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Selenium-containing analogs of SAHA induce cytotoxicity in lung cancer cells

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ABSTRACT

Cancer therapy has moved beyond conventional chemotherapeutics to more mechanism-based targeted approaches. Studies demonstrate that histone deacetylase (HDAC) is a promising target for anticancer agents. Numerous, structurally diverse, hydroxamic acid derivative, HDAC inhibitors have been reported and have been shown to induce growth arrest, differentiation, autophagy, and/or apoptotic cell death by inhibiting multiple signaling pathways in cancer cells. Suberoylanilide hydroxamic acid (SAHA) has emerged as an effective anticancer therapeutic agent and was recently approved by the FDA for the treatment of advanced cutaneous T-cell lymphoma. In our previous study, we reported the development of the novel, potent, selenium-containing HDAC inhibitors (SelSA-1 and SelSA-2). In this study, the effects of SelSA-1 and SelSA-2 on signaling pathways and cytotoxicity were compared with the known HDAC inhibitor, SAHA, in lung cancer cell lines. After 24 h of treatment, SelSA-1 and SelSA-2 inhibited lung cancer cell growth to a greater extent than SAHA in a dose-dependent manner with IC_{50} values at low micromolar concentrations. SelSA-1 and SelSA-2 inhibited ERK and PI3K-AKT signaling pathways while simultaneously increasing in autophagy in A549 cells in a time dependent manner. This preliminary study demonstrates the effectiveness of the selenium-containing analogs of SAHA, SelSA-1, and SelSA-2, as HDAC inhibitors and provides insight into the improvement and/or development of these analogs as a therapeutic approach for the treatment of lung cancer.

Chromatin remodeling is a key step in the regulation of gene expression consequently affecting cell function, differentiation, and proliferation. Chromatin structure affects transcription by regulating the access of transcription factors to their target sequences.[1,2] The key mechanism by which chromatin remodeling occurs is thought to be the modification of the NH_2-terminal tail of histones by histone acetyltransferase (HAT), which contributes to the 'histone code' determining the transcription of target genes.[3] The reversible acetylation of the side chain of specific histone lysine residues, by histone deacetylase (HDACs) and HAT, is one of the most widely studied chromatin modifications.[4] There are approximately 20 human HDACs (HDAC) which fall into four classes:[5,6] Class I and II are zinc-dependent metallohydrolases,[5] class III HDACs are NAD^+-dependent deactylases,[7] and class IV (comprising only HDAC11) exhibits properties of both class I and class II HDAC.[8] HDAC inhibitors (HDACIs) including short chain fatty acid (butyrate and valproate) derivatives, cyclic peptides, depsipeptides (FK-228), and hydroxamic acid derivatives are in preclinical development and/or undergoing clinical trials.[9]

A second generation HDACI, Suberoylanilide hydroxamic acid (SAHA) inhibits secretion of TNF-α, IL-1β, and IFN-γ in LPS induced peripheral blood monocytes cell models, as well as prevents the formation of tumors in mice and rats.[10] SAHA (Vorinostat) has emerged as an effective therapeutic anticancer agent and was recently approved by the FDA for the treatment of advanced cutaneous T-cell lymphoma.[11] SAHA induces apoptosis,[12] decreases expression of cyclin A, B, and D, as well as their respective cyclin dependent kinases (cdks) resulting in G1/S and/or G2/M growth arrest in cancer cells.[13–15] HDACIs can also induce cell differentiation resulting in cessation of growth of cancer cells in vitro[16] and in vivo[17] as a novel approach for the treatment of cancer.[13]

HDAC inhibitors can induce both mitochondria-mediated apoptosis and caspase-independent autophagic cell death. For example, treatment with HDACIs has been shown to induce cancer cell apoptosis as a result of inhibition of both the MAPK and PI3K/AKT signaling pathways in cancer cells[18,20] via a cytochrome C-dependent (mitochondrial) apoptotic signaling mechanism. Furthermore, co-administration of HDAC and Doxorubicin has been shown to synergistically promote apoptosis, in part, through up-regulation of pro-apoptotic Bcl2 molecules such as Bax, Bak, and Bad.[21–25] Separately, studies demonstrate that SAHA-induced cell death is mediated by the induction of autophagy and occurs independent of caspase activation and apoptosis, in HeLa cells.[26] Activation of autophagy by SAHA, in chondrosarcoma cells, induced cell death and resulted in inhibition of tumor growth in mice xenograft models.[27] Thus, initiation of autophagic cell death by HDAC inhibitors has clear therapeutic implications for treatment of cancers with apoptotic defects.[28]

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Hydroxamic acid derivative HDAC inhibitors[29] are proposed to chelate the zinc ion in the active site of HDAC in a bidentate fashion through its CO and OH groups[30] and occasionally have been associated with problems such as poor pharmacokinetics and severe toxicity.[31] Recently, we reported two selenium-containing derivatives of SAHA (Fig. 1), SelSA-1 (selenium dimer) and SelSA-2 (selenocyanide derivative) and both compounds were more potent HDAC inhibitors as compared to SAHA in Hela cell nuclear extracts.[32]

In the present study, we sought to investigate whether these SelSA compounds block cancer cell growth and/or induce apoptosis in a manner similar to SAHA and with improved efficacy relative to SAHA. We further wanted to determine the mechanisms of action of these newly developed selenium-containing analogs, SelSA-1 and SelSA-2, and to identify the signaling pathways involved in their effects on lung cancer cells.

For the current study, SAHA and SelSA compounds were synthesized as described.[32] Stock solutions of SAHA, SelSA-1 and SelSA-2 were prepared in dimethylsulfoxide (DMSO; Sigma, St. Louis, MO) at a concentration of 10 mM. The concentrations of the drugs employed in this study ranged from 0.1 μM to 50 μM. The drugs were dissolved in 100% DMSO and then diluted in the media for experiments. Control cells for all experiments were incubated in media supplemented with DMSO alone. The final concentration of DMSO was maintained at 0.2%. This concentration has no effect on growth and survival of the cells.

Human lung cancer cells were cultured in Dulbecco's modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin at 37 °C in a humidified atmosphere of 5% carbon dioxide. The sulforhodamine B (SRB) assay for cytotoxicity was carried out according to published procedure.[33] IC_{50} values and dose–response curves were determine by non-linear regression analysis using GraphPad Prism 5.0 software. After 24 h treatment of the cells, SelSA-1, SelSA-2, and SAHA caused a dose-dependent inhibition of growth[34] in all tested human lung cancer cell lines (A549, H2126, H1299, H226, H460, H522, H23, and H441) with IC_{50} at low- to sub-micromolar concentrations (Table 1). In A549, H2126, H226, H460, H522, H23, and H441 lung cancer cells, SelSA-1 and SelSA-2 exhibited calculated IC_{50} values lower than those obtained for SAHA. Significantly lower IC_{50} values were also observed for SelSA-2 relative to SAHA in H460 cells (p <0.05) and for SelSA-1 relative to SAHA in H441 cells (p <0.003). In general, SelSA-1 and SelSA-2 exhibited a more potent inhibition of growth activity on the majority lung cancer cells as compared to SAHA. Moreover, SelSA-1 and SelSA-2 had IC_{50} values comparable to SAHA in the normal lung epithelial cell line 16HBE140 (data not shown). These findings are consistent with reports indicating that HDAC inhibitors induce cell cycle growth arrest in both normal and transformed cells.[35,36] The relative resistance of normal cells to SelSA compounds may indicate that these SelSA compounds will have a suitable therapeutic window and will be well tolerated in future clinical trials as compared to SAHA.

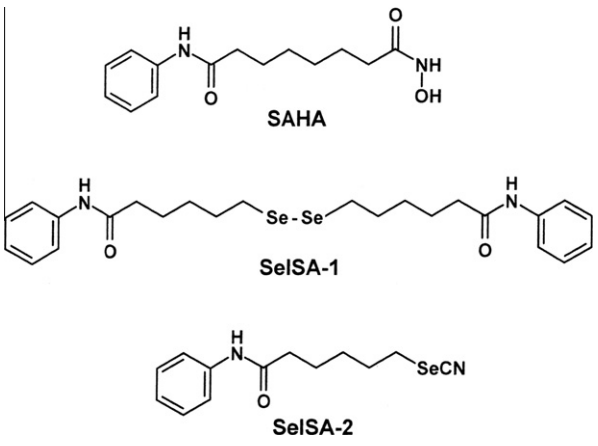

Figure 1. Structures of SAHA, SelSA-1, and SelSA-2.

There are studies indicating that HDAC inhibitors could influence cell proliferation and sensitivity to chemotherapeutic agents by inhibiting the functions of signal transduction networks.[36] Activation of the MAPK signaling pathway has been associated with enhanced proliferation and has been shown to be a feature common to many types of human tumors.[38,39] Additionally, the PI3K/AKT signaling pathway has been associated with cell survival.[40] Inhibition of HDAC has been shown, previously, to inhibit both the MAPK and PI3K/AKT signaling pathways resulting in apoptosis in cancer cells.[18–20] We next examined whether SelSA-1 and SelSA-2 induce cell death by inhibiting the MAPK and PI3K/AKT signaling pathways when compared with SAHA. Time dependent cellular responses for the MAPK and PI3K/AKT signaling pathways were examined in drug resistant A549 lung cancer cells using Western blot analysis.[37] SelSA-1 and SelSA-2 inhibited both ERK and AKT phosphorylation after 24 h at 5 μM (Fig. 2A) without obvious changes in total ERK and AKT levels in comparison to DMSO treated control cells (Fig. 2B). In contrast, SAHA did not inhibit AKT phosphorylation and only modestly inhibited ERK phosphorylation in A549 cells (Fig. 2A). These results are in agreement with previous reports in cancer cells with constitutively active MAPK or PI3K signaling, where inhibition of the ERK[40] or PI3K/AKT[41] enhanced the induction of apoptosis by HDAC inhibitors. Our results are also consistent with the observation that down regulation of both ERK and AKT and hence, elimination of compensatory interaction between these pathways, may be more therapeutically effective than interruption of either pathway alone.[41]

Recent studies have established that autophagy also plays an important role in cancer biology.[42] However, exactly how autophagy intersects with cancer development, disease progression and therapeutic response is controversial. Since autophagy has been reported to play a role in SAHA and butyrate induced cell death,[43] we evaluated the ability of our novel SelSA compounds to induce autophagy in A549 cells. As shown in Figure 2C, both SelSA compounds and SAHA-induced a time dependent increased in autophagy as indicated by increased levels of LC3 II. Interestingly, SelSA-1 and SelSA-2 induced autophagy after 3 h and 24 h whereas SAHA did not induce autophagy after 3 h of treatment relative to A549 cells treated with DMSO alone. It is well documented that in cancer cells with defective apoptotic signaling, autophagy can function as a pro-death process.[44,45] That our novel SelSA compounds induced autophagic cell death more rapidly than SAHA suggests improved efficacy and furthermore indicates their utility as therapeutic agents in treating cancers specifically with apoptotic defects. Additionally, these novel SelSA compounds may have potential research use as pharmacological inducers of autophagy for studying the mechanism of autophagic cell death.

Table 1
Cytotoxicity profiles (IC_{50}) of SAHA, SelSA-1, and SelSA-2 in lung cancer cells

Cell type	SAHA (μM) mean ± SE	SelSA-1 (μM) mean ± SE	SelSA-2 (μM) mean ± SE
A549	0.868 ± 0.03	0.370 ± 0.021	0.653 ± 0.030
H2126	1.319 ± 0.01	1.091 ± 0.018	0.928 ± 0.016
H1299	0.550 ± 0.04	0.438 ± 0.070	0.923 ± 0.040
H226	3.480 ± 0.005	1.732 ± 0.005	1.050 ± 0.007
H460	3.448 ± 0.005**	2.126 ± 0.007	1.461 ± 0.008**
H522	0.832 ± 0.024	0.188 ± 0.0366	0.779 ± 0.0264
H23	0.383 ± 0.028	0.279 ± 0.030	0.271 ± 0.010
H441	0.686 ± 0.057*	0.092 ± 0.048*	0.449 ± 0.079

* p <0.003.
** p <0.005.

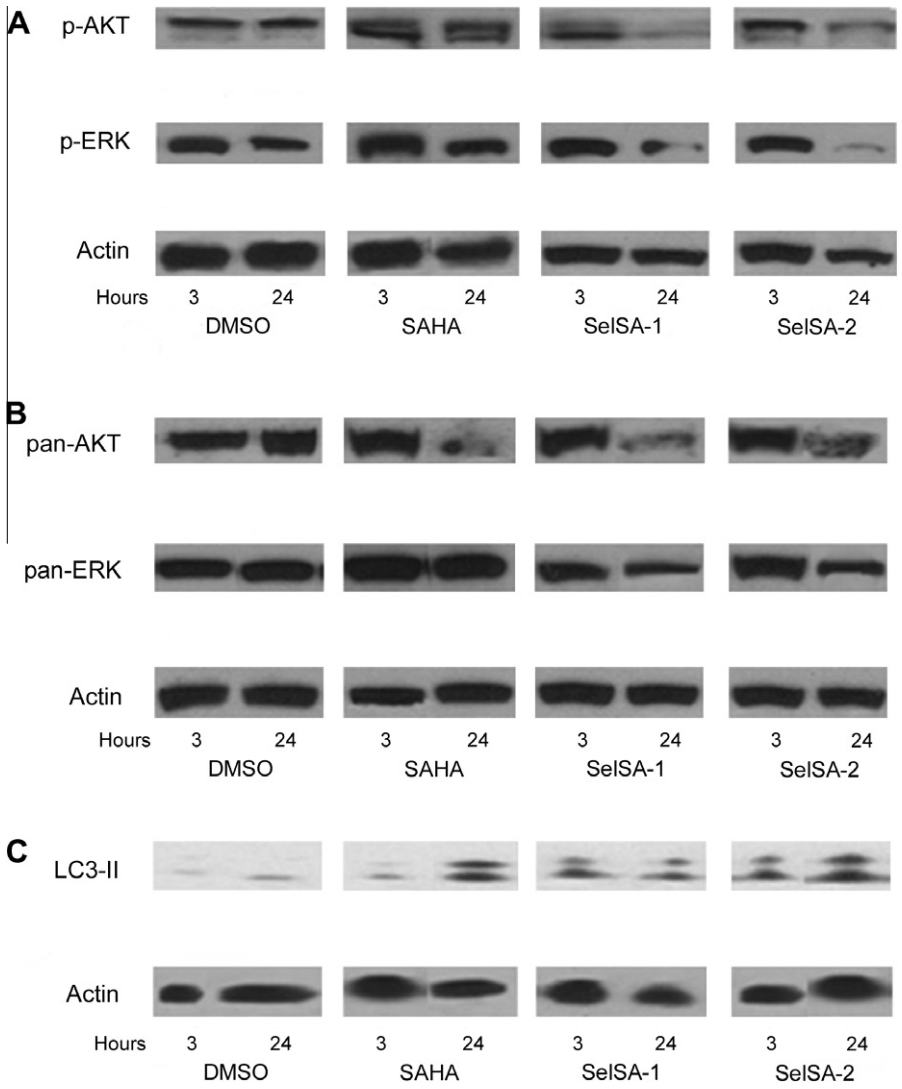

Figure 2. Effects of SelSA-1 and SelSA-2 on MAPK and PI3K/AKT signaling. (A) A549 cells treated with DMSO, SAHA, SelSA-1, and SelSA-2 were analyzed for expression of phospho-AKT and phospho-ERK inhibition after 3 h and 24 h by Western blot analysis using anti-pAKT and anti-pERK antibodies. (B) Total cellular levels of ERK and AKT were determined by Western blot analysis using anti-pan-AKT and anti-pan-ERK antibodies. (C) Induction of autophagy by SAHA and the SelSA compounds was examined by Western blot analysis using anti-LC3 II antibodies.

In summary, our results show that the novel selenium-containing compounds SelSA-1 and SelSA-2 are potent inhibitors of HDAC in vitro, induce cytotoxicity in a panel of lung cancer cell lines, inhibit the MAPK and PI3K/AKT signaling pathways, and induce autophagy. These SelSA compounds are significantly more effective in inducing cytotoxicity (in H460 and H441 cells) and in the inhibition of MAPK signaling and induction of autophagy (in A549 cells) than SAHA. We anticipate that our newly developed SelSA-1 and SelSA-2 HDAC inhibitors will lead to further development of HDAC inhibitors that are safer and more effective against cancer and would be effective as part of a therapeutic strategy addressing epigenomic modifications. Further studies on the pharmacokinetic characteristics of SelSA-1 and SelSA-2 compounds are therefore warranted.

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