Chemosensitization of rhabdomyosarcoma cells by the histone deacetylase inhibitor SAHA

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Abstract

Rhabdomyosarcoma (RMS) is the most common soft-tissue sarcoma in childhood with a dismal prognosis, highlighting the need for novel treatment strategies. Here, we identify a novel synthetic lethal interaction between the histone deacetylase inhibitor (HDACI) SAHA and anticancer drugs in RMS cells. Importantly, SAHA significantly increases chemotherapeutic drug-induced apoptosis in both embryonal and alveolar RMS cell lines, including several anticancer agents that are used in the clinic for the treatment of RMS such as Doxorubicin, Etoposide, Vincristine and Cyclophosphamide. Calculation of combination index (CI) reveals that the interaction of SAHA and Doxorubicin is synergistic. Mechanistically, SAHA causes acetylation of histone H3 protein in RMS cells, indicating that SAHA alters the chromatin context. Also, cotreatment with SAHA and Doxorubicin changes the ratio of pro- and anti-apoptotic Bcl-2 proteins with downregulation of Mcl-1 and Bcl-xL, dephosphorylation of Bcl-2 and upregulation of BimEL, thus shifting the balance towards apoptosis. Consistently, SAHA and Doxorubicin cooperate to stimulate activation of Bax and Bak, caspase activation and caspase-dependent apoptosis. Overexpression of Bcl-2 significantly rescues SAHA/Doxorubicin-mediated apoptosis, underscoring the requirement of the mitochondrial apoptotic pathway for the synergistic induction of apoptosis by SAHA and Doxorubicin. Caspase-dependent apoptotic cell death is confirmed by the use of the broad-range caspase inhibitor N-benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone (zVAD.fmk), which significantly decreases SAHA/Doxorubicin-triggered apoptosis. In conclusion, these findings demonstrate that the HDACI SAHA represents a promising strategy to prime RMS cells for chemotherapy-induced apoptosis and warrants further investigation in combination regimens.

Introduction

RMS is the most common soft-tissue sarcoma in children and comprises two major subtypes, which are associated with distinct genetic alterations and clinical prognosis, i.e. alveolar rhabdomyosarcoma (ARMS) and embryonal rhabdomyosarcoma (ERMS) [1,2]. Despite multimodal treatment regimens and advances in combination chemotherapy, patients with primary metastatic disease and metastatic relapse have a chance for cure of less than 20% [3]. This highlights the high medical need to identify and validate novel molecular targets in RMS that can be exploited for the development of innovative treatment approaches in order to improve the dismal prognosis of these patients. Since conventional therapies and risk stratification have already been optimized in recent years, experimental therapies are likely critical to improve the poor outcome of children with high-risk RMS.

Aberrant expression or activity of histone deacetylases (HDACs) and histone acetyl transferases (HATs) contribute to the abnormal epigenetic state of cancer cells, one of the hallmarks of human cancers [4]. HDACs determine the acetylation status of histones, thereby affecting chromatin topology and gene expression [4]. In addition to histones, HDACs deacetylate many non-histone proteins, for example the DNA repair protein Ku70 or transcription factors such as FOXO proteins, thereby modulating their activity, localization or interaction with other proteins [4]. Since HDACs can revert aberrant epigenetic states in cancer cells, they are considered as promising anticancer therapeutics [5]. The anticancer effects of HDACIs have been largely attributed to their ability to impair the survival of tumor cells and to trigger programmed cell death (apoptosis) [5]. HDACIs can induce apoptosis via two
well-defined apoptotic pathways, i.e. the extrinsic (death receptor) pathway and the intrinsic (mitochondrial) pathway, which both eventually lead to activation of caspases as central death effector molecules [6]. The mitochondrial pathway involves the release of proteins from the mitochondrial intermembrane space into the cytosol, which in turn results in caspase-3 activation and apoptosis [7]. Mitochondrial outer membrane permeabilization is tightly controlled by various factors including proteins of the Bcl-2 family [7]. Bcl-2 proteins consist of both antiapoptotic members, e.g. Bcl-2, Bcl-XL and Mcl-1, as well as proapoptotic molecules such as Bax, Bak and BH3-only domain proteins, e.g. Bim, Bmf and Noxa [8].

Suberoylanilide hydroxamic acid (SAHA, Vorinostat) is the clinically most advanced HDACI and has been approved by the US FDA in 2006 for the treatment of refractory cutaneous T-cell lymphoma [9]. SAHA belongs to the hydroxamic acid family of HDACIs and is a pan-HDAC [9]. While SAHA as single agent has been reported to suppress growth of RMS cell lines [10,11], SAHA displayed limited in vivo activity when it was evaluated against the solid tumor

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Fig. 1. SAHA causes acetylation of histone H3 in RMS cells and sensitizes RMS cells for chemotherapy-induced apoptosis. (A) RMS cells were treated with 2 μM SAHA. Acetylation of histone 3 (Ac-H3) was assessed by Western blot. (B) Cells were treated with indicated concentrations of SAHA and/or Doxorubicin. Apoptosis was determined by analysis of DNA fragmentation of PI-stained nuclei using flow cytometry. Mean and SD of three experiments performed in triplicate are shown. (C) Cells were treated with indicated concentrations of SAHA and/or Etoposide for 72 h. Apoptosis was determined by analysis of DNA fragmentation of PI-stained nuclei using flow cytometry. Mean and SD of three experiments performed in triplicate are shown. (D) Cells were treated with indicated concentrations of SAHA and/or Vincristine for 72 h. Apoptosis was determined by analysis of DNA fragmentation of PI-stained nuclei using flow cytometry. Mean and SD of three experiments performed in triplicate are shown. (E) Cells were treated with indicated concentrations of SAHA and/or Cyclophosphamide for 72 h. Apoptosis was determined by analysis of DNA fragmentation of PI-stained nuclei using flow cytometry. Mean and SD of three experiments performed in triplicate are shown. (F) Cells were treated with indicated concentrations of SAHA and/or Doxorubicin for 24, 48 and 72 h. Apoptosis was determined by analysis of DNA fragmentation of PI-stained nuclei using flow cytometry. Mean and SD of three experiments performed in triplicate are shown; "P < 0.01.
xenograft panel of the pediatric preclinical testing program [11]. A recent phase I clinical trial of SAHA in children with recurrent solid tumors demonstrated that SAHA is well-tolerated, while it displayed little antitumor activity as stand-alone treatment [12]. Currently, SAHA is evaluated in early clinical trials for childhood cancer. Together, these reports indicate that SAHA represents an interesting investigational agent for the treatment of pediatric malignancies including RMS. However, they also indicate that SAHA as single agent might be insufficient to achieve tumor regression, suggesting that SAHA might be best exploited in combination protocols. Since chemotherapy constitutes one of the key pillars of current treatment protocols for children with RMS, anticancer drugs are prime candidates for rational combinations. However, it is not known at present whether or not SAHA can be used to increase chemosensitivity of RMS cells. Therefore, the current study aims at investigating the question whether SAHA sensitizes RMS cells for chemotherapy-induced apoptosis.

Materials and methods

Cell culture and chemicals

RMS cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA) and maintained in RPMI 1640 or DMEM medium (Life Technologies, Inc., Eggenstein, Germany), supplemented with 10% fetal calf serum (FCS) (Biochrom, Berlin, Germany), 1% penicillin/streptomycin (Invitrogen) and 1 mM Sodium Pyruvate (Invitrogen, Karlsruhe, Germany). ZVAD.fmk was purchased from Bachem (Heidelberg, Germany), Vorinostat (SAHA) from Selleck Chemicals (Houston, TX, USA), and all other chemicals from Sigma (Deisenhofen, Germany) unless indicated otherwise.

Determination of cell viability and apoptosis

Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay according to the manufacturer’s instructions (Roche Diagnostics, Mannheim, Germany) or by crystal violet staining (0.75% crystal violet, 50% ethanol, 0.25% NaCl, and 1.57% formaldehyde). Apoptosis was determined by fluorescence-activated cell-sorting (FACScanto II, BD Biosciences, Heidelberg, Germany) analysis of DNA fragmentation of propidium iodide (PI)-stained nuclei as described previously [13].

Transduction

For Bcl-2 overexpression, cells were transduced with murine stem-cell virus (pMSCV, Clontech) vector containing mouse Bcl-2 or empty vector using the packaging cell line 293T (BD Biosciences). Stable cell lines were selected by 10 µg/ml Blasticidin (Invitrogen).

Colony forming assay

To determine colony formation, 200 cells were seeded in a 6-well tissue culture plate and allowed to settle overnight. Cells were treated with 1 µM SAHA for 24 h before 0.015 µg/ml Doxorubicin was added for 1 h. Then, medium was exchanged.

Fig. 1 (continued)
Western blot analysis

Western blot analysis was performed as described previously [13] using the following antibodies: mouse anti-caspase-8, mouse anti-Noxa, rat anti-Bmf (Alexis Biochemicals, Grünberg, Germany), mouse anti-Bcl-2, rabbit anti-Bcl-X,-, mouse anti-Bax, rabbit anti-Bak (BD Transduction Laboratories), rabbit anti-caspase-8, rabbit anti-Bim, mouse anti-poly ADP ribose polymerase (PARP) (Cell Signaling, Beverly, MA), acetylated histone H3 (Upstate Biotechnology, Lake Placid, NY), rabbit anti-Mcl-1 (Stressgen, Victoria, BC), rabbit histone H3 (Abcam, Cambridge, UK), rabbit anti-phospho-histone H3 (pHH3) (Millipore, Darmstadt, Germany). Mouse anti-GAPDH (HyTest, Turku, Finland) or mouse anti-Bak antibody (Ab-1; Calbiochem) and 10 μg/ml mouse anti-Bax anti-body (6A7, Sigma) or anti-Bak antibody (Ab-1; Calbiochem) were used as loading controls. Goat anti-mouse IgG, goat anti-rabbit IgG conjugated to horseradish peroxidase (Santa Cruz Biotechnology, Santa Cruz, CA) were used as secondary antibodies. Enhanced chemiluminescence was used for detection (Amersham Bioscience, Freiburg, Germany). Representative blots of at least two independent experiments are shown.

Determination of activation of Bax and Bak

For detection of active Bax or Bak, cells were lysed in CHAPS lysis buffer (10 mM HEPES pH 7.4; 150 mM NaCl; 1% CHAPS) as previously described [14]. A total of 700–1000 l cell suspensions were incubated with 2 μg/ml mouse anti-Bax antibody (6A7, Sigma) or anti-Bak antibody (Ab-1; Calbiochem) and 10 μg/ml mouse anti-Bax antibody (6A7, Sigma) or anti-Bak antibody (Ab-1; Calbiochem) were used as loading controls. Goat anti-mouse IgG, goat anti-rabbit IgG conjugated to horseradish peroxidase (Santa Cruz Biotechnology, Santa Cruz, CA) were used as secondary antibodies. Enhanced chemiluminescence was used for detection (Amersham Bioscience, Freiburg, Germany). Representative blots of at least two independent experiments are shown.

Statistical analysis

Statistical significance was assessed by Student’s t-test (two-tailed distribution, two-sample, unequal variance). Drug interaction was analyzed by the CI method using CalcuSyn software (Biosoft, Cambridge, UK). CI <0.9 indicates synergism, 0.9–1.1 additivity and >1.1 antagonism.

Table 1

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Combination index (CI) was calculated as described in materials and methods for apoptosis induced by combined treatment for 72 h with indicated concentrations of SAHA and chemotherapeutic drugs.

Results

SAHA causes acetylation of histone H3 in RMS cells

To control that SAHA triggers histone acetylation in RMS cells we monitored the acetylation status of histone H3 in the presence and absence of SAHA. We used both ERMS (RD) and ARMS (Rh30) cell lines to represent the two major histological entities. Exposure to SAHA markedly increased histone H3 acetylation (Fig. 1A).

SAHA sensitizes RMS cells for chemotherapy-induced apoptosis

To investigate the question whether SAHA modulates chemosensitivity of RMS cells, we analyzed the efficacy of several anticancer drugs that are used in the clinic for the treatment of RMS in the presence and absence of SAHA. Importantly, SAHA acted in concert with the topoisomerase II inhibitors Doxorubicin and Etoposide, the vinca alkaloid Vincristine and the alkylating agent Cyclophosphamide to induce apoptosis in RMS cells compared to treatment with either agent alone (Fig. 1B–E). We calculated the CI to explore whether this drug interaction is additive or synergistic. This calculation revealed that SAHA acted in a synergistic manner in particular together with Doxorubicin and Etoposide (Table 1).

To investigate in more detail the molecular mechanisms underlying this synergism of HDACI and chemotherapeutic drugs in RMS, we focused the subsequent experiments on Doxorubicin, since this drug yielded the most pronounced synergistic interaction. A kinetic analysis demonstrated that SAHA enhanced Doxorubicin-induced apoptosis in a time-dependent manner (Fig. 1F). Together,
this set of experiments demonstrates that SAHA sensitizes RMS cells for several chemotherapeutic agents.

**Cotreatment with SAHA/Doxorubicin impairs clonogenicity of RMS cells with little cytotoxicity on non-malignant myoblasts**

To explore whether combined treatment with SAHA and Doxorubicin also affects long-term survival of RMS cells, we performed colony assays. Of note, SAHA/Doxorubicin cotreatment suppressed colony formation of RMS cells, showing that it inhibits long-term survival (Fig. 2A). To examine whether SAHA increases chemotherapy-imposed cytotoxicity against non-malignant cells, we used the myoblast cell line C2C12. The addition of SAHA did not enhance Doxorubicin-induced apoptosis in C2C12 cells (Fig. 2B). These findings demonstrate that cotreatment with SAHA/Doxorubicin impairs clonogenicity of RMS cells, while it exerts little cytotoxicity on non-malignant myoblasts.

**SAHA and Doxorubicin cooperate to induce caspase activation and caspase-dependent apoptosis**

To gain insights into the underlying mechanisms of the synergistic interaction of SAHA and Doxorubicin, we analyzed modulation of apoptosis signaling pathways, since the anticancer effects of HDACIs have been largely attributed to their ability to trigger cell death via apoptosis [6]. To this end, we examined activation of caspases, as they are key effector molecules of apoptosis. SAHA and Doxorubicin acted together to trigger cleavage of caspase-8 into active p43/p18 fragments, cleavage of caspase-9 into active p37/p35 fragments, cleavage of caspase-3 into active p17/p12 fragments and cleavage of PARP into p89 fragment (Fig. 3A). To test whether caspase activity is required for the induction of apoptosis, we used the broad-range caspase inhibitor zVAD.fmK. The addition of zVAD.fmK significantly diminished SAHA/Doxorubicin-induced DNA fragmentation compared to cells that were treated with SAHA/Doxorubicin in the absence of zVAD.fmK (Fig. 3B). In addition, SAHA/Doxorubicin-triggered loss of cell viability was significantly rescued in the presence of zVAD.fmK as determined by MTT or crystal violet assays (Fig. 3C and D). This set of experiments shows that SAHA and Doxorubicin cooperate to trigger caspase activation and caspase-dependent apoptosis.

**SAHA/Doxorubicin cotreatment shifts the balance of pro- and antiapoptotic proteins**

Next, we analyzed the effect of SAHA and Doxorubicin on expression levels of apoptosis regulatory proteins. Cotreatment with SAHA and Doxorubicin resulted in downregulation of Mcl-1...

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**Fig. 2.** Cotreatment with SAHA/Doxorubicin impairs clonogenicity of RMS cells with little cytotoxicity on non-malignant myoblasts. (A) Cells were treated with 1 μM SAHA for 24 h before 0.015 μg/ml Doxorubicin was added for 1 h. Colonies were counted and the percentage of surviving colonies relative to solvent-treated controls was calculated. Mean and SD of three experiments performed in duplicate are shown; "P < 0.01. (B) The myoblast cell line C2C12 was treated with 2 μM SAHA and/or 0.25 μg/ml Doxorubicin for 72 h. Apoptosis was determined by analysis of DNA fragmentation of PI-stained nuclei using flow cytometry. Mean and SD of three experiments performed in triplicate are shown.
Fig. 3. SAHA and Doxorubicin cooperate to induce caspase activation and caspase-dependent apoptosis. (A) Caspase-9, caspase-8, caspase-3 activation and PARP cleavage was measured by Western blot analysis after treatment with 2 μM SAHA and/or 0.25 μg/ml Doxorubicin for 36 h. (B) Cells were treated with 2 μM SAHA and/or 0.25 μg/ml Doxorubicin in the absence and presence of 50 μM zVAD.fmk for 72 h. Apoptosis was determined by analysis of DNA fragmentation of PI-stained nuclei using flow cytometry. Mean and SD of three experiments performed in triplicate are shown. **P < 0.01. (C) Cells were treated with 2 μM SAHA and/or 0.25 μg/ml Doxorubicin in the absence and presence of 50 μM zVAD.fmk. Loss of viability was measured after 48 h by MTT assay. Mean and SD of three experiments performed in triplicate are shown. **P < 0.01. (D) Cells were treated with 2 μM SAHA and/or 0.25 μg/ml Doxorubicin in the absence and presence of 50 μM zVAD.fmk. Loss of viability was measured after 48 h by crystal violet assay. Mean and SD of three experiments performed in triplicate are shown. *P < 0.05; **P < 0.01.
and Bcl-x<sub>L</sub> and upregulation of BimEL (Fig. 4A, lanes 4 and 8). In Rh30 cells, we observed a slight upregulation of Bmf in response to SAHA or SAHA/Doxorubicin cotreatment (Fig. 4A, lane 4). In addition, treatment with Doxorubicin or the combination of Doxorubicin and SAHA caused dephosphorylation of Bcl-2 (Fig. 4A, lanes 3, 4, 7 and 8), an event that has been reported to facilitate its proteasomal degradation and to reduce its antiapoptotic function[15]. Consistent with the notion that Bcl-2 phosphorylation is dynamically regulated during the cell cycle[15], treatment with Doxorubicin caused dephosphorylation of phospho-histone H3, a marker for mitotic cells (Fig. 4B, lanes 3, 4, 7 and 8).

We then explored the question whether this shift in the ratio of pro- and antiapoptotic Bcl-2 proteins leads to engagement of mitochondrial signaling pathways. To this end, we analyzed

Fig. 4. SAHA and Doxorubicin cooperate to shift the balance toward proapoptotic proteins. (A) Cells were treated with 2 μM SAHA and/or 0.25 μg/ml Doxorubicin for 18 h. Expression levels of pro- and antiapoptotic proteins was determined by Western blot. (B) Cells were treated with 2 μM SAHA and/or 0.25 μg/ml Doxorubicin for 18 h. pHH3 was assessed by Western blot. (C) Cells were treated with 2 μM SAHA and/or 0.25 μg/ml Doxorubicin for 24 h. Activation of Bax and Bak was analyzed by immunoprecipitation using active conformation-specific antibodies.
activation of Bax and Bak, two multidomain proapoptotic Bcl-2 proteins that control mitochondrial outer membrane permeabilization. Since activation of Bax and Bak is accompanied by a conformational change that can be detected by specific antibodies, we immunoprecipitated Bax and Bak using conformation-specific antibodies. We found that treatment with SAHA alone stimulated activation of Bax and Bak, which was further increased by combined treatment with SAHA and Doxorubicin (Fig. 4C, lanes 6, 8, 14 and 16).

**Overexpression of Bcl-2 inhibits synergistic induction of apoptosis by SAHA and Doxorubicin**

To further examine the requirement of the mitochondrial apoptotic pathway for the synergistic induction of apoptosis by SAHA and Doxorubicin, we generated RMS cells with overexpression of the antiapoptotic protein Bcl-2 that is known to block mitochondrial apoptosis (Fig. 5A). Importantly, Bcl-2 overexpression significantly decreased SAHA/Doxorubicin-induced DNA fragmentation (Fig. 5B). This underscores the hypothesis that the synergistic induction of apoptosis by SAHA and Doxorubicin involves mitochondrial signaling events.

**Discussion**

The HDACi SAHA is considered as a promising investigational agent for the treatment of childhood cancers including RMS and is currently being evaluated in early clinical trials. However, there is mounting evidence indicating that monotherapy with SAHA might not be sufficient for efficient suppression of RMS growth, highlighting the need to develop SAHA-based combination therapies. In the present study, we identify the synergistic interaction of SAHA together with chemotherapeutic agents to trigger apoptosis in RMS cells. SAHA sensitizes RMS cells for the induction of apoptosis by several anticancer drugs that are commonly used in clinical protocols for the treatment of RMS, including Doxorubicin, Etoposide, Vincristine and Cyclophosphamide, thus underscoring the clinical relevance of this chemosensitization. The most pronounced synergistic drug interaction was found for the topoisomerase II inhibitors Doxorubicin and Etoposide. In contrast to RMS cells, SAHA fails to enhance the cytotoxicity of Doxorubicin against nonmalignant myoblasts, pointing to some tumor selectivity.

Mechanistic studies revealed that the synergism of SAHA and Doxorubicin involves the cooperative induction of apoptotic cell death, as demonstrated by several lines of evidence. SAHA and Doxorubicin act in concert to shift the ratio of pro- and antiapoptotic proteins in favor of apoptosis, thereby facilitating the activation of the mitochondrial gatekeeper proteins Bax and Bak, which in turn leads to activation of caspases and eventually DNA fragmentation. Caspase-dependent apoptotic cell death was confirmed by experiments using the broad-range caspase inhibitor zVAD.fmk, which significantly rescues the synergistic induction of apoptosis by SAHA and Doxorubicin. The observed shift in the ratio of pro- and antiapoptotic proteins in favor of apoptosis by SAHA and Doxorubicin cotreatment involves downregulation of the antiapoptotic mitochondrial proteins Mcl-1 and Bcl-xL as well as upregulation of the proapoptotic protein BimL in line with previous reports showing that HDACIs can both upregulate and downregulate gene expression levels [6]. In addition, Doxorubicin causes dephosphorylation of Bcl-2 protein, which reduces its stability and antiapoptotic properties [15]. Together, these changes in the ratio of pro- and antiapoptotic Bcl-2 proteins facilitate conformational activation of Bax and Bak. Also, treatment with SAHA alone stimulates activation of Bax and Bak, possibly via increased acetylation of Ku70, a well-known non-histone substrate of HDAC that has been described to interfere with Bax activation by binding to Bax [16,17]. We previously reported that HDACIs trigger the release of Bax from its binding to Ku70 by increasing Ku70 acetylation, thereby facilitating Bax activation and priming medulloblastoma cells for chemotheraphy-induced apoptosis [14,18–20]. Furthermore, activation of Bak in response to DNA damage has been linked to the translocation of nuclear histone H1.2 protein to the cytoplasm, where it was shown to induce conformational activation and oligomerization of Bak [21]. The notion that signaling via the mitochondrial pathway of apoptosis is required for the synergistic activation of cell death is underscored by rescue experiments showing that Bcl-2 overexpression provides a significant protection against SAHA/Doxorubicin-induced apoptosis. It is interesting to note that engagement of the mitochondrial signaling pathway has previously been implicated as the predominant mechanism of HDACI-mediated tumor cell death [6]. SAHA as single agent has previously been shown to suppress the growth of RMS cell lines in vitro [10,11]. However in vivo, SAHA as monotherapy was described to elicit no objective responses in a
panel of pediatric solid tumor xenografts including RMS [11]. While SAHA was reported to enhance the radiosensitivity of RMS cells [22], there are no studies yet on SAHA-based combinations with chemotherapeutics. Against this background, our study is the first to demonstrate that SAHA primes RMS cells for anticancer drug-triggered cell death. Since chemotherapeutics represents a central element of current treatment protocols for RMS, this synergistic interaction is of special interest for future clinical translation.

In previous studies, we reported that HDACIs, e.g. SAHA, MS275 or valproic acid, sensitize different pediatric cancers including medulloblastoma, neuroblastoma and glioblastoma to chemotherapy- or death receptor-induced apoptosis [14,18–20]. These reports underscore the notion that SAHA may provide a mean to broadly sensitize several childhood cancers for the induction of apoptosis. Beyond pediatric malignancies, in other neoplasms HDACIs have also been shown to act in concert with different cancer therapies including DNA-damaging drugs [23], underlining the wider relevance of rational combinations with HDACIs.

Our present study demonstrates a synergistic interaction of SAHA together with established anticancer agents and has therefore important clinical implications for the development of new therapeutic strategies for RMS. It will be the subject of future studies to examine the antitumor activity of SAHA/chemotherapy combination treatment in preclinical in vivo models of RMS. The application of SAHA in pediatric cancers is encouraged by data showing that SAHA is in general well-tolerated by children [12]. Currently, SAHA as monotherapy and in combination regimens is under evaluation in phase I/II clinical trials for the treatment of RMS. It will be the subject of future studies to examine the antitumor activity of SAHA/chemotherapy combination treatment in preclinical in vivo models of RMS. The application of SAHA in pediatric cancers is encouraged by data showing that SAHA is in general well-tolerated by children [12]. Currently, SAHA as monotherapy and in combination regimens is under evaluation in phase I/II clinical trials for the treatment of RMS.

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Disclosure

The authors declare that there is nothing to disclose.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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