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## Ubiquitin-specific proteases as therapeutic targets in paediatric primary bone tumours?

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**Abstract:** In children and young adults, primary malignant bone tumours are mainly composed of osteosarcoma and Ewing's sarcoma. Despite advances in treatments, nearly 40% of patients succumb to these diseases. In particular, the clinical outcome of metastatic osteosarcoma or Ewing's sarcoma remains poor, with less than 30% of patients who develop metastases surviving five years after initial diagnosis. Over the last decade, the cancer research community has shown considerable interest in the processes of protein ubiquitination and deubiquitination. In particular, a growing number of studies show the relevance to target the ubiquitin-specific protease (USP) family in various cancers. This review provides an update on the current knowledge regarding the implication of these USPs in the progression of bone sarcoma: osteosarcoma and Ewing's sarcoma.

**Keywords:** Ubiquitin-specific proteases, Osteosarcoma, Ewing's sarcoma, Cancer development, Therapeutic targets

**Running title:** USPs and paediatric primary bone tumours

## 1. Introduction

Although Gideon Goldstein discovered ubiquitin—in 1975 by Gideon Goldstein [1], research on protein ubiquitination gained ground in the 1980s [2,3]. Various studies have highlighted the crucial role of protein ubiquitination in the regulation of numerous cellular functions by regulating their cellular localization, activity, interaction with other proteins, or degradation by the proteasome, for example [4–6]. This ubiquitination process is a reversible reaction due to the action of enzymes, the deubiquitinases (DUBs), which hydrolyse the bonds between ubiquitin molecules within polyubiquitin chains or between ubiquitin and the target protein [7–10]. The crucial role of DUBs in the control of various cellular processes, including cellular homeostasis, suggests that aberrant expression or activity of these enzymes may be involved in the development of pathologies such as cancers.

## 2. Ubiquitination

Ubiquitination is a post-translational modification that allows the binding of ubiquitin moieties to a target protein. Ubiquitin consists of 26 amino acid polypeptides with a C-terminal tail signature consisting of a di-glycine (GG) sequence and seven lysine amino acids (K6, K11, K27, K29, K33, K48, and K63) [11]. Ubiquitin is ubiquitously present in eukaryotic organisms and is highly conserved with only three amino acid differences between yeast and human proteins. Four genes in the human genome encode for linear chains of ubiquitin: PS27A, UBB, UBC, and UBA52 [12].

More than 80% of proteins undergo this ubiquitination process [13], which occurs in three steps: the initial ATP-dependent activation step catalysed by an E1 activating enzyme, the second step in which the ubiquitin molecule is linked to an E2 conjugating enzyme, and the final step in which the ubiquitin moiety is covalently attached to its target protein by an E3 ligase (Figure 1A) [4,14].

The first step activates the C-terminus of ubiquitin by inducing a 3D conformational change. This process is ATP-dependent and involves two steps: the formation of a ubiquitin/adenylate intermediate complex followed by the interaction of this complex with an E1 cysteine residue to form an E1/Ubiquitin complex. Two E1 enzymes have been described in humans: the UBE1 or UBA1 enzyme and the UBE1L2 or UBA6 enzyme. The second step allows the transfer of the activated ubiquitin to the thiol group of the active cysteine of the conjugating enzyme E2. Approximately 40 E2 enzymes

have been identified in mammals. In the third step, the E3 ligase enzyme allows the transfer of ubiquitin molecules to the target protein via a covalent isopeptide bond between the C-terminal glycine residue (G76) of the ubiquitin molecule and a lysine residue of the target protein. Nearly 700 E3 enzymes have been identified in mammals, and E3 enzymes are schematically classified into two main categories: E3 ligases that form a thioester bond with ubiquitin molecule and participate directly in the transfer of ubiquitin to the substrate (HECT family), and those that act as an adaptor between the E2s and the substrates in order to promote the transfer of ubiquitin (E3 RING (Really Interesting New Gene) and the U-BOX) [15–17].

The diversity of enzymes illustrates the complexity of ubiquitination mechanisms. Ubiquitin contains seven lysine residues (K6, K11, K27, K29, K33, K48, and K63) and a methionine (M1) at its N-terminus, which lead to the formation of many types of ubiquitination. Thus, mono-ubiquitination occurs when a single ubiquitin molecule binds the target protein, multi-ubiquitination occurs when multiple ubiquitin molecules bind the protein at different lysine residues, and poly-ubiquitination occurs when a poly-ubiquitin chain binds one lysine residue of the target protein (Figure 1B) [6,18–20]. The fate of a protein modified by ubiquitin molecules depends, in part, on the nature of the ubiquitination or the length and type of ubiquitin chains formed. For example, mono-ubiquitination appears to be involved in various cellular processes, including endocytosis, histone regulation, DNA repair, and protein transport. The best-characterized ubiquitin polymers to date are the K48 and K63 type chains in which the ubiquitin molecules are linked via their lysine 48 or 63, respectively. The K48 chains induce the degradation of the targeted protein by the proteasome. On the other hand, the attachment of K63 chains has a role in the activation of proteins involved in different cellular processes such as DNA repair, endocytosis, or intracellular signalling. K63 chains also play a particularly important role in the regulation of the activation of proteins involved in NF- $\kappa$ B (nuclear factor-kappa B) signalling pathways (Figure 1C) [21–26]. This type of ubiquitin chain exerts its regulatory function in particular in the assembly and stability of kinase complexes of NF- $\kappa$ B cascade [27].

### **3. Deubiquitination**

#### *3.1. Deubiquitinases (DUBs)*

About 100 DUBs encoded by the human genome have been identified. According to the structure of their catalytic domain, DUBs are classified into six families: ubiquitin-specific proteases (USP), ubiquitin C-terminal hydrolases (UCH), ovarian tumour domain proteases (OTU), Machado-Joseph domain proteases (MJD), JAB1/MPN/Mov37 metalloproteases (JAMM), and a recently identified family of DUBs: the motif interacting with ubiquitin (MIU)-containing novel DUB family (MINDY) (Figure 2A) [28].

The DUB families can be classified into two classes according to their catalytic mechanism: USPs, UCHs, OTUs, MJDs, and MCPiPs are cysteine proteases, whose enzymatic activity depends on the thiol group of cysteine in their active site, and zinc metalloproteases JAMM (Figure 2B) [28–30].

### *3.2. Ubiquitin-Specific Proteases*

The USP family (UBP in yeast) is the largest family of DUBs (Table 1). Yeast has 16 USP/UBP family members, while humans are thought to have more than 50 [28,29]. USPs belong to the family of cysteine proteases whose enzymatic activity resides on the thiol group of a central cysteine. One of the main characteristics of these enzymes is the presence of two short and conserved motifs, the "histidine box" and the "cysteine box" containing the residues involved in ubiquitin proteolysis [31]. Hydrolysis is a three-step mechanism combining enzyme/substrate binding, acylation, and deacylation steps (Figure 2C).

Despite a strong homology between USPs at the cysteine and histidine boxes, the 3D structures of USPs show distinct differences in the accessibility of the catalytic pocket. The first X-ray structure of a USP protein was that of USP7 [32,33]. Experiments indicate that the hydrolysis of bound substrates by USP7 is controlled by three domains: the finger, the palm, and the thumb, with the active site located in a deep catalytic cleft between the palm and the thumb while the ubiquitin moiety is coordinated by the finger [34].

## **4. USPs and cancer**

This section focuses on the role of USPs in cancer development by examining some key functions in primary tumour development such as cell proliferation or death, and the modulation of signalling pathways involved in the tumour metastatic process, such as the TGF- $\beta$  signalling cascade.

#### 4.1. USPs and cell proliferation

The control of cell proliferation is a major event in the development of cancers, in particular, by controlling the growth of the primary tumour. In this context, several USPs have been identified as participating in the control of cell proliferation through various mechanisms of action. For example, USP1, by interacting with the ribosomal protein RPS16 (ribosomal protein 16), participates in the control of liver cancer cell proliferation and USP1 knockdown reduces hepatic tumour growth *in vivo* [35]. In breast cancer, USP1 appears to be involved in controlling cell proliferation via ER $\alpha$  (oestrogen receptor  $\alpha$ ) activity [36] or regulation of the Hippo/TAZ (Hippo/transcriptional coactivator PDZ-binding motif) pathway [37]. USP10 has also been identified as being able to regulate the cell cycle via the Hippo/YAP/TAZ (Hippo/yes-associated protein/TAZ) cascade as, for example, in hepatocellular carcinoma cells [38]. Early work involving USP2 in cell proliferation showed that USP2 regulates the p53 pathway by interacting with MDM2 (murine double minute 2) [39]. Gu and Coll also showed that USP2 specifically targets cyclin D1 [40]. In this regard, the use of a small molecule inhibitor of USP2, ML364, results in the cell cycle arrest of colorectal cancer cells [41]. In addition, cisplatin treatment of testicular embryonal carcinoma cells results in decreased USP2 expression and induction of the cell cycle inhibitor p21 [42]. USP3 has recently been shown to promote cell proliferation by targeting RBM4 (RNA binding motif 4) [43] or KLF5 (Krüppel-like factor 5) [44] in non-small cell lung and breast cancer cells, respectively. USP5 stimulates ovarian cancer cell proliferation via the deubiquitination of HDAC2 (histone deacetylase 2) [45] or the modulation of cycle regulators in pancreatic cancer cells [46]. It has been shown that USP7 promotes the proliferation of many cancer cells such as hepatoblastoma cells [47], renal cancer cells [48], or papillary thyroid carcinoma cells [49], for example. USP7 seems to act on cell proliferation via various mechanisms, such as via the stabilization of Ki67 in non-small cell lung cancer cells [50], the PI3K/AKT (phosphatidylinositol 3-kinase/protein kinase B) signalling pathway in hepatoblastoma [47], ARMC5 (armadillo repeat containing 5) stabilization in renal cancer cells [48], or p57(KIP2) repression [49]. Other USPs-besides USP7 seem

to regulate cell proliferation via AKT or MAPK (microtubule-associated protein kinase) signalling pathways such as USP10 or USP12 in hepatocellular carcinoma [51–53], USP13 in lung cancer [54], USP18 in cervical cancer [55], USP21 in hepatocellular carcinoma [56], or USP46 in lung cancer [57]. Another major mechanism of action of USPs in cell cycle regulation appears to be the ability of some to regulate the expression of c-Myc, an oncogene involved in cell cycle control. For example, USP2, USP22, USP28, and USP37 stabilize c-Myc expression and, thus, stimulate the proliferation of many tumour cells [58–61].

Interestingly, it has been shown that some USPs are able to target some oncogenes produced as a result of chromosomal rearrangements leading to the expression of fusion genes. For example, BCR-ABL known to be a major oncogene in chronic myelogenous leukaemia is the target of USP25 and USP7. It has been shown that USP25 or USP7 silencing leads to increased levels of ubiquitinated BCR-ABL, stimulates BCR-ABL degradation and thus reduces leukaemia cells proliferation or death by apoptosis [62,63]. Off note, the role of USPs in the stability of the EWS-FLI1 fusion protein is discussed in the chapter on paediatric bone tumours.

#### *4.2. USPs and apoptosis*

USPs regulate various factors related to apoptosis. In particular, it has been shown in recent years that different USPs are closely associated with the p53/MDM2 signalling pathway.

The p53 protein regulates cell death by apoptosis [64,65] schematically through its ability to interact with different members of the Bcl-2 (B-cell lymphoma 2) family or by regulating the expression of genes encoding anti- or pro-apoptotic proteins involved in the main apoptosis signalling pathways as well as the intrinsic or extrinsic pathways.

In this context, several USPs have been shown to regulate p53 degradation and, thus, apoptosis [66]. For example, USP2 and USP7 stabilize MDM2 to degrade p53, resulting in an anti-apoptotic phenotype. USP7, one of the most studied USPs in this context, has been shown to play a key role in preventing the ubiquitination of MDM2 and MDM4 (murine double minute 4) and, thus, prevents their degradation by the proteasome. In many cancers, inhibition of USP7 leads to cell cycle arrest and cell death by apoptosis [67–69]. Thus, USP7 has been identified as a potential anti-cancer target in various tumours. For example, the compound XL188, a potent and selective USP7 inhibitor, induces

p53-dependent growth suppression in Ewing's sarcoma cell lines [70]. Other USPs participate in the regulation of apoptosis via the p53 axis. For example, USP10 can interact with G3BP2 (Ras GTPase-activating protein-binding protein 2) to block p53-signalling and, thus, contributes to a poor prognosis in prostate cancer [71]. Furthermore, Beclin1 controls p53 levels by regulating the activity of USP10 [72]. USP15 stabilizes MDM2 to mediate cancer cell survival and inhibit antitumor T-cell responses [73]. USP22 promotes non-small cell lung cancer and retinoblastoma tumorigenesis via p53 inhibition [74,75]. As another example, knocking down USP39 inhibits non-small cell lung cancer cell proliferation by activating the p53 pathway [76].

#### *4.3. USPs and metastatic development*

One of the key events in metastatic development is the epithelial-mesenchymal transition (EMT). This process, which promotes cancer cell invasiveness and contributes to the development of CTCs (circulating tumour cells) [77,78], involves molecular and cellular changes, including a loss of intercellular interactions associated with a loss or decrease in the expression of intercellular junction components such as E-cadherin, claudins, occludins, and desmosomes. In parallel, an increase in the expression of mesenchymal markers, such as N-cadherin, fibronectin, and vimentin, is observed. These gene expression changes are regulated by different transcription factors, such as Snail-1, Snail-2 (Slug), ZEB-1 (zinc finger E-box binding homeobox 1), and ZEB-2 (zinc finger E-box binding homeobox 2), or Twist [78,79].

Several USPs have been described as participating in the control of EMT. For example, USP3, USP11, and USP29 stimulate EMT in glioblastoma cells, as well as in ovarian or gastric cancer cells, respectively, by stabilizing Snail [80–82]. USP22 regulates the ability of colon cancer cells to migrate via EMT stimulation [83]. USP34 and USP37 stimulate EMT in mammary epithelial cells [84,85]. USP17 and USP39 stimulate EMT in ovarian cancer cells and, thus, their ability to migrate [86,87].

One of the main mechanisms by which USPs stimulate metastatic development is their ability to modulate various pro-metastatic signalling pathways, such as the TGF- $\beta$  (transforming growth factor beta) pathway. Schematically, activated TGF- $\beta$  binds to two membrane receptors: type I (TBR1) and type II (TBR2). This binding induces a signalling cascade via Smads proteins. The activated TBR1 receptor phosphorylates the receptor-regulated Smads (R-Smads), including Smad2 and Smad3,



which then associate with Smad4. This protein complex is translocated into the nucleus and regulates the expression of target genes. TGF- $\beta$  signalling can be controlled by various inhibitory mechanisms. Among them, Smad7 is able to recruit E3-ubiquitin ligases to the activated TBRI, leading to receptor degradation [88,89]. Historically, the two USPs identified as able to modulate the TGF- $\beta$  pathway are USP4 and USP15 [90–92]. For example, USP4 drives breast cancer cell invasion via Relaxin/TGF- $\beta$ 1/Smad2/MMP-9 (matrix metalloproteinase 9) signalling [93] and promotes hepatocellular carcinoma metastasis by enhancing TGF- $\beta$  signalling-induced EMT [94]. Schematically, these two USPs directly deubiquitinylate TBRI and drive TGF- $\beta$  responsiveness (Figure 3) [95,96]. It may be noted that USP4 modulates the pathways of various TGF- $\beta$  family members, such as BMPs (Bone morphogenetic proteins) and activin, by directly ubiquitinating Smad4 and, thus, preventing its degradation [97]. Other USPs besides USP4 and USP15 have been identified as able to modulate the TGF- $\beta$  response. For example, USP47 stimulates TGF- $\beta$ -induced EMT in mammary epithelial cells [98].

## **5. Paediatric primary bone tumours: Osteosarcoma and Ewing's sarcoma**

Paediatric bone sarcomas belong to a family of very heterogeneous tumours of bone mesenchymal origin. These tumours represent less than 0.2% of the malignant tumours registered in the EURO CARE database and are considered rare cancers and orphan tumours [99]. These tumours are mainly represented by osteosarcoma (OS) and Ewing's sarcoma (ES) (Table 2).

### *5.1. Osteosarcoma*

OS is the most common primary bone tumour in children, adolescents, and young adults and is the third most common in adults [100,101]. The overall incidence is 3.4 per million per year worldwide [102]. OS primarily affects children and young adults between 10 and 30 years. Specifically, OS is characterized by a two-peak age distribution with the first peak at 15-19 years (8 cases/million/year) and the second at 75-79 years (6 cases/million/year) [103]. The first peak of OS incidence corresponds to the period of growth during puberty, suggesting a close relationship between rapid growth and disease development. Different degrees of differentiation of OS cells can be found in this pathology. In general, there seems to be an inverse correlation between the degree of differentiation

and the aggressiveness of the tumours. Among paediatric cancers, OS ranks eighth after lymphomas and brain tumours.

This malignant bone tumour is characterized by the formation of an osteoid matrix or immature bone most commonly at the metaphysis and diaphysis of long bones such as the femur, tibia, and humerus [104]. Three major histological subtypes of OS have been identified: osteoblastic (50%), chondroblastic (25%), and fibroblastic (25%). OS presents with a wide variety of lesions that are distinct in their clinical and radiographic presentation, microscopic appearance, and evolution, leading to the definition of this pathology as a highly heterogeneous tumour. Areas of osteoformation may be accompanied by significant areas of osteolysis, highlighting a very heterogeneous tumour [105].

Although the cell of OS origin is not clearly defined, many studies suggest that OS arises from mesenchymal stem cells (MSC) differentiated or not into osteoblasts. The expression of osteoblastic markers such as RUNX2 (Runt-related transcription factor 2), ALP (alkaline phosphatase), OCN (osteocalcin), and BSP (bone sialoprotein) by OS cells suggests that this tumour originates from the deregulation of the MSC differentiation program [106–108]. Regardless of the cellular origin, OS cells are genetically unstable cells characterized by a complex karyotype and a high frequency of genetic alterations with copy number variations or multiple fusion sequences in chromosomes [109]. For example, whole-genome sequencing analyses have shown that *p53* and *Rb1* (retinoblastoma 1) have recurrent somatic alterations in concordant studies, suggesting that they may be key players in the oncogenesis of these tumours [110–114]. Another factor in genomic instability is alternative telomere lengthening (ALT), which prevents telomere shortening and induces senescence [115].

Although the exact origin of OS remains unknown, various pathologies may be involved in the origin and development of OS [116]. Among these predispositions, we can mention the Li-Fraumeni syndrome that leads to the development of multiple tumours, like—such as OS [117,118]. Retinoblastoma linked to a mutation of both alleles of the *Rb* gene is also predisposing for OS, occurring 100 times more frequently than in the general population [119]. Rothmund-Thomson syndrome, linked to a mutation on chromosome 8, is characterized by an increased risk of OS [120]. Paget's disease, which combines abnormalities of bone architecture and marrow fibrosis, also increases the risk of developing OS as a result of pathological changes in bone remodelling [121].

One of the major problems is that OS is an aggressive tumour, with up to 20% of patients developing metastases at diagnosis, most often to the lungs. Bone and lymph node metastases may

also develop. In addition, it has been estimated that undetectable metastases at diagnosis are present in 80% of cases, particularly in the lungs.

The current curative treatment combines surgical resection with preoperative and postoperative treatment using, classically, four chemotherapy agents: cisplatin, doxorubicin, methotrexate, and ifosfamide [122]. Although this conventional treatment has led to a significant increase in patient survival in the 1980s, no effective treatment option is yet available for metastatic or chemotherapy-refractory OS [123]. While the five-year survival rate is greater than 78% in the absence of metastases at diagnosis or for good responders, this survival rate drops to 25% for metastatic or recurrent OS [124–127]. For isolated lung metastases, surgery is the standard treatment and should involve complete resection of all metastases [122,128].

## *5.2. Ewing's sarcoma*

ES was first described by James Ewing in a 14-year-old girl as a new bone tumour entity called "diffuse endothelioma of bone" [129].

ES is the second most common primary malignant bone tumour after OS with an incidence of 1.5 cases per million, which has remained unchanged for decades [130]. This tumour primarily affects children, adolescents, and young adults. The median age of patients with ES is 15 years [131], with approximately 30% of cases occurring in children younger than 10 years, and 30% in adults older than 20 years [132]. A few cases of ES have been described in newborn children [133,134]. Men appear to be more affected than women, with a sex ratio of 3:2 [130]. Although a few rare familial cases have been noted [135], this disease is not associated with a strong hereditary predisposition. To date, no environmental exposures have been linked to the oncogenesis of ES [131,136]. Nevertheless, ES is much more common in Caucasians and is almost absent in the African population [130,137,138].

Approximately 85% of ES are located in the bones, mainly in the diaphysis of long bones such as femur, tibia, fibula, or humerus. They can also be located in pelvic bones and the chest cavity with variable frequency [139]. In about 20% of cases, extra-osseous tumours may occur in many organs. These extra-osseous SE are more common in adults than in children [140].

ES development is associated with a chromosomal translocation between a member of the RNA-binding protein family FET and a member of the ETS transcription factor family. The first translocation,

t(11;22)(q24;q12), was identified in 1983 [141,142]. In 85% of cases, this translocation gives rise to the transcription factor EWS-FLI1 [141–143]. In about 10% of cases, a translocation between EWS and ERG, t(21;22)(q22;q12), is observed [144–149]. EWS-FLI1 binds to DNA through the DNA-binding domain of FLI1. FLI1 and EWS-FLI1 bind to the same canonical ETS binding motif consisting of a GGAA site [150–152]. Thus, EWS-FLI1 can bind to GGAA sequences located on GGAA microsatellites [151–153]. The binding of EWS-FLI1 to DNA modulates the expression of target genes involved in cell proliferation and metastatic dissemination [131,136].

ES cellular origin is not established and remains contested, with two hypotheses suggesting an origin of primary cells from either the neural crest or MSCs. ES cells have thus been shown to express neural crest stem cell markers such as CD57, ENO2 (Enolase 2), and various Notch pathway genes [154–156]. Furthermore, expression of EWS-FLI1 induces a neural crest-like phenotype and abrogates the existing cell differentiation program [157,158]. Furthermore, expression of EWS-FLI1 in MSCs blocks their differentiation, demonstrating the role of EWS-FLI1 on the highly undifferentiated status of ES cells [159]. Ectopic expression of EWS-FLI1 in murine MSCs led to cell transformation and development of tumours with ES-like properties, such as CD99 expression [160,161]. In addition, comparison of transcriptomic profiles of ES cells depleted for EWS-FLI1 possess MSC properties capable of differentiating into adipocytes or osteoblasts [162].

Like OS, ES is a very aggressive tumour. Approximately 25% of patients develop metastases at the time of diagnosis. The presence of metastases is one of the major factors associated with a very poor survival rate. Only 20% of patients with metastases at diagnosis survive after five years, compared to 70% for patients with localized tumours [163–165]. Patient survival rates depend on other factors such as response to chemotherapy, tumour site and volume, patient age, and extent and severity of disease [166–168].

Treatment for ES includes neoadjuvant chemotherapy, surgery, and/or radiation therapy. Chemotherapy is given before surgery to reduce the overall size of the tumour and target potential micro-metastases. Conventional chemotherapy in Europe combines ifosfamide, doxorubicin, and etoposide [169,170]. To date, studies using immunotherapy have been disappointing. Indeed, ES is considered a "cold tumour" due to a very small fraction of tumours showing immune infiltration [171]. Furthermore, ES cells do not express the PDL1 (programmed cell death 1 ligand 1) molecule [172,173].

## 6. USPs and paediatric primary bone tumours

Regarding OS, the most studied USP is USP1. Following immunohistochemistry on thirty biopsies from patients with OS, Liu and Coll showed that this USP is overexpressed in tumour tissues compared to its expression in healthy tissues. By silencing USP1, the authors showed a decrease in the proliferation of U2OS OS cells and a decrease in their ability to form colonies in the absence of anchoring (soft agar). The authors associated these results with a decrease in the protein level of different actors able to directly or indirectly modulate cell proliferation, such as SIK2 (salt inducible kinase 2), MMP-2 (matrix metalloproteinase 2), GSK3 $\beta$  (glycogen synthase kinase 3 beta), Bcl-2 (B-cell lymphoma 2), Stat3 (signal transducer and activator of transcription 3), cyclin E1 or A1, Notch1, and Wnt-1 [174]. In a more recent study, Zhou and Coll showed that miR-192-5p, whose expression was significantly decreased in 25 biopsies from OS patients and in the OS cell lines U2OS and 143B, directly target USP1. Taken together, the authors showed that miR-192-5p could suppress OS cell proliferation and migration by targeting USP1 and, therefore, that the miR-192-5p/USP1 axis may function as a novel therapeutic target in OS. Interestingly, it appears that USP1 is also involved in the response of OS cells to cisplatin [175]. Apart from an action on the proliferation and migration of OS cells, it seems that USP1 is able to stimulate the maintenance of the undifferentiated state of OS cells. Indeed, Williams and Coll showed that USP1, by binding to DNA binding inhibitors (IDIs), promotes their stability and, thus, stem cell-like characteristics in OS. Therefore, USP1 silencing in OS cells leads to the decreased stability of IDIs proteins and increased osteoblastic differentiation. Conversely, ectopic expression of USP1 in MSCs stabilizes IDIs proteins and inhibits osteoblastic differentiation. The authors concluded that the involvement of USP1 in the preservation of the stem cell state of OS identifies USP1 as a target for potential therapy [176]. It seems that USP1, by acting on the proliferation, migration, or differentiation of OS cells, could constitute a target for the definition of a new treatment. In this context, Liang and Coll showed in an earlier study that ML323, a USP1 inhibitor, potentiates cisplatin cytotoxicity in OS cells [177]. In addition to USP1, the role of other USPs has been studied in OS. For example, Zeng and Coll demonstrated that USP7 expression is increased in OS tumour tissues compared to the expression measured in surrounding healthy tissues. Moreover, this expression is correlated with the stage of the disease and the presence of metastasis.

Furthermore, USP7 silencing results in the decreased ability of OS cells to migrate and invade. The authors linked these properties of USP7 to modulate the response of cells to the Wnt/ $\beta$ -catenin pathway and to the ability of this signalling cascade to modulate EMT [178]. Chen and Coll showed that USP9x knockdown inhibits OS cell proliferation and colony formation. The authors associated this effect with the ability of USP9 to control SOX2 (SRY-box transcription factor 2) stability [179]. Zhang and Coll demonstrated that USP22 greatly increased expression in OS tissues and cell lines. USP22 silencing inhibits the proliferation, invasion, and EMT of OS cells *in vitro*. In addition, USP22 silencing blocks OS tumour growth and metastasis *in vivo*. The authors linked these properties to the ability of USP22 to modulate the PI3K/Akt signalling pathway [180]. As for USP1, it seems that USP22 is the target of miRNA. Thus, in 65 OS samples, Liu and Coll showed an increase in USP22 expression associated with a decrease in miR-140 expression. In addition, overexpression of miR-140 inhibits the proliferation, migration, and invasion of OS cells by directly targeting USP22, suggesting that the miR-140/USP22 axis may represent a novel therapeutic target in OS [181] (Figure 4). Finally, Gan and Coll showed that deletion of USP39 in U2OS cells reduces cell proliferation and blocks their ability to form colonies. The authors associated the effects of USP39 silencing with the arrest of OS cells in the G2/M phase and the induction of apoptosis of U2OS cells by cleavage of PARP [182]. Very recently, Lavaud and Coll identified four new USPs, USP6, USP27x, USP41, and USP43, whose expression is increased in HOS, U2OS, and MG63 cell lines. Interestingly, Kaplan-Meier analysis shows that the expression of USP6 and USP41 is correlated with patient survival. Finally, *in vivo* experiments using a preclinical OS model demonstrate that PR619, a pan USP inhibitor, reduces primary OS tumour growth and the development of lung metastases [183].

Concerning ES, the literature is very limited. Two studies mention the role of USPs in the development of ES. Henrich and Coll showed that USP6 modulates the IFN (interferon) response in ES cells by potentiating the activation of STAT1 and STAT3 [184]. Interestingly, Gierisch and Coll showed that USP19 regulates the stability of EWS-FLI1. Indeed, the extinction of USP19 leads to a decrease of EWS-FLI1 protein levels and a reduction of the activity of the protein. In addition, USP19 silencing leads to a decrease in cell growth and colony-forming ability *in vitro* and delays tumour growth *in vivo* [185].

## **7. Conclusion and perspectives**

Accumulating evidence has thus demonstrated the relevance of targeting USPs in ES and OS. Depending on the protein targeted, targeting this family of ubiquitin proteases could inhibit not only primary tumour growth but also metastatic development. Indeed, it appears that some USPs, whose expression is increased in the OS, participate in the control of OS cells proliferation and their migration, and invasion capacities. Some mechanisms of action of these USPs have been identified, such as the ability to modulate some signalling pathways involved in OS development such as the Wnt pathway [186] or the TGF- $\beta$  pathway [88,89]. In addition to metastatic development, one of the major problems in the treatment of OS and ES is a resistance to chemotherapy that prevents complete remission in some patients. In this context, it is interesting to note that the silencing of certain USPs potentiates the response of OS to chemotherapies such as cisplatin.

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## References

- [1] D.H. Schlesinger, G. Goldstein, Molecular conservation of 74 amino acid sequence of ubiquitin between cattle and man, *Nature*. 255 (1975) 423–424. <https://doi.org/10.1038/255423a0>.
- [2] A. Hershko, A. Ciechanover, H. Heller, A.L. Haas, I.A. Rose, Proposed role of ATP in protein breakdown: conjugation of protein with multiple chains of the polypeptide of ATP-dependent proteolysis, *Proc Natl Acad Sci U S A*. 77 (1980) 1783–1786. <https://doi.org/10.1073/pnas.77.4.1783>.
- [3] A. Varshavsky, The early history of the ubiquitin field, *Protein Sci*. 15 (2006) 647–654. <https://doi.org/10.1110/ps.052012306>.
- [4] A. Hershko, A. Ciechanover, The ubiquitin system, *Annu Rev Biochem*. 67 (1998) 425–479. <https://doi.org/10.1146/annurev.biochem.67.1.425>.
- [5] X.-J. Yang, Multisite protein modification and intramolecular signaling, *Oncogene*. 24 (2005) 1653–1662. <https://doi.org/10.1038/sj.onc.1208173>.
- [6] L. Hicke, Protein regulation by monoubiquitin, *Nat Rev Mol Cell Biol*. 2 (2001) 195–201. <https://doi.org/10.1038/35056583>.
- [7] D. Komander, M.J. Clague, S. Urbé, Breaking the chains: structure and function of the deubiquitinases, *Nat Rev Mol Cell Biol*. 10 (2009) 550–563. <https://doi.org/10.1038/nrm2731>.
- [8] A.Y. Amerik, M. Hochstrasser, Mechanism and function of deubiquitinating enzymes, *Biochim Biophys Acta*. 1695 (2004) 189–207. <https://doi.org/10.1016/j.bbamcr.2004.10.003>.
- [9] T.E.T. Mevissen, D. Komander, Mechanisms of Deubiquitinase Specificity and Regulation, *Annu Rev Biochem*. 86 (2017) 159–192. <https://doi.org/10.1146/annurev-biochem-061516-044916>.
- [10] M.J. Clague, S. Urbé, D. Komander, Breaking the chains: deubiquitylating enzyme specificity begets function, *Nat Rev Mol Cell Biol*. 20 (2019) 338–352. <https://doi.org/10.1038/s41580-019-0099-1>.
- [11] C.M. Pickart, D. Fushman, Polyubiquitin chains: polymeric protein signals, *Curr Opin Chem Biol*. 8 (2004) 610–616. <https://doi.org/10.1016/j.cbpa.2004.09.009>.
- [12] Y. Kimura, K. Tanaka, Regulatory mechanisms involved in the control of ubiquitin homeostasis, *J Biochem*. 147 (2010) 793–798. <https://doi.org/10.1093/jb/mvq044>.
- [13] K. Husnjak, I. Dikic, Ubiquitin-binding proteins: decoders of ubiquitin-mediated cellular functions, *Annu Rev Biochem*. 81 (2012) 291–322. <https://doi.org/10.1146/annurev-biochem-051810-094654>.
- [14] C.M. Pickart, M.J. Eddins, Ubiquitin: structures, functions, mechanisms, *Biochim Biophys Acta*. 1695 (2004) 55–72. <https://doi.org/10.1016/j.bbamcr.2004.09.019>.
- [15] S.J.L. van Wijk, H.T.M. Timmers, The family of ubiquitin-conjugating enzymes (E2s): deciding between life and death of proteins, *FASEB J*. 24 (2010) 981–993. <https://doi.org/10.1096/fj.09-136259>.
- [16] Y. Sun, E3 ubiquitin ligases as cancer targets and biomarkers, *Neoplasia*. 8 (2006) 645–654. <https://doi.org/10.1593/neo.06376>.
- [17] B.A. Schulman, J.W. Harper, Ubiquitin-like protein activation by E1 enzymes: the apex for downstream signalling pathways, *Nat Rev Mol Cell Biol*. 10 (2009) 319–331. <https://doi.org/10.1038/nrm2673>.
- [18] C.M. Pickart, Ubiquitin enters the new millennium, *Mol Cell*. 8 (2001) 499–504. [https://doi.org/10.1016/s1097-2765\(01\)00347-1](https://doi.org/10.1016/s1097-2765(01)00347-1).
- [19] C.M. Pickart, Mechanisms underlying ubiquitination, *Annu Rev Biochem*. 70 (2001) 503–533. <https://doi.org/10.1146/annurev.biochem.70.1.503>.
- [20] M. Hochstrasser, Lingering mysteries of ubiquitin-chain assembly, *Cell*. 124 (2006) 27–34. <https://doi.org/10.1016/j.cell.2005.12.025>.
- [21] I. Martinez-Forero, A. Rouzaut, A. Palazon, J. Dubrot, I. Melero, Lysine 63 polyubiquitination in immunotherapy and in cancer-promoting inflammation, *Clin Cancer Res*. 15 (2009) 6751–6757. <https://doi.org/10.1158/1078-0432.CCR-09-1225>.
- [22] R. Varadan, M. Assfalg, A. Haririnia, S. Raasi, C. Pickart, D. Fushman, Solution conformation of Lys63-linked di-ubiquitin chain provides clues to functional diversity of polyubiquitin signaling, *J Biol Chem*. 279 (2004) 7055–7063. <https://doi.org/10.1074/jbc.M309184200>.
- [23] Z.J. Chen, L.J. Sun, Nonproteolytic functions of ubiquitin in cell signaling, *Mol Cell*. 33 (2009) 275–286. <https://doi.org/10.1016/j.molcel.2009.01.014>.
- [24] B.A. Malynn, A. Ma, Ubiquitin makes its mark on immune regulation, *Immunity*. 33 (2010) 843–852. <https://doi.org/10.1016/j.immuni.2010.12.007>.

- [25] K.D. Wilkinson, Ubiquitin: a Nobel protein, *Cell*. 119 (2004) 741–745. <https://doi.org/10.1016/j.cell.2004.12.001>.
- [26] J. Giles, Chemistry Nobel for trio who revealed molecular death-tag, *Nature*. 431 (2004) 729. <https://doi.org/10.1038/431729a>.
- [27] V.G. Bhoj, Z.J. Chen, Ubiquitylation in innate and adaptive immunity, *Nature*. 458 (2009) 430–437. <https://doi.org/10.1038/nature07959>.
- [28] S.M.B. Nijman, M.P.A. Luna-Vargas, A. Velds, T.R. Brummelkamp, A.M.G. Dirac, T.K. Sixma, R. Bernards, A genomic and functional inventory of deubiquitinating enzymes, *Cell*. 123 (2005) 773–786. <https://doi.org/10.1016/j.cell.2005.11.007>.
- [29] F.E. Reyes-Turcu, K.D. Wilkinson, Polyubiquitin binding and disassembly by deubiquitinating enzymes, *Chem Rev*. 109 (2009) 1495–1508. <https://doi.org/10.1021/cr800470j>.
- [30] M.J. Clague, I. Barsukov, J.M. Coulson, H. Liu, D.J. Rigden, S. Urbé, Deubiquitylases from genes to organism, *Physiol Rev*. 93 (2013) 1289–1315. <https://doi.org/10.1152/physrev.00002.2013>.
- [31] Y. Ye, H. Scheel, K. Hofmann, D. Komander, Dissection of USP catalytic domains reveals five common insertion points, *Mol Biosyst*. 5 (2009) 1797–1808. <https://doi.org/10.1039/b907669g>.
- [32] M. Hu, P. Li, M. Li, W. Li, T. Yao, J.-W. Wu, W. Gu, R.E. Cohen, Y. Shi, Crystal structure of a UBP-family deubiquitinating enzyme in isolation and in complex with ubiquitin aldehyde, *Cell*. 111 (2002) 1041–1054. [https://doi.org/10.1016/s0092-8674\(02\)01199-6](https://doi.org/10.1016/s0092-8674(02)01199-6).
- [33] M. Hu, P. Li, L. Song, P.D. Jeffrey, T.A. Chenova, K.D. Wilkinson, R.E. Cohen, Y. Shi, Structure and mechanisms of the proteasome-associated deubiquitinating enzyme USP14, *EMBO J*. 24 (2005) 3747–3756. <https://doi.org/10.1038/sj.emboj.7600832>.
- [34] L. Daviet, F. Colland, Targeting ubiquitin specific proteases for drug discovery, *Biochimie*. 90 (2008) 270–283. <https://doi.org/10.1016/j.biochi.2007.09.013>.
- [35] Y. Liao, Z. Shao, Y. Liu, X. Xia, Y. Deng, C. Yu, W. Sun, W. Kong, X. He, F. Liu, Z. Guo, G. Chen, D. Tang, H. Gan, J. Liu, H. Huang, USP1-dependent RPS16 protein stability drives growth and metastasis of human hepatocellular carcinoma cells, *J Exp Clin Cancer Res*. 40 (2021) 201. <https://doi.org/10.1186/s13046-021-02008-3>.
- [36] Z. Niu, X. Li, S. Feng, Q. Huang, T. Zhuang, C. Yan, H. Qian, Y. Ding, J. Zhu, W. Xu, The deubiquitinating enzyme USP1 modulates ER $\alpha$  and modulates breast cancer progression, *J Cancer*. 11 (2020) 6992–7000. <https://doi.org/10.7150/jca.50477>.
- [37] A. Mussell, H. Shen, Y. Chen, M. Mastro, K.H. Eng, W. Bshara, C. Frangou, J. Zhang, USP1 Regulates TAZ Protein Stability Through Ubiquitin Modifications in Breast Cancer, *Cancers (Basel)*. 12 (2020) E3090. <https://doi.org/10.3390/cancers12113090>.
- [38] H. Zhu, F. Yan, T. Yuan, M. Qian, T. Zhou, X. Dai, J. Cao, M. Ying, X. Dong, Q. He, B. Yang, USP10 Promotes Proliferation of Hepatocellular Carcinoma by Deubiquitinating and Stabilizing YAP/TAZ, *Cancer Res*. 80 (2020) 2204–2216. <https://doi.org/10.1158/0008-5472.CAN-19-2388>.
- [39] L.F. Stevenson, A. Sparks, N. Allende-Vega, D.P. Xirodimas, D.P. Lane, M.K. Saville, The deubiquitinating enzyme USP2a regulates the p53 pathway by targeting Mdm2, *EMBO J*. 26 (2007) 976–986. <https://doi.org/10.1038/sj.emboj.7601567>.
- [40] J. Shan, W. Zhao, W. Gu, Suppression of cancer cell growth by promoting cyclin D1 degradation, *Mol Cell*. 36 (2009) 469–476. <https://doi.org/10.1016/j.molcel.2009.10.018>.
- [41] M.I. Davis, R. Pragani, J.T. Fox, M. Shen, K. Parmar, E.F. Gaudio, L. Liu, C. Tanega, L. McGee, M.D. Hall, C. McKnight, P. Shinn, H. Nelson, D. Chattopadhyay, A.D. D'Andrea, D.S. Auld, L.J. DeLucas, Z. Li, M.B. Boxer, A. Simeonov, Small Molecule Inhibition of the Ubiquitin-specific Protease USP2 Accelerates cyclin D1 Degradation and Leads to Cell Cycle Arrest in Colorectal Cancer and Mantle Cell Lymphoma Models, *J Biol Chem*. 291 (2016) 24628–24640. <https://doi.org/10.1074/jbc.M116.738567>.
- [42] T. Bonacci, M.J. Emanuele, Dissenting degradation: Deubiquitinases in cell cycle and cancer, *Semin Cancer Biol*. 67 (2020) 145–158. <https://doi.org/10.1016/j.semcancer.2020.03.008>.
- [43] X.-H. Liao, Y. Wang, B. Zhong, S.-Y. Zhu, USP3 promotes proliferation of non-small cell lung cancer through regulating RBM4, *Eur Rev Med Pharmacol Sci*. 24 (2020) 3143–3151. [https://doi.org/10.26355/eurrev\\_202003\\_20681](https://doi.org/10.26355/eurrev_202003_20681).
- [44] Y. Wu, J. Qin, F. Li, C. Yang, Z. Li, Z. Zhou, H. Zhang, Y. Li, X. Wang, R. Liu, Q. Tao, W. Chen, C. Chen, USP3 promotes breast cancer cell proliferation by deubiquitinating KLF5, *J Biol Chem*. 294 (2019) 17837–17847. <https://doi.org/10.1074/jbc.RA119.009102>.
- [45] Y. Du, J. Lin, R. Zhang, W. Yang, H. Quan, L. Zang, Y. Han, B. Li, H. Sun, J. Wu, Ubiquitin specific peptidase 5 promotes ovarian cancer cell proliferation through deubiquitinating HDAC2, *Aging (Albany NY)*. 11 (2019) 9778–9793. <https://doi.org/10.18632/aging.102425>.

- [46] B.P. Kaistha, A. Krattenmacher, J. Fredebohm, H. Schmidt, D. Behrens, M. Widder, T. Hackert, O. Strobel, J.D. Hoheisel, T.M. Gress, M. Buchholz, The deubiquitinating enzyme USP5 promotes pancreatic cancer via modulating cell cycle regulators, *Oncotarget*. 8 (2017) 66215–66225. <https://doi.org/10.18632/oncotarget.19882>.
- [47] M. Ye, J. He, J. Zhang, B. Liu, X. Liu, L. Xie, M. Wei, R. Dong, K. Li, D. Ma, K. Dong, USP7 promotes hepatoblastoma progression through activation of PI3K/AKT signaling pathway, *Cancer Biomark*. 31 (2021) 107–117. <https://doi.org/10.3233/CBM-200052>.
- [48] G. Yan, N. Liu, J. Tian, Y. Fu, W. Wei, J. Zou, S. Li, Q. Wang, K. Li, J. Wang, Deubiquitylation and stabilization of ARMC5 by ubiquitin-specific processing protease 7 (USP7) are critical for RCC proliferation, *J Cell Mol Med*. 25 (2021) 3149–3159. <https://doi.org/10.1111/jcmm.16306>.
- [49] P. Xie, H. Wang, J. Xie, Z. Huang, S. Chen, X. Cheng, X. Zhang, F. Liu, Y. Li, D. Huang, USP7 promotes proliferation of papillary thyroid carcinoma cells through TBX3-mediated p57KIP2 repression, *Mol Cell Endocrinol*. 518 (2020) 111037. <https://doi.org/10.1016/j.mce.2020.111037>.
- [50] C. Zhang, J. Lu, Q.-W. Zhang, W. Zhao, J.-H. Guo, S.-L. Liu, Y.-L. Wu, B. Jiang, F.-H. Gao, USP7 promotes cell proliferation through the stabilization of Ki-67 protein in non-small cell lung cancer cells, *Int J Biochem Cell Biol*. 79 (2016) 209–221. <https://doi.org/10.1016/j.biocel.2016.08.025>.
- [51] C. Lu, Z. Ning, A. Wang, D. Chen, X. Liu, T. Xia, D.S. Tekcham, W. Wang, T. Li, X. Liu, J. Liu, H. Qi, H. Luo, J. Du, C. Ma, Q. Yan, J. Liu, G. Xu, H.-L. Piao, G. Tan, USP10 suppresses tumor progression by inhibiting mTOR activation in hepatocellular carcinoma, *Cancer Lett*. 436 (2018) 139–148. <https://doi.org/10.1016/j.canlet.2018.07.032>.
- [52] C. Liu, X. Li, G. Feng, M. Cao, F. Liu, G. Zhang, Y. Lu, Downregulation of USP12 inhibits tumor growth via the p38/MAPK pathway in hepatocellular carcinoma, *Mol Med Rep*. 22 (2020) 4899–4908. <https://doi.org/10.3892/mmr.2020.11557>.
- [53] U.L. McClurg, E.E. Summerscales, V.J. Harle, L. Gaughan, C.N. Robson, Deubiquitinating enzyme Usp12 regulates the interaction between the androgen receptor and the Akt pathway, *Oncotarget*. 5 (2014) 7081–7092. <https://doi.org/10.18632/oncotarget.2162>.
- [54] Y. Wu, Y. Zhang, C. Liu, Y. Zhang, D. Wang, S. Wang, Y. Wu, F. Liu, Q. Li, X. Liu, M.Y. Zaky, D. Yan, S. Liu, Amplification of USP13 drives non-small cell lung cancer progression mediated by AKT/MAPK signaling, *Biomed Pharmacother*. 114 (2019) 108831. <https://doi.org/10.1016/j.biopha.2019.108831>.
- [55] W. Diao, Q. Guo, C. Zhu, Y. Song, H. Feng, Y. Cao, M. Du, H. Chen, USP18 promotes cell proliferation and suppressed apoptosis in cervical cancer cells via activating AKT signaling pathway, *BMC Cancer*. 20 (2020) 741. <https://doi.org/10.1186/s12885-020-07241-1>.
- [56] W. Li, K. Cui, E.V. Prochownik, Y. Li, The deubiquitinase USP21 stabilizes MEK2 to promote tumor growth, *Cell Death Dis*. 9 (2018) 482. <https://doi.org/10.1038/s41419-018-0523-z>.
- [57] W. Wang, M. Chen, H. Xu, D. Lv, S. Zhou, H. Yang, USP46 Inhibits Cell Proliferation in Lung Cancer through PHLPP1/AKT Pathway, *Biomed Res Int*. 2020 (2020) 2509529. <https://doi.org/10.1155/2020/2509529>.
- [58] B. Benassi, R. Flavin, L. Marchionni, S. Zanata, Y. Pan, D. Chowdhury, M. Marani, S. Strano, P. Muti, G. Blandino, M. Loda, MYC is activated by USP2a-mediated modulation of microRNAs in prostate cancer, *Cancer Discov*. 2 (2012) 236–247. <https://doi.org/10.1158/2159-8290.CD-11-0219>.
- [59] D. Kim, A. Hong, H.I. Park, W.H. Shin, L. Yoo, S.J. Jeon, K.C. Chung, Deubiquitinating enzyme USP22 positively regulates c-Myc stability and tumorigenic activity in mammalian and breast cancer cells, *J Cell Physiol*. 232 (2017) 3664–3676. <https://doi.org/10.1002/jcp.25841>.
- [60] Z. Weili, L. Zhikun, W. Jianmin, T. Qingbao, Knockdown of USP28 enhances the radiosensitivity of esophageal cancer cells via the c-Myc/hypoxia-inducible factor-1 alpha pathway, *J Cell Biochem*. 120 (2019) 201–212. <https://doi.org/10.1002/jcb.27305>.
- [61] J. Pan, Q. Deng, C. Jiang, X. Wang, T. Niu, H. Li, T. Chen, J. Jin, W. Pan, X. Cai, X. Yang, M. Lu, J. Xiao, P. Wang, USP37 directly deubiquitinates and stabilizes c-Myc in lung cancer, *Oncogene*. 34 (2015) 3957–3967. <https://doi.org/10.1038/onc.2014.327>.
- [62] N. Shibata, N. Ohoka, G. Tsuji, Y. Demizu, K. Miyawaza, K. Ui-Tei, T. Akiyama, M. Naito, Deubiquitylase USP25 prevents degradation of BCR-ABL protein and ensures proliferation of Ph-positive leukemia cells, *Oncogene*. 39 (2020) 3867–3878. <https://doi.org/10.1038/s41388-020-1253-0>.
- [63] S. Jiang, X. Wang, Y. He, H. Huang, B. Cao, Z. Zhang, J. Liu, Q. Wang, Z. Huang, X. Mao, Suppression of USP7 induces BCR-ABL degradation and chronic myelogenous leukemia cell apoptosis, *Cell Death Dis*. 12 (2021) 456. <https://doi.org/10.1038/s41419-021-03732-6>.

- [64] K.H. Vousden, C. Prives, Blinded by the Light: The Growing Complexity of p53, *Cell*. 137 (2009) 413–431. <https://doi.org/10.1016/j.cell.2009.04.037>.
- [65] K.T. Bieging, L.D. Attardi, Deconstructing p53 transcriptional networks in tumor suppression, *Trends Cell Biol.* 22 (2012) 97–106. <https://doi.org/10.1016/j.tcb.2011.10.006>.
- [66] C.L. Brooks, W. Gu, p53 regulation by ubiquitin, *FEBS Lett.* 585 (2011) 2803–2809. <https://doi.org/10.1016/j.febslet.2011.05.022>.
- [67] S. Bhattacharya, D. Chakraborty, M. Basu, M.K. Ghosh, Emerging insights into HAUSP (USP7) in physiology, cancer and other diseases, *Signal Transduct Target Ther.* 3 (2018) 17. <https://doi.org/10.1038/s41392-018-0012-y>.
- [68] J. Zhou, J. Wang, C. Chen, H. Yuan, X. Wen, H. Sun, USP7: Target Validation and Drug Discovery for Cancer Therapy, *Med Chem.* 14 (2018) 3–18. <https://doi.org/10.2174/1573406413666171020115539>.
- [69] S.-M. Qi, G. Cheng, X.-D. Cheng, Z. Xu, B. Xu, W.-D. Zhang, J.-J. Qin, Targeting USP7-Mediated Deubiquitination of MDM2/MDMX-p53 Pathway for Cancer Therapy: Are We There Yet?, *Front Cell Dev Biol.* 8 (2020) 233. <https://doi.org/10.3389/fcell.2020.00233>.
- [70] B. Stolte, A.B. Iniguez, N.V. Dharia, A.L. Robichaud, A.S. Conway, A.M. Morgan, G. Alexe, N.J. Schauer, X. Liu, G.H. Bird, A. Tsherniak, F. Vazquez, S.J. Buhrlage, L.D. Walensky, K. Stegmaier, Genome-scale CRISPR-Cas9 screen identifies druggable dependencies in TP53 wild-type Ewing sarcoma, *J Exp Med.* 215 (2018) 2137–2155. <https://doi.org/10.1084/jem.20171066>.
- [71] K.-I. Takayama, T. Suzuki, T. Fujimura, S. Takahashi, S. Inoue, Association of USP10 with G3BP2 Inhibits p53 Signaling and Contributes to Poor Outcome in Prostate Cancer, *Mol Cancer Res.* 16 (2018) 846–856. <https://doi.org/10.1158/1541-7786.MCR-17-0471>.
- [72] J. Liu, H. Xia, M. Kim, L. Xu, Y. Li, L. Zhang, Y. Cai, H.V. Norberg, T. Zhang, T. Furuya, M. Jin, Z. Zhu, H. Wang, J. Yu, Y. Li, Y. Hao, A. Choi, H. Ke, D. Ma, J. Yuan, Beclin1 controls the levels of p53 by regulating the deubiquitination activity of USP10 and USP13, *Cell.* 147 (2011) 223–234. <https://doi.org/10.1016/j.cell.2011.08.037>.
- [73] Q. Zou, J. Jin, H. Hu, H.S. Li, S. Romano, Y. Xiao, M. Nakaya, X. Zhou, X. Cheng, P. Yang, G. Lozano, C. Zhu, S.S. Watowich, S.E. Ullrich, S.-C. Sun, USP15 stabilizes MDM2 to mediate cancer-cell survival and inhibit antitumor T cell responses, *Nat Immunol.* 15 (2014) 562–570. <https://doi.org/10.1038/ni.2885>.
- [74] F. Ding, C. Bao, Y. Tian, H. Xiao, M. Wang, X. Xie, F. Hu, J. Mei, USP22 promotes NSCLC tumorigenesis via MDMX up-regulation and subsequent p53 inhibition, *Int J Mol Sci.* 16 (2014) 307–320. <https://doi.org/10.3390/ijms16010307>.
- [75] D. Zhou, P. Liu, D.-W. Sun, Z.-J. Chen, J. Hu, S.-M. Peng, Y.-L. Liu, USP22 down-regulation facilitates human retinoblastoma cell aging and apoptosis via inhibiting TERT/P53 pathway, *Eur Rev Med Pharmacol Sci.* 21 (2017) 2785–2792.
- [76] J. Yuan, G. Zhang, X. Li, Q. Ma, W. Cheng, W. Wang, B. Zhang, T. Hu, G. Song, Knocking down USP39 Inhibits the Growth and Metastasis of Non-Small-Cell Lung Cancer Cells through Activating the p53 Pathway, *Int J Mol Sci.* 21 (2020) E8949. <https://doi.org/10.3390/ijms21238949>.
- [77] B. Costanza, I.A. Umelo, J. Bellier, V. Castronovo, A. Turtoi, Stromal Modulators of TGF- $\beta$  in Cancer, *J Clin Med.* 6 (2017) E7. <https://doi.org/10.3390/jcm6010007>.
- [78] X.-X. Jie, X.-Y. Zhang, C.-J. Xu, Epithelial-to-mesenchymal transition, circulating tumor cells and cancer metastasis: Mechanisms and clinical applications, *Oncotarget.* 8 (2017) 81558–81571. <https://doi.org/10.18632/oncotarget.18277>.
- [79] C.D. Morrison, J.G. Parvani, W.P. Schiemann, The relevance of the TGF- $\beta$  Paradox to EMT-MET programs, *Cancer Lett.* 341 (2013) 30–40. <https://doi.org/10.1016/j.canlet.2013.02.048>.
- [80] L. Fan, Z. Chen, X. Wu, X. Cai, S. Feng, J. Lu, H. Wang, N. Liu, Ubiquitin-Specific Protease 3 Promotes Glioblastoma Cell Invasion and Epithelial-Mesenchymal Transition via Stabilizing Snail, *Mol Cancer Res.* 17 (2019) 1975–1984. <https://doi.org/10.1158/1541-7786.MCR-19-0197>.
- [81] W. Wang, J. Wang, H. Yan, K. Zhang, Y. Liu, Upregulation of USP11 promotes epithelial-to-mesenchymal transition by deubiquitinating Snail in ovarian cancer, *Oncol Rep.* 41 (2019) 1739–1748. <https://doi.org/10.3892/or.2018.6924>.
- [82] W. Qian, Q. Li, X. Wu, W. Li, Q. Li, J. Zhang, M. Li, D. Zhang, H. Zhao, X. Zou, H. Jia, L. Zhang, X.-D. Yang, Z. Hou, Deubiquitinase USP29 promotes gastric cancer cell migration by cooperating with phosphatase SCP1 to stabilize Snail protein, *Oncogene.* 39 (2020) 6802–6815. <https://doi.org/10.1038/s41388-020-01471-0>.

- [83] Y. Li, Y. Yang, J. Li, H. Liu, F. Chen, B. Li, B. Cui, Y. Liu, USP22 drives colorectal cancer invasion and metastasis via epithelial-mesenchymal transition by activating AP4, *Oncotarget*. 8 (2017) 32683–32695. <https://doi.org/10.18632/oncotarget.15950>.
- [84] E. Oh, J.Y. Kim, D. Sung, Y. Cho, N. Lee, H. An, Y.-J. Kim, T.-M. Cho, J.H. Seo, Inhibition of ubiquitin-specific protease 34 (USP34) induces epithelial-mesenchymal transition and promotes stemness in mammary epithelial cells, *Cell Signal*. 36 (2017) 230–239. <https://doi.org/10.1016/j.cellsig.2017.05.009>.
- [85] T. Qin, B. Li, X. Feng, S. Fan, L. Liu, D. Liu, J. Mao, Y. Lu, J. Yang, X. Yu, Q. Zhang, J. Zhang, B. Song, M. Li, L. Li, Abnormally elevated USP37 expression in breast cancer stem cells regulates stemness, epithelial-mesenchymal transition and cisplatin sensitivity, *J Exp Clin Cancer Res*. 37 (2018) 287. <https://doi.org/10.1186/s13046-018-0934-9>.
- [86] N. Yildirim, G.C. Kocal, Z. Isik, B. Saatli, U. Saygili, T. Uysal, C. Ulukus, M. Koyuncuoglu, H. Ellidokuz, Y. Basbinar, Ubiquitin-Proteasome Axis, Especially Ubiquitin-Specific Protease-17 (USP17) Gene Family, is a Potential Target for Epithelial-Mesenchymal Transition in High-Grade Serous Ovarian Cancer, *Reprod Sci*. 26 (2019) 794–805. <https://doi.org/10.1177/1933719118799189>.
- [87] C. Yan, J. Yuan, J. Xu, G. Zhang, X. Li, B. Zhang, T. Hu, X. Huang, Y. Mao, G. Song, Ubiquitin-specific peptidase 39 regulates the process of proliferation and migration of human ovarian cancer via p53/p21 pathway and EMT, *Med Oncol*. 36 (2019) 95. <https://doi.org/10.1007/s12032-019-1308-7>.
- [88] F. Verrecchia, F. R dini, Transforming Growth Factor- $\beta$  Signaling Plays a Pivotal Role in the Interplay Between Osteosarcoma Cells and Their Microenvironment, *Front Oncol*. 8 (2018) 133. <https://doi.org/10.3389/fonc.2018.00133>.
- [89] A. Lamora, J. Talbot, M. Mullard, B. Brounais-Le Royer, F. Redini, F. Verrecchia, TGF- $\beta$  Signaling in Bone Remodeling and Osteosarcoma Progression, *J Clin Med*. 5 (2016) E96. <https://doi.org/10.3390/jcm5110096>.
- [90] J. Zhang, X. Zhang, F. Xie, Z. Zhang, H. van Dam, L. Zhang, F. Zhou, The regulation of TGF- $\beta$ /SMAD signaling by protein deubiquitination, *Protein Cell*. 5 (2014) 503–517. <https://doi.org/10.1007/s13238-014-0058-8>.
- [91] K. Aggarwal, J. Massagu , Ubiquitin removal in the TGF- $\beta$  pathway, *Nat Cell Biol*. 14 (2012) 656–657. <https://doi.org/10.1038/ncb2534>.
- [92] T. Das, E.J. Song, E.E. Kim, The Multifaceted Roles of USP15 in Signal Transduction, *Int J Mol Sci*. 22 (2021) 4728. <https://doi.org/10.3390/ijms22094728>.
- [93] W.-H. Cao, X.-P. Liu, S.-L. Meng, Y.-W. Gao, Y. Wang, Z.-L. Ma, X.-G. Wang, H.-B. Wang, USP4 promotes invasion of breast cancer cells via Relaxin/TGF- $\beta$ 1/Smad2/MMP-9 signal, *Eur Rev Med Pharmacol Sci*. 20 (2016) 1115–1122.
- [94] C. Qiu, Y. Liu, Y. Mei, M. Zou, Z. Zhao, M. Ye, X. Wu, Ubiquitin-specific protease 4 promotes metastasis of hepatocellular carcinoma by increasing TGF- $\beta$  signaling-induced epithelial-mesenchymal transition, *Aging (Albany NY)*. 10 (2018) 2783–2799. <https://doi.org/10.18632/aging.101587>.
- [95] L. Zhang, F. Zhou, Y. Drabsch, R. Gao, B.E. Snaar-Jagalska, C. Mickanin, H. Huang, K.-A. Sheppard, J.A. Porter, C.X. Lu, P. ten Dijke, USP4 is regulated by AKT phosphorylation and directly deubiquitylates TGF- $\beta$  type I receptor, *Nat Cell Biol*. 14 (2012) 717–726. <https://doi.org/10.1038/ncb2522>.
- [96] P.J.A. Eichhorn, L. Rod n, A. Gonz lez-Junc , A. Dirac, M. Gili, E. Mart nez-S ez, C. Aura, I. Barba, V. Peg, A. Prat, I. Cuartas, J. Jimenez, D. Garc a-Dorado, J. Sahuquillo, R. Bernards, J. Baselga, J. Seoane, USP15 stabilizes TGF- $\beta$  receptor I and promotes oncogenesis through the activation of TGF- $\beta$  signaling in glioblastoma, *Nat Med*. 18 (2012) 429–435. <https://doi.org/10.1038/nm.2619>.
- [97] F. Zhou, F. Xie, K. Jin, Z. Zhang, M. Clerici, R. Gao, M. van Dinther, T.K. Sixma, H. Huang, L. Zhang, P. Ten Dijke, USP4 inhibits SMAD4 monoubiquitination and promotes activin and BMP signaling, *EMBO J*. 36 (2017) 1623–1639. <https://doi.org/10.15252/embj.201695372>.
- [98] V.C. Silvestrini, C.H. Thom , D. Albuquerque, C. de Souza Palma, G.A. Ferreira, G.P. Lanfredi, A.P. Masson, L.E.A. Delsin, F.U. Ferreira, F.C. de Souza, L.M.F. de Godoy, A. Aquino, E. Carrilho, R.A. Panepucci, D.T. Covas, V.M. Fa a, Proteomics analysis reveals the role of ubiquitin specific protease (USP47) in Epithelial to Mesenchymal Transition (EMT) induced by TGF $\beta$ 2 in breast cells, *J Proteomics*. 219 (2020) 103734. <https://doi.org/10.1016/j.jprot.2020.103734>.

- [99] C.A. Stiller, A.W. Craft, I. Corazziari, EURO CARE Working Group, Survival of children with bone sarcoma in Europe since 1978: results from the EURO CARE study, *Eur J Cancer*. 37 (2001) 760–766. [https://doi.org/10.1016/s0959-8049\(01\)00004-1](https://doi.org/10.1016/s0959-8049(01)00004-1).
- [100] L. Mirabello, R. Pfeiffer, G. Murphy, N.C. Daw, A. Patiño-Garcia, R.J. Troisi, R.N. Hoover, C. Douglass, J. Schüz, A.W. Craft, S.A. Savage, Height at diagnosis and birth-weight as risk factors for osteosarcoma, *Cancer Causes Control*. 22 (2011) 899–908. <https://doi.org/10.1007/s10552-011-9763-2>.
- [101] M. Kansara, M.W. Teng, M.J. Smyth, D.M. Thomas, Translational biology of osteosarcoma, *Nat Rev Cancer*. 14 (2014) 722–735. <https://doi.org/10.1038/nrc3838>.
- [102] L. Mirabello, R.J. Troisi, S.A. Savage, International osteosarcoma incidence patterns in children and adolescents, middle ages and elderly persons, *Int J Cancer*. 125 (2009) 229–234. <https://doi.org/10.1002/ijc.24320>.
- [103] A.M. Czarnecka, K. Synoradzki, W. Firlej, E. Bartnik, P. Sobczuk, M. Fiedorowicz, P. Grieb, P. Rutkowski, *Molecular Biology of Osteosarcoma*, *Cancers (Basel)*. 12 (2020) E2130. <https://doi.org/10.3390/cancers12082130>.
- [104] L. Mirabello, R.J. Troisi, S.A. Savage, Osteosarcoma incidence and survival rates from 1973 to 2004: data from the Surveillance, Epidemiology, and End Results Program, *Cancer*. 115 (2009) 1531–1543. <https://doi.org/10.1002/cncr.24121>.
- [105] J. Ritter, S.S. Bielack, Osteosarcoma, *Ann Oncol*. 21 Suppl 7 (2010) vii320-325. <https://doi.org/10.1093/annonc/mdq276>.
- [106] A.J. Mutsaers, C.R. Walkley, Cells of origin in osteosarcoma: mesenchymal stem cells or osteoblast committed cells?, *Bone*. 62 (2014) 56–63. <https://doi.org/10.1016/j.bone.2014.02.003>.
- [107] A.B. Mohseny, K. Szuhai, S. Romeo, E.P. Buddingh, I. Briaire-de Bruijn, D. de Jong, M. van Pel, A.-M. Cleton-Jansen, P.C.W. Hogendoorn, Osteosarcoma originates from mesenchymal stem cells in consequence of aneuploidization and genomic loss of Cdkn2, *J Pathol*. 219 (2009) 294–305. <https://doi.org/10.1002/path.2603>.
- [108] W. Xiao, A.B. Mohseny, P.C.W. Hogendoorn, A.-M. Cleton-Jansen, Mesenchymal stem cell transformation and sarcoma genesis, *Clin Sarcoma Res*. 3 (2013) 10. <https://doi.org/10.1186/2045-3329-3-10>.
- [109] S. Lorenz, T. Barøy, J. Sun, T. Nome, D. Vodák, J.-C. Bryne, A.-M. Håkelién, L. Fernandez-Cuesta, B. Möhlendick, H. Rieder, K. Szuhai, O. Zaikova, T.C. Ahlquist, G.O.S. Thomassen, R.I. Skotheim, R.A. Lothe, P.S. Tarpey, P. Campbell, A. Flanagan, O. Myklebost, L.A. Meza-Zepeda, Unscrambling the genomic chaos of osteosarcoma reveals extensive transcript fusion, recurrent rearrangements and frequent novel TP53 aberrations, *Oncotarget*. 7 (2016) 5273–5288. <https://doi.org/10.18632/oncotarget.6567>.
- [110] M. Bousquet, C. Noirot, F. Accadbled, J. Sales de Gauzy, M.P. Castex, P. Brousset, A. Gomez-Brouchet, Whole-exome sequencing in osteosarcoma reveals important heterogeneity of genetic alterations, *Ann Oncol*. 27 (2016) 738–744. <https://doi.org/10.1093/annonc/mdw009>.
- [111] C.C. Lau, C.P. Harris, X.-Y. Lu, L. Perlaky, S. Gogineni, M. Chintagumpala, J. Hicks, M.E. Johnson, N.A. Davino, A.G. Huvos, P.A. Meyers, J.H. Healy, R. Gorlick, P.H. Rao, Frequent amplification and rearrangement of chromosomal bands 6p12-p21 and 17p11.2 in osteosarcoma, *Genes Chromosomes Cancer*. 39 (2004) 11–21. <https://doi.org/10.1002/gcc.10291>.
- [112] X. Chen, A. Bahrami, A. Pappo, J. Easton, J. Dalton, E. Hedlund, D. Ellison, S. Shurtleff, G. Wu, L. Wei, M. Parker, M. Rusch, P. Nagahawatte, J. Wu, S. Mao, K. Boggs, H. Mulder, D. Yergeau, C. Lu, L. Ding, M. Edmonson, C. Qu, J. Wang, Y. Li, F. Navid, N.C. Daw, E.R. Mardis, R.K. Wilson, J.R. Downing, J. Zhang, M.A. Dyer, St. Jude Children's Research Hospital–Washington University Pediatric Cancer Genome Project, Recurrent somatic structural variations contribute to tumorigenesis in pediatric osteosarcoma, *Cell Rep*. 7 (2014) 104–112. <https://doi.org/10.1016/j.celrep.2014.03.003>.
- [113] J. Toguchida, K. Ishizaki, M.S. Sasaki, Y. Nakamura, M. Ikenaga, M. Kato, M. Sugimoto, Y. Kotoura, T. Yamamuro, Preferential mutation of paternally derived RB gene as the initial event in sporadic osteosarcoma, *Nature*. 338 (1989) 156–158. <https://doi.org/10.1038/338156a0>.
- [114] J.S. Wunder, N. Gokgoz, R. Parkes, S.B. Bull, S. Eskandarian, A.M. Davis, C.P. Beauchamp, E.U. Conrad, R.J. Grimer, J.H. Healey, D. Malkin, D.C. Mangham, M.J. Rock, R.S. Bell, I.L. Andrulis, TP53 mutations and outcome in osteosarcoma: a prospective, multicenter study, *J Clin Oncol*. 23 (2005) 1483–1490. <https://doi.org/10.1200/JCO.2005.04.074>.
- [115] A.J. Cesare, R.R. Reddel, Alternative lengthening of telomeres: models, mechanisms and implications, *Nat Rev Genet*. 11 (2010) 319–330. <https://doi.org/10.1038/nrg2763>.

- [116] G. Ottaviani, N. Jaffe, The etiology of osteosarcoma, *Cancer Treat Res.* 152 (2009) 15–32. [https://doi.org/10.1007/978-1-4419-0284-9\\_2](https://doi.org/10.1007/978-1-4419-0284-9_2).
- [117] J.M. Varley, D.G. Evans, J.M. Birch, Li-Fraumeni syndrome--a molecular and clinical review, *Br J Cancer.* 76 (1997) 1–14. <https://doi.org/10.1038/bjc.1997.328>.
- [118] A. Carnevale, E. Lieberman, R. Cárdenas, Li-Fraumeni syndrome in pediatric patients with soft tissue sarcoma or osteosarcoma, *Arch Med Res.* 28 (1997) 383–386.
- [119] L. Chauveinc, V. Mosseri, E. Quintana, L. Desjardins, P. Schlienger, F. Doz, B. Dutrillaux, Osteosarcoma following retinoblastoma: age at onset and latency period, *Ophthalmic Genet.* 22 (2001) 77–88. <https://doi.org/10.1076/opge.22.2.77.2228>.
- [120] A. Leonard, A.W. Craft, C. Moss, A.J. Malcolm, Osteogenic sarcoma in the Rothmund-Thomson syndrome, *Med Pediatr Oncol.* 26 (1996) 249–253. [https://doi.org/10.1002/\(SICI\)1096-911X\(199604\)26:4<249::AID-MPO5>3.0.CO;2-J](https://doi.org/10.1002/(SICI)1096-911X(199604)26:4<249::AID-MPO5>3.0.CO;2-J).
- [121] M.F. Hansen, M. Seton, A. Merchant, Osteosarcoma in Paget's disease of bone, *J Bone Miner Res.* 21 Suppl 2 (2006) P58-63. <https://doi.org/10.1359/jbmr.06s211>.
- [122] P.G. Casali, S. Bielack, N. Abecassis, H.T. Aro, S. Bauer, R. Biagini, S. Bonvalot, I. Boukovinas, J.V.M.G. Bovee, B. Brennan, T. Brodowicz, J.M. Broto, L. Brugières, A. Buonadonna, E. De Álava, A.P. Dei Tos, X.G. Del Muro, P. Dileo, C. Dhooge, M. Eriksson, F. Fagioli, A. Fedenko, V. Ferraresi, A. Ferrari, S. Ferrari, A.M. Frezza, N. Gaspar, S. Gasperoni, H. Gelderblom, T. Gil, G. Grignani, A. Gronchi, R.L. Haas, B. Hassan, S. Hecker-Nolting, P. Hohenberger, R. Issels, H. Joensuu, R.L. Jones, I. Judson, P. Jutte, S. Kaal, L. Kager, B. Kasper, K. Kopeckova, D.A. Krákorová, R. Ladenstein, A. Le Cesne, I. Lugowska, O. Merimsky, M. Montemurro, B. Morland, M.A. Pantaleo, R. Piana, P. Picci, S. Piperno-Neumann, A.L. Pousa, P. Reichardt, M.H. Robinson, P. Rutkowski, A.A. Safwat, P. Schöffski, S. Sleijfer, S. Stacchiotti, S.J. Strauss, K. Sundby Hall, M. Unk, F. Van Coevorden, W.T.A. van der Graaf, J. Whelan, E. Wardelmann, O. Zaikova, J.Y. Blay, ESMO Guidelines Committee, PaedCan and ERN EURACAN, Bone sarcomas: ESMO-PaedCan-EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow-up, *Ann Oncol.* 29 (2018) iv79–iv95. <https://doi.org/10.1093/annonc/mdy310>.
- [123] H.T. Ta, C.R. Dass, P.F.M. Choong, D.E. Dunstan, Osteosarcoma treatment: state of the art, *Cancer Metastasis Rev.* 28 (2009) 247–263. <https://doi.org/10.1007/s10555-009-9186-7>.
- [124] N. Gaspar, B.-V. Occean, H. Pacquement, E. Bompas, C. Bouvier, H.J. Brisse, M.-P. Castex, N. Cheurfa, N. Corradini, J. Delaye, N. Entz-Werlé, J.-C. Gentet, A. Italiano, C. Lervat, P. Marec-Berard, E. Mascard, F. Redini, L. Saumet, C. Schmitt, M.-D. Tabone, C. Verite-Goulard, M.-C. Le Deley, S. Piperno-Neumann, L. Brugieres, SFCE (Société Française des Cancers de l'Enfant et l'adolescent), GSF-GETO (Groupe Sarcome Français), UNICANCER sarcoma group, Results of methotrexate-etoposide-ifosfamide based regimen (M-EI) in osteosarcoma patients included in the French OS2006/sarcome-09 study, *Eur J Cancer.* 88 (2018) 57–66. <https://doi.org/10.1016/j.ejca.2017.09.036>.
- [125] T. Heare, M.A. Hensley, S. Dell'Orfano, Bone tumors: osteosarcoma and Ewing's sarcoma, *Curr Opin Pediatr.* 21 (2009) 365–372. <https://doi.org/10.1097/MOP.0b013e32832b1111>.
- [126] D.D. Moore, H.H. Luu, Osteosarcoma, *Cancer Treat Res.* 162 (2014) 65–92. [https://doi.org/10.1007/978-3-319-07323-1\\_4](https://doi.org/10.1007/978-3-319-07323-1_4).
- [127] B. Kempf-Bielack, S.S. Bielack, H. Jürgens, D. Branscheid, W.E. Berdel, G.U. Exner, U. Göbel, K. Helmke, G. Jundt, H. Kabisch, M. Kevric, T. Klingebiel, R. Kotz, R. Maas, R. Schwarz, M. Semik, J. Treuner, A. Zoubek, K. Winkler, Osteosarcoma relapse after combined modality therapy: an analysis of unselected patients in the Cooperative Osteosarcoma Study Group (COSS), *J Clin Oncol.* 23 (2005) 559–568. <https://doi.org/10.1200/JCO.2005.04.063>.
- [128] I. Ługowska, A. Pieńkowski, A. Szumera-Ciećkiewicz, H. Kosęła-Paterczyk, P. Teterycz, M. Głogowski, K. Kozak, A. Klimczak, S. Falkowski, P. Rutkowski, [The long-term treatment outcomes of adult osteosarcoma], *Pol Merkur Lekarski.* 42 (2017) 158–164.
- [129] J. Ewing, Classics in oncology. Diffuse endothelioma of bone. James Ewing. Proceedings of the New York Pathological Society, 1921, *CA Cancer J Clin.* 22 (1972) 95–98. <https://doi.org/10.3322/canjclin.22.2.95>.
- [130] M.U. Jawad, M.C. Cheung, E.S. Min, M.M. Schneiderbauer, L.G. Koniaris, S.P. Scully, Ewing sarcoma demonstrates racial disparities in incidence-related and sex-related differences in outcome: an analysis of 1631 cases from the SEER database, 1973-2005, *Cancer.* 115 (2009) 3526–3536. <https://doi.org/10.1002/cncr.24388>.
- [131] T.G.P. Grünewald, F. Cidre-Aranaz, D. Surdez, E.M. Tomazou, E. de Álava, H. Kovar, P.H. Sorensen, O. Delattre, U. Dirksen, Ewing sarcoma, *Nat Rev Dis Primers.* 4 (2018) 5. <https://doi.org/10.1038/s41572-018-0003-x>.

- [132] S. Durer, H. Shaikh, Ewing Sarcoma, in: StatPearls, StatPearls Publishing, Treasure Island (FL), 2021. <http://www.ncbi.nlm.nih.gov/books/NBK559183/> (accessed August 27, 2021).
- [133] S.Y. Kim, M. Tsokos, L.J. Helman, Dilemmas associated with congenital ewing sarcoma family tumors, *J Pediatr Hematol Oncol.* 30 (2008) 4–7. <https://doi.org/10.1097/MPH.0b013e31815cf71f>.
- [134] H. van den Berg, U. Dirksen, A. Ranft, H. Jürgens, Ewing tumors in infants, *Pediatr Blood Cancer.* 50 (2008) 761–764. <https://doi.org/10.1002/pbc.21292>.
- [135] R.L. Randall, S.L. Lessnick, K.B. Jones, L.G. Gouw, J.E. Cummings, L. Cannon-Albright, J.D. Schiffman, Is There a Predisposition Gene for Ewing’s Sarcoma?, *J Oncol.* 2010 (2010) 397632. <https://doi.org/10.1155/2010/397632>.
- [136] N. Riggi, M.L. Suvà, I. Stamenkovic, Ewing’s Sarcoma, *N Engl J Med.* 384 (2021) 154–164. <https://doi.org/10.1056/NEJMra2028910>.
- [137] J.F. Fraumeni, A.G. Glass, Rarity of Ewing’s sarcoma among U.S. Negro children, *Lancet.* 1 (1970) 366–367. [https://doi.org/10.1016/s0140-6736\(70\)90754-3](https://doi.org/10.1016/s0140-6736(70)90754-3).
- [138] J. Worch, J. Cyrus, R. Goldsby, K.K. Matthay, J. Neuhaus, S.G. DuBois, Racial differences in the incidence of mesenchymal tumors associated with EWSR1 translocation, *Cancer Epidemiol Biomarkers Prev.* 20 (2011) 449–453. <https://doi.org/10.1158/1055-9965.EPI-10-1170>.
- [139] M.L. Bernstein, M. Devidas, D. Lafreniere, A.-K. Soud, P.A. Meyers, M. Gebhardt, K. Stine, R. Nicholas, E.J. Perlman, R. Dubowy, I.W. Wainer, P.S. Dickman, M.P. Link, A. Goorin, H.E. Grier, Pediatric Oncology Group, Children’s Cancer Group Phase II Study 9457, Children’s Oncology Group, Intensive therapy with growth factor support for patients with Ewing tumor metastatic at diagnosis: Pediatric Oncology Group/Children’s Cancer Group Phase II Study 9457--a report from the Children’s Oncology Group, *J Clin Oncol.* 24 (2006) 152–159. <https://doi.org/10.1200/JCO.2005.02.1717>.
- [140] A.D. Lynch, F. Gani, C.F. Meyer, C.D. Morris, N. Ahuja, F.M. Johnston, Extraskelatal versus Skeletal Ewing Sarcoma in the adult population: Controversies in care, *Surg Oncol.* 27 (2018) 373–379. <https://doi.org/10.1016/j.suronc.2018.05.016>.
- [141] A. Aurias, C. Rimbaut, D. Buffe, J. Dubouset, A. Mazabraud, [Translocation of chromosome 22 in Ewing’s sarcoma], *C R Seances Acad Sci III.* 296 (1983) 1105–1107.
- [142] C. Turc-Carel, I. Philip, M.P. Berger, T. Philip, G. Lenoir, [Chromosomal translocation (11; 22) in cell lines of Ewing’s sarcoma], *C R Seances Acad Sci III.* 296 (1983) 1101–1103.
- [143] O. Delattre, J. Zucman, B. Plougastel, C. Desmaze, T. Melot, M. Peter, H. Kovar, I. Joubert, P. de Jong, G. Rouleau, Gene fusion with an ETS DNA-binding domain caused by chromosome translocation in human tumours, *Nature.* 359 (1992) 162–165. <https://doi.org/10.1038/359162a0>.
- [144] I.S. Jeon, J.N. Davis, B.S. Braun, J.E. Sublett, M.F. Roussel, C.T. Denny, D.N. Shapiro, A variant Ewing’s sarcoma translocation (7;22) fuses the EWS gene to the ETS gene ETV1, *Oncogene.* 10 (1995) 1229–1234.
- [145] Y. Kaneko, K. Yoshida, M. Handa, Y. Toyoda, H. Nishihira, Y. Tanaka, Y. Sasaki, S. Ishida, F. Higashino, K. Fujinaga, Fusion of an ETS-family gene, EIAF, to EWS by t(17;22)(q12;q12) chromosome translocation in an undifferentiated sarcoma of infancy, *Genes Chromosomes Cancer.* 15 (1996) 115–121. [https://doi.org/10.1002/\(SICI\)1098-2264\(199602\)15:2<115::AID-GCC6>3.0.CO;2-6](https://doi.org/10.1002/(SICI)1098-2264(199602)15:2<115::AID-GCC6>3.0.CO;2-6).
- [146] T.L. Ng, M.J. O’Sullivan, C.J. Pallen, M. Hayes, P.W. Clarkson, M. Winstanley, P.H.B. Sorensen, T.O. Nielsen, D.E. Horsman, Ewing sarcoma with novel translocation t(2;16) producing an in-frame fusion of FUS and FEV, *J Mol Diagn.* 9 (2007) 459–463. <https://doi.org/10.2353/jmoldx.2007.070009>.
- [147] M. Peter, J. Couturier, H. Pacquement, J. Michon, G. Thomas, H. Magdelenat, O. Delattre, A new member of the ETS family fused to EWS in Ewing tumors, *Oncogene.* 14 (1997) 1159–1164. <https://doi.org/10.1038/sj.onc.1200933>.
- [148] D.C. Shing, D.J. McMullan, P. Roberts, K. Smith, S.-F. Chin, J. Nicholson, R.M. Tillman, P. Ramani, C. Cullinane, N. Coleman, FUS/ERG gene fusions in Ewing’s tumors, *Cancer Res.* 63 (2003) 4568–4576.
- [149] J. Zucman, T. Melot, C. Desmaze, J. Ghysdael, B. Plougastel, M. Peter, J.M. Zucker, T.J. Triche, D. Sheer, C. Turc-Carel, Combinatorial generation of variable fusion proteins in the Ewing family of tumours, *EMBO J.* 12 (1993) 4481–4487.
- [150] V. Boeva, D. Surdez, N. Guillon, F. Tirode, A.P. Fejes, O. Delattre, E. Barillot, De novo motif identification improves the accuracy of predicting transcription factor binding sites in ChIP-Seq data analysis, *Nucleic Acids Res.* 38 (2010) e126. <https://doi.org/10.1093/nar/gkq217>.



- [151] K. Gangwal, S. Sankar, P.C. Hollenhorst, M. Kinsey, S.C. Haroldsen, A.A. Shah, K.M. Boucher, W.S. Watkins, L.B. Jorde, B.J. Graves, S.L. Lessnick, Microsatellites as EWS/FLI response elements in Ewing's sarcoma, *Proc Natl Acad Sci U S A.* 105 (2008) 10149–10154. <https://doi.org/10.1073/pnas.0801073105>.
- [152] N. Guillon, F. Tirode, V. Boeva, A. Zynovyev, E. Barillot, O. Delattre, The oncogenic EWS-FLI1 protein binds in vivo GGAA microsatellite sequences with potential transcriptional activation function, *PLoS One.* 4 (2009) e4932. <https://doi.org/10.1371/journal.pone.0004932>.
- [153] K.M. Johnson, C. Taslim, R.S. Saund, S.L. Lessnick, Identification of two types of GGAA-microsatellites and their roles in EWS/FLI binding and gene regulation in Ewing sarcoma, *PLoS One.* 12 (2017) e0186275. <https://doi.org/10.1371/journal.pone.0186275>.
- [154] F. Baliko, T. Bright, R. Poon, B. Cohen, S.E. Egan, B.A. Alman, Inhibition of notch signaling induces neural differentiation in Ewing sarcoma, *Am J Pathol.* 170 (2007) 1686–1694. <https://doi.org/10.2353/ajpath.2007.060971>.
- [155] A. Franchi, G. Pasquinelli, G. Cenacchi, C. Della Rocca, C. Gambini, M. Bisceglia, G.N. Martinelli, M. Santucci, Immunohistochemical and ultrastructural investigation of neural differentiation in Ewing sarcoma/PNET of bone and soft tissues, *Ultrastruct Pathol.* 25 (2001) 219–225.
- [156] J. Wahl, L. Bogatyreva, P. Boukamp, M. Rojewski, F. van Valen, J. Fiedler, N. Hipp, K.-M. Debatin, C. Beltinger, Ewing's sarcoma cells with CD57-associated increase of tumorigenicity and with neural crest-like differentiation capacity, *Int J Cancer.* 127 (2010) 1295–1307. <https://doi.org/10.1002/ijc.25163>.
- [157] S. Hu-Lieskovan, J. Zhang, L. Wu, H. Shimada, D.E. Schofield, T.J. Triche, EWS-FLI1 fusion protein up-regulates critical genes in neural crest development and is responsible for the observed phenotype of Ewing's family of tumors, *Cancer Res.* 65 (2005) 4633–4644. <https://doi.org/10.1158/0008-5472.CAN-04-2857>.
- [158] M.A. Teitell, A.D. Thompson, P.H. Sorensen, H. Shimada, T.J. Triche, C.T. Denny, EWS/ETS fusion genes induce epithelial and neuroectodermal differentiation in NIH 3T3 fibroblasts, *Lab Invest.* 79 (1999) 1535–1543.
- [159] E.C. Torchia, S. Jaishankar, S.J. Baker, Ewing tumor fusion proteins block the differentiation of pluripotent marrow stromal cells, *Cancer Res.* 63 (2003) 3464–3468.
- [160] Y. Castellero-Trejo, S. Eliazar, L. Xiang, J.A. Richardson, R.L. Ilaria, Expression of the EWS/FLI-1 oncogene in murine primary bone-derived cells Results in EWS/FLI-1-dependent, ewing sarcoma-like tumors, *Cancer Res.* 65 (2005) 8698–8705. <https://doi.org/10.1158/0008-5472.CAN-05-1704>.
- [161] N. Riggi, L. Cironi, P. Provero, M.-L. Suvà, K. Kaloulis, C. Garcia-Echeverria, F. Hoffmann, A. Trumpp, I. Stamenkovic, Development of Ewing's sarcoma from primary bone marrow-derived mesenchymal progenitor cells, *Cancer Res.* 65 (2005) 11459–11468. <https://doi.org/10.1158/0008-5472.CAN-05-1696>.
- [162] F. Tirode, K. Laud-Duval, A. Prieur, B. Delorme, P. Charbord, O. Delattre, Mesenchymal stem cell features of Ewing tumors, *Cancer Cell.* 11 (2007) 421–429. <https://doi.org/10.1016/j.ccr.2007.02.027>.
- [163] N. Gaspar, D.S. Hawkins, U. Dirksen, I.J. Lewis, S. Ferrari, M.-C. Le Deley, H. Kovar, R. Grimer, J. Whelan, L. Claude, O. Delattre, M. Paulussen, P. Picci, K. Sundby Hall, H. van den Berg, R. Ladenstein, J. Michon, L. Hjorth, I. Judson, R. Luksch, M.L. Bernstein, P. Marec-Bérard, B. Brennan, A.W. Craft, R.B. Womer, H. Juergens, O. Oberlin, Ewing Sarcoma: Current Management and Future Approaches Through Collaboration, *J Clin Oncol.* 33 (2015) 3036–3046. <https://doi.org/10.1200/JCO.2014.59.5256>.
- [164] M. Paulussen, S. Ahrens, S. Burdach, A. Craft, B. Dockhorn-Dworniczak, J. Dunst, B. Fröhlich, W. Winkelmann, A. Zoubek, H. Jürgens, Primary metastatic (stage IV) Ewing tumor: survival analysis of 171 patients from the EICESS studies. *European Intergroup Cooperative Ewing Sarcoma Studies*, *Ann Oncol.* 9 (1998) 275–281. <https://doi.org/10.1023/a:1008208511815>.
- [165] H.L. Spraker, S.L. Price, A. Chaturvedi, J.D. Schiffman, K.B. Jones, S.L. Lessnick, M. Beckerle, R.L. Randall, The clone wars - revenge of the metastatic rogue state: the sarcoma paradigm, *Front Oncol.* 2 (2012) 2. <https://doi.org/10.3389/fonc.2012.00002>.
- [166] S.J. Cotterill, S. Ahrens, M. Paulussen, H.F. Jürgens, P.A. Voûte, H. Gadner, A.W. Craft, Prognostic factors in Ewing's tumor of bone: analysis of 975 patients from the European Intergroup Cooperative Ewing's Sarcoma Study Group, *J Clin Oncol.* 18 (2000) 3108–3114. <https://doi.org/10.1200/JCO.2000.18.17.3108>.
- [167] M. Paulussen, S. Ahrens, J. Dunst, W. Winkelmann, G.U. Exner, R. Kotz, G. Amann, B. Dockhorn-Dworniczak, D. Harms, S. Müller-Wehrich, K. Welte, B. Kornhuber, G. Janka-

- Schaub, U. Göbel, J. Treuner, P.A. Voûte, A. Zoubek, H. Gadner, H. Jürgens, Localized Ewing tumor of bone: final results of the cooperative Ewing's Sarcoma Study CESS 86, *J Clin Oncol.* 19 (2001) 1818–1829. <https://doi.org/10.1200/JCO.2001.19.6.1818>.
- [168] G. Bacci, S. Ferrari, F. Bertoni, S. Rimondini, A. Longhi, P. Bacchini, C. Forni, M. Manfrini, D. Donati, P. Picci, Prognostic factors in nonmetastatic Ewing's sarcoma of bone treated with adjuvant chemotherapy: analysis of 359 patients at the Istituto Ortopedico Rizzoli, *J Clin Oncol.* 18 (2000) 4–11. <https://doi.org/10.1200/JCO.2000.18.1.4>.
- [169] C. Juergens, C. Weston, I. Lewis, J. Whelan, M. Paulussen, O. Oberlin, J. Michon, A. Zoubek, H. Juergens, A. Craft, Safety assessment of intensive induction with vincristine, ifosfamide, doxorubicin, and etoposide (VIDE) in the treatment of Ewing tumors in the EURO-E.W.I.N.G. 99 clinical trial, *Pediatr Blood Cancer.* 47 (2006) 22–29. <https://doi.org/10.1002/psc.20820>.
- [170] R. Ladenstein, U. Pötschger, M.C. Le Deley, J. Whelan, M. Paulussen, O. Oberlin, H. van den Berg, U. Dirksen, L. Hjorth, J. Michon, I. Lewis, A. Craft, H. Jürgens, Primary disseminated multifocal Ewing sarcoma: results of the Euro-EWING 99 trial, *J Clin Oncol.* 28 (2010) 3284–3291. <https://doi.org/10.1200/JCO.2009.22.9864>.
- [171] T.G. Grünewald, M. Alonso, S. Avnet, A. Banito, S. Burdach, F. Cidre-Aranaz, G. Di Pompo, M. Distel, H. Dorado-Garcia, J. Garcia-Castro, L. González-González, A.E. Grigoriadis, M. Kasan, C. Koelsche, M. Krumbholz, F. Lecanda, S. Lemma, D.L. Longo, C. Madrigal-Esquivel, Á. Morales-Molina, J. Musa, S. Ohmura, B. Ory, M. Pereira-Silva, F. Perut, R. Rodriguez, C. Seeling, N. Al Shaaili, S. Shaabani, K. Shrivastava, S. Sinha, E.M. Tomazou, M. Trautmann, M. Vela, Y.M. Versleijen-Jonkers, J. Visgauss, M. Zalacain, S.J. Schober, A. Lissat, W.R. English, N. Baldini, D. Heymann, Sarcoma treatment in the era of molecular medicine, *EMBO Mol Med.* 12 (2020) e11131. <https://doi.org/10.15252/emmm.201911131>.
- [172] I. Machado, J.A. López-Guerrero, K. Scotlandi, P. Picci, A. Llombart-Bosch, Immunohistochemical analysis and prognostic significance of PD-L1, PD-1, and CD8+ tumor-infiltrating lymphocytes in Ewing's sarcoma family of tumors (ESFT), *Virchows Arch.* 472 (2018) 815–824. <https://doi.org/10.1007/s00428-018-2316-2>.
- [173] C. Spurny, S. Kailayangiri, S. Jamitzky, B. Altvater, E. Wardelmann, U. Dirksen, J. Harges, W. Hartmann, C. Rossig, Programmed cell death ligand 1 (PD-L1) expression is not a predominant feature in Ewing sarcomas, *Pediatr Blood Cancer.* 65 (2018). <https://doi.org/10.1002/psc.26719>.
- [174] J. Liu, H. Zhu, N. Zhong, Z. Jiang, L. Xu, Y. Deng, Z. Jiang, H. Wang, J. Wang, Gene silencing of USP1 by lentivirus effectively inhibits proliferation and invasion of human osteosarcoma cells, *Int J Oncol.* 49 (2016) 2549–2557. <https://doi.org/10.3892/ijo.2016.3752>.
- [175] S. Zhou, M. Xiong, G. Dai, L. Yu, Z. Zhang, J. Chen, W. Guo, MicroRNA-192-5p suppresses the initiation and progression of osteosarcoma by targeting USP1, *Oncol Lett.* 15 (2018) 6947–6956. <https://doi.org/10.3892/ol.2018.8180>.
- [176] S.A. Williams, H.L. Maecker, D.M. French, J. Liu, A. Gregg, L.B. Silverstein, T.C. Cao, R.A.D. Carano, V.M. Dixit, USP1 deubiquitinates ID proteins to preserve a mesenchymal stem cell program in osteosarcoma, *Cell.* 146 (2011) 918–930. <https://doi.org/10.1016/j.cell.2011.07.040>.
- [177] Q. Liang, T.S. Dexheimer, P. Zhang, A.S. Rosenthal, M.A. Villamil, C. You, Q. Zhang, J. Chen, C.A. Ott, H. Sun, D.K. Luci, B. Yuan, A. Simeonov, A. Jadhav, H. Xiao, Y. Wang, D.J. Maloney, Z. Zhuang, A selective USP1-UAF1 inhibitor links deubiquitination to DNA damage responses, *Nat Chem Biol.* 10 (2014) 298–304. <https://doi.org/10.1038/nchembio.1455>.
- [178] Q. Zeng, Z. Li, X. Zhao, L. Guo, C. Yu, J. Qin, S. Zhang, Y. Zhang, X. Yang, Ubiquitin-specific protease 7 promotes osteosarcoma cell metastasis by inducing epithelial-mesenchymal transition, *Oncol Rep.* 41 (2019) 543–551. <https://doi.org/10.3892/or.2018.6835>.
- [179] X. Chen, X. Zhang, H. Cai, W. Yang, H. Lei, H. Xu, W. Wang, Q. Zhu, J. Kang, T. Yin, W. Gu, Y.-L. Wu, Targeting USP9x/SOX2 axis contributes to the anti-osteosarcoma effect of neogambogic acid, *Cancer Lett.* 469 (2020) 277–286. <https://doi.org/10.1016/j.canlet.2019.10.015>.
- [180] D. Zhang, F. Jiang, X. Wang, G. Li, Downregulation of Ubiquitin-Specific Protease 22 Inhibits Proliferation, Invasion, and Epithelial-Mesenchymal Transition in Osteosarcoma Cells, *Oncol Res.* 25 (2017) 743–751. <https://doi.org/10.3727/096504016X14772395226335>.
- [181] W. Liu, D. Wang, L. Liu, L. Wang, M. Yan, miR-140 inhibits osteosarcoma progression by impairing USP22-mediated LSD1 stabilization and promoting p21 expression, *Mol Ther Nucleic Acids.* 24 (2021) 436–448. <https://doi.org/10.1016/j.omtn.2021.01.029>.
- [182] Z. Gan, K. Han, S. Lin, H. Hu, Z. Shen, D. Min, Knockdown of ubiquitin-specific peptidase 39 inhibited the growth of osteosarcoma cells and induced apoptosis in vitro, *Biol Res.* 50 (2017) 15. <https://doi.org/10.1186/s40659-017-0121-z>.

- [183] M. Lavaud, M. Mullard, R. Tesfaye, J. Amiaud, M. Legrand, G. Danieau, R. Brion, S. Morice, L. Regnier, M. Dupuy, B. Brounais-Le Royer, F. Lamoureux, B. Ory, F. Rédini, F. Verrecchia, Overexpression of the Ubiquitin Specific Proteases USP43, USP41, USP27x and USP6 in Osteosarcoma Cell Lines: Inhibition of Osteosarcoma Tumor Growth and Lung Metastasis Development by the USP Antagonist PR619, *Cells*. 10 (2021) 2268. <https://doi.org/10.3390/cells10092268>.
- [184] I.C. Henrich, R. Young, L. Quick, A.M. Oliveira, M.M. Chou, USP6 Confers Sensitivity to IFN-Mediated Apoptosis through Modulation of TRAIL Signaling in Ewing Sarcoma, *Mol Cancer Res*. 16 (2018) 1834–1843. <https://doi.org/10.1158/1541-7786.MCR-18-0289>.
- [185] M.E. Gierisch, G. Pedot, F. Walser, L.A. Lopez-Garcia, P. Jaaks, F.K. Niggli, B.W. Schäfer, USP19 deubiquitinates EWS-FLI1 to regulate Ewing sarcoma growth, *Sci Rep*. 9 (2019) 951. <https://doi.org/10.1038/s41598-018-37264-5>.
- [186] G. Danieau, S. Morice, F. Rédini, F. Verrecchia, B.B.-L. Royer, New Insights about the Wnt/ $\beta$ -Catenin Signaling Pathway in Primary Bone Tumors and Their Microenvironment: A Promising Target to Develop Therapeutic Strategies?, *Int J Mol Sci*. 20 (2019) E3751. <https://doi.org/10.3390/ijms20153751>.

## Legends of figures

**Fig. 1:** Schematic representation of the ubiquitination process. A) Enzymatic mechanisms of ubiquitin (Ub) transfer to a target. The initial ATP-dependent activation step catalysed by E1 (activating enzymes) is followed by the second step in which the ubiquitin is covalently linked to an E2 (conjugating enzyme), and the final step in which the ubiquitin is covalently attached to its target protein amino group facilitated by an E3 ligase enzyme. B) Different types of ubiquitination: Monoubiquitination, multiubiquitination, and polyubiquitination. C) Main physiological roles associated with protein ubiquitination.

**Fig. 2:** The DUBs family. A) Different members of the DUB family: ubiquitin-specific proteases (USP), ubiquitin C-terminal hydrolases (UCH), ovarian tumour domain proteases (OTU), Machado-Joseph domain proteases (MJD), the motif interacting with ubiquitin (MIU)-containing novel DUB family (MINDY), and JAB1/MPN/Mov37 metalloproteases (JAMM). B) Protease mechanisms: cysteine protease (left panel) and metalloprotease (right panel) (adapted from Erez et al., Nature, 2009). C) Schematic representation of the mechanism of action of USPs in three steps: After binding of USP to its substrate, the catalytic cysteine, deprotonated by a histidine residue, undergoes a nucleophilic attack on the carbonyl carbon atom of glycine 76 of ubiquitin to generate an acyl-enzyme intermediate. The hydrolysis of this intermediate allows the release of ubiquitin (adapted from Daviet and Colland, Biochemistry, 2008).

**Fig. 3:** Schematic representation of the action of USP4 and USP15 on the metastatic process via activation of the TGF- $\beta$  pathway. TGF- $\beta$  binds to two membrane receptors, type I (TBRI) and type II (TBRII). The activated TBRI receptor phosphorylates Smad3, which then associate with Smad4. The Smad3/4 complex is translocated into the nucleus and regulates the expression of target genes. Smad7 recruits E3-ubiquitin ligases (Smurf2) to the activated TRI, leading to receptor degradation. USP4 and USP15 directly deubiquitinate TBRI and, thus, drive TGF- $\beta$  responsiveness.

**Fig. 4:** Schematic representation of USPs action on key functions in osteosarcoma progression.

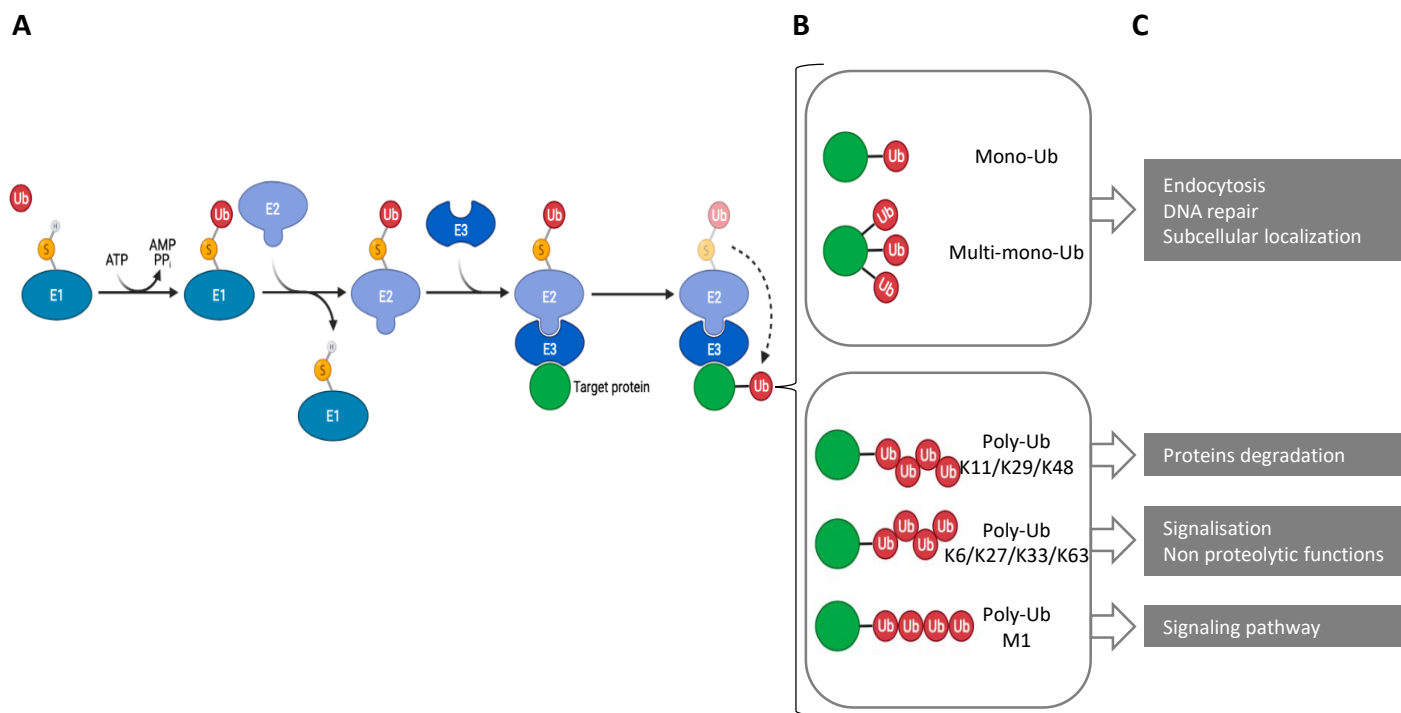
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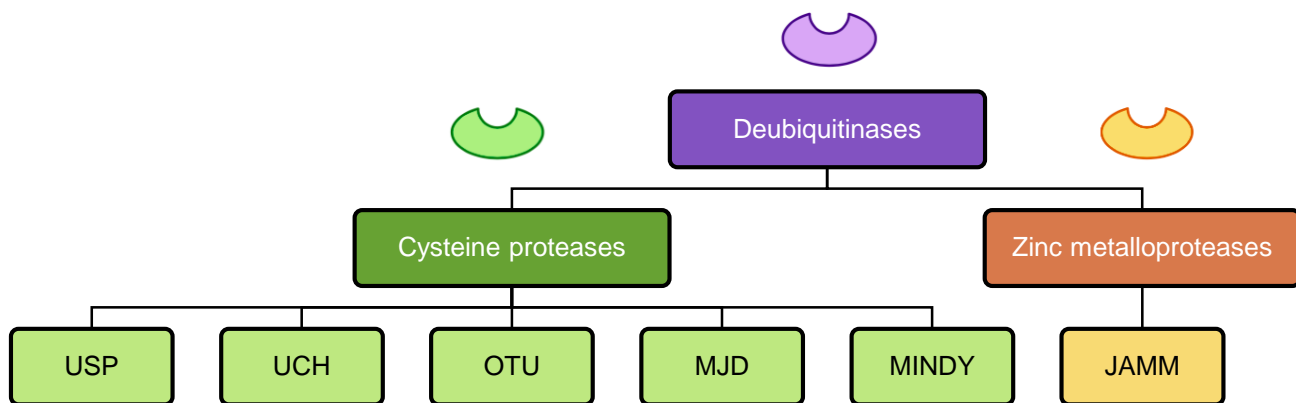
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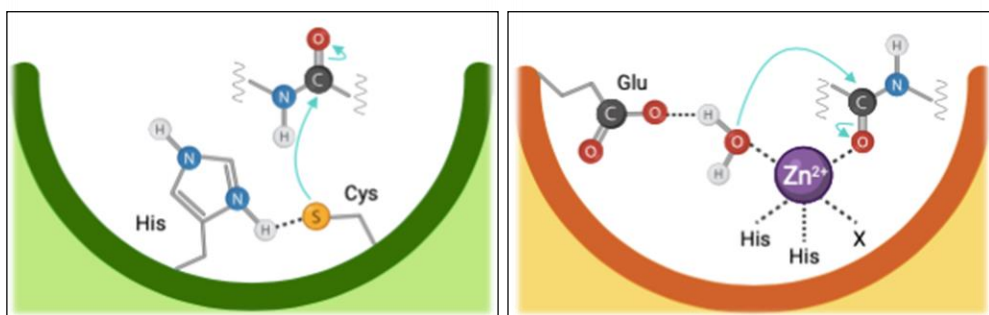


**Figure 1**

A



B



C

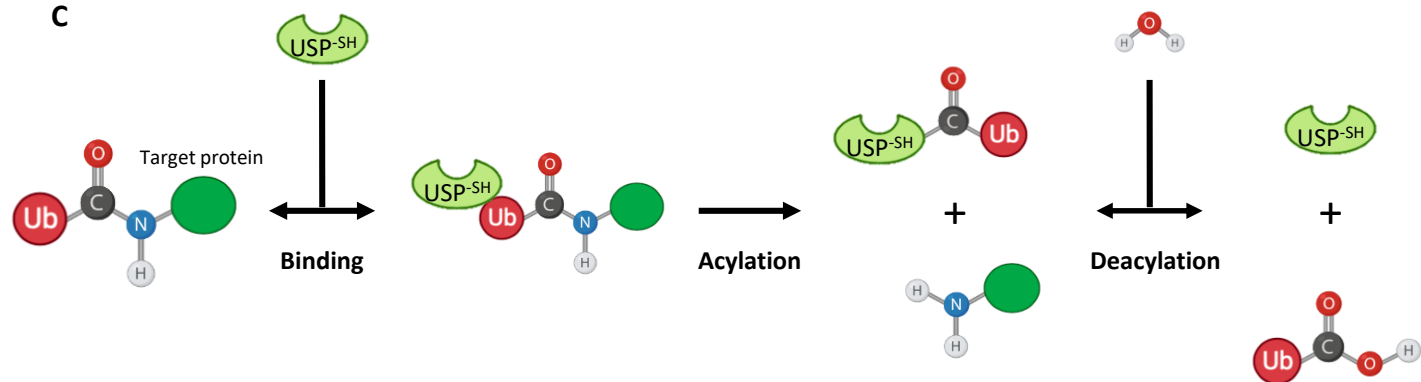


Figure 2

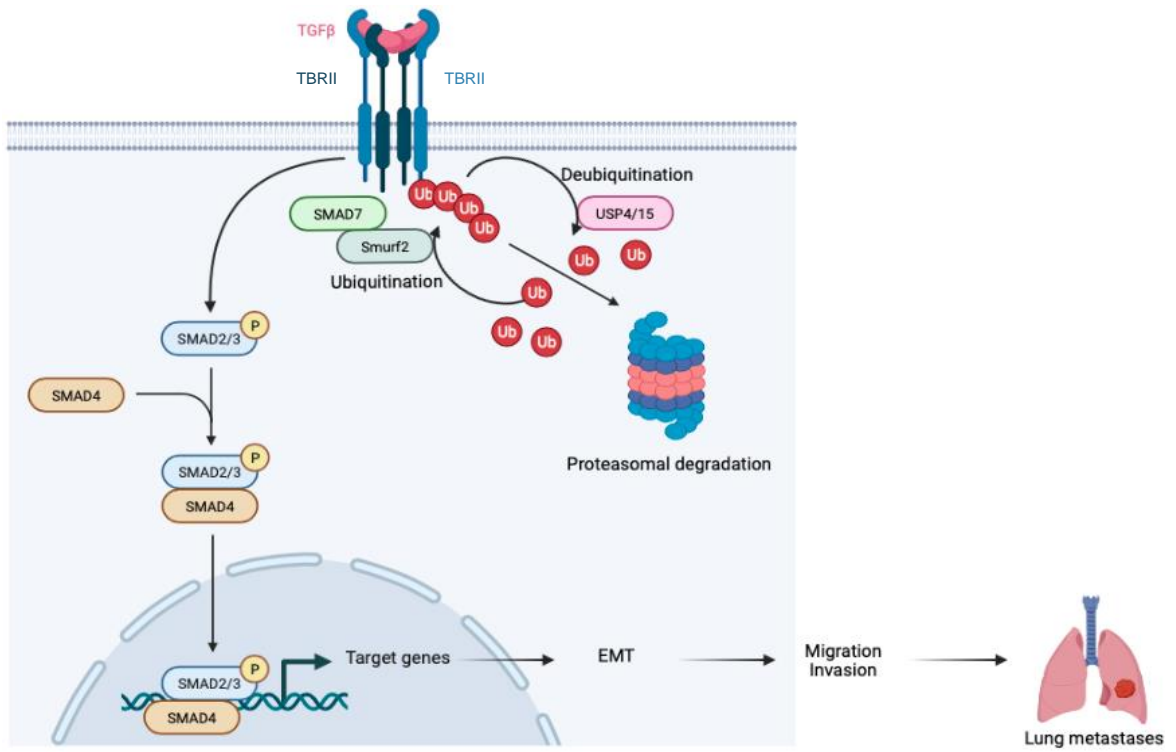
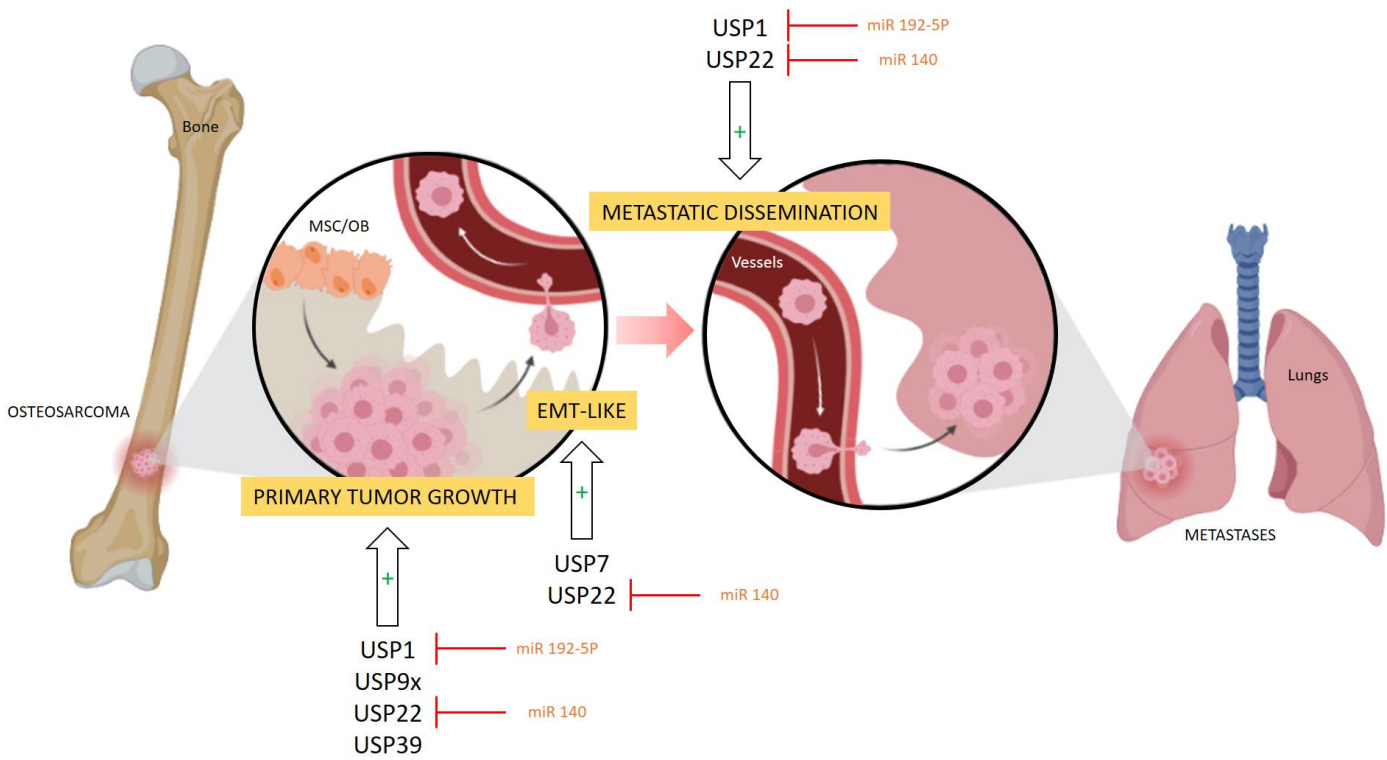


Figure 3





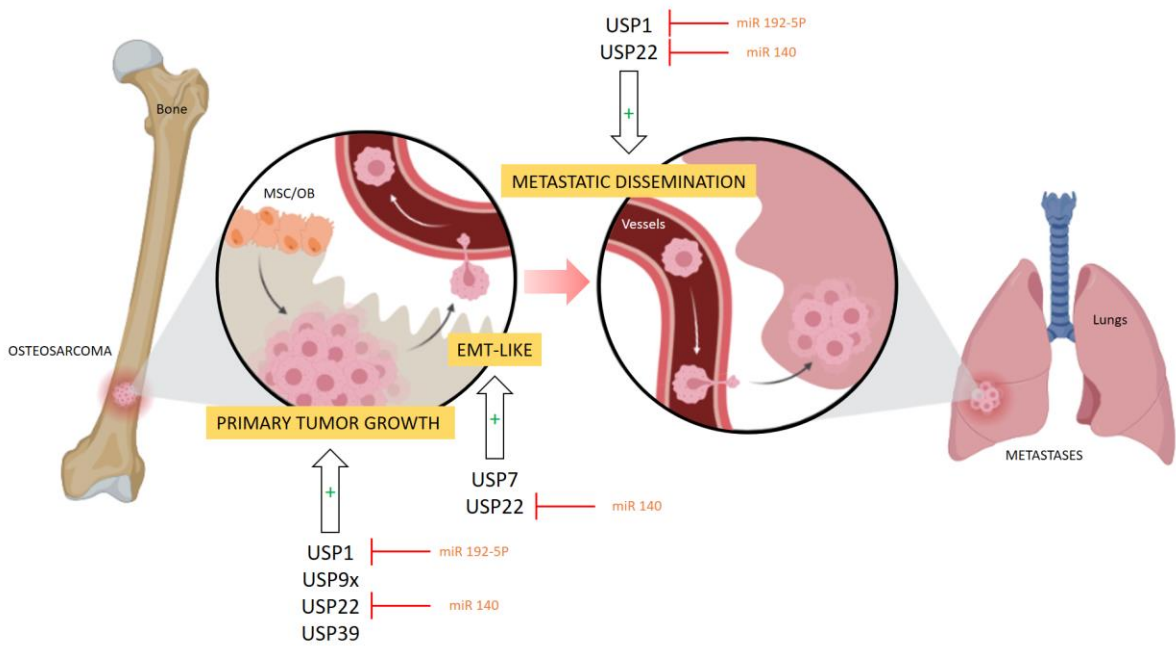
**Figure 4**

Protein	Gene	Length (AA)	Chromosomal location
Ubiquitin carboxyl-terminal hydrolase 1	USP1	785	1p31.3
Ubiquitin carboxyl-terminal hydrolase 2	USP2	605	11q23.3
Ubiquitin carboxyl-terminal hydrolase 3	USP3	520	15q22.31
Ubiquitin carboxyl-terminal hydrolase 4	USP4	963	3p21.31
Ubiquitin carboxyl-terminal hydrolase 5	USP5	858	12p13
Ubiquitin carboxyl-terminal hydrolase 6	USP6	1406	17p13.2
USP6 N-terminal-like protein	USP6NL	828	10p14
Ubiquitin carboxyl-terminal hydrolase 7	USP7	1102	16p13.2
Ubiquitin carboxyl-terminal hydrolase 8	USP8	1118	15q21.2
Probable ubiquitin carboxyl-terminal hydrolase FAF-X	USP9X	2554	Xp11.4
Ubiquitin carboxyl-terminal hydrolase 10	USP10	798	16q24.1
Ubiquitin carboxyl-terminal hydrolase 11	USP11	963	Xp11.3
Ubiquitin carboxyl-terminal hydrolase 12	USP12	370	13q12.13
Ubiquitin carboxyl-terminal hydrolase 13	USP13	863	Xp11.3
Ubiquitin carboxyl-terminal hydrolase 14	USP14	494	18p11.32
Ubiquitin carboxyl-terminal hydrolase 15	USP15	981	12q14.1
Ubiquitin carboxyl-terminal hydrolase 16	USP16	823	21q21
Ubiquitin carboxyl-terminal hydrolase 17	USP17L2	530	8p23.1
Ubl carboxyl-terminal hydrolase 18	USP18	372	22q11.2
Ubiquitin carboxyl-terminal hydrolase 19	USP19	1318	3p21.31
Ubiquitin carboxyl-terminal hydrolase 20	USP20	914	9q34.2
Ubiquitin carboxyl-terminal hydrolase 21	USP21	565	1q23.3
Ubiquitin carboxyl-terminal hydrolase 22	USP22	525	17p11.2
Ubiquitin carboxyl-terminal hydrolase 24	USP24	2620	1p32.3
Ubiquitin carboxyl-terminal hydrolase 25	USP25	1055	21q21.1
Ubiquitin carboxyl-terminal hydrolase 26	USP26	913	Xq26.2
Ubiquitin carboxyl-terminal hydrolase 27	USP27X	438	Xp11.23
Ubiquitin carboxyl-terminal hydrolase 28	USP28	1077	11q23.2
Ubiquitin carboxyl-terminal hydrolase 29	USP29	922	19q13.43
Ubiquitin carboxyl-terminal hydrolase 30	USP30	517	12q24.11
Ubiquitin carboxyl-terminal hydrolase 31	USP31	1352	16p12.2
Ubiquitin carboxyl-terminal hydrolase 32	USP32	1604	17q23.3
Ubiquitin carboxyl-terminal hydrolase 33	USP33	942	1p31.1
Ubiquitin carboxyl-terminal hydrolase 34	USP34	3546	2p15
Ubiquitin carboxyl-terminal hydrolase 35	USP35	1018	11q14.1
Ubiquitin carboxyl-terminal hydrolase 36	USP36	1123	17q25.3
Ubiquitin carboxyl-terminal hydrolase 37	USP37	979	2q35
Ubiquitin carboxyl-terminal hydrolase 38	USP38	1042	4q31.21
U4/U6.U5 tri-snRNP-associated protein 2	USP39	565	2p11.2
Ubiquitin carboxyl-terminal hydrolase 40	USP40	1235	2q37.1
Putative ubiquitin carboxyl-terminal hydrolase 41	USP41	358	22q11.21
Ubiquitin carboxyl-terminal hydrolase 42	USP42	1324	7p22.1
Ubiquitin carboxyl-terminal hydrolase 43	USP43	1123	17p13.1
Ubiquitin carboxyl-terminal hydrolase 44	USP44	712	12q22
Ubiquitin carboxyl-terminal hydrolase 45	USP45	814	6q16.2
Ubiquitin carboxyl-terminal hydrolase 46	USP46	366	4q12
Ubiquitin carboxyl-terminal hydrolase 47	USP47	1375	11p15.3
Ubiquitin carboxyl-terminal hydrolase 48	USP48	1035	1p36.12
Ubiquitin carboxyl-terminal hydrolase 49	USP49	688	6p21.1
Inactive ubiquitin carboxyl-terminal hydrolase 50	USP50	339	15q21.1
Ubiquitin carboxyl-terminal hydrolase 51	USP51	711	Xp11.21
Inactive ubiquitin carboxyl-terminal hydrolase 53	USP53	1073	4q26
Inactive ubiquitin carboxyl-terminal hydrolase 54	USP54	1684	10q22.2

Table 1 : List of human USPs adapted from Uniprot (<https://www.uniprot.org/>) et GeneCards (<https://www.genecards.org/Search/Keyword?queryString=USP53>)

	<b>Osteosarcoma</b>	<b>Ewing's sarcoma</b>
<b>Primary bone paediatric tumor ranking</b>	<i>1<sup>st</sup></i>	<i>2<sup>nd</sup></i>
<b>Incidence (worldwide)</b>	<i>3.4 / million / year</i>	<i>1.5 / million / year</i>
<b>Age</b>	<i>15-19 &amp; 75-79 years old</i>	<i>15 years old</i>
<b>Localization</b>	<i>Long bones</i>	<i>Long bones &amp; extra-osseous</i>
<b>Cellular origin</b>	<i>Mesenchymal stem cells</i>	<i>Mesenchymal stem cells &amp; primary cells from neural crest</i>
<b>Genetic origin</b>	<i>Complex karyotype</i>	<i>EWS-FLI1 fusion gene (85%) EWS-ERG fusion gene (10%)</i>
<b>Histological aspects</b>	<i>Osteoblastic / Chondroblastic / Fibroblastic</i>	<i>Osteoblastic</i>
<b>Radiographic aspects</b>	<i>Osteoformation / Osteolysis</i>	<i>Osteolysis</i>
<b>Main metastases localization</b>	<i>Lungs</i>	<i>Lungs</i>
<b>Treatment</b>	<i>Chemotherapy -&gt; Surgery -&gt; Chemotherapy</i>	<i>Chemotherapy -&gt; Surgery -&gt; Chemotherapy and/or radiotherapy</i>
<b>5 years survival rates after diagnosis</b>	<i>78% localized tumor ; 25% metastases</i>	<i>70% localized tumor ; 20% metastases</i>

Table 2 : Main clinical characteristics of osteosarcoma and Ewing's sarcoma



**Graphical abstract : Roles of USPs in the regulation of key functions in osteosarcoma development (primary tumour growth and metastatic development).**