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Review

SETDB1 amplification in osteosarcomas: Insights from its role in healthy tissues and other cancer types

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ABSTRACT

Epigenetic modifications, which reversibly regulate gene expression without altering the DNA sequence, are increasingly described in the literature as essential elements in the processes leading to cancer development. SETDB1 regulates histone 3 (H3) K9 di- and trimethylation, promoting heterochromatin formation, and plays a key role in gene silencing. Epigenetic deregulation of *SETDB1* expression appears to be involved in different cancers types, particularly in aggressive, relapsing or treatment-resistant subtypes. Despite advances in research, the full range of mechanisms through which this protein acts remains unclear; however, it is evident that SETDB1 has a pivotal role, particularly in the mesenchymal stem cells differentiation, tumor evasion and treatment resistance. Its role in genetically complex sarcomas, such as osteosarcoma, has not been fully explored, although recent Omics analyses suggest its presence and amplification in osteosarcoma. Given its involvement in osteoblastogenesis and adipogenesis, we discuss the potential of SETDB1 as a key target for new therapeutic strategies in osteosarcoma.

INTRODUCTION

Advances in biotechnology and genetics have provided a deeper understanding of the various mechanisms that can lead to oncogenesis and tumor aggressiveness. However, some tumor types, such as sarcomas, remain poorly understood and involve complex genetic processes, including multiple rearrangements and few recurrent somatic mutations. These complexities are often associated with limited or no therapeutic options [1]. Researchers are investigating the involvement of microenvironment, intercellular signals, immune system interactions, and global homeostasis in tumorigenesis. Tumor cells can manipulate and control these elements to promote their proliferation and dissemination [2–5]. For instance, recent studies have shown, that tumors can modulate neuroendocrine secretions to create a more favorable environment for their growth [6]. Sarcomas are a heterogeneous group of tumors of mesenchymal origin, accounting for about 15% of all cancers in children and young adults. Among sarcomas, osteosarcoma is the most prevalent malignant bone tumor in adolescents and young adults [7]. It is an aggressive tumor, and its current treatment consists of multidrug chemotherapy and surgical resection. Despite extensive efforts to understand the complex genetics of osteosarcoma or its microenvironment, no new, effective, therapeutic strategies have emerged [8, 9]. The survival rate remained stagnant over the past few decades (70% for localized forms and less than 30% for metastatic or treatment-resistant forms at diagnosis) due to chemotherapy resistance and metastatic recurrence.

Several recent studies on the biology and genetics of sarcomas, including osteosarcoma, have highlighted the role of epigenetics as a key component of tumor cell

plasticity and the regulation of neoantigen expression [1, 10]. Numerous genes involved in epigenetic regulation have been identified, which are capable of controlling transcription and, consequently, cell fate at various levels, particularly in mesenchymal tumors [11]. The definition of epigenetics encompasses DNA modifications such as acetylation, methylation, phosphorylation, ubiquitination, and sumoylation, which reversibly regulate gene expression. DNA methylation catalyzes chromatin changes leading to heterochromatin formation. Two distinct groups of proteins are involved: the PRMT1 family and the SET domain-containing protein family (which affect arginine and lysine residues) [12, 13].

Whole exome sequencing of osteosarcoma samples from both diagnosis and relapses has highlighted several factors, including *SETDB1*, that are amplified in the most aggressive forms of the disease [14]. In addition, current analyses show a correlation between *SETDB1* presence, DNA methylation levels of epigenomic targets, tumor aggressiveness, and response to treatments [15]. In this work, we will discuss the role of SETDB1 in the healthy state, in mesenchymal differentiation, and how its amplification may contribute to the plasticity and immune escape of osteosarcoma cells.

After reviewing the literature on the complex genetics of sarcomas, with a particular focus on osteosarcoma, we shifted our attention to the mechanisms of epigenetic regulation. In this review, only articles specifically describing SETDB1 were retained, sourced from bibliographic databases. The following keywords (SETDB1 osteosarcoma, epigenetic regulation in sarcoma, EMT, mesenchymal stem cells, immune escape, etc.) allowed us to categorize the articles, first focusing on the description of the protein itself, and then expanding to its role in the various related domains. Articles that did not specifically mention SETDB1 were excluded.

SETDB1: STRUCTURE AND INTERACTIONS

The "SET domain-bifurcated histone lysine methyltransferase 1" (SETDB1, also known as ESET, "ERG-associated protein with SET domain") catalyzes di- and tri-methylation of histone H3 at lysine 9 (H3-K9), resulting in two distinct histone marks: H3K9me2 and H3K9me3 [16–18]. This modification leads to heterochromatin formation, by creating a binding site for heterochromatin protein 1 (HP1), with the primary consequence of gene expression silencing. These essential epigenetic modifications play a key role in the repression of satellite repeats and transposable elements [19, 20].

The main known and described functions of SETDB1 include embryonic and postnatal development, promyelocytic leukemia nuclear body (PML-NB) formation, retroelement silencing, modulation of cell fate and proliferation, and immune cell regulation (Figure 1).

The SETDB1 protein structure, which includes three isoforms generated through alternative splicing, features a bifurcated SET domain and conserved amino acids found in other species, forming an interacting chain. The C-terminus of SETDB1 is responsible for the methylation reaction, while the N-terminus interacts with chromatin modification enzymes, such as DNA methyltransferases, particularly DNMT3, through its methyl-CpG-binding domain (MBD), leading to trimethylation of H3K9. Two Tudor domains located in the N-terminus facilitate the formation of complexes with other regulatory factors (Figure 2) [21, 22].

H3K9me3, generated by SETDB1, interacts with ATF7IP (activating transcription factor 7-interacting protein 1), a cofactor that facilitates the recruitment of HP1. The resulting complex plays a key role in forming heterochromatin by altering the spatial conformation of euchromatin from an "open" to a "closed" state, which restricts gene accessibility. Consequently, SETDB1 has a mostly suppressive role on gene expression [23], affecting indirectly other histone modifications, through mechanisms that remain poorly understood [21].

Beyond its activity on H3K9me3, SETDB1 also regulates cell fate and cell lineage by controlling the association of Cohesin with unique topological domains, recently identified as DiSCs (domains involving SETDB1 and Cohesin). Within the genome, this system represents a non-canonical model for SETDB1 binding [24].

Under physiological conditions, particularly during embryonic differentiation and development of the central nervous system (CNS), SETDB1 plays a key role [25]. It is also essential for maintaining X chromosome silencing in female mammalian cells [26]. Loss of H3K9me3 and dysregulation of SETDB1 have been associated with aging, neurological disorders, obesity, altered tissue integrity, and, ultimately, carcinogenesis [27–29].

SETDB1 ROLE IN DIFFERENTIATION OF MESENCHYMAL STEM CELLS

During embryogenesis and postnatal development, SETDB1 regulates cell stemness and cell fate by controlling the differentiation of MSCs through the modulation of transcription factors. During embryonic skeletal development, SETDB1/ESET, as a repressor, regulates the transactivating ability of RUNX2 (a hypertrophy-promoting transcription factor) indispensable for osteoblast differentiation [30]. SETDB1 can bind to and inhibit RUNX2 activity through its association with histone deacetylase 4, forming a multi-protein complex that activates SETDB1 intrinsic methyltransferase activity. This suppresses RUNX2-mediated transactivation of the osteocalcin gene [31]. Mesenchymal stem cells lose their ability to differentiate into osteoblasts in *ESET* knockout, which instead promotes the hypertrophic differentiation and apoptosis of articular chondrocytes. This is explained

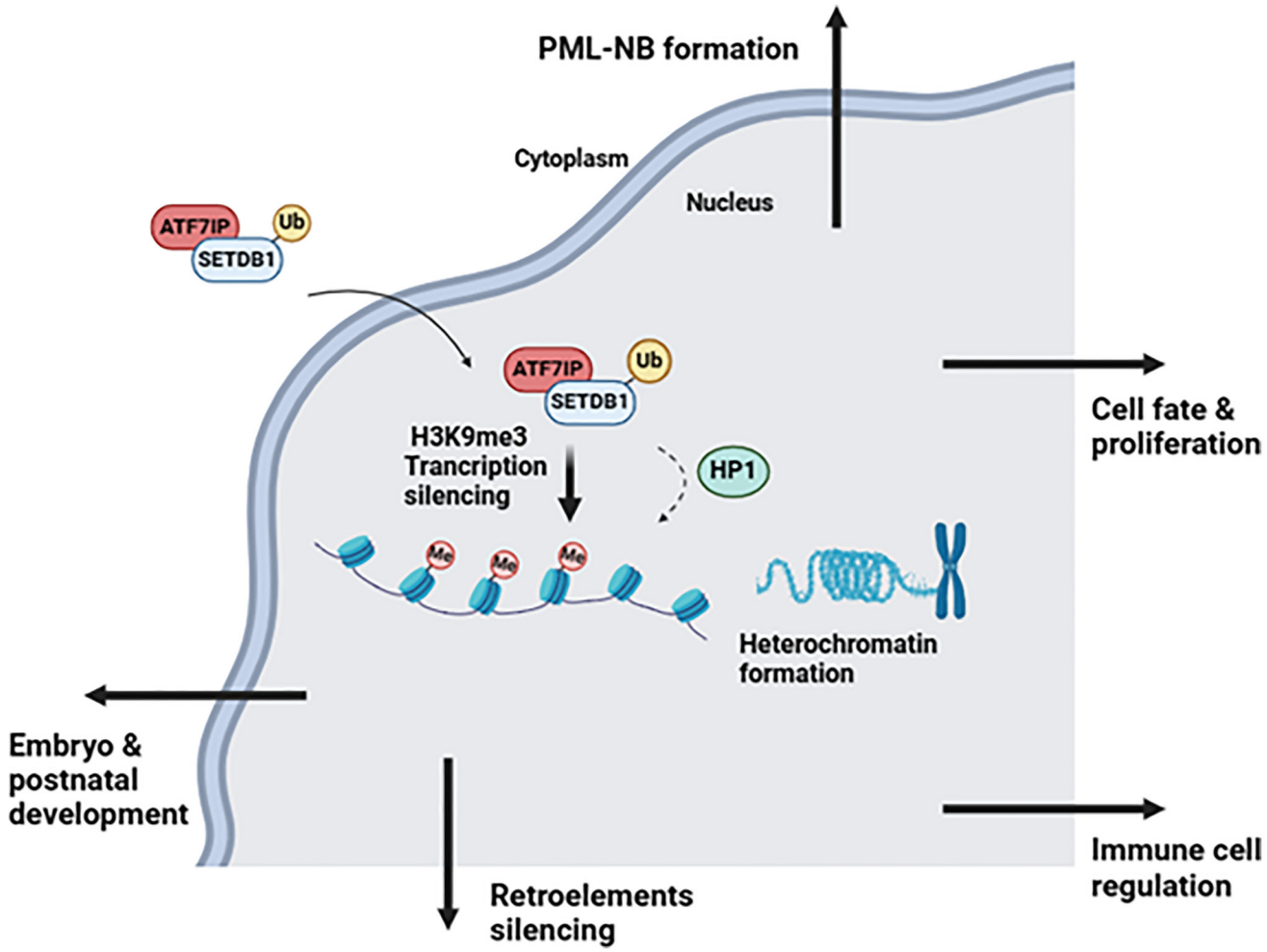

Figure 1: SETDB1 main functions, generated with BioRender (http://biorender.com).

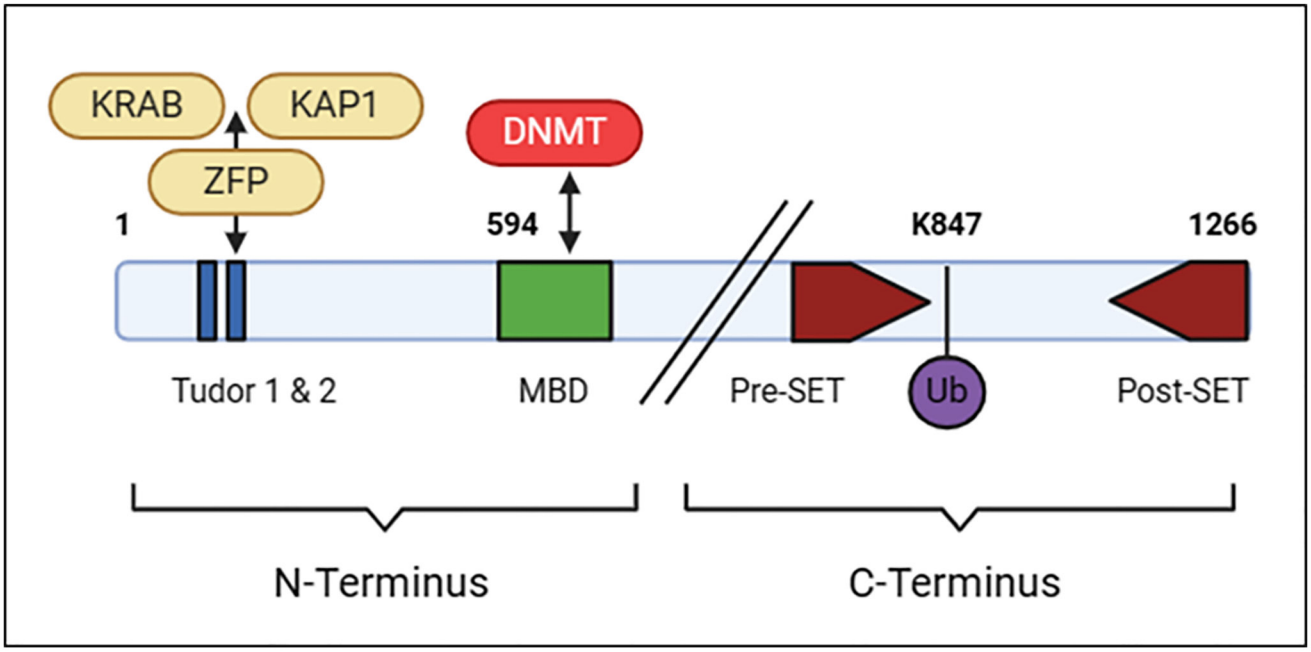

Figure 2: SETDB1 protein structure generated with BioRender (http://biorender.com).

by the upregulation of matrix metalloproteinases (MMPs) and disintegrin and metalloproteinase with thrombospondin motifs (ADAMTs), enzymes responsible for matrix degradation and typically regulated by RUNX2 [30]. In addition to the disorganization of growth plate chondrocytes, *SETDB1* seems to regulate epiphyseal plate formation and, when deleted, disrupts long bone growth [31, 32]. In the same way, through its action on the transcription factor peroxisome proliferator-activated receptor gamma PPARɣ - an inhibitor of osteoblastogenesis and inducer of adipogenesis - *SETDB1* regulates adipogenic differentiation of MSCs [33]. Two signaling pathways coexist: the canonical Wnt/beta-catenin pathway, which inhibits PPARɣ mRNA expression and the noncanonical Wnt pathway, which activates SETDB1, leading to PPARɣ repression via histone H3K9 methylation of its target genes [34]. In addition, another cellular pathway involving SETDB1, but independent of its methyltransferase activity, seems to repress adipogenic genes and inhibit pre-adipocyte differentiation in an independent DNA methylation way. Researchers suggest that SETDB1 ubiquitination, necessary for its activity, is not fully present during gene repression, implying the existence of an alternative pathway [35].

SETDB1, by inhibiting the expression of the endogenous retroviruses (ERVs) family of transposable elements, plays a crucial role in maintaining muscle tissue integrity and facilitating repair following injury. ERVs are known to trigger muscle cell death after once these cells exit their quiescent state. *In vivo*, in muscle stem cells (MuSCs), *SETDB1* absence leads to an uncontrolled inflammatory response mediated by the interferon pathway and cytokine release, ultimately causing necrosis [36]. This pro-inflammatory response occurs through the activation of the DNA-sensing cGAS-STING pathway. These two SETDB1-regulated events, are essential to maintaining a delicate balance between tissue regeneration and inflammatory response.

SETDB1 appears to be a key regulator of mesenchymal stem cell fate, influencing both adipogenesis and osteoblastogenesis. Considering the pathogenicity of osteosarcoma and its MSC-related origin, this protein could play a pivotal factor in tumor proliferation.

SETDB1 IN TUMORIGENESIS

During oncogenesis, *SETDB1* transcription is significantly upregulated, associated with tumorigenesis promotion and modulation of genes involved in various oncologic signaling pathways. *SETDB1* gain-of-function mutations promote proliferation, invasion and migration of cancer cells [37–39].

Depending on the cellular context, *SETDB1* can act either as a proto-oncogene, by silencing tumor suppressor genes such as APOE, p53 and HoxA, or as a tumor suppressor through the downregulation of the oncogene ANXA2 [18]. For example, under severe hypoxic conditions, *SETDB1* regulates p53-induced apoptosis [40], whereas in others conditions, it promotes tumorigenesis by increasing the expression of cancer-related genes. *SETDB1* is involved in multiple signaling pathways, including WNT, focal adhesion, MAPK, insulin, Toll-like receptors (TLR), and JAK-STAT pathways [29]. Additionally, *SETDB1* can promote AKT1 signaling and repress pro-apoptotic genes transcription. Given that AKT1 hyperactivation is associated with poor prognosis in several cancers, this role of *SETDB1* further underscores its importance in cancer progression [23].

Epigenetic deregulation of *SETDB1* expression seems to be involved in various types of cancers, including melanoma [37], hepatocellular carcinoma, ovarian [41], lung cancer, colorectal [42] and breast cancer [29, 43]. *SETDB1* is notably overexpressed in aggressive types of diseases and is often implicated in the epigenetic regulation of tumor progression and metastasis. In breast tumors, *SETDB1* plays a significant role in promoting metastasis by facilitating the acquisition of stem-cell-like properties and activating EMT programs [23]. *In vitro*, loss of *SETDB1* blocks cell invasion and migration, resulting *in vivo* into a reduction of lung metastasis. On the contrary, through a direct binding to the promoter of the transcription factor *SNAIL1,* overexpression of *SETDB1* enhances cell invasiveness by acting as an EMT inducer [44]. In the same way, *SETDB1* downregulates MiR7 leading to *STAT3* suppression, which inhibits BCSCs metastasis *in vivo* [45]. It also suppresses *FOXA2* expression, a key metastasis regulator, promoting NSCLC (non-small cell lung cancer) cells invasion and migration [39]. In ovarian cancer cells, knockdown of *SETBD1* prevents cells migration and motility by regulating *SF3B4* expression and influencing the tumor immune microenvironment [41]. In colorectal cancer, *SETDB1* drives tumor development and proliferation by downregulating the tumor suppressor factor p21 and promoting EMT regulation [42]. In myeloma cells, treatment resistance has been associated with the role of *SETDB1* in PI3K/AKT pathway and its involvement in epithelial-mesenchymal transition processes [46].

In pediatric high-grade gliomas (pHGG), gene silencing of *SETDB1* leads to a significant reduction in cell viability and proliferation, while promoting apoptosis. *SETDB1* silencing also reduces the expression of mesenchymal markers and decreases migration capacity of pHGG cells. Analysis of EMT markers following *SETDB1* silencing revealed a downregulation of CDH2, the MARCKS gene, and reduced Snail levels [47]. At the opposite, experiments on lung cancer cells have shown that inhibition of *SETDB1* using the CRISPR/Cas9 system decreases proliferation capacity but unexpectedly increases migration and transformation activities. In *SETDB1* knock out studies, researchers observed downregulation of B-catenin and E-cadherin expression,

modifications in E-cadherin cellular localization, and increased levels of STAT3 and Akt [48].

To date, very few studies describe the role of SETDB1 in sarcomas, and in osteosarcoma specifically, despite genetic analyses suggesting its involvement in osteosarcoma pathophysiology [14]. Through the epigenetic regulation of *GRIK2*, a known tumor-suppressor gene identified in gastric cancer, *SETDB1* appears to enhance cell proliferation, migration, and apoptosis resistance in osteosarcoma [49, 50].

SETDB1 AND IMMUNE PATHWAYS

SETDB1 appears to suppress tumor-intrinsic immunogenicity. One of the most prominent regulation of immune response mediated by *SETDB1* is its ability to prevent tumor cells from evading innate immune sensing by limiting endogenous retrotransposon expression. The first report of this function in cancer cells was published by Cuellar TL et al. in 2017 when the authors demonstrated that *SETDB1* silencing, by decreasing H3K9me3 at repetitive loci in AML cells, elevates the expression of IFN-β and interferon-stimulated genes [51]. More recently, Johnson et al. provided further insight into the diverse mechanisms by which *SETDB1* regulates the immune system, particularly in tumors. Specifically, *SETDB1* is involved in the methylation of the promoter regions of interleukins 2 and 7, modulates T cell function and development, and interferes with B lineage differentiation through endogenous retroviruses (ERVs) repression [52]. Human ERVs, derived from ancestral infections, account for approximately 8% of our genetic heritage and play a pathogenic role in immune diseases [53–55]. Beyond cancer, SETDB1 appears to be involved in immune modulation observed in autoimmune diseases such as immune thrombocytopenia (ITP), where the transcriptional levels of human ERVs correlate with those of *TRIM28/SETDB1* [56]. Similar to KDM5B, an H3K4 demethylase, *SETDB1* can function as an epigenetic checkpoint, preventing the presentation of antigenic determinants derived from transposable elements (TEs). KDM5B recruits SETDB1 to induce epigenetic silencing and ensures its retention in the cell nucleus. In invasive melanoma cells, this mechanism interferes with cancer stem cell-targeting responses by limiting the exposure of antigenic determinants, immunostimulatory cytokines secretion and other pro-inflammatory signals [57, 58]. When *SETDB1* is lost, the repression of transposable elements is disrupted, enabling the production of major histocompatibility complex class I (MHC-I) peptides, the encoding of viral proteins, and the triggering of T-cell responses. Similarly, in human tumors, immune exclusion and resistance to checkpoint blockade are associated with *SETDB1* amplification [58]. Overall, the deletion of *SETDB1* appears to enhance antitumor immune responses: antigen expression and presentation are increased, and T-cell activation is stimulated. Additionally, SETDB1 deficiency has been linked to impaired B-cell development [59].

Furthermore, during T-cell maturation, selection, and lineage development, SETDB1 influences various intracellular signaling genes through its H3K9me3 activity. Upon TCR stimulation, SETDB1 silencing in thymocytes results in impaired ERK activation, which is partially explained by the ectopic expression of the inhibitor FcγRIIB [60].

All these recent discoveries suggest that a balance must be achieved in SETDB1 expression: reducing its pro-tumoral action while preserving its pro-immunity function. Furthermore, three major repressive complexes-KRAB-ZFP, HUSH and KAP1/TRIM28-have been recently identified and shown to mediate immune modulation by SETDB1, depending on the cellular context. HUSH can recruit SETDB1 to silence genes, KRAB-ZFP activates H3K9me3 by binding to specific DNA motifs, and TRIM28, as a major corepressor protein, can bind to the KRAB domain and recruit *SETDB1*. In leukocytes, ERV repression is modulated by the SETDB1-KAP1 complex, whereas in melanocytes, KDM5B recruits SETDB1 [52].

How SETDB1 influences the tumor immunogenicity of osteosarcoma remains to be further investigated. In osteosarcoma, the tumor microenvironment-and particularly its immune infiltration-appears to be predominantly composed of T cells and macrophages [61, 62]. While immunotherapy has proven effective in certain types of cancer, it has not yet shown significant benefits in osteosarcoma. For instance, the pro-tumoral role of interferons (IFN) described in osteosarcoma did not translate into clinical benefit for patients treated with IFN-α2b maintenance therapy, as demonstrated in the EURAMOS-1 clinical trial [63]. Given SETDB1's role in T cell regulation, it could represent a promising therapeutic target. Its dual role in preventing antigenic expression and promoting immune exclusion warrants further investigation in osteosarcoma, a cancer which, despite its abundant chromosomal rearrangements, fails to express antigens.

SETDB1 AS A THERAPEUTIC TARGET

The current standard treatment for osteosarcoma consists of chemotherapy and surgery. Unfortunately, little progress has been made over the past forty years in improving survival rates for refractory or inoperable patients. Despite advancements in modern biology, no specific therapeutic targets have been identified, highlighting the need to investigate the role of protein such as SETDB1. New therapies under investigation include anti-PD1 therapies, inhibitors of the VEGF (vascular endothelial growth factor) and PDGF (platelet-derived growth factor) pathway, PI3K/mTOR pathway inhibitors, MYC oncogene inhibitors, and IGF (insulin-like growth

Table 1: SETDB1 inhibitor molecules

Inhibitors	Action	Limitations
DZNep	**Epigenetic regulation** Inhibites S-adenosyl homocystein hydrolase: H3K27me3 inhibitor	No specific and limited action
Paclitaxel	**Cell cycle** Chemotherapy inhibiting tubulin depolymerization **Epigenetic regulation** Down-regulates *SETDB1* gene expression in a p53 dependent manner	No specific action; Unknown epigenetic mechanism of action
MiR-7	**Gene translation blocking** Directly targets the 3'UTR of SETDB1	Fragile, unstable molecules, requiring a vector for action
Mithramycin A	**Transcriptional regulation** binding actions and competes for GC-rich promoter regions of SETDB1	Limited clinical activity due to tumor heterogeneity
SETDB1-TTD-IN-1 TFA	Selective endogenous binder-competitive small-molecule SETDB1 tandem tudor domain (TTD) inhibitor	Fundamental research only, under study

factor) inhibitors. Although some current studies show promising results , their efficacy remains imperfect [8].

Until now, no specific inhibitors have been used in SETDB1 studies. Instead, 3'-deazaneplanocin A (DZNep), paclitaxel, mithramycin A, and microRNA therapeutics have been primarily explored as inhibitory strategies targeting SETDB1 [23, 29, 45, 64, 65].

In lung cancer cells, the non-specific inhibitor DZNep targets various HMTases, reduces their expression levels, and promotes apoptosis. However, its principal limitation is its lack of specificity [23, 66].

Paclitaxel, an anticancer molecule produced by endophytic fungi, downregulates SETDB1 expression by binding to its promoter region and also enhances p53 expression [64].

MicroRNA therapeutics represent promising candidates for replacing or enhancing the activity of underexpressed tumor-suppressor microRNAs in tumor cells. For example, as proposed by Zhang et al. [45], miR-7 might be introduced to control tumorigenesis via SETDB1 regulation. However, to date, these therapeutic molecules have shown limited clinical benefit.

Mithramycin A, an antitumor antibiotic, is a transcription factor inhibitor that recognizes and binds to GC-rich promoter regions of oncogenic genes, such as *SETDB1*. A study demonstrated that mithramycin A has significant effects on H3K9me3 signatures and *SETDB1* expression [65]. Although mithramycin A exhibits potent anticancer activity, it is associated with severe side effects due to its non-specific action. To overcome this limitation, combinatorial biosynthesis has led to the development of several mithramycin analogs, referred to as "Mithralogs", which demonstrate superior antitumor efficacy an improved toxicity profile [29].

Recently, a selective cell-active inhibitor of *SETDB1* Tudor Domain was discovered. SETDB1-TTD-IN-1 TFA is a potent, competitive and selective small-molecule inhibitor that targets the *SETDB1* tandem Tudor domain (SETDB1-TTD) [67]. By competing with endogenous binders, this molecule disrupts the interaction of SETDB1-TTD with methylated lysine residues, thereby blocking its recognition. This discovery offers a valuable tool for scientists to better understand the precise biological functions of SETDB1-TTD. All these molecules are summarized in Table 1.

It is well established that SETDB1 regulates gene expression by interacting with multiple factors. Therefore, targeting downstream proteins regulated by SETDB1 may offer a strategy to increase the specificity of therapeutic molecules. For example, SETDB1 modulates the transcriptional activity of PPARγ, a lipid-binding nuclear receptor involved in osteoblastogenesis mechanisms. PPARγ is targetable by T0070907, an available irreversible inverse agonist, which has shown potential interest in osteosarcoma (OS) progression [68–70].

Despite the promise raised by such specific inhibitors, studies on similar types of molecules have yet to demonstrate clear clinical benefits and thus require further investigation.

The identification of a SETDB1 inhibitor could provide new hope for the treatment of osteosarcoma by modulating its anti-tumor immune activity and altering its microenvironment, potentially enhancing tumor sensitivity to therapeutic approaches, such as radiotherapy.

SETDB1 AND RADIO-SENSITIVITY

In osteosarcoma, radiotherapy is typically used for unresectable disease to achieve better local control. However, in most cases, this treatment is ineffective due to unknown mechanisms. Osteosarcoma often requires high doses of radiation to achieve disease control [71, 72]. Cellular exposure to ionizing radiation can activate the type I interferon response, which plays a crucial role in tumor response to radiotherapy through the cGAS/STING signaling pathway. DNA methyltransferase

inhibitors have been shown to promote ERV activation, leading to the production of type I interferons by inducing a viral mimicry state. This is achieved through the generation of cytoplasmic double-stranded RNAs (dsRNA) and the activation of the RIG-I–MDA5-MAVS signaling pathway [73, 74]. SETDB1 plays a crucial role in suppressing ERV activation by maintaining heterochromatin. Radiation can promote ERV activation by significantly attenuating *SETDB1* expression, which leads to a downregulation of H3K9 trimethylation. Furthermore, *SETDB1* loss could significantly enhance ERV activation and type I interferon production, thereby sensitizing murine tumors to radiotherapy, which is dependent on cytotoxic T cells and type I interferons [59]. Tumor radiosensitivity appears to be inversely correlated with *SETDB1* expression, through mechanisms that influence cellular immune infiltration, including T cells, macrophages, NK cells, and dendritic cells [52, 75]. These findings are consistent with its role in immune escape and the regulation of various pro-inflammatory actors involved in therapeutic responses in oncology, particularly in osteosarcoma.

SETDB1 AMPLIFICATION MIGHT PLAY A MAJOR ROLE IN OSTEOSARCOMA TREATMENT AND IMMUNE ESCAPE

Altogether, studies on SETDB1 paint the picture of a key protein involved in tumor progression, resistance to therapeutics and MSC commitment [18, 23, 29, 44], although its role in osteosarcoma remains poorly characterized [49, 50]. *SETDB1* amplification is associated with increased aggressiveness and therapeutic tolerance through several mechanisms, particularly in tumor immunogenicity [51, 57, 58]. *SETDB1* expression, through the mechanisms described above, influences the response to anti-cancer therapies, including radiotherapy [52, 59, 75].

In a recent study, we confirmed, through whole exome sequencing of osteosarcoma samples from diagnosis and relapses, as well as their derived PDX models, the presence of *SETDB1* amplification, predominantly in relapses [14]. Given its involvement in osteoblastogenesis and adipogenesis [30–32], this protein could play a significant role in the formation and proliferation of osteosarcomas, as well as in promoting migration and metastasis (Figure 3).

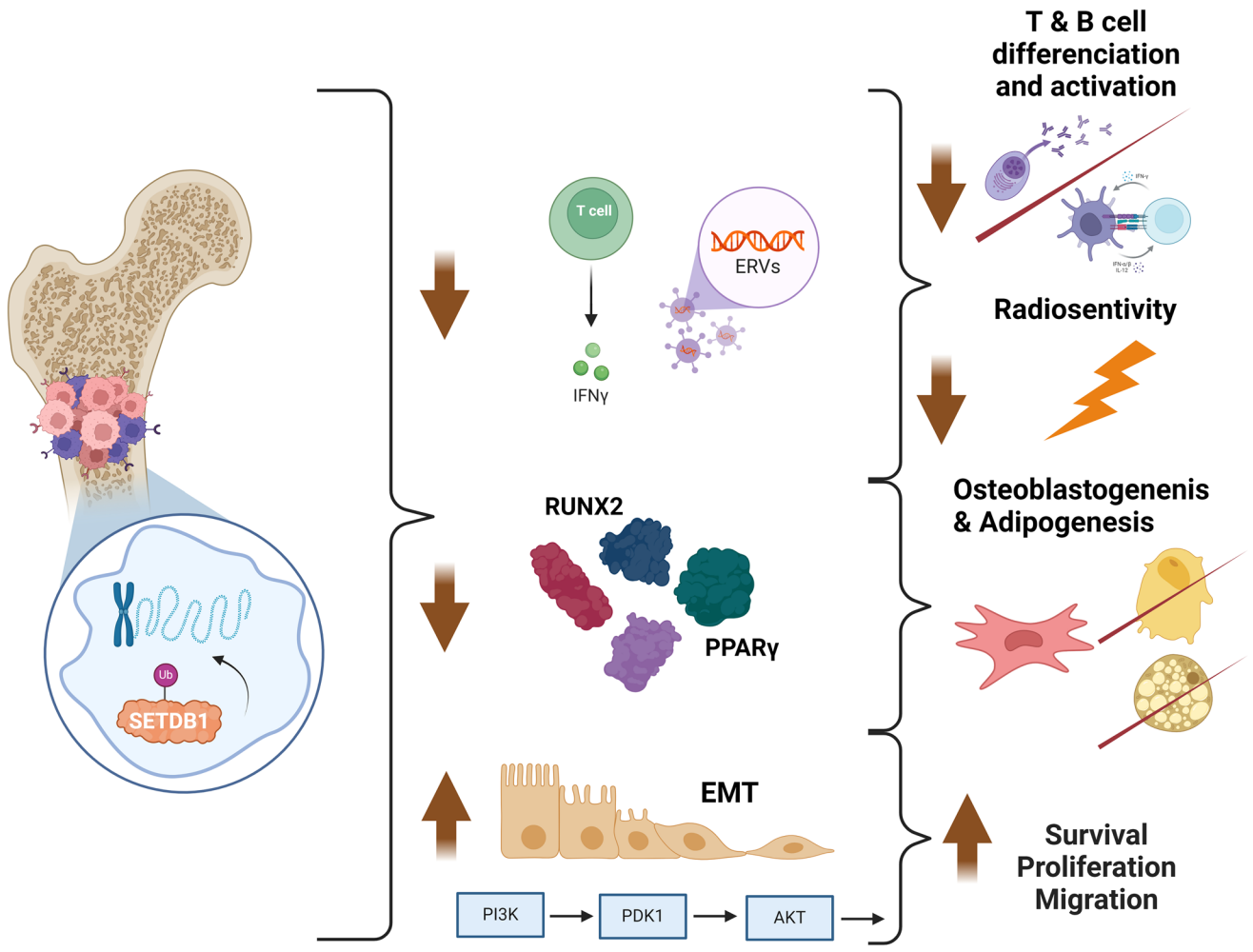

Figure 3: Proposed summary of the role of SETDB1 in osteosarcoma (http://biorender.com).

The challenge of specifically targeting this protein is twofold: to block its action on MSC differentiation and to reinstate the cytotoxic activity of the immune system. The recent discovery of a specific inhibitor, SETDB1-TTD-IN-1 TFA, provides a valuable opportunity to study SETDB1's action on tumor cells.

As mentioned above, there are few, if any, studies describing SETDB1 in osteosarcoma, and we are only beginning to understand how this protein can shape the expression of downstream targets.

CONCLUSIONS AND FUTURE PERSPECTIVE

Osteosarcoma is characterized by a complex genetic profile that leads to significant genetic instability, which contributes to therapeutic resistance. This is one of the reasons why there have been no new therapeutic breakthroughs in recent decades, and survival rates remain stable, with the persistent problem of refractory or relapsed disease. Technological advances have enabled a detailed description of the genetic landscape of tumors, thus thereby deepening our understanding of the origins of tumorigenesis. A stratification of osteosarcomas has been developed based on these methods, opening up new and, better-targeted therapeutic avenues [76].

In recent years, epigenetics has emerged as a key mechanism in oncogenesis and cancer aggressiveness. SETDB1, through its role in histone methylation, is a major player in heterochromatin formation, and thus promotes or prevents the expression of numerous genes linked to carcinogenesis. Its involvement in various cancers has been well studied, but its role in osteosarcoma remains unclear. Our team has demonstrated the presence of *SETDB1* abnormalities, particularly its amplification, through WES analysis of osteosarcoma human samples and cell lines [14]. Therefore, it is now essential to continue research on the role of *SETDB1* in osteosarcoma and investigate whether its inhibition could provide a pathway to improve patient care.

AUTHOR CONTRIBUTIONS

E. Verdier designed and wrote the article; all the others authors supervised, reviewed and approved the article.

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CONFLICTS OF INTEREST

Authors have no conflicts of interest to declare.

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