Serine Proteases in Histone Deacetylase Inhibitor–Induced Apoptosis – Letter

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Histone deacetylase inhibitors (HDACi) are a new group of targeted antitumor agents (1). They exert their activity against cancer cells by stimulating differentiation, by inhibiting proliferation, and by inducing apoptosis. Although the latter has been extensively studied, our understanding of how HDACi harness the apoptotic machinery is incomplete. In particular, the significance of caspases and other proteases in the apoptotic pathway mediated by HDACi has not yet been conclusively resolved. In a recent article, the hydroxamic acid HDACi trichostatin A (TSA) and vorinostat [also called suberoylanilide hydroxamic acid (SAHA)] have been reported to induce caspase-independent, but serine protease-dependent, apoptosis in pancreatic cancer cells (2). It was shown that the serine protease inhibitor 4-(2-aminoethyl)-benzenesulfonylfluoride (AEBSF; also called Pefabloc; and called ISP in ref. 2) was able to block TSA-induced apoptosis, leading to the conclusion that the latter depended on serine protease activity. [AEBSF functions by sulfonylating the active-site serine residue in serine proteases (3), but it may also covalently modify other proteins (4).]

We have reservations about this conclusion, because we have evidence that AEBSF is capable of directly inactivating the HDAC inhibitory action of hydroxamic acids. In an effort to identify proteases involved in HDACi-elicited apoptosis, we have also employed AEBSF. We observed that AEBSF abolished TSA- or vorinostat-induced apoptosis, whereas it had no effect on apoptosis triggered by the short-chain fatty acid HDACi sodium butyrate (NaB). In fact, AEBSF fully suppressed the cytotoxic effect of TSA under otherwise extremely toxic conditions (i.e., continuous exposure to 1 μmol/L TSA for 10 days in clonogenic assays). To us, this effect of AEBSF seemed too strong to be due to a specific inhibition of proteases and, thus, prompted speculation that it was, rather, due to a direct inactivation of TSA by AEBSF.

If this was the case, AEBSF should impede the effect of TSA on HDAC enzymatic activity. First, we tested this idea by measuring HDAC activity in intact cells (5). We found that AEBSF potently impaired the HDAC inhibitory effect of TSA and vorinostat, whereas it left NaB action unscathed (Fig. 1A). For comparison, the pan-caspase inhibitor z-VAD-fmk did not affect TSA action. In line with these results, AEBSF also prevented TSA- and vorinostat-induced, but not NaB-induced, histone hyperacetylation, as determined on Western blots (data are not shown). Second, we measured recombinant HDAC1 activity using a cell-free assay: AEBSF impaired the effect of TSA in this system, too (Fig. 1B). Therefore, our finding suggests that AEBSF directly inactivates TSA and vorinostat and, in this way, restrains these HDACi from inhibiting HDAC activity, and, in turn, from inducing apoptosis.

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Figure 1. A, HDAC activity was measured in SK-OV-3 cells using Boc-K(Ac)-AMC; mean ± SEM of three experiments in duplicate. Similar results were obtained in A549 cells. B, recombinant human HDAC1 (Biomol International) activity was measured using Boc-K(Ac)-AMC; mean ± SEM of three experiments.

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Beyond the effect of AEBSF on HDACi, our observation raises the concern that AEBSF might also directly modify other compounds. Use of AEBSF as sole serine protease inhibitor, as for example, in an article that reported that serine proteases have a critical role in etoposide-induced apoptosis (6). In conclusion, a potential direct action of AEBSF on small molecule compounds should be taken into account in future studies on serine proteases.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

Correction: Serine Proteases in Histone Deacetylase Inhibitor–Induced Apoptosis – Letter

In this Letter to the Editor (Mol Cancer Ther 2010;9:2440–1), which was published in the August 2010 issue of Molecular Cancer Therapeutics (1), the authors were not listed in the correct order. The correct order is Jürgen Sonnemann, Chithra D. Palani, Lénia Ferrão Beck, Bettina Appel, and James F. Beck. The grant support was also not given. Grant support was received from Wilhelm Sander-Stiftung, Neustadt/Donau. The online article has been changed to reflect these corrections and no longer matches the print.

Reference

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