[Cell Cycle 4:6, 741-743; June 2005]; ©2005 Landes Bioscience

Extra Views

Mechanisms of Selective Anticancer Action of Histone Deacetylase Inhibitors

Alessandra Insinga[1]
Saverio Minucci[1,2,*]
Pier Giuseppe Pelicci[1,3,*]

[1]Department of Experimental Oncology; European Institute of Oncology; Milan, Italy

[2]Department of Biomolecular Sciences and Biotechnology; University of Milan; Milan, Italy

[3]IFOM-FIRC Institute; Milan, Italy

*Correspondence to: Pier Giuseppe Pelicci; Department of Experimental Oncology; European Institute of Oncology; Via Ripamonti 435; 20141 Milan, Italy; Tel.: +39/02/57489868; Fax: +39.02.57489851; Email: piergiuseppe.pelicci@ifom-ieo-campus.it; or Saverio Minucci; Department of Experimental Oncology; European Institute of Oncology; Via Ripamonti 435; 20141 Milan, Italy; Tel.: +39.02.57489832; Fax: +39.02.57489851; Email: saverio.minucci@ifom-ieo-campus.it

Received 04/05/05; Accepted 04/07/05

Previously published online as a *Cell Cycle* E-publication: http://www.landesbioscience.com/journals/cc/abstract.php?id=1717

KEY WORDS

apoptosis, death receptors, p53, leukemia, oncogenic fusion protein, mouse model

ABBREVIATIONS

AMLs	acute myeloid leukemias
APL	acute promyelocytic leukemia
RAR	retinoic acid receptor
HDACs	histone deacetylases
HDACI	histone deacetylase inhibitors
TRAIL	TNF-related apoptosis inducing ligand
FasL	Fas ligand
DR	death receptors

ABSTRACT

Histone deacetylases (HDACs) regulate transcription and specific functions, such as tumor suppression by p53, and are frequently altered in cancer. Inhibitors of HDACs (HDACI) possess anti-tumor activity and are well tolerated, suggesting that they might develop into a specific strategy for cancer treatment. Indeed, HDACIs have successfully entered clinical trials, but the molecular basis for their selective anti-tumor activities is not clear. Recent work on leukemias expressing the PML-RAR or AML1-ETO oncogenes, known to initiate leukemogenesis through deregulation of HDACs, shows that HDACIs induce massive blast cell apoptosis. Interestingly, the pro-apoptotic activity of the drug is not due to the relief of oncogene-mediated inhibition of the p53 tumor-suppressor pathway but, instead, relies on the selective upregulation of the death receptors DR5 and Fas and their cognate ligands TRAIL and FasL. Significantly, normal myeloid progenitors are not sensitive to HDACI-induced apoptosis and oncogene expression is not sufficient to confer HDACI-sensitivity to normal cells, demonstrating that sensitivity to HDACI is a property of the fully transformed phenotype. In principle, our findings could thus apply to other cancers, where the contribution of HDACs to tumorigenesis is not yet defined.

Epigenetic modifications of DNA (methylation) and chromatin (acetylation and methylation of histones) underlie regulation of all genome functions, including gene expression, DNA replication and genome stability.[1-4] Among those, gene silencing is associated with hypoacetylated histones, often found within regions of DNA methylation and/or methylation of specific histone residues (e.g., lysine 9 of histone 3), while gene activation is associated with acetylated histones and methylation of lysine 4 of histone 3. Histone deacetylases (HDACs) and histone acetyl-transferases (HAT) play a key role in maintaining the balance between acetylated and deacetylated states of chromatin, that appears to be essential for normal cell growth.[5,6]

Aberrant regulation of gene expression due to chromatin alterations is a hallmark of many forms of cancer. Alterations in the structure or function of epigenome-modifying enzymes have been documented in cancer.[7-12] Genes that encode HAT enzymes (p300, PCAF and CBP) are aberrantly expressed or mutated in a variety of human tumors -both hematological and epithelial- and there is growing evidence to support a role for HATs in regulating tumorigenesis.[8] HDACs are overexpressed or associated with oncogenic transcription factors in a number of cancers, due to chromosomal translocations or aberrant physical associations, making them attractive targets for anticancer therapies.[7]

A range of structurally diverse HDACI have been purified from natural sources or synthetically produced, and can be subdivided into four groups (hydroxamates, cyclic peptides, aliphatic acids and benzamides).[13-16] All these groups of compounds, despite their differences in structure and active concentrations, are rather unique as they couple broad antitumor activity and low toxicity, as revealed on cell lines as well as animal model systems.[13-15,17] Preliminary results from clinical trials using several HDACI as anticancer agents also confirmed their activity against different types of cancers at relatively well tolerated doses.[13,15,16] As a consequence, there are now intense efforts to understand the molecular basis of the high and selective sensitivity of tumor cells to HDACI.[15]

To this end, we have used PML-RAR and AML1-ETO leukemias, not to study leukemia therapy per se, but to exploit systems where altered gene expression through aberrant histone deacetylation is clearly linked to disease onset and progression.[12,18,19] Indeed, using murine model systems of PML-RAR and AML1-ETO leukemias, we found that the HDACI VPA is biologically active and enhances survival of the treated mice. However, when we examined the underlying biological effects, we found that this drug does not relieve the PML-RAR- (or AML1-ETO-) mediated transcriptional silencing of

target promoters and does not induce terminal differentiation of the leukemia blasts. This is not surprising, if one considers that PML-RAR-mediated transcriptional repression also involves recruitment of DNA methyltransferases and DNA methylation at target promoters. Furthermore, we found that VPA induces apoptosis of the leukemic blasts and that, although it increases acetylation/stabilization of p53,[20] its apoptotic effect is clearly p53-independent. Therefore, despite the relevance of HDACs in oncogenic transformation, the sensitivity of leukemic cells to HDACI is not due to inhibition of the HDAC-dependent activities of the leukemia-associated fusion proteins, but is, instead, a feature of the fully transformed leukemic phenotype. In principle, our findings could thus apply to other cancers, where the contribution of HDACs to tumorigenesis is not yet defined. Accordingly, we have recently identified a subset of breast cancer tumors where HDACI induce apoptosis (manuscript in preparation).

To further investigate the mechanisms of the selective anticancer action of HDACI, we focused on death-receptor mediated apoptosis. It was previously reported that HDACI induce apoptosis of cancer cell lines in vitro through activation of pro-apoptotic genes, as Fas and Bak.[21] Furthermore, in leukemic cells, the clinically efficacious drug ATRA induces apoptosis through up regulation of the tumor-selective death ligand TRAIL.[22] Our studies show that VPA induces nonredundant transcriptional up regulation of TRAIL/DR5 and FasL/Fas, which is required for its apoptogenic effect in vivo.

Pharmacologically, a range of different HDACI is now available. When we tested TSA, this compound induced the up regulation of TRAIL/Fas and massive apoptosis of leukemic cells in vitro. In vivo, instead, TSA was clinically inactive (most likely due to unfavorable pharmacokinetic properties) and induced expression of Fas, but not of TRAIL. In line with these observations, it has been recently reported that in vivo treatment of transplanted tumors with TSA, at the same concentrations, is unable to inhibit tumor growth. However, if the same cells are pretreated with TSA in vitro, they fail to implant.[23] Our results predict, therefore, that the apoptogenic activity of HDACI depends on the simultaneous activation of members of the TNF superfamily and suggest that the transcriptional activation properties of these drugs must be carefully monitored during in vivo treatment to adjust proper treatment schedules.

What happens in normal and preleukemic cells, that are VPA-insensitive? Our data establish a link between tumor cell apoptosis and the tumor-selective death ligand TRAIL. In vitro, TRAIL induces rapid apoptosis of a variety of tumor cell lines, independent of the p53 status, but not of normal cell lines. Although two death receptors and three decoy receptors have been identified which bind TRAIL, the molecular mechanism underlying the resistance or sensitivity of normal/transformed cells remains poorly understood. However, our experiments reveal that the basis for the selective toxicity of HDACI towards cancer cells instead relies on the selective induction of TRAIL, DR5, FasL and Fas in fully transformed cells. And hence, they raise the possibility that global chromatin changes occurring late during oncogenic transformation might impose different mechanisms of silencing of these promoters. Presumably, these chromatin-affecting events are common to many tumors, and account for the broad anticancer activity of HDACI. Yet, further studies are needed to dissect the mechanisms underlying the differential regulation of these relevant HDACI-target promoters in normal/preleukemic and cancer cells.

The fact that VPA-induced apoptosis of leukemic cells can be prevented when Fas and TRAIL are simultaneously inhibited, suggests that HDACI might possess a much broader anti-tumor activity than soluble TRAIL. Results from preclinical studies using soluble TRAIL have been very promising for cancer treatment; however numerous reports have demonstrated resistance of various tumor cells to TRAIL.[24] Our data raise the possibility that an HDACI-based therapy might be more effective in these cases. Alternatively, those HDACI that induce activation of Fas only, as TSA does in vivo, might act synergistically with TRAIL in TRAIL-resistant cancers. Consistent with this idea, it has been recently demonstrated that HDACI can sinergize with soluble TRAIL in vitro.[25]

To what extent do the murine PML-RAR or AML1-ETO leukemias reflect the behavior of human tumors? Our in vitro data on acute myeloid leukemia patients' blasts are in agreement with the mechanisms uncovered by use of the preclinical models: (1) VPA induces apoptosis and expression of TRAIL/Fas in blasts from PML-RAR and AML1-ETO patients; (2) a sizable fraction of AMLs, regardless of their primary genetic lesion and not including PML-RAR and AML1-ETO cases, is also VPA-sensitive; (3) VPA increased expression of TRAIL/Fas only in patients' blasts where it also induced apoptosis. In the patient blasts VPA also increased expression of caspase 8 (at variance with the mouse leukemic cells where this effect was significantly milder; data not shown); up regulation of caspase 8, one of the effectors of TRAIL/Fas-induced apoptosis, might further facilitate death receptor-mediated eradication of leukemia blasts. Clinical studies are now needed to validate our approach. Which patients will benefit from HDACI-treatment? Based on our results, in vitro tests on primary cells aimed at revealing up-regulation of TRAIL/Fas might predict in vivo sensitivity, thereby allowing for a molecular identification of those patients that will benefit from an HDACI-based therapy. Clearly, a molecular understanding of the mechanisms of action of HDACI will be helpful for an effective use of these drugs as "single agents" as well as in "combination therapies".

References

1. Jenuwein T, Allis CD. Translating the histone code. Science 2001; 293:1074-80.
2. Turner BM. Cellular memory and the histone code. Cell 2002; 111:285-91.
3. Bird AP, Wolffe AP. Methylation-induced repression-belts, braces, and chromatin. Cell 1999; 99:451-4.
4. Jaenisch R, Bird A. Epigenetic regulation of gene expression: How the genome integrates intrinsic and environmental signals. Nat Genet 2003; 33:245-54.
5. Johnson CA, Turner BM. Histone deacetylases: Complex transducers of nuclear signals. Semin Cell Dev Biol 1999; 10:179-88.
6. Roth SY, Denu JM, Allis CD. Histone acetyltransferases. Annu Rev Biochem 2001; 70:81-120.
7. Minucci S, Nervi C, Lo Coco F, Pelicci PG. Histone deacetylases: A common molecular target for differentiation treatment of acute myeloid leukemias? Oncogene 2001; 20:3110-5.
8. Phillips AC, Vousden KH. Acetyltransferases and tumour suppression. Breast Cancer Res 2000; 2:244-6.
9. Jones PA, Laird PW. Cancer epigenetics comes of age. Nat Genet 1999; 21:163-7.
10. Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. Nat Rev Genet 2002; 3:415-28.
11. Sellers WR, Loda M. The EZH2 polycomb transcriptional repressor-a marker or mover of metastatic prostate cancer? Cancer Cell 2002; 2:349-50.
12. Minucci S, Maccarana M, Cioce M, De Luca P, Gelmetti V, Segalla S, Di Croce L, Giavara S, Matteucci C, Gobbi A, Bianchini A, Colombo E, Schiavoni I, Badaracco G, Hu X, Lazar MA, Landsberger N, Nervi C, Pelicci PG. Oligomerization of RAR and AML1 transcription factors as a novel mechanism of oncogenic activation. Mol Cell 2000; 5:811-20.
13. Marks P, Rifkind RA, Richon VM, Breslow R, Miller T, Kelly WK. Histone deacetylases and cancer: Causes and therapies. Nat Rev Cancer 2001; 1:194-202.
14. Johnstone RW. Histone-deacetylase inhibitors: Novel drugs for the treatment of cancer. Nat Rev Drug Discov 2002; 1:287-99.
15. Johnstone RW, Licht JD. Histone deacetylase inhibitors in cancer therapy: Is transcription the primary target? Cancer Cell 2003; 4:13-8.
16. Kelly WK, O'Connor OA, Marks PA. Histone deacetylase inhibitors: From target to clinical trials. Expert Opin Investig Drugs 2002; 11:1695-713.

17. Marks PA, Richon VM, Rifkind RA. Histone deacetylase inhibitors: Inducers of differentiation or apoptosis of transformed cells. J Natl Cancer Inst 2000; 92:1210-6.
18. Grignani F, De Matteis S, Nervi C, Tomassoni L, Gelmetti V, Cioce M, Fanelli M, Ruthardt M, Ferrara FF, Zamir I, Seiser C, Lazar MA, Minucci S, Pelicci PG. Fusion proteins of the retinoic acid receptor-alpha recruit histone deacetylase in promyelocytic leukaemia. Nature 1998; 391:815-8.
19. Lin RJ, Evans RM. Acquisition of oncogenic potential by RAR chimeras in acute promyelocytic leukemia through formation of homodimers. Mol Cell 2000; 5:821-30.
20. Insinga A, Monestiroli S, Ronzoni S, Carbone R, Pearson M, Pruneri G, Viale G, Appella E, Pelicci P, Minucci S. Impairment of p53 acetylation, stability and function by an oncogenic transcription factor. Embo J 2004; 23:1144-54.
21. Johnstone RW, Ruefli AA, Lowe SW. Apoptosis: A link between cancer genetics and chemotherapy. Cell 2002; 108:153-64.
22. Altucci L, Rossin A, Raffelsberger W, Reitmair A, Chomienne C, Gronemeyer H. Retinoic acid-induced apoptosis in leukemia cells is mediated by paracrine action of tumor-selective death ligand TRAIL. Nat Med 2001; 7:680-6.
23. Maecker HL, Yun Z, Maecker HT, Giaccia AJ. Epigenetic changes in tumor Fas levels determine immune escape and response to therapy. Cancer Cell 2002; 2:139-48.
24. LeBlanc HN, Ashkenazi A. Apo2L/TRAIL and its death and decoy receptors. Cell Death Differ 2003; 10:66-75.
25. Rosato RR, Almenara JA, Dai Y, Grant S. Simultaneous activation of the intrinsic and extrinsic pathways by histone deacetylase (HDAC) inhibitors and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) synergistically induces mitochondrial damage and apoptosis in human leukemia cells. Mol Cancer Ther 2003; 2:1273-84.