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Deacetylase Inhibitors Dissociate the Histone-Targeting ING2 Subunit from the Sin3 Complex

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SUMMARY

Histone deacetylase (HDAC) inhibitors are in clinical development for several diseases, including cancers and neurodegenerative disorders. HDACs 1 and 2 are among the targets of these inhibitors and are part of multisubunit protein complexes. HDAC inhibitors (HDACis) block the activity of HDACs by chelating a zinc molecule in their catalytic sites. It is not known if the inhibitors have any additional functional effects on the multisubunit HDAC complexes. Here, we find that suberoylanilide hydroxamic acid (SAHA), the first FDA-approved HDACi for cancer, causes the dissociation of the PHD-finger-containing ING2 subunit from the Sin3 deacetylase complex. Loss of ING2 disrupts the in vivo binding of the Sin3 complex to the *p21* promoter, an important target gene for cell growth inhibition by SAHA. Our findings reveal a molecular mechanism by which HDAC inhibitors disrupt deacetylase function.

INTRODUCTION

Histone deacetylases (HDACs) remove acetyl groups from histones as well as nonhistone proteins. Histone hyperacetylation is generally correlated with gene expression, and HDACs often work to repress gene expression. Inhibitors of HDACs (HDACis) show promise as anticancer agents as well as in therapies for neurodegenerative diseases (Khan and La Thangue, 2008; Wiech et al., 2009). The hydroxamic acid SAHA is currently used as a treatment for advanced and refractory cutaneous T cell lymphoma (CTCL) (Khan and La Thangue, 2008; Mann et al., 2007). A second HDACi, Istodax (also known as romidepsin, depsipeptide, and FK228), has also recently been approved for CTCL treatment (http://www.fda.gov/AboutFDA/CentersOffices/CDER/ucm189466.htm). HDACis can inhibit cancer progression through a number of mechanisms, including inducing apoptosis, arresting cells in G1/S or G2/M, and causing cells to differentiate (Frew et al., 2009; Marks and Xu, 2009; Smith and Workman, 2009). One of the mechanisms by which HDACis work is through modulation of gene expression by acetylation of histones, to produce a transcriptional program that is favorable for cell cycle arrest or apoptosis (Frew et al., 2009; Marks and Xu, 2009; Smith and Workman, 2009). Overall, HDACis cause a small percentage of genes to be misregulated transcriptionally, and, in this subset of genes, some are upregulated, whereas some are downregulated (Smith, 2008; Van Lint et al., 1996). In addition, HDACis mediate the acetylation of many nonhistone proteins, although this also appears to be a rather small subset of all possible acetylated proteins (Choudhary et al., 2009; Spange et al., 2009).

There are four classes of HDACs. Classes I, II, and IV are zinc-dependent hydrolases, whereas Class III HDACs are nicotinamide adenine dinucleotide (NAD^+)-dependent enzymes called sirtuins (Yang and Seto, 2008). There are 11 known zinc-dependent HDACs (Class I: HDACs 1–3 and 8; Class II: HDACs 4–7, 9, and 10; Class IV: HDAC 11) (Yang and Seto, 2008). Many inhibitors being tested as anticancer agents affect several of these enzymes. Crystal structures have been solved for a bacterial Class I homolog and for human HDACs 7 and 8 in complex with the hydroxamic acid inhibitors trichostatin A (TSA) and SAHA (Finnin et al., 1999; Schuetz et al., 2008; Vannini et al., 2004). These inhibitors work by chelating a zinc molecule in the active site of the HDACs through their hydroxamic acid moieties (Finnin et al., 1999; Schuetz et al., 2008; Vannini et al., 2004). Because these molecules contain aliphatic chains that extend out through the normal acetyl lysine-binding pockets in the HDACs, they also may inhibit binding of the HDAC to their normal acetyl lysine substrates (Finnin et al., 1999; Schuetz et al., 2008; Vannini et al., 2004). Many inhibitors in clinical development affect several HDACs; therefore, work has recently focused on understanding which HDACs are needed to mediate the anticancer effects of the inhibitors (Balasubramanian et al., 2009; Witt et al., 2009). The goal is to obtain cancer cell growth-inhibiting properties while maximizing the selectivity of the inhibitors. Studies suggest that, in vivo, HDACs 1 and 2 play a role in mediating cell growth arrest by these molecules (Glaser et al., 2003; Haberland et al., 2009).

However, HDACs1 and 2 do not work alone; rather, they reside in multisubunit chromatin modifying complexes, of which three have been characterized: Mi-2/NuRD, which contains HDAC, histone demethylase, and chromatin remodeling activities; CoREST, which can repress neuronal-specific genes in non-neuronal cells; and Sin3, which has been implicated in cell cycle control (Wang et al., 2009; Yang and Seto, 2008). Residency in these complexes is important for full activity and specificity of these HDACs in the cell (Alland et al., 2002; Denslow and Wade, 2007). However, it is not known if HDACis act directly on

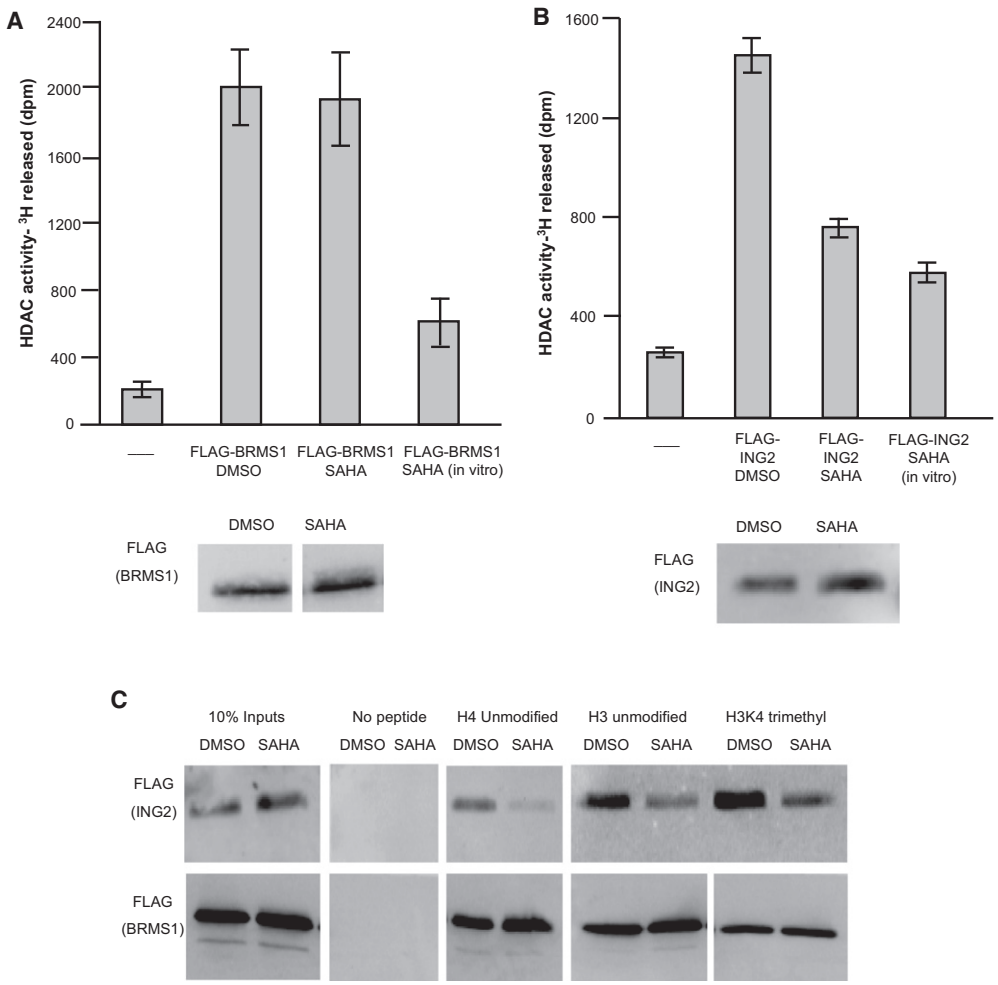

Figure 1. SAHA Alters the Biochemical Properties of the Sin3 Complex

(A and B) HDAC assays were performed on ^{3}H acetylated core histones with (A) FL-BRMS1-purified complexes (top panel) or (B) FL-ING2-purified complexes (top panel). DMSO and SAHA labels indicate that complexes were purified from 293T cells treated for 9 hr with these compounds. The SAHA (in vitro) label indicates that complexes were purified from untreated cells and that SAHA was added directly to the deacetylation reaction. Error bars in (A) and (B) represent ± standard deviation. Amounts of complex used in the assays were normalized to levels of (A) FL-BRMS1 (lower panel) or to levels of (B) FL-ING2 (lower panel).

(C) Western blot analysis of FLAG-tagged proteins bound to histone peptides.

these multisubunit complexes. The Sin3 complex is a 1.2 MDa complex implicated in cell cycle control through its interactions with the tumor suppressor protein Rb and can repress E2F-mediated transcription to prevent progression to S phase (Lai et al., 2001). The Sin3 complex is also implicated in controlling progression through the G2 phase of the cell cycle (David et al., 2003; Pile et al., 2002). Therefore, this complex is among the potential targets of HDACis that could mediate the growth arrest by these molecules. We set out to determine if HDACis had any effects on the multisubunit Sin3 complex, and if the complex was still intact after the HDACs were bound to the inhibitors.

RESULTS

ING2-Purified Complexes Are Altered by HDACis

To determine if HDACis alter the properties of the Sin3 complex, we purified the complex from 293T cells that stably expressed tagged subunits. We used two different known subunits of the Sin3 complex as baits for these purifications, inhibitor of growth 2 (ING2), which binds to H3K4 that is di- and trimethylated through its PHD finger, and breast cancer metastasis suppressor 1 (BRMS1), which has an unknown function in the complex (Doyon et al., 2006; Meehan et al., 2004; Shi et al., 2006). The *ING2* gene is deleted in some head and neck carcinomas, whereas BRMS1 is important for suppressing cancer metastasis, suggesting that their roles in the Sin3 complex could be related to cell growth and cancer progression (Seraj et al., 2000; Sironi et al., 2004). We performed purifications from cells treated with the HDAC inhibitor SAHA (7.5 μM) or DMSO and tested if there were differences in the HDAC activities of the complexes. Sin3 complexes purified through the BRMS1 subunit from SAHA-treated cells still had HDAC activity on acetylated core histones, suggesting that the inhibitor was lost during the purification (Figure 1A). This result is consistent with kinetic analyses of these competitive inhibitors (Sekhavat et al., 2007). These complexes were