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REVIEW ARTICLE

Assessment of Synergistic Contribution of Histone Deacetylases in Prognosis and Therapeutic Management of Sarcoma

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Abstract

Sarcomas are a rare group of neoplasms with a mesenchymal origin that are mainly characterized by the abnormal growth of connective tissue cells. The standard treatment for local control of sarcomas includes surgery and radiation, while for adjuvant and palliative therapy, chemotherapy has been strongly recommended. Despite the availability of multimodal therapies, the survival rate for patients with sarcoma is still not satisfactory. In recent decades, there has been a considerable effort to overcome chemotherapy resistance in sarcoma cells. This has led to the investigation of more cellular compounds implicated in gene expression and transcription processes. Furthermore, it has been discovered that histone acetylation/deacetylation equilibrium is affected in carcinogenesis, leading to a modified chromatin structure and therefore changes in gene expression. In addition, histone deacetylase inhibition is found to play a key role in limiting the tumor burden in sarcomas, as histone deacetylase inhibitors act on well-described oncogenic signaling pathways. Histone deacetylase inhibitors disrupt the increased cell motility and invasiveness of sarcoma cells, undermining their metastatic potential. Moreover, their activity on evoking cell arrest has been extensively described, with histone deacetylase inhibitors regulating the reactivation of tumor suppressor genes and induction of apoptosis. Promoting autophagy and increasing cellular reactive oxygen species are also included in the antitumor activity of histone deacetylase inhibitors. It should be noted that many studies revealed the synergy between histone deacetylase inhibitors and other drugs, leading to the enhancement of an antitumor effect in sarcomas. Therefore, there is an urgent need for therapeutic interventions modulated according to the distinct clinical and molecular characteristics of each sarcoma subtype. It is concluded that a better understanding of histone deacetylase and histone deacetylase inhibitors could provide patients with sarcoma with more targeted and efficient therapies, which may contribute to significant improvement of their survival potential.

1 Introduction

Sarcomas are a rare group of neoplasms with a mesenchymal origin that are mainly characterized by the abnormal growth of connective tissue cells. They can be classified into two major categories, bone sarcomas and soft-tissue sarcomas (STSs) and they represent 1% of malignancies [1, 2]. The incidence of new cases of STSs is estimated at 4–5 cases per 100,000 per year, while that of bone sarcomas is 1 case per 100,000 per year [3]. Sarcomas are more common among children as they account for over 20% of all pediatric solid neoplasms [4]. Moreover, STSs can be considered mainly as a neoplastic disorder of adolescents and young adults, as 18% of all STSs are diagnosed between the ages of 18 and 40 years [5, 6]. Furthermore, STSs can occur in various anatomical sites of the body and over 110 different histological subtypes have been described in the literature [7, 8]. The cause of sarcomas remains unknown while carcinogens,

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Key Points

Histone acetylation/deacetylation equilibrium is affected in carcinogenesis, leading to an altered chromatin structure and therefore changes in gene expression.

Inhibition of histone deacetylase promotes a variety of anticancer processes in sarcoma; a rare group of neoplasms with a mesenchymal origin that are mainly characterized by the abnormal growth of connective tissue cells.

A better understanding of histone deacetylase and histone deacetylase inhibitors could provide patients with sarcoma with more targeted and efficient therapies, which may contribute to a significant improvement of their survival potential.

viruses, ionizing radiation, co-existence of familial cancer syndromes, such as Li Fraumeni syndrome, retinoblastoma, Gardner's syndrome, Werner's syndrome, and neurofibromatosis type 1, and some immunodeficiency conditions may play a pivotal role in their development [9–11]. The standard treatment for local control of sarcomas includes surgery and radiotherapy, while for adjuvant and palliative therapy, chemotherapy is strongly recommended [12]. Therefore, there is a growing necessity to discover new therapeutic strategies for the treatment of sarcomas.

In recent decades, there has been a considerable effort focused on overcoming chemotherapy resistance in tumor cells. This has led to the investigation of more cellular compounds implicated in gene expression and transcription processes. Furthermore, it has been discovered that histone acetylation/deacetylation equilibrium is affected in carcinogenesis, leading to a modified chromatin structure and therefore changes in gene expression [13]. A large amount of data has provided evidence that histone deacetylases (HDACs) influence diverse cellular processes and contribute to sarcoma growth and progression by multiple mechanisms [12]. The aim of this review article is to elucidate the molecular identity and mechanism of action of HDAC inhibitors as well as verify their potential utility as anti-cancer agents. We especially focus on and critically review the relevant literature of HDAC implications in sarcoma development and treatment.

2 Histone Deacetylase (HDAC) Molecular Identity and Mechanism of Action

The regulation of gene expression in eukaryotic cells is linked directly to the structure of chromatin, an organized and dynamic protein–DNA complex [14]. The fundamental subunit of chromatin is the nucleosome, which consists of a 146-base pair segment of DNA wrapped around eight histone proteins, two H2A/H2B dimers, and a H3/H4 tetramer [15–17]. There are several histone post-translational modifications, such as acetylation, methylation, ubiquitination, and phosphorylation, which can modify the accessibility of various proteins to the DNA, altering its expression [16]. Acetylation and deacetylation are histone modifications mediated through histone acetyltransferases and HDACs, respectively [18] (Fig. 1b). Histone deacetylases, having the ability to remove acetyl groups from histones, increase chromatin condensation, rendering DNA less accessible to various factors that can promote gene transcription [19] (Fig. 1d). Histone deacetylase activity has been linked to transcriptional regulation, chaperone function, modulation of apoptosis, DNA repair, autophagy, metabolism, senescence, and angiogenesis [20] (Fig. 1e).

2.1 Classification of Histone Acetylation/Deacetylation

Important functions in a cell, such as transcriptional regulation, cell cycle, and apoptosis, co-exist normally when a specific equilibrium between histone acetyltransferases and HDACs is established. Therefore, elevated activity of HDACs has been shown to have an upstream effect on genes regulating cell proliferation, migration, and metastasis [21]. Histone deacetylases have been classified into four classes, according to their yeast analogs. Class I consists of nuclear HDACs 1, 2, 3, and 8. Class IIa includes HDACs 4, 5, 7, and 9, and HDACs 6 and 10 belong to Class IIb. Class II HDACs are located both in the nucleus and the cytoplasm. Class IV includes HDAC 11. Class I, II, and IV are Zn2 + dependent. Class III HDACs are NAD + -dependent homologs of the yeast sirtuin proteins [22, 23].

2.2 HDAC Inhibition in Cancer Cells

Histone deacetylase inhibitors have been classified into five groups: (I) hydroxamic acids (i.e., trichostatin A); (II) aliphatic acid compounds (phenylbutyrate and valproic acid); (III) benzamides (i.e., MS275); (IV) cyclic tetrapeptides (i.e., trapoxin B); and (V) sirtuin inhibitors, including the pan-inhibitor nicotinamide and SIRT1 and SIRT2 inhibitors sirtinol and cambinol, respectively [21]. Histone deacetylase inhibitors have been extensively studied in clinical trials,

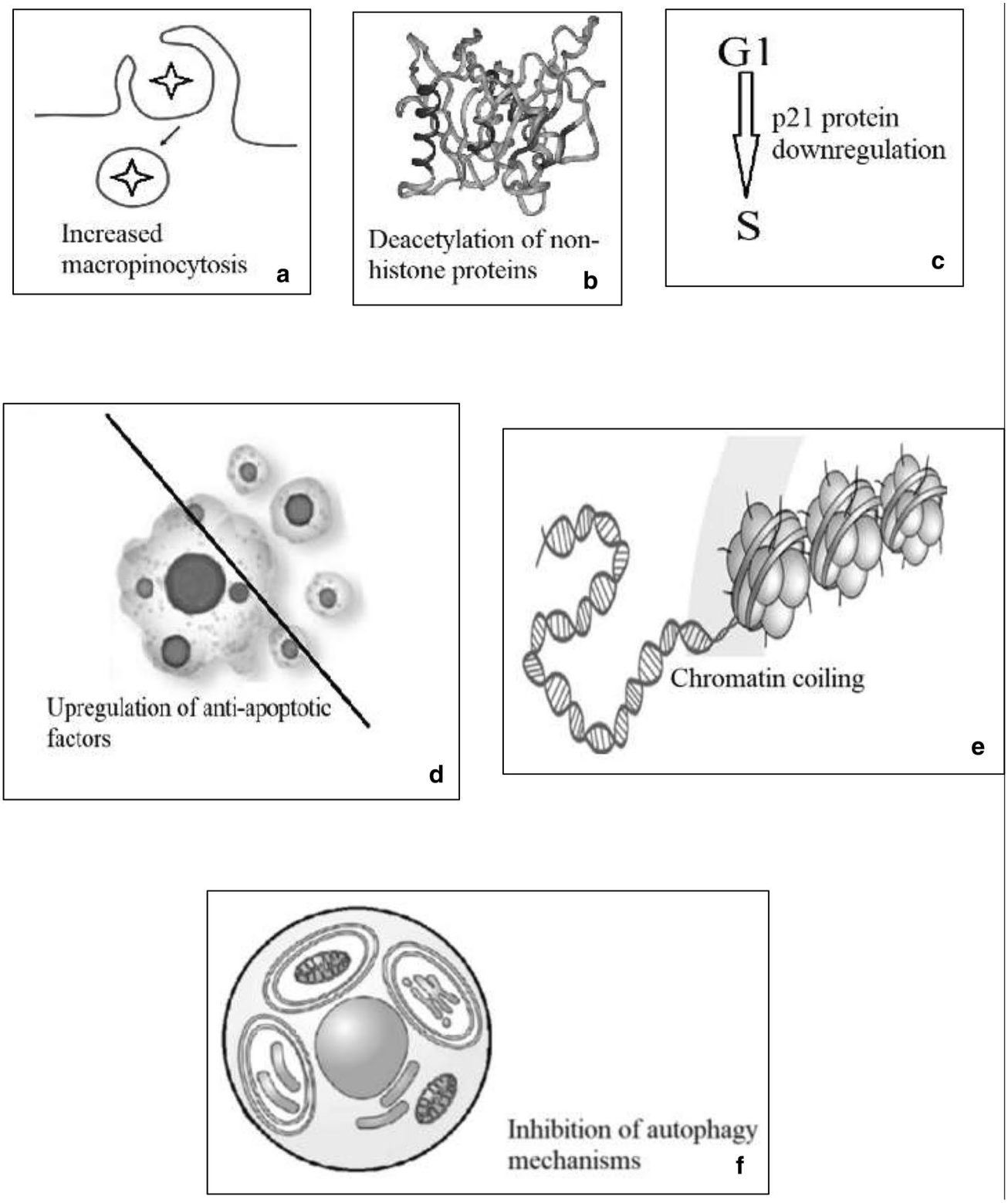

Fig. 1 Histone deacetylase implication and mechanism of action in carcinogenesis process

though only four have been approved in clinical practice. Vorinostat and romidepsin are being administered for the treatment of advanced primary cutaneous T cell lymphoma, belinostat for the therapy of peripheral T-cell lymphoma, and panobinostat has been recommended for the treatment of multiple myeloma. Thrombocytopenia, neutropenia, diarrhea, nausea, vomiting, and fatigue are the most commonly reported toxicities [24].

The anticancer effects of HDAC inhibitors are mostly mediated by cell-cycle arrest, apoptosis and autophagy induction, alteration of non-coding RNA expression, and inhibition of angiogenesis [24]. Histone deacetylase inhibitors regulate the expression of a variety of cell-cycle-related genes, promoting cell-cycle arrest and induction of cell differentiation. It has also been suggested that they induce the upregulation of proteins p21 and p53, which have a synergistic function and promote cell-cycle arrest and apoptosis [25, 26] (Fig. 1c). Another promising therapeutic anticancer strategy is autophagy by HDAC inhibitors. The balance between histone acetyltransferases and HDACs regulates the acetylation of many autophagy-related proteins, including the product of the autophagy-related genes [24, 27]. Unfortunately, the exact pathways involved have still not been elucidated as there are differences in the models applied, cancer cells, HDAC inhibitors, and their doses. Moreover, it has been proposed that increased macropinocytosis in cancer cells is induced by HDAC overexpression and is highly related to cellular migration and substantial metastatic potential [28] (Fig. 1a).

It is common knowledge that HDAC inhibitors modify non-coding RNA expression, and therefore alter cell cycle and differentiation. In accordance with recent research, long non-coding RNAs and important cellular transcriptional and post-transcriptional molecules regulate histones that alter complexes to target loci that are either activated or silenced [24, 29, 30]. In addition, HDAC inhibitors can decrease cancer angiogenesis by controlling the cellular stress response pathways. Inhibition of Class II HDACs intensifies the population and emphasizes the function of regulatory T cells, while Class I HDAC inhibitors boost the functions of natural killer cells and CD8 T cells [31].

Finally, HDAC inhibitors have been identified as having a synergistic effect when administered alongside with DNA targeting chemotherapeutic agents, such as inhibitors of topoisomerases or DNA synthesis, DNA intercalators, and agents covalently modifying DNA (i.e., doxorubicin, etoposide, 5-fluorouracil, cisplatin, melphalan, temozolomide, and ellipticine). It has been also reported that combined treatment with HDAC inhibitors and chemotherapy or radiotherapy can result in an increased anticancer efficacy [32]. Results are summarized in Table 1.

3 HDAC Inhibition in Sarcoma Development and Treatment

3.1 HDAC Contribution in the Management of Osteosarcoma

Osteosarcoma is the most common primary malignancy of bone that occurs more often in adolescents and young adults, with a higher incidence in men [6, 33]. The 5-year survival rate for patients with primary or metastatic tumors is 65% and 20%, respectively, making osteosarcoma a malignancy with a poor prognosis [34]. Metastasis occurs frequently, is present in over 80% of affected patients, and is detected most commonly in the lungs [35]. Therefore, osteosarcoma is one of the most biologically virulent cancers and is difficult to cure by conventional procedures. Elucidation of its molecular basis may prove useful in developing and identifying prognostic biomarkers.

The identification of strong metastatic potential in highly metastatic murine osteosarcoma cells has been shown to be limited in vitro by the use of HDAC inhibitors, such as vorinostat [33]. Limitations in cell motility and invasiveness, as well as downregulation of gene expression of mammalian target of rapamycin, ALDH1, and PGC-1 have been reported, suggesting vorinostat as a potential treatment for highly metastatic osteosarcoma [33]. Suberanilohydroxamic acid (vorinostat) was also shown to severely reduce the vitality of osteosarcoma cells and therefore, local cytotoxic therapy in the treatment of osteosarcoma was proposed to decrease the rate of metastasis and enhance overall survival [36]. Interestingly, in human osteosarcoma tissues, HDAC2 was found strongly expressed in the nucleus, indicating that HDAC2 can be considered a potential therapeutic target

Table 1 Histone deacetylase (HDAC) classification and potential association with cancer development

HDAC class	HDACs involved	Cellular location	Correlation with cancer development and therapeutic management
Class 1	HDAC 1, 2, 3, 8	Nucleus (zinc related)	Prostate, lung, gastrointestinal, cervical, pancreatic, and breast cancer, neuroblastoma, leukemia, melanoma
Class 2	HDAC 4, 5, 6, 7, 9, 10	Nucleus cytoplasm (zinc related)	Breast, colorectal, and gastric cancer, myeloma, sarcoma, lymphoma
Class 3	Sirtuins (SIRT1-7)	Mitochondria (NAD + dependent)	Breast and gastric cancer, cholangiocarcinoma
Class 4	HDAC 11	Nucleus cytoplasm (zinc related)	Ovarian and liver cancer, rhabdomyosarcoma

[34]. Additionally, AR-42, a potent HDAC inhibitor, was documented to reduce cell viability and evoke an even stronger apoptotic effect compared with vorinostat when used at the same concentrations in both canine and human osteosarcoma cells [35].

3.2 HDAC Inhibition in Ewing Sarcoma Therapeutic Approach

Ewing sarcoma (EWS) is the second most common, primary solid bone malignancy in children and young adults, after osteosarcoma [37]. Currently, patients with EWS demonstrate a survival rate of 50–65% at 5 years. However, approximately 20–25% of patients appear with metastases at diagnosis with the survival rate being less than 30%, whereas patients with an isolated pulmonary metastasis have an overall survival of 50% [38, 39]. Despite recent research on the therapeutic strategies against EWS, surgical resection appears the only potentially curative approach. Unfortunately, surgical removal of EWS does not necessarily guarantee a patient's long-term recovery. Alternative therapies such as radiotherapy and chemotherapy have proved on occasion to be insufficient. Therefore, further investigation into the therapeutic management of this malignancy is strongly needed.

Recently, researchers found that EWS cell lines are sensitive to a lysine (K)-specific demethylase 1A (KDM1A) blockade with the small-molecule inhibitor SP-250940. Through FAD-dependent oxidative reaction, KDM1A specifically removes histone H3K4me2 to H3K4me1 or H3K4me0. When forming a complex with an androgen receptor, KDM1A changes its substrates to H3K9me2. Therefore, the KDM1A complex mediates a coordinated histone modification switch through enzymatic activities as well as histone modification readers. Unfortunately, prolonged long-term exposure of the SP-2509 hypersensitive A673 was demonstrated with generation of a SP-2509 drug-resistant cell line. It is believed that resistance is primarily driven through epigenetic avenues and HDAC inhibitors are promising drugs that can be used as an adjunct to chemotherapy. According to the recent literature, HDAC inhibitors, and specifically vorinostat and entinostat, could be used to overcome initial SP-2509 drug-resistant cell populations [40]. Furthermore, the class I HDAC inhibitor, MS-275 (entinostat) increases reactive oxygen species in sarcoma cells and blocks their capacity for invasion and metastasis in vivo. In accordance with a recent investigation, MS-275 induces acetylation within the YB-1 CSD to block binding of target messenger RNAs. Therefore, it decreases the antioxidant factor NRF2 that reduces NFE2L2 translation and synthesis of NRF2 to increase cellular reactive oxygen species. Based on the above, MS-275 can be administered to reduce the metastatic capacity [41].

3.3 HDAC Inhibition in Chondrosarcoma-Affected Patients

Chondrosarcomas (CS) are represented by a heterogeneous group of primary bone malignancies and are characterized by the formation of hyaline cartilaginous neoplastic tissue. Chondrosarcomas affect mostly adults and prognosis depends on the histologic grading and the wide oncological margins. It is noteworthy that grade I CS treated with surgical resection followed by filling the cavity with a bone graft shows long-term local control and in the majority of cases do not metastasize. Unfortunately, CS is resistant to conventional chemotherapy and radiotherapy and there is no curative treatment for metastatic disease or non-resectable sites, such as the skull or pelvis [42]. Therefore, new targeted treatments need to be developed to achieve positive results in the treatment of CS as conventional chemotherapeutics are not effective.

Researchers suggest that adding HDAC inhibitors to bone cement presents a positive effect in the treatment of CS. They supported that adding 50 mg of valproic acid per clot can cause a nearly 100% reduction in tumor cell activity counting from the first day of measurement [36]. Moreover, in CS cells, vorinostat can induce apoptosis in the cell line SW1353 with a cleaved-PARP expression and sub-G1 fragmentation according to a flow cytometric analysis, and can promote autophagy in Rat Chondrosarcoma Cell (RCS) and OUMS-27 cell lines, as proved by the detection of an autophagosome-specific protein and specific ultrastructural morphology in the cytoplasm. Additionally, vorinostat can inhibit the tumor growth of CS cells in an in vivo xenograft model [43].

3.4 HDAC Contribution in Prognosis and Treatment of Soft-Tissue Sarcoma

Malignant STSs are a diverse group of malignancies that arise in mesenchymal tissues, such as muscle and fat, currently classified into ~ 50 distinct histological subtypes. Soft-tissue sarcomas represent < 1% of all malignant tumors, affect approximately one per 50 million people annually, with 50% of patients succumbing to the disease [44, 45]. Surgery and radiotherapy are the main treatment options, while the wide variety of STS characterization prevents the development of targeted therapies [46]. A recent survey implicated hypoxia inducible factor 2a levels as a biomarker for vorinostat efficacy in STS, as most STSs expressed lower levels of hypoxia inducible factor 2a relative to normal tissue and vorinostat specifically increases hypoxia inducible factor 2a in multiple STS subtypes [47]. Moreover, machine learning algorithms predicted that trichostatin A could potentially be efficient in all STS subtypes, while vorinostat was efficient only for undifferentiated pleomorphic sarcoma and

leiomyosarcoma (LMS) [gynecologic subtype] [44]. Though HDAC inhibition using vorinostat was deemed to be short term and linked to limited efficacy in patients with STS, the combination of vorinostat and doxorubicin induced cell death in fibrosarcoma xenografts [45, 48].

3.5 HDAC Inhibition in Rhabdomyosarcoma

Rhabdomyosarcoma (RMS) is the most common STS in children and comprises two major subtypes, embryonal RMS and alveolar RMS [49]. Despite different approaches to therapy, which consists of surgery, chemotherapy, and radiotherapy, RMS is a malignancy with a poor prognosis [50]. This therapeutic limitation requires a search for new targeted therapies. Targeting HDACs represents a promising therapeutic option in RMS, as treatment with pan-HDAC inhibitors, trichostatin A and vorinostat, has demonstrated an anti-tumor effect in embryonal RMS in vitro and in vivo [51]. A number of HDAC inhibitors, such as quisinostat, vorinostat, entinostat, and panobinostat, when administered with BET inhibitors, synergistically induce mitochondrial apoptosis by shifting the equilibrium of pro- and antiapoptotic BCL-2 proteins towards apoptosis [52]. The same synergistic effect leading to mitochondrial apoptosis in RMS has been reported using combined treatment with LSD1 and HDAC inhibitors [50]. The novel HDAC inhibitor OBP-801 also induced M-phase arrest followed by apoptosis via mitotic catastrophe in RMS cells [53]. Finally, synergistic efficacy of combination treatment of valproic acid and caffeine has been described in RMS cells in vitro [54]. Another approach to treating RMS included CRISPR-mediated knockout of HDAC3, which is a major suppressor of myogenic differentiation, leading to decreased tumor cell growth [50]. Moreover, synergistic antitumor activity of entinostat (HDAC3 inhibitor) with vincristine was apparent in both embryonal RMS and alveolar RMS [55, 56].

Core regulatory transcription factors (CR TFs) orchestrate the placement of super-enhancers (SEs) to activate transcription of cell-identity specifying gene networks and promote malignant transformation. In a recent survey, the CR circuitry of RMS has been investigated and critical CR TF dependencies have been defined. These CR TFs build SEs that present the largest levels of histone acetylation, yet paradoxically SEs also harbor the highest amounts of HDACs. In accordance with research findings, hyperacetylation selectively halts CR TF transcription, removes RNA Pol2 from core regulatory genetic elements, and eliminates RNA-Pol2, but not BRD4 phase condensates. Therefore, this study identified essential regulatory networks underlying childhood RMS in primary tumors and cell lines and used a relevant disease context to mechanistically interrogate the consequences of hyperacetylation at the chromatin template. An associated SE-specific requirement for balancing histone modification states to maintain SE architecture and CR TF transcription has been accomplished [57].

In the same direction, Gryder et al. sought to identify small molecules capable of selectively disabling CR circuitry, initiating PAX3-FOXO1 fusion oncogene-positive RMS as a model system. Using both large agnostic screening and 77 mechanistically curated epigenetic and transcriptional probes, they reported that SE-driven transcription had a rapid and selective dependence on readers, writers, and erasers of histone acetylation, while small-molecule modulators of histone methylation had almost no impact within a 24-h window. RNA-sequencing screening further confirmed that acetylation-axis-perturbing probes selectively ablate transcription of CR networks. In addition, bromodomains, which assemble to the genome by binding the acetyl-lysine histone scaffold associated with active enhancers and promoters, are essential for CR TF-dependent transcription 2. Moreover, HDAC enzymes are also essential for CR transcription, exposing a new mechanism underlying the long appreciated phenotypic consequences of chemical probes inhibiting the enzymatic activity of various HDAC isoforms. Therefore, the researchers revealed that nuclear Class I HDACs 1, 2, and 3 but not HDAC8 are co-essential for CR transcription, and simultaneous inhibition of HDAC1/2/3 disrupts CR TF chromatin architecture [58].

3.6 HDAC Inhibition in the Treatment of Leiomyosarcoma

Leiomyosarcoma is a STS and its incidence increases with age reaching a peak at the seventh decade of life. It is estimated that approximately 10–20% of all newly diagnosed STSs are LMSs. Leiomyosarcoma is the predominant sarcoma arising from large vessels and the most common is uterine sarcoma. With regard to sex incidence, retroperitoneal and inferior vena cava LMSs are more common in women, while non-cutaneous soft-tissue sites and cutaneous LMSs are more frequent in men. The treatment of patients with localized LMS consists mainly of surgical resection. However, when patients present with advanced metastatic disease, therapeutic alternatives are limited [59]. Two new drugs approved from the US Food and Drug Administration, the multityrosine kinase inhibitor, pazopanib and the DNA binder trabectedin, were not indicative of any improvement in overall survival of LMS-affected patients [60]. Therefore, discovery of additional treatment options is warranted.

According to recent investigations, nearly 30% of LMSs express high levels of class IIa HDACs and HDAC9, suggesting that HDAC9 inhibitors can be initiated as possible onco-immunological drugs against LMSs [61]. Moreover, mocetinostat, an HDAC inhibitor, combined with the frequently administered chemotherapeutic agent gemcitabine possess a synergistic effect in LMS cells in vitro. More

specifically, mocetinostat can reduce the expression of gemcitabine-resistance markers RRM1 and RRM2 and can potentially increase the expression of gemcitabine-sensitivity marker hENT1 in LMS cells [62]. Additionally, LMSs show adequate sensitivity with HDAC inhibitors quisinostat and trichostatin A [44].

3.7 HDAC Contribution in Retroperitoneal Sarcoma Therapeutic Management

Liposarcoma (LPS) is a highly morbid mesenchymal tumor of adipocytic differentiation and is classified into four principal subtypes: well-differentiated LPS, dedifferentiated LPS, myxoid LPS, and pleomorphic LPS. Those four categories present distinct molecular and clinical characteristics, thus making a subtype-tailored treatment approach realistic [63, 64]. Targeting HDAC2 in vitro, by treating DDLPS cell lines with the HDAC inhibitors MI-192 (HDAC2/3 inhibitor) or romidepsin (HDAC1/2 inhibitor), induced the process of apoptosis [64]. Moreover, administration of a murine DDLPS xenograft model with romidepsin reduced tumor growth and led to *TP53* reactivation [64]. Both in vitro and in vivo experiments concluded that targeting HDAC2 has a potential role in reducing *MDM2* expression and subsequently diminishing the oncogenicity of DDLPS tumors [64].

In contrast, synovial sarcoma (SS) is an aggressive high-grade soft-tissue malignancy arising most frequently in the extremities of adolescents and young adults [65]. In recent decades, several cases of retroperitoneal SS (RSS) have been also described in the literature [66]. Wide surgical removal of the lesion and neoadjuvant or adjuvant radiotherapy are the standard treatments for SS, with conventional cytotoxic therapy, including doxorubicin and ifosfamide, providing limited benefit [67, 68]. Despite the availability of multimodal therapies, the mortality rate remains approximately 50% within 10 years of diagnosis, making mandatory the need for targeted therapies against SS [68]. Histone deacetylase inhibition by quisinostat significantly decreased cell viability in human SS cell lines, induced neuronal differentiation, response to oxygen-containing species, as well as cell-cycle arrest by reactivating tumor suppressor genes (*CDKN2A*) and pro-apoptotic factors (BIK, BIM, and BMF) [68]. Moreover, treating a mouse model of SS with quisinostat induced apoptosis and decreased tumor burden [68]. Quisinostat in combination with proteasome-targeting agents decreases cell viability and induces apoptosis in a murine model of SS. This synergistic effect involved activation of pro-apoptotic proteins BIM and BIK, phosphorylation of BCL-2, elevation of endoplasmic reticulum stress, increase in the levels of reactive oxygen species, and suppression of tumor growth [65].

3.8 HDAC Administration in Patients with Endometrial Stromal Sarcoma

According to the latest classification of the World Health Organization, endometrial stromal tumors can be subdivided into four subtypes: endometrial stromal nodule, low-grade endometrial stromal sarcoma (ESS), high-grade ESS, and undifferentiated endometrial sarcoma (UES). Low-grade ESS, high-grade ESS, and UES account for less than 10% of uterine sarcomas and less than 1% of all primary malignant tumors of the uterus [69]. For uterine sarcomas, the average age at diagnosis is 60 years. More specifically, ESS is most commonly diagnosed in women of 40–55 years, while UES is diagnosed usually in postmenopausal women. The cornerstone of treatment for early stage (I or II) disease includes hysterectomy and bilateral salpingo-oophorectomy [70]. Although hormonal therapy is efficacious in some progressive and metastatic cases of ESS, it is not effective when the tumor does not express the respective steroid receptor [71]. Because of the rarity of the disease and associated poor outcome, the necessity of new therapeutic targeted drugs is highly warranted.

In accordance with a recent study based on cell culture, the pan-HDAC inhibitor vorinostat, combined with inhibition of PI3K and mammalian target of rapamycin could be a promising treatment option for patients with ESS. Vorinostat alone induces ESS cell death by mitosis failure or apoptosis, thus demonstrating favorable therapeutic behavior [72]. Moreover, valproic acid, which is an anti-epileptic drug and an HDAC inhibitor, showed inhibition of tumor growth in ESS. With regard to UES, HDAC1/2/4/6/7/8 showed strong immunoreactivity in a high percentage of cases. It was also found that the HDAC family is strongly expressed in a large portion of patients with UES. Based on the above, vorinostat seems to be a promising drug in the treatment of UES [73].

3.9 HDACs and Rare Tumors of Mesenchymal Origin

Myeloid sarcoma (MS) is an extramedullary tumor of immature granulocytic cells. It is an extremely rare nosologic entity and may occur at any age. Myeloid sarcoma is commonly encountered in the lymph nodes, skin, and soft tissues. It usually occurs in association with acute myeloid leukemia (AML), but it can also be isolated with an incidence of two cases per million adults. Systemic chemotherapy is the cornerstone of treatment for the disease and is usually combined with surgery and radiotherapy; yet it is not always effective [74]. Because of the rarity of the disease, more studies are required to establish the ideal treatment for MS. However, HDAC inhibitors are currently being tested for AML and may change treatment strategies in the different subgroups of patients with MS [75]. Histone deacetylase inhibitors such as panobinostat, vorinostat, and trichostatin

A have been shown to promote cell death, autophagy, apoptosis, or growth arrest in preclinical AML models, but seem to be working more efficiently in combination with other drugs. Moreover, clinical trials with pracinostat, in combination with DNA hypomethylating agents or chemotherapy, showed encouraging results in the treatment of AML implicating their potential beneficial contribution in a therapeutic approach for MS [76] (see Table 2).

4 Clinical Trials of HDAC Inhibitors in Sarcoma

Treatment options for advanced sarcomas remain limited. Multiple preclinical studies have been useful in identifying the potential activity of HDAC inhibitors in several sarcoma models, thus providing significant evidence that they could be considered as promising therapeutic agents for this

Table 2 Studies on histone deacetylase (HDAC) association with bone sarcomas

Author	Year	HDACi studied	Samples used	Associated parameters and results
Tonak et al. [36]	2014	VPA, SAHA	Osteosarcoma cell lines	Using anti-neoplastic bone cement PMMA loaded with VPA and SAHA could improve the outcome Local cytotoxic therapy might improve the rate of metastasis and survival of patients
Xiaodong et al. [33]	2015	Vorinostat	Metastatic K7M2 osteosarcoma cell lines	Reduced proliferation and metastatic potential of the cells Gene expressions of mTOR, ALDH1, and PGC-1 were downregulated by vorinostat treatment
Murahari et al. [35]	2017	AR-42, SAHA	Human and canine osteosarcoma cell lines	AR-42 induced a greater apoptotic response compared with SAHA AR-42 with doxorubicin potent inhibition of cell viability and synergistic effect AR-42 and SAHA induced cell death via the activation of the intrinsic mitochondrial pathway through activation of caspase 3/7
La Noce et al. [34]	2018	VPA, DAC	Osteosarcoma cell lines	Decreased repressive histone markers, and increased active markers Increase of acetylation of histones H3, a decrease of DNA global methylation, HDAC2, and DNMT3a
Pishas et al. [40]	2018	Vorinostat, entinostat	Ewing sarcoma cell lines	SP-2509 drug-resistant cells exhibited elevated expression levels of the multi-drug resistance genes *ABCB1*, *ABCC3*, and *ABBC5* and decreased expression of the transcriptional repressor RCOR1/CoREST
El-Naggar et al. [41]	2019	Entinostat (MS-275)	Ewing sarcoma cell lines and human tissue	MS-275 inhibits YB-1 deacetylation YB-1 potent metastatic driver in high-risk childhood bone sarcomas MS-275 dramatically reduces sarcoma metastasis in vivo
Yamamoto et al. [44]	2008	SAHA	Chondrosarcoma cell lines	SAHA inhibited the growth of chondrosarcoma cell lines and induced apoptosis in SW1353 SAHA induced autophagy-associated cell death SAHA inhibited tumor growth in an in vivo xenograft model
Tonak et al. [43]	2014	VPA, SAHA	Chondrosarcoma cell lines	Loaded PMMA with VPA or SAHA could improve the outcome No changes in stability and architecture of the cement clots loaded with chemotherapeutic drugs

DAC demethylating agent, 5-azacytidine, *HDACi* histone deacetylase inhibitor, *mTOR* mammalian target of rapamycin, *PMMA* polymethylmethacrylate, *SAHA* suberoylanilidehydroxamic acid, *VPA* valproic acid

disease and has led to their investigation in several clinical trials [12] (Table 3). In a phase II trial, the efficacy of pracinostat (SB939), a novel oral HDAC 1, 2, and 4 inhibitor, was examined in patients with recurrent/metastatic translocation-associated sarcoma. Among 14 patients evaluated and confirmed as carrying specific chromosomal translocations, eight achieved stable disease (SD) with a median duration of 5 months. A 3-month progression-free survival rate of 49% was also observed [77]. In addition, a single-arm, open-label, phase II trial of oral panobinostat was conducted in 47 patients with advanced pretreated STS, but showed limited efficacy; SD was demonstrated in 36% of included patients. Nine patients were free of progression at 3 months and six at 6 months [78].

The efficacy and safety of vorinostat as a single agent in refractory STS have been investigated in another phase II study. In a cohort of 40 heavily pretreated patients, response to vorinostat was low. Median progression-free survival was 3.2 months and overall survival was 12.3 months. However, a small subgroup of six patients presented with long-lasting disease stabilization [79]. Based on the modest clinical outcome of HDAC inhibitor monotherapy for sarcomas, a rational design of combination treatment became challenging to improve the response rate to therapy and overcome intrinsic and acquired resistance. In a phase I trial, the combination of vorinostat with bortezomib was proved feasible at doses that achieved clinical benefit in patients with relapsed/refractory sarcoma. It was also observed that bortezomib did not affect the pharmacokinetics of vorinostat [80]. In a subsequent study, evaluating an intermittent dosing schedule of vorinostat with bortezomib, SD was observed in two pretreated patients with sarcoma [81].

Another phase I study, evaluating the effect of pulse-dose vorinostat combined with the cyclin-dependent kinase inhibitor flavopiridol, provides evidence that combination therapy, using intermittent high doses of vorinostat, enhances the efficacy of the regimen [82]. Furthermore, a phase I study was conducted to evaluate the combination of oral abexinostat with doxorubicin in patients with metastatic sarcoma. In a cohort of 21 patients, including ten who had previously developed disease progression on prior treatment with doxorubicin, SD was observed in 7 out of 17 assessable patients who received at least five cycles of the combination. Supportive administration of granulocyte colony-stimulating factor increased the maximum tolerated dose of combination therapy [83].

The combination of belinostat with doxorubicin was also tested in a phase I/II clinical trial, demonstrating a response rate of 15% in the group of patients with STS who received the highest dose level, and 18% when they excluded chondrosarcomas. Time to progression was superior when compared with that of some reports of the first-line single-agent doxorubicin in STS [84]. Additionally, panobinostat in combination with epirubicin was evaluated for the treatment of doxorubicin-refractory sarcoma in a dose escalation phase I trial. The treatment was proved to be well tolerated and suggested a method to reverse doxorubicin resistance. Among 20 enrolled patients, 11 maintained prolonged SD and one patient responded partially. Moreover, peripheral blood histone acetylation level and neutrophil count correlated with clinical benefit [85].

Tumor metastasis depends on angiogenesis. Based on the hypothesis that the efficacy of anti-angiogenic agents is improved in combination with other anticancer drugs and epigenetic modifiers, the HDAC inhibitor valproic

Table 3 Clinical trials of histone deacetylase (HDAC) inhibitors in sarcomas

HDAC inhibitor	Combination therapy	Study phase	Types of sarcoma	No. of patients
Pracinostat [75]		II	Endometrial stromal tumor, desmoplastic small round blue cell tumor, translocation-associated sarcoma, adenosarcoma, fibrosarcoma, alveolar soft part sarcoma, malignant peripheral nerve sheath tumor	38
Panobinostat [76]		II	Ewing sarcoma, rhabdomyosarcoma, synovial sarcoma	47
Vorinostat [77]		II	Leiomyosarcoma, endometrial stromal tumor, uterine carcinosarcoma	40
Entinostat [86]		I	Ewing sarcoma, sarcoma, leiomyosarcoma	27
Abexinostat [81]	Doxorubicin	I	Liposarcoma, leiomyosarcoma, fibrosarcoma, others	21
Belinostat [82]	Doxorubicin	I/II	Liposarcoma, leiomyosarcoma chondrosarcoma, angiosarcoma, myxofibrosarcoma, undifferentiated pleomorphic sarcoma, rhabdomyosarcoma, fibrosarcoma, myogenic sarcoma, synovial sarcoma	41
Panobinostat [83]	Epirubicin	I	Sarcoma, leiomyosarcoma, chondrosarcoma, liposarcoma	20
Valproic acid [84]	Bevacizumab, gemcitabine, docetaxel	I/II	Angiosarcoma, carcinosarcoma, epithelioid sarcoma, extrauterine leiomyosarcoma, undifferentiated uterine sarcoma, liposarcoma, malignant peripheral nerve sheath, malignant solitary fibrous tumor, rhabdomyosarcoma	46
Abexinostat [85]	Pazopanib	I	Sarcoma	6

acid, combined with the vascular endothelial growth factor inhibitor bevacizumab, and standard chemotherapy with gemcitabine and docetaxel was evaluated to enhance the cytotoxic effects against sarcomas. In this pilot study, combination therapy was moderately safe. Interestingly, in 61% of patients who had not responded to gemcitabine/docetaxel administration, partial or complete response was demonstrated with addition of the two drugs. It is worth mentioning that considerable responses were apparent in patients with epithelioid sarcoma and carcinosarcoma [86].

The hypothesis of inhibiting HDAC to enhance response and reverse resistance to angiogenesis inhibitors has been evaluated in a phase I study by using abexinostat in combination with pazopanib. A long-term benefit was observed for pretreated patients and response was associated with higher HDAC2 expression in peripheral blood, an increased histone acetylation level, as well as modulation of vascular endothelial growth factor [87]. An additional study assessed the oral HDAC inhibitor entinostat (MS-275) in patients with advanced solid malignancies and lymphomas. Two out of 27 treated patients showed partial remissions, and six patients presented with prolonged disease stabilization. Peripheral blood mononuclear cell histone acetylation levels appeared to be increased in responders [88].

5 Future Prospective

Despite the availability of multimodal therapies, consisting of surgery, chemotherapy, and radiotherapy, the survival rate for patients with sarcoma is still not satisfactory. Therefore, there is an urgent need for targeted therapies, modulated according to the distinct clinical and molecular characteristics of each sarcoma subtype. Histone deacetylase inhibition is found to play a key role in limiting the tumor burden in sarcomas, as HDAC inhibitors act on well-described oncogenic signaling pathways. Histone deacetylase inhibitors disrupt the increased cell motility and invasiveness of sarcoma cells, undermining their metastatic potential [33, 89]. Moreover, their activity on evoking cell arrest has been extensively described, with HDAC inhibitors regulating the reactivation of tumor suppressor genes and induction of apoptosis [35, 67]. Promoting autophagy and increasing cellular reactive oxygen species are also included in the antitumor activity of HDAC inhibitors [41, 44]. It should be noted that many studies revealed the synergy between HDAC inhibitors and other drugs, leading to the enhancement of the antitumor effect in sarcomas. Frequently administered chemotherapeutic agents, as well as proteasome targeting agents in combination with HDAC inhibitors, suppress tumor growth and restrict the metastatic potential of sarcomas [46, 53–55, 61, 64].

The increasing need for targeted therapies in oncology shifted research interest to new emerging targets, such as HDACs. Those research efforts delivered promising results by providing us with a broad spectrum of information regarding HDACs' role in carcinogenesis and their potential role as targets of antitumor therapy. Nevertheless, HDACs' functions should be further studied both in normal cells and cancer cells to clarify their contribution under normal conditions in the cell and elucidate their involvement in carcinogenesis. Investigating HDACs' molecular interactions and epigenetic modifications could pave the way towards more individualized approaches in the treatment of malignancies. Furthermore, HDAC inhibitors' synergistic or additive effect, when combined with chemotherapeutic agents on the suppression of tumor growth, could enhance the effectiveness of treatment options in cancer. Ongoing clinical trials endeavor to clarify this synergistic effect by combining vorinostat with chemotherapeutic agents in the treatment of sarcoma [73, 74]. It is concluded that a better understanding of HDACs and HDAC inhibitors could provide patients with sarcoma with more targeted and efficient therapies, which may contribute to a significant survival improvement for the patients.

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Declarations

Conflicts of interest No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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