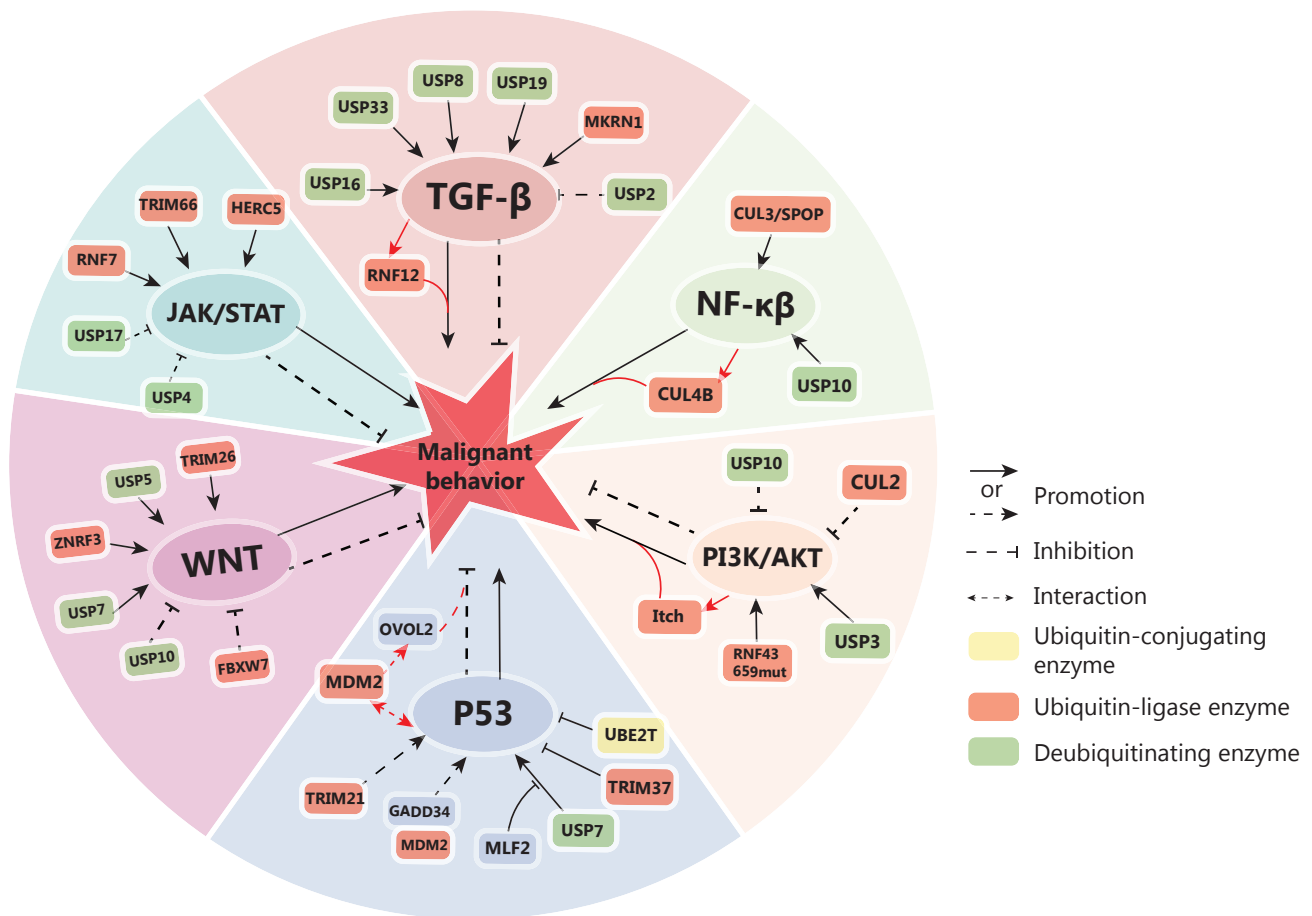
Figure 2 Ubiquitin-mediated regulation of key signaling pathways in tumor cells. Ubiquitin ligases and deubiquitinases have pivotal roles in modulating the activation or inhibition of TGF-β, NF-κβ, WNT, PI3K/AKT, JAK/STAT, and P53 signaling pathways in tumor cells. These pathways, in turn, influence the malignant behavior of tumors. Notably, the activation of some signaling pathways can also lead to the activation of downstream ubiquitin ligases, further impacting tumor progression. **TGF-β**: deubiquitinases ubiquitin-specific protease 16 (USP16), USP33, USP8, USP19, and the makorin ring finger protein 1 (MKRN1) activate the TGF-β signaling pathway. The activation of TGF-β further activates ring finger protein 12 (RNF12). These events collectively contribute to the malignant behavior of tumors. Conversely, USP2 inhibits TGF-β signaling, suppressing tumor growth. **NF-κβ**: USP10 and cullin-3/ speckle-type POZ protein (CUL3/SPOP) activate the NF-κβ signaling pathway, promoting tumor formation. NF-κβ activation also upregulates CUL4B, further driving tumor growth. **WNT**: USP7, USP5, and zinc and ring finger 3 (ZNRF3), tripartite motif containing 26 (TRIM26) activate the WNT pathway, promoting tumorigenesis. Conversely, USP10 and recombinant F-box and WD repeat domain containing protein 7 (FBXW7) inhibit tumorigenesis by suppressing the WNT pathway. **PI3K/ AKT**: RNF43 and USP3 activate the PI3K/AKT pathway, promoting tumorigenesis. Conversely, CUL2 and USP10 inhibit PI3K/AKT, suppressing tumorigenesis. Activated PI3K/AKT can further activate ubiquitin ligase Itch, contributing to tumorigenesis. **JAK/STAT**: RNF7, TRIM66, and HERC5 activate the JAK/STAT pathway, promoting tumorigenesis. USP17 and USP4 inhibit the JAK/STAT pathway, suppressing tumorigenesis. **p53**: TRIM21 activates the p53 pathway, while growth arrest and DNA-damage-inducible gene 34 (GADD34) competitively binds to mouse-double minute 2 (MDM2) to promote p53 expression. These events collectively inhibit tumorigenesis. TRIM37 and ubiquitin-conjugating enzyme E2T (UBE2T) inhibit p53 expression, while myeloid leukemia factor 2 (MLF2) inhibits the binding of USP7 to p53, further promoting tumorigenesis. Additionally, p53 interacts with MDM2, which in turn promotes the expression of OVO-like zinc finger 2 (OVOL2) and inhibits tumorigenesis.

switchboard, dictating the fate of proteins through targeted attachment and removal of ubiquitin molecules. In the following sections, we delve deeper into the specific effects of

ubiquitination on tumor development, exploring how ubiquitination manipulates key signaling networks to promote cancer hallmarks.

P53 signaling pathway

The p53 pathway, a critical tumor suppressor, is tightly regulated by ubiquitination and deubiquitination enzymes, offering promising opportunities for therapeutic intervention. Researchers have investigated the biological function of growth arrest and DNA-damage-inducible gene 34 (GADD34) by transfecting U2OS human OS cells. MDM2 was shown to mediate GADD34 ubiquitination. Interestingly, GADD34 acts as a ubiquitination competitor, reducing p53 ubiquitination and increasing p53 protein levels, thereby suppressing OS proliferation[37]. In breast and rectal cancers, TRIM21 acts as a gatekeeper, inhibiting mutant p53 accumulation and suppressing tumor growth[38]. Conversely, in hepatocellular carcinoma, TRIM37 promotes p53 ubiquitination and degradation, hindering cancer progression[39]. These contrasting examples highlight the context-dependent nature of ubiquitination in p53 regulation. Exploiting ubiquitination for targeted therapy presents exciting possibilities. In pancreatic cancer, UBE2T-mediated p53 degradation confers gemcitabine resistance, highlighting the potential of manipulating this pathway to overcome drug resistance[40]. Colorectal cancer (CRC) progression relies on myeloid leukemia factor 2 (MLF2), which disrupts the USP7-p53 deubiquitination complex, promoting tumorous growth[41]. Additionally, in breast cancer, p53 directly binds mousedouble minute 2 (MDM2), preventing ubiquitination of OVO-like zinc finger 2 (OVOL2), a transcriptional suppressor of metastasis. This interaction makes it possible to exploit the p53-MDM2-OVOL2 axis for therapeutic benefit[42].

JAK/STAT signaling pathway

The JAK/STAT signaling pathway intricately collaborates with ubiquitination to influence tumor cell behavior. Destruction of the DUBs (usp17 and usp4) targeting PDGFR β/STAT3 in osteosarcoma will lead to the interruption of signal transduction and the uncontrolled proliferation of OS cells[43]. Silencing TRIM66 reduces Janus kinase2 (JAK2) activation and signal transducer and activator of transcription 3 (STAT3) phosphorylation in rectal cancer, leading to suppressed cell proliferation, migration, and invasion[44]. Conversely, under glucose starvation, protein tyrosine phosphatase mitochondrial 1 (PTPMT1)-mediated dephosphorylation of recombinant human eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1) safeguards it from HECT and RLD domain

containing E3 ubiquitin protein ligase 5 (HERC5)-induced ubiquitination and degradation. Stabilized 4EBP1 promotes lung cancer cell apoptosis by competing with Jak and ERK for binding to STAT3[45]. Interestingly, ring finger protein 7 (RNF7) activates STAT3 signaling by ubiquitinating recombinant suppressors of cytokine signaling (SOCS1) in renal cell carcinoma, thereby inhibiting apoptosis, promoting glycolysis, and reducing sensitivity to sunitinib[46]. These diverse examples highlight the multifaceted role of ubiquitination in modulating the JAK/STAT pathway in tumors.

PI3K/AKT signaling pathway

The PI3K/AKT pathway, a central hub controlling numerous cellular processes through the vast network of downstream effectors and intricate crosstalk with other signaling cascades, has a crucial role in cancer development and progression[47]. This multifaceted pathway has been extensively studied in various cancers, revealing the diverse and context-dependent influence. USP3 leads to activation of the PI3K/AKT signaling pathway in OS by binding to EPHA2, then reducing protein degradation. Overexpression of USP3 significantly increased OS cell proliferation, migration, and invasion[48]. A mutated form of RNF43 (RNF43 659mut) binds to p85 in rectal cancer, promoting ubiquitination and degradation, ultimately leading to enhanced PI3K signaling and increased tumor activity[49]. Conversely, USP10 deubiquitinates K63-linked ubiquitin chains on Akt in non-small cell lung cancer, weakening downstream signaling and inhibiting tumor cell proliferation[50]. USP10 further exerts a tumor suppressive role by stabilizing adenosine 5′monophosphate-activated protein kinase alpha (AMPKα) and phosphatase and tension homologue deleted from chromosome 10 (PTEN) in liver cancer, leading to mammalian target of rapamycin complex 1 (mTORC1) inhibition and reduced Akt phosphorylation[51]. Breast cancer presents another example of how ubiquitination modulates the PI3K/AKT pathway. Recombinant peptidyl prolyl cis/trans isomerase NIMA interacting protein 1 (Pin1), an isomerase activated by Akt phosphorylation, promotes the interaction between salt-induced protein kinase 1 (Sik1) and E3 ligase Itch, leading to Sik1 ubiquitination and degradation, ultimately contributing to tumorigenesis[52]. Interestingly, the N6-methyladenosine (m6A) modification (WEE2-AS1) stabilizes RPN2 protein by preventing Cul2-mediated K322 ubiquitination, thereby activating PI3K/AKT and promoting glioblastoma progression[53].

NF-κβ signaling pathway

The NF-κβ signaling pathway has a pivotal role in tumor development and resistance to therapies. In osteosarcoma, the ubiquitin ligase, cullin 4B protein (CUL4B), orchestrated by NF-κβ subunits, promotes invasion by degrading the CDK inhibitor, p21[54]. The CUL3/SPOP E3 ligase promotes growth by targeting DRAK1 for degradation in paclitaxel-resistant cervical cancer cells, leading to enhanced NF-κβ activity[55]. USP10 deubiquitinates NLRP7 protein in CRC, stabilizing NLRP7 and prompting M2 macrophage polarization *via* NF-κβ-dependent secretion of monocyte chemoattractant protein-1 (MCP-1). This pro-tumorigenic cascade fuels CRC proliferation and metastasis[56].

WNT signaling pathway

The WNT signaling pathway, which has an essential role in many cancers, presents promising therapeutic opportunities through modulation of ubiquitination and deubiquitination. USP7, a DUB, promotes migration by activating WNT signaling *via* EMT/β-catenin in OS[57]. For example, the development of proteolysis-targeting chimeras (PROTACs) targeting zinc and ring finger 3 (ZNRF3), a WNT-responsive ligase, leading to targeted degradation of transmembrane proteins and CRC regression in cell culture and animal models[58]. The interaction between prospero homeobox 1 (PROX1) and heterogeneous nuclear ribonucleoprotein H (hnRNPH) inhibits ubiquitination of breast cancer, promoting WNT pathway activation and tumor metastasis[59]. USP5 deubiquitinates and stabilizes β-catenin, thereby activating the WNT/β-catenin pathway and promoting stem cell characteristics in lung cancer[60].

Conversely, Let-7b-5p acts as a tumor suppressor in colon cancer by blocking the ubiquitination-mediated degradation of adenomatous polyposis coli (APC) and NKD1, crucial molecules in the WNT pathway[61]. Similarly, the 185aa isoform of circFBXW7 regulates the WNT pathway by ubiquitinating and inhibiting β-catenin, effectively suppressing lung adenocarcinoma stem cell activity and reversing resistance to tyrosine kinase inhibitors[62]. Finally, the LKB1/AMPK pathway interacts with WNT/β-catenin, with USP10 acting as a central hub. By modulating metabolism and cell proliferation, the LKB1/AMPK axis inhibits tumor growth through USP10-mediated regulation[63].

TGF-β signal pathway

The TGF-β signaling pathway presents a perplexing conundrum in cancer, acting as both a tumor suppressor and promoter depending on the specific cellular context[64]. This paradoxical role hinges on the delicate balance between ubiquitination and deubiquitination of key pathway components, highlighting the potential of targeting these enzymes for therapeutic intervention. USP8 promotes tumor progression and T cell depletion in breast cancer by deubiquitinating the TGF-β receptor, TβRII[65]. Targeting USP19 splicing or its enzymatic activity offers a promising avenue, as shown by the splicing regulator, herboxidiene, which suppresses cell migration by upregulating the anti-migratory USP19 isoform[66]. Dysregulation of deubiquitination can also drive cancer development, as exemplified in hepatocellular carcinoma (HCC) in which elevated recombinant diacylglycerol kinase gamma (DGKG) recruits USP16, leading to zinc finger E-box-binding homeobox 2 (ZEB2) deubiquitination and subsequent TGF-β1 upregulation, which promotes angiogenesis and regulatory T cell differentiation[67]. Similarly, makorin ring finger protein 1 gene (MKRN1) in colon cancer fosters TGF-β signaling and metastasis by ubiquitinating and degrading Smad nuclear interacting protein 1 (SNIP1)-β[68]. Adding another layer of complexity, Akt-mediated phosphorylation further intertwines ubiquitination with TGF-β signaling, as evident in RNF12, which promotes breast cancer metastasis upon Akt activation[69]. Deubiquitination enzymes also have crucial roles. USP33, for example, promotes pancreatic cancer malignancy through the TGFBR2/TGF-β pathway[70], while inhibiting USP2 expression conversely activates TGF-β signaling and fuels glioblastoma development[71].

Impact of ubiquitination on the malignant OS phenotype

Ubiquitination, a process attaching a small protein tag to other proteins, has a central role in regulating various signaling pathways that profoundly influence the biological behavior of OS (**Figure 3**). The impact extends beyond mere cell proliferation, intricately modulating invasion, migration, and the threat of metastasis. This complex regulatory network presents a wealth of potential targets for therapeutic intervention, offering hope for improved outcomes in this challenging cancer.
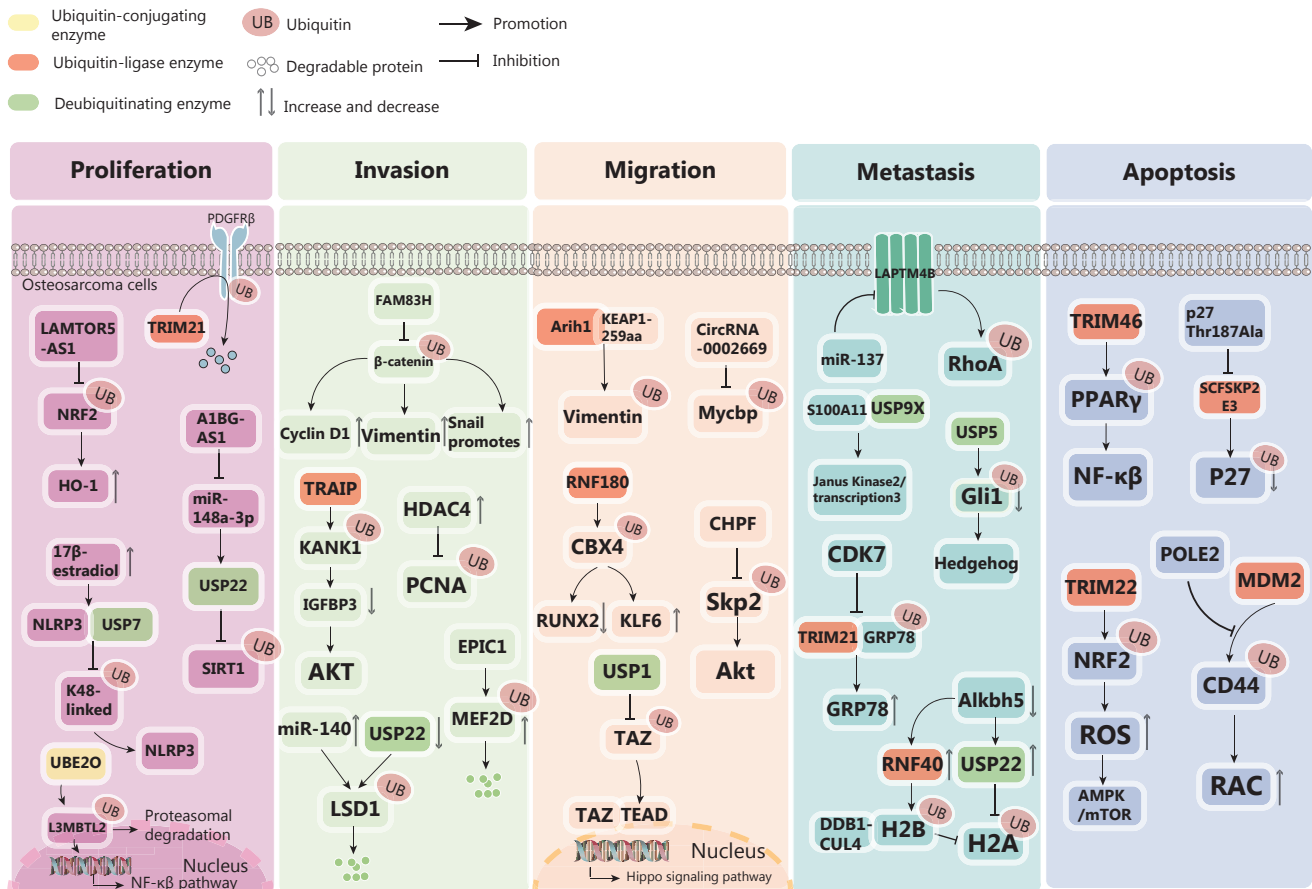
Figure 3 Dysregulated ubiquitination pathway drives osteosarcoma malignancy. **Proliferation**: Proliferation of osteosarcoma is regulated by intricate ubiquitination processes. UBE2O ubiquitinates L3MBTL2, bolstering NF-κβ expression and tumor growth. LAMTOR5-AS1 halts proliferation by blocking NRF2 ubiquitination, augmenting heme oxygenase-1 levels. TRIM21 reduces PDGFRβ *via* ubiquitination, inhibiting tumor expansion. A1BG-AS1 enhances proliferation by inhibiting miR-148a-3p and upregulating USP22, inhibiting SIRT1 ubiquitination. High E2 levels facilitate USP7-NLRP3 interaction, removing K48-linked ubiquitination, boosting NLRP3 inflammasome and proliferation. **Invasion**: Invasion is curbed by EPIC1-mediated MEF2D ubiquitination and degradation. TRAIP promotes Kank1 polyubiquitination, downregulating IGFBP3 and activating Akt, fostering invasion. FAM83H stabilizes β-catenin, upregulating cyclin D1, vimentin, and snail, promoting invasion. HDAC4 overexpression elevates PCNA while decreasing ubiquitinated PCNA, enhancing invasion. miR-140 or USP22 knockdown triggers LSD1 ubiquitination and degradation, inhibiting invasion. **Migration**: KEAP1-259aa interacts with ARIH1 to promote the proteasomal degradation of cytoplasmic vimentin, inhibiting osteosarcoma cell migration. CircRNA_0002669 promotes osteosarcoma cell migration by preventing MYCBP ubiquitination. USP1 interacts with TAZ, activating the Hippo signaling pathway and promoting cell migration. CHPF inhibits SKP2 ubiquitination and activates the Akt signaling pathway, promoting osteosarcoma migration. RNF180 ubiquitinates Cbx4, upregulating KLF6 and downregulating Runx2, inhibiting cell migration. **Metastasis**: S100A11 interacts with USP9X to stimulate the JAK2/STAT3 signaling pathway, promoting lung metastasis in osteosarcoma patients. USP5 deubiquitinates GLI1, activating the Hedgehog signaling pathway and promoting osteosarcoma metastasis. ALKBH5 increases the expression of USP22 and RNF40, inhibiting histone H2A single ubiquitination. RNF40, ubiquitinated on H2B, inhibits H2A ubiquitination in tumors, interacting with the DDB1-CUL4-based ubiquitin E3 ligase complex to promote osteosarcoma metastasis. CDK7 inhibits TRIM21-mediated GRP78 ubiquitination, and promoting osteosarcoma metastasis. miR-137 can inhibit osteosarcoma metastasis by targeting LAPTM4B to stabilize RhoA protein. **Apoptosis**: POLE2 enhances CD44 expression by inhibiting MDM2-mediated ubiquitination, subsequently activating the RAC signaling pathway to promote osteosarcoma cell apoptosis. TRIM22 inhibits osteosarcoma progression through Nrf2-mediated (ROS) imbalance. The p27 Thr187Ala knock-in mutation disrupts SCFSkp2 E3 ligase-mediated p27 ubiquitination, leading to p27 Thr187Ala accumulation and promoting osteosarcoma cell apoptosis. TRIM46 interacts with PPAR, ubiquitinates PPAR, activates the NF-κβ signaling pathway, and induces apoptosis.

Proliferation

Cell proliferation, the fundamental process driving primary tumor growth, is meticulously regulated within OS through a complex interplay of ubiquitination pathways. These pathways exert diverse and often opposing influences, underscoring the intricate regulatory network governing this critical biological process. Several ubiquitination axes have emerged as pivotal contributors. A negative correlation exists between lethal (3) malignant brain tumor-like protein 2 (L3MBTL2) and UBE2O within OS tissue. UBE2O ubiquitinates L3MBTL2, targeting L3MBTL2 for proteasomal degradation. The UBE2O/L3MBTL2 axis is indispensable for OS growth. Elevated UBE2O and diminished L3MBTL2 expression correlate with adverse clinical outcomes in OS patients. Pharmacologic inhibition of UBE2O using arsenic trioxide potentiates L3MBTL2-induced aggregates, consequently suppressing OS growth. Consequently, the UBE2O-L3MBTL2 axis is posited as a promising therapeutic target for OS[72]. High-throughput sequencing identified a novel long non-coding RNA (lncRNA) termed long non-coding RNA LAMTOR5 antisense RNA 1 (LAMTOR5-AS1). This lncRNA enhances nuclear factor erythroid 2-related factor 2 (NRF2) levels by impeding NRF2 ubiquitination and degradation, although the transcriptional capacity of NRF2 is compromised. Subsequently, elevated NRF2 upregulates the downstream gene, heme oxygenase-1 (HO-1). Moreover, NRF2 autoregulates expression by inducing LAMTOR5-AS1 transcription. LAMTOR5-AS1 significantly inhibits OS cell proliferation[73]. The E3 ubiquitin ligase, TRIM21, is characterized by a tripartite motif, facilitates platelet-derived growth factor receptor beta (PDGFRβ) ubiquitination within the U2OS OS cell line, thereby regulating basal PDGFRβ levels. siRNA-mediated depletion of TRIM21 attenuates PDGF-BB-induced PDGFRβ ubiquitination, promoting OS cell growth[74].

Studies have revealed high expression of A1BG antisense RNA 1 (A1BG-AS1) and USP22 in addition to low miR-148a-3p levels in OS tissues and cells. Downregulation of A1BG-AS1 and USP22 or upregulation of miR-148a-3p inhibits the malignant behavior of OS cells. A1BG-AS1 functions as a miR-148a-3p sponge, while miR-148a-3p targets USP22, thereby suppressing its expression. Conversely, USP22 upregulation reverses the phenotypic inhibition of OS cells induced by A1BG-AS1 inhibition. Mechanistically, USP22 influences OS cell biology by deubiquitinating silent mating type information regulation 2 homolog-1 (SIRT1)[75]. In OS patients with

high 17β-estradiol (E2) levels, E2 activates mTORC1, promoting USP7 stability. USP7 interacts with NLRP3 and removes K48-linked ubiquitination, enhancing the NLRP3 inflammasome pathway and promoting proliferation of OS cells[76].

Invasion

OS invasion is meticulously regulated by ubiquitination-mediated protein degradation, with several key factors influencing this process. Notably, several proteins that suppress invasion are targeted for degradation. Researchers observed a significant increase in myocyte enhancer factor 2D (MEF2D) protein ubiquitination in EPIC1-overexpressing OS cells. Co-transfection of pCDNA-EPIC1 and pCDNA-MEF2D rescued the cell viability and invasion inhibition induced by EPIC1 overexpression. These findings indicated that EPIC1 suppresses OS cell survival and invasion by promoting MEF2D ubiquitination[77]. TRAIP, a key differentially expressed gene with prognostic significance in OS, was identified. TRAIP promotes Kank1 polyubiquitination and subsequent degradation in OS cells, downregulates insulin-like growth factor binding protein 3 (IGFBP3), and activates the Akt pathway, thereby fostering invasion. These results highlight the critical role of the TRAIP/Kank1/IGFBP3/Akt signaling axis in OS progression[78]. Family with sequence similarity 83 member H (FAM83H) inhibits β-catenin ubiquitination in OS, thereby stabilizing β-catenin. Consequently, increased expression of cyclin D1, vimentin, and snail promotes cellular invasion[79]. Overexpressed histone deacetylase 4 (HDAC4) increases PCNA protein levels without affecting PCNA mRNA levels, while decreasing ubiquitinated PCNA. HDAC4 promotes invasion, while HDAC4 knockout exhibits the opposite effect. Direct binding between HDAC4 and PCNA was confirmed[80]. In OS tissues and cells, miR-140 and p21 expression is decreased, while USP22 and lysine-specific demethylase 1 (LSD1) expression is increased. Overexpressing miR-140 or knocking down USP22 induces LSD1 ubiquitination and degradation, inhibiting OS cell invasion[81].

Migration

Researchers discovered that Kelch-like ECH-associated protein 1-259aa (KEAP1-259aa) interacts with the E3 ubiquitin ligase, Arih1, to bind cytoplasmic vimentin, promoting proteasomal degradation and inhibiting OS cell migration[82]. CircRNA_0002669 directly binds to c-Myc binding protein

888

Shen et al. Unraveling ubiquitination role in osteosarcoma

(MYCBP), a positive regulator of c-Myc, preventing MYCBP ubiquitination and proteasomal degradation. Consequently, circRNA_0002669 promotes OS cell migration by safeguarding MYCBP from protein degradation and disrupting the miR-889-3p-mediated pathway[83]. USP1 directly interacts with the transcription co-activator, TAZ, in OS cell lines. Mechanistic analysis revealed that USP1 inhibition of the anti-OS effect is partially attributed to TAZ instability, reduced nuclear accumulation, and subsequent downregulation of Hippo signaling pathway genes. Similarly, the pharmacologic USP1 inhibitor, ML323, exerts analogous effects on the Hippo signaling pathway, inhibiting OS migration *in vitro* and *in vivo*[84]. Chondroitin polymerizing factor (CHPF), a glycosyltransferase strongly expressed in OS tissues and cells, promotes OS migration by counteracting s-phase kinase-associated protein 2 (SKP2) ubiquitination and activating the Akt signaling pathway[85]. The novel E3 ubiquitin ligase, RNF180, promotes chromobox homolog 4 (Cbx4) ubiquitination, upregulates Kruppel-like factor 6 (KLF6), and downregulates runt-related transcription factor 2 (Runx2) in OS cells, inhibiting cell migration[86].

Metastasis

OS cell-derived extracellular vesicles (EVs) activate pulmonary interstitial macrophages by secreting the chemokine, CXCL2, and initiating an influx of granulocyte-macrophage colony-stimulating factor (GM-CSF)-mobilized dendritic cells (GM-DCs). Proteomic analysis of EVs revealed that S100A11, packaged within EVs, stimulates the Janus kinase 2/signal transducer and activator of transcription 3 signaling pathway in macrophages through interaction with USP9X. High expression of S100A11 or circulating GM-DCs is associated with lung metastasis and poor prognosis in OS patients[87]. USP5 promotes OS metastasis by activating the Hedgehog (Hh) signaling pathway, as demonstrated in cultured cells and animal models. Mechanistically, USP5 stabilizes and deubiquitinates GLI family zinc finger protein 1 (GLI1), a key mediator of the Hh pathway. The oncogenic effects of USP5 in OS are contingent on GLI1 stability[88]. Alkbh5-mediated m6A deficiency in OS leads to increased USP22 and RNF40 expression, inhibiting histone H2A mono-ubiquitination and inducing key oncogenes, thereby driving uncontrolled cell cycle progression, continuous replication, and DNA repair. RNF40, ubiquitinated on H2B, inhibits H2A ubiquitination in cancer by interacting with the DDB1-CUL4-based ubiquitin

E3 ligase complex, affecting DDB1-CUL4-based ubiquitin E3 ligase complex stability and promoting OS metastasis[89]. The E3 ubiquitin ligase, TRIM21, binds and targets GRP78 for ubiquitination and degradation, while cyclin-dependent kinase 7 (CDK7) phosphorylates G protein-coupled receptor 78 (GRP78) at T69, inhibiting TRIM21 recruitment and stabilizing GRP78. Notably, THZ1, a specific CDK7 inhibitor, suppresses OS growth and metastasis. Combined treatment with CDK7 and GRP78 inhibitors exhibits a synergistic effect on OS growth and progression[90]. MiR-137 targets LAPTM4B, a lysosomal-associated protein transmembrane 4B. LAPTM4B stabilizes RhoA protein by inhibiting ubiquitin-mediated proteasomal degradation, regulating stress fiber organization and promoting OS cell metastasis[91].

Apoptosis

Researchers discovered that DNA polymerase epsilon 2 (POLE2) enhances CD44 expression by inhibiting MDM2-mediated ubiquitination, subsequently activating the RAC signaling pathway to promote OS cell apoptosis[92]. TRIM22 interacts with Nrf2, accelerating degradation through Nrf2 ubiquitination. This process is dependent on TRIM22 E3 ligase activity and independent of Kelch-like ECH-associated protein 1 (Keap1), ultimately activating the ROS/AMPK/mTOR/autophagy signaling axis and inducing apoptosis. Mechanistically, TRIM22 inhibits OS progression *via* Nrf2-mediated intracellular reactive oxygen species (ROS) imbalance. Overexpressed TRIM22 significantly enhances ROS production, while inhibiting mitochondrial potential, thereby activating the AMPK/mTOR signaling cascade[93]. The p27 Thr187Ala knock-in mutation disrupts SCFSkp2 E3 ligase-mediated p27 ubiquitination and degradation, leading to p27 Thr187Ala accumulation and promoting OS cell apoptosis[94]. As an oncogene in OS, TRIM46 interacts with peroxisome proliferator-activated receptor α (PPAR), ubiquitinates PPAR, activates the NF-κβ signaling pathway, and induces apoptosis[95].

Application of ubiquitination in the treatment of OS

As our understanding of the intricate dance between ubiquitination and OS deepens, the potential for clinical diagnosis and treatment expands exponentially. Notably, tumor drug

resistance remains a formidable foe in OS therapy, significantly impacting both treatment efficacy and patient outcomes. Overcoming this resistance, predicting OS progression with accuracy, and effectively curbing tumor growth and metastasis represent the holy grail in current treatment strategies. Exploiting ubiquitination pathways offers a glimmer of hope, a promising avenue to address these challenges and potentially revolutionize OS management (**Figure 4**).
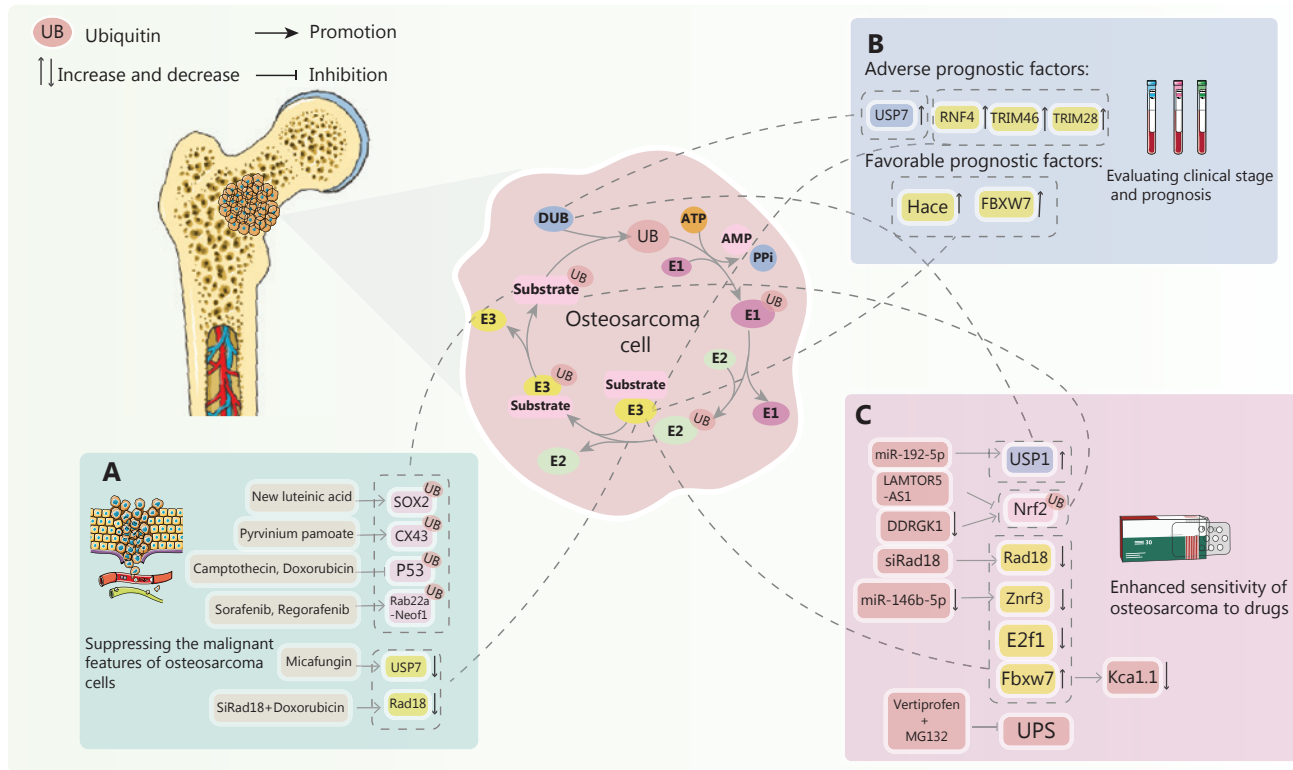

Figure 4 The potential of ubiquitination modulation in osteosarcoma treatment. In the context of osteosarcoma, targeting ubiquitination pathways has emerged as a promising therapeutic strategy. Recent research has yielded significant advancements in prognostic assessment, overcoming drug resistance, and inhibiting tumor development through ubiquitination-based interventions. (A) New Luteinic acid interacts with USP9X to directly target the ubiquitination and degradation of the key transcription factor, sex determining region Y box protein 2 (SOX2), significantly inhibiting osteosarcoma cell proliferation. Pyrvinium pamoate, a casein kinase 1 (CK1α) activator, promotes the ubiquitination and degradation of connexin 43 (CX43), hindering osteosarcoma metastasis. Sorafenib and remifentanil ubiquitinate and degrade RAB22A-NEOF1, blocking RAB22A-NEOF1-driven lung metastasis. Camptothecin or adriamycin treatment reduces p53 ubiquitination, stabilizing p53 protein levels, and inhibiting osteosarcoma cell proliferation. Micafungin exerts its anti-tumor effect on osteosarcoma by downregulating USP7. Adriamycin, encapsulated in nanoparticles and combined with siRNA targeting RAD18, significantly inhibits tumor growth and metastasis by reducing RAD18 expression. (B) Research has demonstrated that in osteosarcoma patients, elevated levels of ubiquitin ligases RNF4, TRIM46, TRIM28, and the deubiquitinase USP47 are associated with poor prognosis, whereas elevated levels of ubiquitin ligases HACE and FBXW7 indicate a favorable prognosis. (C) Mir-192-5p enhances the susceptibility of osteosarcoma cells to cisplatin by downregulating USP1 expression. Lamtor5-as1 increases chemosensitivity by inhibiting Nrf2 ubiquitination, leading to decreased Nrf2 transcriptional activity and disruption of the pro-survival loop in drug-resistant cells. Targeting DDRGK1 can reduce its expression and further promote Nrf2 ubiquitination, enhancing tumor drug sensitivity. RGD exosome-delivered SIRAD18 effectively silences the ubiquitin ligase RAD18, potentially enhancing anti-tumor drug activity. Inhibiting E2F1-mediated retinoic acid receptor α ubiquitination can improve drug efficacy in osteosarcoma treatment. Vertiprofen (VP), in combination with the proteasome inhibitor, MG132, simultaneously inhibits the ubiquitin-proteasome system (UPS) and enhances chemotherapy sensitivity by regulating autophagy and protein homeostasis. Inhibiting miR-146b-5p and downregulating the cell surface E3 ligase, ZNRF3, ultimately sensitizes osteosarcoma cells to chemotherapeutic drugs. The E3 ligase, FBXW7, downregulates the calcium-activated potassium channel, KCa1.1, overcoming osteosarcoma drug resistance.

890

Shen et al. Unraveling ubiquitination role in osteosarcoma

Judging clinical staging and prognosis

RNF4 as a potential prognostic marker across various sarcomas, including OS. High levels of RNF4, but not the RNF4 partner, BMP6, correlate with significantly shorter survival times for patients[96]. Interestingly, elevated RNF4 alone predicts poorer outcomes, suggesting the independent prognostic value. Unlike RNF4, HACE1 appears to act as a tumor suppressor. Lower HACE1 expression is associated with both reduced survival rates and advanced disease stages[97]. Our research has shown significantly higher USP7 expression in advanced and metastatic OS compared to early-stage counterparts[57]. This positive correlation with tumor stage and metastasis strengthens the potential as a prognostic marker.

Fbxw7 serves as a guardian against OS. Lower expression of Fbxw7 in OS tissues is linked to worse outcomes, highlighting the potential as an independent prognostic marker[98]. TRIM46 leads to a contrasting outcome. An elevated expression of TRIM46 in OS suggests a pro-tumor role and patients with high TRIM46 levels exhibit significantly lower survival rates[95]. The PVT1/TRIM28 complex promotes tumorigenesis by enhancing the degradation of a tumor suppressor protein, ultimately activating mTOR signaling and promoting stem cell characteristics. Notably, high PVT1 expression is associated with a poor prognosis and decreased survival, underlining the potential of PVT1 as a therapeutic target[99].

These diverse examples showcase the complex interplay of ubiquitination in regulating OS prognosis. Understanding these intricate mechanisms holds immense potential for developing novel prognostic tools and therapeutic strategies to improve patient outcomes.

Application in OS drug therapy

The notorious resistance of OS to chemotherapy poses a significant challenge in patient treatment. However, recent research offers promising avenues for overcoming this hurdle by targeting key pathways involved in drug resistance. DDRGK1, a protein stabilizing NRF2, emerges as a potential target. Disabling DDRGK1 destabilizes NRF2, unleashing a tide of ROS and enhancing sensitivity to drugs, such as doxorubicin and etoposide[35]. miR-192-5p, for example, bolsters OS cell susceptibility to cisplatin by targeting USP1 expression[100]. Rad18, implicated in adriamycin resistance, can be effectively silenced by chemically modified siRad18 loaded onto RGD

exosomes, significantly enhancing the anti-tumor activity of adriamycin[101].

LAMTOR5-AS1, an lncRNA, enhances chemotherapy sensitivity by boosting NRF2 activity and HO-1 expression, disrupting the pro-survival loop in resistant cells and leaving cells vulnerable to drug attack[73]. The calcium-activated potassium channel, KCa1.1, is linked to tumor progression and can be downregulated *via* Fbxw7, an E3 ligase, overcoming resistance to paclitaxel, adriamycin, and cisplatin[102]. Vertiprofen (VP), exhibiting dose-dependent cytotoxicity by inducing protein ubiquitination, can be combined with the proteasome inhibitor, MG132, to selectively target p53 for degradation, enhancing chemotherapy sensitivity by modulating autophagy and protein homeostasis[103]. miR-146b-5p, promoting tumor progression and chemoresistance, can be inhibited to upregulate MMP16 protein and downregulate Znrf3, a cell surface E3 ligase, ultimately sensitizing OS cells to chemotherapy drugs[104]. Inhibiting E2F1-mediated ubiquitination of retinoic acid receptorαcan amplify the efficacy of all-trans retinoic acid (ATRA) in OS treatment[105].

Inhibition of malignant OS biological behavior

The aggressive growth and metastatic spread of OS are fueled by complex protein interactions, presenting promising targets for novel therapeutic interventions. New luteinic acid, a natural compound, interacts with USP9X, directly targeting the key transcription factor, Sox2, for ubiquitination and degradation, significantly inhibiting OS cell proliferation[106]. Pyrvinium pamoate, a CK1α activator, promotes CX43 phosphorylation at T437, triggering CX43 ubiquitination and degradation, thereby hindering OS metastasis[107]. Similarly, the kinase, PINK1, phosphorylates Rab22a-Neof1, marking it for ubiquitination and degradation by sorafenib and regorafenib, thus blocking Rab22a-Neof1-driven lung metastasis[108]. Proteins, like Gadd34, can act as "ubiquitination baits," protecting other key proteins from degradation. Gadd34 upregulation following treatment with camptothecin or doxorubicin reduces p53 ubiquitination, thereby stabilizing p53 protein levels and inhibiting OS cell proliferation[109].

Recent studies have demonstrated that micafungin effectively inhibits epithelial-mesenchymal transition (EMT) in OS cells by reducing the levels of ubiquitin-specific protease 7 (USP7), phospho-Akt, and phospho-GSK-3β. *In vivo* experiments using a xenograft tumor mouse model revealed

significant tumor growth inhibition following daily intra-peritoneal micafungin administration compared to the control group. These findings suggest that micafungin exerts its anti-tumor effects on OS by suppressing EMT *via* the USP7/Akt/GSK-3β pathway. USP7, a DUB, is a key oncogenic factor that stabilizes oncoproteins and inhibits tumor suppressors. Downregulation of USP7 by micafungin highlights USP7 potential as a therapeutic target. While preclinical studies have demonstrated promising anti-tumor effects of micafungin, rigorous human clinical trials are essential to evaluate safety and efficacy. Phase I trials will establish optimal dosage and identify potential adverse reactions, followed by phase II and III trials to assess therapeutic efficacy in OS patients. Given the complex nature of OS, combination therapies targeting USP7 in conjunction with chemotherapy or immunotherapy enhance anti-tumor responses and overcome drug resistance. Identifying predictive biomarkers of response to USP7 inhibitors is crucial for patient selection and treatment optimization[110].

Another study demonstrated the potential of nanoparticle-based drug delivery for overcoming OS chemotherapy resistance. By encapsulating doxorubicin within nanoparticles and incorporating siRNA targeting Rad18, an E3 ubiquitin ligase implicated in doxorubicin resistance, the researchers achieved significant tumor growth inhibition and reduced metastasis. This approach also induced immunogenic cell death, suggesting a synergistic effect with immunotherapy. While these preclinical findings are encouraging, safety, efficacy, cost, and biomarker development must be addressed before clinical use. Rigorous clinical trials and innovative approaches to reduce production costs are essential to ensure widespread access to this promising therapy[111].

Future research directions in clinical treatment

Ubiquitination holds significant potential for the treatment of OS. Addressing the challenges of drug specificity, resistance, and delivery is imperative for translating this potential into improved patient outcomes. Research focused on identifying and overcoming mechanisms of drug resistance is crucial for long-term therapeutic success. Additionally, identifying biomarkers predictive of patient response to ubiquitin-targeted therapy can personalize treatment strategies and enhance patient prognosis. The development of ubiquitination-related, OS-specific biomarkers is a promising future research direction. Common targets in OS treatment include VEGFR, MDM2, mTOR, and HDAC[112]. The development of specific inhibitors against MDM2 or other oncogenic E3 ligases can prevent p53 degradation and reactivate the tumor-suppressive function[113]. However, identifying highly selective inhibitors that avoid disrupting essential cellular processes remains a challenge. Conversely, drugs that activate specific DUBs can stabilize p53 and enhance anti-tumor effects in OS. Similar to E3 ligase inhibitors, achieving drug specificity remains an obstacle. The future holds promise for developing specific drugs targeting E3 ligases or DUBs that drive tumor growth or suppress its elimination. Additionally, combining these therapies with conventional approaches like chemotherapy could offer a multifaceted attack on the disease. Identifying patients most likely to benefit through ubiquitination-based biomarkers could further personalize treatment strategies. Targeted protein degradation (TPD) and other emerging technologies utilize engineered E3 ligase chimeras to target specific proteins for ubiquitination and degradation. This approach may eliminate oncogenic proteins lacking natural E3 ligase recognition motifs. While still in the preclinical stage, TPD is anticipated to target key proteins driving OS progression that are currently intractable to treatment[114]. Ensuring drug specificity to avoid unintended cellular disruptions, overcoming potential resistance mechanisms, and developing efficient delivery systems to tumor sites remain significant hurdles. Overall, by addressing these challenges, research on ubiquitination holds the key to unlocking novel and effective therapeutic strategies for OS.

Conclusions

The propensity of OS for distant metastases and resistance to targeted drugs remains a major obstacle, leading to poor patient outcomes. Deciphering the molecular mechanisms driving its development and progression is crucial for improving treatment efficacy and survival rates. Recent research has highlighted the pivotal role of ubiquitination, a post-translational protein modification, in this aggressive cancer. This review delves into the multifaceted impact of ubiquitination on OS cell biology and the potential for clinical application. Ubiquitin ligases and DUBs, the enzymes responsible for adding and removing ubiquitin tags, meticulously regulate key signaling pathways, such as Ras/PI3K/mTOR and NF-κβ. This intricate relationship impacts proteins within the WNT pathway, ultimately influencing cell proliferation, invasion, migration, and apoptosis. Furthermore, ubiquitination modulates

the expression of genes, such as p53 and c-Myc, further shaping OS cell behavior.

The clinical spotlight shines brightly on the role of ubiquitination in OS resistance to chemotherapy. While targeted therapies offer high specificity and reduced side effects, drug resistance remains a formidable hurdle. Ubiquitination has a crucial role in regulating tumor drug sensitivity by influencing drug transport pathways, the apoptosis pathway, and target protein expression within the tumor microenvironment. Additionally, the levels of specific ubiquitin ligase and DUB expression hold promise as prognostic indicators for patients. Exciting recent advances in CRISPR/Cas9 library screening provide a powerful tool to identify genes essential for cancer cell survival, proliferation, migration, and drug resistance. This technology significantly bolsters research and development efforts aimed at overcoming resistance in OS targeted therapy, offering a beacon of hope for improved patient outcomes[115].

Despite the promising potential of exploiting ubiquitination for targeted therapies, significant knowledge gaps remain that hinder progress. While downregulating TRIM7 shows promise in inhibiting metastasis and resensitizing tumors to chemotherapy, the exact role of abnormal m6A modifications in TRIM7 and the impact on OS progression and resistance are largely unexplored[36]. Similarly, the mechanisms by which TRIM22 suppresses tumor growth and metastasis in OS require further investigation[93]. Beyond TRIM7 and TRIM22, other intriguing targets exist. The identification of Bmi1, Ring1b, and USP22 as "cancer death" markers predicting poor prognosis highlights the potential of histone ubiquitination, but the intricate regulatory mechanisms in cancer remain unclear[116]. Additionally, the USP13/Mettl3 axis promoting OS progression through autophagy warrants further exploration, given the complex and multifaceted roles of autophagy in tumor biology[117]. Delving deeper into these knowledge gaps holds immense potential for unlocking novel therapeutic avenues. By elucidating the mechanisms underlying the observed effects of TRIM7, TRIM22, histone ubiquitination, and the USP13/Mettl3 axis, researchers can develop targeted therapies that effectively overcome drug resistance and improve clinical outcomes for OS patients. The rapid emergence of drug resistance during chemotherapy necessitates personalized treatment strategies. Tailoring future regimens based on individual patient bioindicators can minimize unnecessary drug exposure and maximize clinical benefit. In this context, leveraging ubiquitination modifications present an exciting avenue

for enhancing the efficacy of existing targeted therapies while mitigating systemic toxicity.

While existing research has established that ubiquitination influences the malignant behavior of OS cells and is a critical factor in the disease, the precise identity and function of many ubiquitinated substrates remain unclear. Moreover, many studies have focused on a single OS subtype, disregarding disease heterogeneity. Consequently, the impact of ubiquitination across different cell subtypes remains largely unexplored. Furthermore, studies often rely solely on bioinformatics or basic *in vitro* experiments, hindering the comprehensive elucidation of the ubiquitin-mediated signaling network and its translation into clinical applications or drug development. Although progress has been made, most studies concentrate on a limited number of proteins, neglecting the broader ubiquitination landscape within OS cells. A comprehensive understanding of ubiquitination dynamics across different cellular compartments is still lacking. The complex interplay of ubiquitination with other post-translational modifications (e.g., methylation, glycosylation, and phosphorylation) complicates the isolation of its specific role in OS pathogenesis. Despite the identification of numerous ubiquitin-related proteins associated with OS, translating these findings into effective therapeutic targets remains a significant challenge. Additionally, the paucity of clinical data on ubiquitin-based therapies for OS patients hampers the development of treatment regimens. Addressing these knowledge gaps and pursuing focused research directions will be essential for advancing our understanding of OS and developing more effective treatment strategies.

Future studies should employ proteomic methods to identify novel ubiquitinated proteins in OS, providing fresh insights into disease mechanisms. However, research should extend beyond protein identification to encompass detailed investigations into the enzymatic activity, substrate specificity, and regulatory mechanisms of ubiquitin ligases and DUBs. Given the heterogeneity of OS and the complex intracellular environment, future research should encompass multiple cell subtypes and explore the interplay between ubiquitination and other post-translational modifications. By elucidating how these modifications synergistically regulate protein function in OS, groundbreaking discoveries may be achieved. In the clinical realm, developing specific inhibitors or activators of ubiquitin ligases or DUBs for OS treatment is essential. Furthermore, exploring the synergistic effects of targeting ubiquitination in combination with other therapies holds promise for improved

patient outcomes. Ubiquitination represents a promising avenue for unraveling the complexities of OS, thereby enabling the development of novel therapeutic strategies and enhancing patient prognosis.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

Author contributions

Conceived and designed the analysis: Huan Liu, Cheng Zhang, and Jianlin Shen.
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894

Shen et al. Unraveling ubiquitination role in osteosarcoma

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