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Histone deacetylases 1/2 as critical factors in protein synthesis and tumorigenesis

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Abstract
Histone deacetylases (HDACs) co-repress transcription by deacetylating histone tails. HDAC1 and HDAC2, as highly related proteins, have crucial functions in physiology and development. HDAC1 has premier role in mediation of cyclin-dependent kinase inhibitor p21, and HDAC1-knockout mice are embryonic lethal. Silencing of HDAC1 leads to disturb of the cell cycle with mitotic cell loss. HDAC2 also deacetylases non-histone proteins. Cap binding protein Eukaryotic translation initiation factor 4E (eIF4E) binds the 5' cap structure of messenger RNAs (mRNAs). The sumoylation of eIF4E is promoted by HDAC2, the sumoylation of eIF4E is independent from its deacetylating activity. The sumoylation of eIF4E promotes the formation of eIF4F translation complex that induces translation of a series of important proteins that are essential for cell proliferation and preventing cell apoptosis. Disruption of eIF4E sumoylation leads to abrogation of mRNA translation of the series of proteins. These data implies it is meaningful to study sumoylation as a novel regulatory mechanism for protein synthesis and eIF4E as a critical factor for potential oncological study. HDAC2 is overexpressed in a number of tumor types, implying its role in tumorigenesis. As understood the tumor suppressor factor p53 in human tumors is often silenced, p53 gene is often mutated. The molecular reason for p53 inactivation remains to be defined. Remarkably, recent findings show the overexpression of HDAC2 in tumor cells has links to the inactivation of p53. Here we show in solid and hematopoeotic cancers overexpressed HDAC2 has links with p53 silence *in vivo* and *in vitro*. Further study of the relationship of p53 and HDAC2 can help bring new ideas to cancer therapy.

Key words: Histone deacetylases 1/2, sumoylation, eIF4E, tumorgenesis

Introduction
The transcription of many crucial genes were co-repressed by histone deacetylases (HDACs). HDACs have many subtypes, of which, HDAC1 and 2, hold important roles in bi ological processes. HDAC1 and HDAC2 share over 85% homologue. HDAC2 promotes do wnstream mRNA translation by catalyzing Eukaryotic translation initiation factor 4E (eIF4E) and HDAC2 is overexpressed in many cancer types.The function and structure of HDAC2 a nd HDAC1 will be elucidated in this article.

HDAC2 in eIF4E sumoylation mediated mRNA translation
Over 95% proteins in eukaryotes are synthesized by cap dependent mRNA translation. In this process, one control step that impacts the rate is the formation of eIF4F translation complex. The complex is formed up by eIF4G, eIF4A and eIF4E. eIF4G is scaffold protein for the frame of the complex, eIF4A is ATP-dependent mRNA helicase and eIF4E is cap-binding protein (1, 2). The binding of eIF4E to cap structure is inhibited by 4E-BPs. 4E-BP1 has multiple phosphorylation sites and the phosphorylation induces dissociation of 4E-BPs from eIF4E, hence leading to the formation of eIF4F complex and the downstream mRNA translation (3). The regulation of protein function can be through covalent modification of small ubiquitin-related modifier (SUMO). There are four SUMO molecules, sequencing comparison shows *SUMO2* and *SUMO3* are 97% identical, however, *SUMO1* shares only 50% with *SUMO2* and *SUMO3*. Also, whilst *SUMO1, 2 and 3* are universally expressed, *SUMO4* mainly expresses in kidney, lympg node and spleen. The sumoylation of eIF4E by HDAC2 is mediated by E1, E2 and E3 enzyme. E1 enzyme functions as activating enzyme, E2 enzyme functions as conjugating enzyme and E3 enzyme functions as ligases (4). Ubc9 is the solo conjugating enzyme that has be identified. Also, several sequence-specific E3 ligases have been identified as well (5). The identified E3 ligases include protein inhibitor of activated STAT (PIAS),

RanBP2, polycomb group protein Pc2, and Mms21-Nse1 (6). Furthermore, HDAC4 and HDAC7 promote sumoylation of myocyte enhancer factor 2 (MEF2) and promyelocytic leukemia protein, respectively (7-9). Human HDACs is a family with 18 enzymes that can be grouped into 4 classes according to their sequence homology. Only HDAC4 and HDAC7 have been reported to have E3 activity. eIF4E is modified by SUMO1 conjugation, phosphorylation of Ser209 of eIF4E promotes sumoylation, eIF4E after sumoylation does not impact cap binding translation directly, but rather, induce the dissociation of 4E-BP1 and eIF4E, leading to the formation of eIF4F complex and mRNA translation. The study of sumoylation of eIF4E by HDAC2 and its regulation can largely broaden our view on HDAC related oncogenesis pathways.

Histone deacetylase 2 as an epigenetic regulator

In cencer cells deacetylases are often dysfunctional (10, 11) and cell cycle control and differentiation are disturbed by HDAC overexpression (12, 13). HDAC2 is the most widely overexpressed factor of all HDAC family in cancer (12). Monitoring HDAC2 expression can observe the efficiency of histone deacetylases inhibitors (HDACi), in clinic, HDAC2 expression level is a meaningful indicator (14, 15). The deacetylation of lysine residues in proteins are catalyzed by HDAC2 (10, 11, 16, 17). HDAC2 is mediated by a number of posttranslational modifications (PTMs) and HDAC2 expression is mediated by transcriptional and posttranscriptional mechanism, the responsible enzymes for several PTMs regulating HDAC2 are found (10, 11). HDACi are broad compounds which can be divided into two groups: pan-HDACi and isoenzyme-selective HDACi (18, 19). Both are considered as promising future agents of tumor therapy (Table.1) (20). Treatment with HDACi leads to hyper-acetylated protein accumulation (21). Therefore, cells exposed to these agents altered gene expression and signaling nodes (22, 23). The process to search for HDAC2 specific-targeting drugs is ongoing (24). Such drugs are expected to have positive functions whilst with less side effects (25).

Table 1: HDACi with substances inactivating HDAC2.

HDACi	Inhibitory profile	Substance class	Indications	References
Vorinostat/SAHA (Suberoylanilide hydroxamic acid)	Pan-HDACi	Hydroxamic acid	CTCL approval, for other cancer types in clinical trials	26
Romidepsin/FK288	Pan-HDACi	Depsipeptide	Same as above	27
VPA (valproic acid)	Class I HDAC selective	Short fatty acid	In clinical trial phase III	28
Entinostat/MS-275	Class I HDAC selective (HDAC1>2>3)	Benzamide	In clinical trial phase II	29, 30
Butyrate	Pan-HDACi	Short fatty acid		31, 32
Panobinostat/LBH-589	Pan-HDACi	Hydroxamic acid		33, 34
Trichostatin A (TSA)	Pan-HDACi	Hydroxamic acid	Model HDACi not considered for clinical use	35, 36
BRD8430	Class I HDAC selective (HDAC1>2>3)	Lactam, ortho-amino aniline group		37
CG-1521 (JW-1521)	N/a	Hydroxamic acid		38
OSU-HDAC42 (AR-42)	N/a	Phenylbutyrate based hydroxamic acid		39

The interactions between tumor suppressor p53 and HDAC2

The widely know tumor-suppressive transcription factor p53 is currently the most studied target protein of HDAC2. P53 tetramers target to a number of genes which mediate growth arrest, senescence, and cell apoptosis to limit cellular transformation (40). Additionally, p53 influences the generation of ROS (reactive oxygen species) and metabolism (41). P53 is inactivate in most cancers due to the loss of human p53 encoding gene TP53, TP53 mutations, or signaling pathway errors (42). Post translational modifications like phosphorylation, acetylation, ubiquitinylation, and sumoylation regulate p53 activities in vivo (43). HDAC2 and p53 are recently found heavily connected in terms of mediating each other in tumorigenesis. HDAC2 is recruited by p53 dependent target genes promoters to repress the transcription. HDAC2 PTMs manage interactions with particular targets (44). HDAC2 also coordinates with peptidylarginine deiminase 4 (PAD4) to mediate expression of p53 target gene. PAD4 takes part in lysine methylation mediation by triggering arginine citrullination. Together HDAC2 and PAD4 corepress p53 (45). When they bound to promoters, the histone acetylation and methylation are suppressed. When HDAC2 and PAD4 are released from the promoters of p53 target genes, the target genes gain increased histone acetylation, methylation and transcription. The target genes include cyclin-dependent kinase inhibitor 1 (CDKN1A), growth arrest and DNA-damage-inducible (GADD45A), p53 upregulated modulator of apoptosis (PUMA/BBC3) (45). In addition, HDAC2 may also regulate p53 target genes without the presence of p53. For instance, HDAC2 is recruited to two binding sites by metastasis-associated protein 1 (MTA1) in the absence of p53 (46). P53 acetylation is indispensable from its function (47, 48). At least 13 lysine residues were found to be acetylated (47), and p53 is controlled by HDAC2 mediated deacelyation. K320 acetylation was suggested by evidence to be related for p53 signaling. HDAC2 deacelyates lysine residue K320 of p53 in colon tumor cells. HDAC2-p53 sumoylation dependent interaction results in acetylation of K320 of p53, lower DNA binding to p53 and target gene impaired transcription regulation.p53 C-terminal including K320 rather than N-terminal interacts more strongly with HDAC2 (45). In melanoma cells p53 expression is also regulated by HDAC2. These regulations allow cancer cells to hamper p53-dependent apoptosis.

HDAC1 and HDAC2 are critical for cancer pathogenesis

Malignancies are usually characterized by chromosomal translocations in hematopoietic system (48-50). In fusion protein, DNA binding domain of one trancription factor is

fused into a protein which recruits epigenetic silencing factors including HDACs. Examples include PML-RARα and AML1-ETO. These factors induce transcription silence and a downstream blocking of the differentiation of hematopoietic precursors. The HDACs inhibition and PML-RARα elimination lead the cell differentiation and apoptosis (51). HDAC1 and HDAC2 are also crucial in development of immune cells and hematopoiesis. Previous study showed mice without *HDAC1* and *HDAC2* presented a termination of thymocyte proliferation (52, 53) and an *Hdac1/2 knock-out* late T cells impairs CD4+ T cell lineage maintenance (54). Double knock-out mature mice causes thrombocytopenia by megakaryocytes premature apoptosis (55). However, single knock out of either *Hdac1*or *Hdac2* cannot generate hematopoietc phenotypes (56). *Hdac1* and *Hdac2* have divided immunoglobulin loci recombination effects in B cells (57). They control cell cycle progression by supressing *p21* expression and block in development of B cell (58). HDAC2 expression level was observed with elevation in acute myoloid leukemia (AML) patients' cells (59) with large variability. By using *Lck-Cre* thymocyte-specific knock-out systemm, deletion of *Hdac1* and *Hdac2* alleles showed increased lymphoma frequency in mice (60). SiRNA induced reduction of *Hdac1/2* levels in pre-leukemic stages quickly triggers leukemia onset (61). However, *Hdac2* knock down in p53 absence background did not lead to a higher leukemia incidence in mice (61). In conclusion, HDAC1 and HDAC2 are critical factors for hematopoietic cell development.

HDAC1 and HDAC2 are highly related whilst sharing differences

HDAC1 and HDAC2 were duplication of the same ancient gene (62). They share 85% homologue in DNA sequence, but this identity highly focuses on dimerization and catalytic domains (AA 1-325) with identity of 92% whilst C-terminal shares only 72% identity. The deletion of C-terminal greatly impairs the activity of HDAC1 (63). Apart from structural similarities, HDAC1 and HDAC2 share a lot of binding partners, however, their functions in various biological processes are redundant. Deletion of either HDAC1 or HDAC2 in many cases is not lethal for cells whilst double deletion of both HDAC1 and HDAC2 results in cell death and mitotic collapse (64). In neural system, neuronal precursors' differentiation into neurons requires either HDAC1 or HDAC2, indicating a substitute role of HDAC1/2 (65). Coherent with the observation, consume of either HDAC1 or 2 by RNAi in tumor cells results in upregulation of both HDAC1 and HDAC2 (66). In a wider picture, HDAC1 and HDAC2 are not compensatory in other physiological systems. HDAC1 knock-out mice perished at embryonic phase while HDAC2 knock-out mice survive til perinatal phase. HDAC2 interference by RNAi in Hela cervical cells leads to $p21^{CIP/Waf1}$ expression upregulation (67). But in ostersarcoma U2OS cells, $p21^{CIP/Waf1}$ expression is increased after HDAC1 consume as well (64). Last but not least, HDAC1 rather than HDAC2 controls ES differentiation. Specific consume of HDAC1 leads to reduced activity of CoREST, NuRD and Sin3A complexes (68). HDAC1 and 2 are both regulated by PTMs, while some PTMs happen in catalytic domain, majority of them happen in C-terminal domain. One of the best studied PTMs of HDAC1 and HDAC2 is phosphorylation. In eukaryotes phosphorylation consists of adding PO_4 group to tyrosine, threonine and serine under the catalysis of specific kinases. This process is balanced by dephosphorylation with the catalysis of phosphatases (69). HDAC1 is a phosphorylation substrate of casein kinase II (CKII) and PKA *in vitro* (70). HDAC2 is a phosphorylation substrated of CKII but not PKA (71). CKII phosphorylates HDAC1 on S421 and S423, another phosphorylation site T221 was discovered by MS. CKII phosphorylates HDAC2 on S422 and S424 and S394 (72).

Conclusion

HDAC1 and HDAC2 have broad expression pattern in a wide range of tissues and play important roles in cell cycle and proliferation (73). In many tumor tissues HDAC1 and HDAC2 are disregulated also appearing to be the major deacetylases taken part in aberrant pathways of cancer cells. HDAC1 overexpression has been reported to be existence in hepatocelluar, colorectal, pancreatic, prostatic, and gastric (74-80). Overexpression of HDAC2 is observed already at the polypstage (81). HDACi are scanned for potential anti-tumor therapy and *in vitro* experiments have been commenced showing HDACi indeed have potent in anti-tumor therapy. HDACi introduce cell cycle arrest, angiogenesis blocking, apoptosis and increase of antigenicity to tumor cells (82). Two types of HDACi have been approved by US FDA for T-cell cutaneous lymphoma for treatment (83, 84). At the same time, side effects and restrictions have also been reported (85). The reason for side effects might be there are various isotypes of HDACi, the HDACi subtype used for treatment might not be specific for the target cancer and clinical settings. HDAC1 and 2 are not only related in tumorgenesis but also in other pathological processes, like Huntington disease (86), diabetes (87), COPD and asthma (88).

References

1. Sonenberg N, Hinnebusch AG. *Cell* 2009; 136:731-745.
2. Gingras AC, Raught B, Sonenberg N. Annu Rev Biochem 1999; 68:913-963.
3. Gingras AC, Raught B, Gygi SP, Niedzwieka A, Miron M, Burley SK *et al.* Genes Dev 2001; 15:2852-2864.
4. Di Bacco A, Gill G. Cell Cycle 2006; 5:2310-2313.
5. Geiss-Friedlander R, Melchoir F. Nat Rev Mol Cel Bio 2007; 8:947-956.
6. Gregoire S, Yang XJ. Mol Cell Biol 2005; 25:2273-2287.
7. Zhao X, Sternsdorf T, Bolger TA, Evans RM, Yao TP. Mol Cell Biol 2005; 25:8456-8464.
8. Gao C, Ho CC, Reineke E, Lam M, Cheng X, Stanya *et al.* Mol Cell Biol 2008; 28:5658-5667.
9. Vatsyayan J, Qing G, Xiao G, Hu J. EMBO Rep 2008; 9:885-890.
10. Brandl A, Heinzel T, Krämer OH. Biol Cell 2009; 101:193-225.
11. Krämer OH. Trends Pharmacol Sci 2009; 30:647-655.
12. Weichert W. Cancer Lett 2009; 280:168-176.
13. Witt O, Deubzer HE, Milde T, Oehme I. Cancer Lett 2009; 277; 8-21.
14. Bug G, Ritter M, Wassmann B, Schoch C, Heinzel T, Schwarz K *et al. Cancer* 2005; 104:2717-2725.
15. Munster PN, Marchion D, Thomas S, Egorin M, Minton S, Springett G *et al.* Br J-Cancer 2009; 101:1044-1050.
16. Botrugno OA, Santoro F, Minucci S. Cancer Lett 2009; 280:134-144.
17. Yang XJ, Seto E. Nat Rev Mol Cell Biol 2008; 9:206-218.
18. Bradner JE, West N, Grachan ML, Greenberg EF, Haggarty ST, Warnow T *et al.* Nat Chem Biol 2010;

6:238-243.
19. Minucci S, Pelicci PG. Nat Rev Cancer 2006; 6:38-51.
20. Müller S, Krämer OH. Curr Cancer Drug Targets 2010; 10:210-228.
21. Spange S, Wagner T, Heinzel T, Krämer OH. Int J Biochem Cell Biol 2009; 41:185-198.
22. Buchwald M, Krämer OH, Heinzel T. Cancer Lett 2009; 280:160-167.
23. Mattews GM, Newbold A, Johnstone RW. Adv Cancer Res 2012; 116:165-197.
24. Bradner JE, Mak R, Tanguturi SK, Mazitschek R, Haggarty SJ, Ross K *et al.* Proc Natl Acad Sci USA 2010; 107:12617-12622.
25. Mercurio C, Minucci S, Pelicci PG. Pharmacol Res 2010; 62:18-34.
26. Beckman R, Prives C. Perspect Biol, 2010, 2 a000935.
27. Richon VM, Emiliani S, Verdin E, Webb Y, Breslow R, Rifkind PA *et al.* Marks A. Proc Natl Acad Sci USA 1998; 95:3003-3007.
28. Nakajima H, Kim YB, Terano H, Yoshida M, Horinouchi S. Exp Cell Res 1998; 241:126-133.
29. Göttlicher M, Minucci S, Zhu P, Krämer OH, Schimpf A, Giavara S *et al.* EMBO J 2001; 20:6969-6968.
30. Suzuki T, Ando T, Tsuchiya K, Fukazawa N, Saito A, Mariko Y *et al.* J Med Chem 1999; 42:3001-3003.
31. Hess-Strumpp H, Bracker TU, Henderson D, Politz O. Int J Biochem Cell Biol 2007:39:1388-1405.
32. Candido EP, Reeves R, Davie JR. Cell 1978; 14:105-113.
33. Davie JR. J Nutr 2003; 133:24855-24935.
34. Gray JE, Haura E, Chiappori A, Tanvetyanon T, Williams CC, Pinder-Schenck M *et al.* Clin Cancer Res 2014; 20:1644-1655.
35. Atadja P. Cancer Lett 2009; 280:233-241.
36. Yoshida M, Kijima M, Akita M, Beppu M. J Biol Chem 1990; 265:17174-17179.
37. Vanhaecke T, Papeleu P, Elaut G, Rogiers V. Curr Med Chem 2004; 11:1629-1643.
38. Lu Q, Wang DS, Chen CS, Hu YD, Chen CS. *J Med Chem* 2005; 48:5530-5535.
39. Gu B, Zhu WG. Int J Biol Sci 2012; 8; 672-684.
40. Gottlieb E, Vousden KH. Perspect Biol, 2010, 2, A001040.
41. Rivlin N, Brosh R, Oren M, Rotter V. Genes Cnacer 2011; 2:466-474.
42. Meek DW, Anderson CW. Perspect Biol 2009, 1, A000950.
43. Schneider G, Krämer OH. Biochim Biophys Acta 2011; 1815:90-103.
44. Vousden KH, Prives C. Cell 2009; 137:413-431.
45. Li P, Wang D, Yao H, Doret P, Hao G, Shen Q *et al.* Oncogene 2010; 29:3153-3162.
46. Li DQ, Pakala SB, Reddy SD, Ohshiro K, Peng SH, Lian Y*et al.* J Biol Chem 2010; 285:10044-10052.
47. Kim Y, Park H, Park D, Lee YS, Choe J, Hahn JH *et al.* J Biol Chem *et al.* 2010; 285:25957-25968.
48. Martens JH, Stunnenberg HG. FESB Lett 2010; 584: 2662-2669.
49. Saeed S, Logie C, Stunnenberg HG, Martens JH. Br J Cancer 2011; 104:554-558.
50. Mitelman F, Johansson B, Mertens F. Nat Rev Cancer 2007; 7:233-245.
51. Krämer OH, Stauber RH, Bug G, Hartkamp J, Knauer SK. Leukemia 2013; 27:792-802.
52. Heideman MR, Wilting RH, Yanover E, Velds A, Jong J de, Kerkhoven RM *et al.* Blood 2013; 121:2038-2050.
53. Dovey OM, Foster CT, Conte N, Edwards SA, Edwards JM, Sigh R *et al.* Blood 2013; 121:1335-1344.
54. Boucheron N, Tschismarov R, Goeschl L, Moser MA, Lagger S, Sakaguchi S *et al.* Nat Immunol 2014; 15:439-448.
55. Wilting RH, Yanover E, Heideman MR, Jacobs H, Horner J, van der Torre J *et al.* EMBO J 2010; 29:2586-2597.
56. Kurosawa K, Lin W, Ohta K. J Biochem 2010; 148:201-207.
57. Yamaguchi T, Cubizolles F, Zhang Y, Reichert N, Kohler H, Seiser C *et al.* Genes Dev 2010; 24:455-469.
58. Kurosawa K, Lin W, Ohta K. J Biochem 2010; 148:201-207.
59. Bradbury CA, Khanim FL, Hayden R, Bunce CM, White DA, Dryson MT *et al. Leukemia* 2005; 19:1751-1759.
60. Matthias P. Blood 2013; 121:1930-1931.
61. Santoro F, Botrugno OA, Dal Zuffo R, Pallavicini I, Mattews GM, Cluse L *et al.* Blood 2013; 121:3459-3468.
62. De Ruijter AJM, Van Gennip AH, Caron HN, Kemp S, Van Kuilenburg ABP. Biochem J 2003; 370:737-749.
63. Hassig CA, Fleischer TC, Billin AN, Schreiber SL, Ayer DE. *Cell* 1997; 89:341-347.
64. Montgomery RL, Davis CA, Potthoff MJ, Harberland M, Fielitz J, Qi X *et al.* Genes Dev 2007; 21:1790-1802.
65. Montgometry RL, Hsieh J, Barbosa AC, Richardson JA, Olsen EN. Proc Natal Acad Sci USA 2009; 19:7876-7881.
66. Senese S, Zaragoza K, Minardi S, Muradore I, Ronzoni S, Passafaro A *et al.* Mol Cell Biol 2007; 27:4784-4795.
67. Huang BH, Laban M, Leung CHW, Lee L, Lee CK, Salto-Tellez M *et al.* Cell Death Differ 2005; 12:395-404.
68. Dovey OM, Foster CT, Cowley SM. Proc Natl Acad Sci USA 2010; 107:8242-8247.
69. Deribe YL, Pawson T, Dikic I. Natl Struc Mol Biol 2010; 17:666-672.
70. Cai R, Kwon P, Yan-Neale Y, Sambuccetti L, Fischer D, Cohen D. Biochem Biophy Res Comm 2001; 283:445-453.
71. Rush J, Moritz A, Lee KA, Guo A, Goss VL, Spek EJ *et al.* Nat Biotechnol 2005; 23:94-101.
72. Tsai SC, Seto E. Journal Biol Chem 2002; 277:31826-31833.
73. Lagger G, O'Carroll D, Rembold M, Khier H, Tischler J, Weitzer G *et al.* EMBO J 2002; 21:2672-2681.
74. Choi JH, Kwon HJ, Yoon BI, Kim JH, Han SU, Joo HJ *et al.* Jpn J Cancer Res 2001; 92:1300-1304.
75. Weichert W, Röske A, Gekeler V, Beckers T, Ebert MP, Pross M *et al.* Lancet Oncol, 2008, 139-48, doi: 10.1016/S1470-2045(08)70004-4.
76. Miyake K, Yoshizumi T, Imura S, Suqimoto K, Batmunkh E, Kanemura H 2008; 36:e1-9. doi: 10.1097/MPA.0b013e31815f2c2a.
77. Weicher W, Röske A, Gekeler V, Beckers T, Stephan C, Jung K. Br J Cancer 2008; 12:604-610.
78. Weichert W, Röske A, Niesporek S, Noske A, Buckendahl AC, Dietel M *et al. Clin Cancer Res* 14, 1669-1677.
79. Rikimaru T, Taketomi A, Yamashita Y, Shirabe K, Hamatsu T, Shimada M, Maehara H. Oncology 2007; 72:69-74.
80. Halkodou K, Gaughan L, Cook S, Leung HY, Neal DE, Robson CN. Prostate 2004; 59:177-189.

81. Mariadason JM. Gastroenterology 2008; 135:1457-1459.
82. Marks PA, Rifkind RA, Richon M, Breslow R, Miller T, Kelly WK. Natur Rev Cancer 2001; 1:194-202.
83. Grant S, Easley C, Kirkpatrick P. Natur Rev Drug Discov 2007; 6:21-22.
84. StatBite: FDA oncology drug product approved in 2009. Journal Natl Cancer Insti 2010; 102:219.
85. Botrugno OA, Santoro F, Minucci S. Cancer Lett 2009; 280:134-144.
86. Landles C, Bates GP. EMBO Reports 2004; 5:958-963.
87. Noh H, Eun YO, Seo JY, Yu MR, Kim YQ, Ha H, Lee HB. Am J Physiol Renal Physiol 2009; 297, 729-739.
88. Barnes PJ. Therap Advan Resp Diesea 2009; 3:235-243.