Cell Cycle

ISSN: 1538-4101 (Print) 1551-4005 (Online) Journal homepage: http://www.tandfonline.com/loi/kccy20

Specific and Redundant Functions of Histone Deacetylases in Regulation of Cell Cycle and Apoptosis

Ping Zhu, Eva Maria Huber, Franz Kiefer & Martin Göttlicher

To cite this article: Ping Zhu, Eva Maria Huber, Franz Kiefer & Martin Göttlicher (2004) Specific and Redundant Functions of Histone Deacetylases in Regulation of Cell Cycle and Apoptosis, Cell Cycle, 3:10, 1240-1242, DOI: 10.4161/cc.3.10.1195

To link to this article: http://dx.doi.org/10.4161/cc.3.10.1195

Published online: 12 Aug 2004.

[Cell Cycle 3:10, e115-e117, EPUB Ahead of Print: http://www.landesbioscience.com/journals/cc/abstract.php?id=1195; October 2004]; ©2004 Landes Bioscience

Extra Views

Specific and Redundant Functions of Histone Deacetylases in Regulation of Cell Cycle and Apoptosis

Ping Zhu[1,†]
Evamaria Huber[2]
Franz Kiefer[2]
Martin Göttlicher[2,3,*]

[1]Forschungszentrum Karlsruhe; Institute of Toxicology and Genetics; Eggenstein, Germany

[2]GSF National Research Center for Environment and Health; Institute of Toxicology; Neuherberg, Germany

[3]Technical University Munich; Institute of Toxicology; München, Germany

[†]Present address: Howard Hughes Medical Institute; University of California; San Diego, California USA; 9500 Gilman Drive; CMM-W, Room 345, La Jolla, California 92093

*Correspondence to: Martin Göttlicher;GSF National Research Center for Environment and Health; Institute of Toxicology; Landstrabe 1; Neuherberg D-85764 Germany; Tel.: +49.89.3187.2446; Fax: +49.89.3187.2494; Email: martin.goettlicher@gsf.de

Received 08/20/04; Accepted 08/26/04

This manuscript has been published online, prior to printing for Cell Cycle, Volume 3, Issue 10. Definitive page numbers have not been assigned. The current citation is: Cell Cycle 2004; 3(10):
http://www.landesbioscience.com/journals/cc/abstract.php?id=1195
Once the issue is complete and page numbers have been assigned, the citation will change accordingly.

KEY WORDS

cell cycle, apoptosis, cancer treatment, histone deacetylases, HDAC inhibitors, colon cancer, myeloid leukemia

ACKNOWLEDGEMENTS

The help of Dr. Beisker with flowcytrometric analysis is greatfully acknowledged.

Studies were supported by the Forschungszentrum Karlsruhe (fellowship to P.Z.), the AICR, St. Andrews, UK (grant to M.G.), and the EC in the sixth framework programme through the CASCADE NoE, contract No 506319.

ABSTRACT

Inappropriate control of expression of genetic information is the cause of many forms of cancer. Aberrant transcriptional repression by recruitment of histone deacetylases (HDACs) is a key step in pathogenesis of myeloid leukemia. We recently reported that development of colonic cancer involves alterations in the transcriptional repression machinery by increased expression of HDAC2 upon loss of the APC tumor suppressor. Increased expression of HDAC2 is essential for prevention of apoptosis of HT-29 colonic cancer cells. We now discuss whether HDAC2 also plays a role for aberrant cell cycle regulation and expression of the $p21^{Cip/Waf}$ cell cycle inhibitor. Whereas inhibition of HDACs by valproic acid or trichostatin A increases p21 expression, selective interference with HDAC2 by siRNA transfection or reconstitution of wildtype APC does not affect p21 expression. Likewise, treatment of HT-29 cells with the HDAC inhibitor valproic acid leads to a moderate inhibition of cell cycle progression in the G1 phase whereas interference with HDAC2 expression does not. Thus, HDAC2 appears to serve a preferential role in the prevention of apoptosis and not in cell cycle control similar to the specific importance of HDAC1 for cell cycle regulation or HDAC 9 for the stress response of the heart.

Carcinogenesis is a multistep process during which cells acquire several critical features due to genetic instability and alterations in gene expression.[1] Inappropriate control of gene expression depends in many cases on defects in individual transcription factors or components of signal transduction pathways. Also inappropriate function of more general components of the transcriptional machinery that control gene expression by covalent modifications of chromatin has been described in many cases.[2] The concepts that histone acetylation plays an essential role in carcinogenesis and that inhibitors of histone deacetylases (HDACs) may serve as cancer therapeutic agents had initially been developed from the finding that several forms of acute myeloid leukaemia are caused by expression of HDAC dependent aberrant transcriptional repressors from the fusion points of chromosomal translocations.[3,4] Exposure of cells or treatment of patients with HDAC inhibitors induces cell cycle arrest, apoptosis and differentiation.[3,5] Overcoming transcriptional repression by inhibition of HDACs aberrantly recruited to genes that are required for myeloid differentiation has been considered as one plausible mechanism behind the beneficial effects of HDAC inhibitors. However, not only myeloid leukaemic cells but also many cell lines derived from carcinomas and development of tumors of nonhaematopoietic origin respond to treatment with HDAC inhibitors by initiation of differentiation, cell cycle arrest of apoptosis. In most cases, no reason for aberrant transcriptional repression during pathogenesis of the disease is obvious. Mechanisms of induction of the different cellular fates and conditions that determine the specific response of a certain type of cancer cell are only partially known. It is, in most cases, not clear whether inhibition of HDACs reverts aberrant transcriptional repression that has been acquired during cellular transformation into the tumor cell or whether some cancer cells are for other yet unknown reasons more sensitive to HDAC inhibition than normal cells of the adult organism.

HDACs constitute a family of almost 20 isoenzymes that is divided into three classes;[6] the class I enzymes HDAC1, 2, 3 and 8 are homologs of the yeast RPD3 protein. Class II enzymes HDAC 4, 5, 6, 7, 9 and 10 are homologs of yeast Hda1. HDAC11 shows limited homologies to, both, class I and II enzymes. The third class is constituted by the Sir2 like proteins, SIRTs. Limited knowledge on specific functions of individual HDACs may be derived from studies in knockout mice. HDAC1 appears to serve a role in control of proliferation during embryogenesis[7] and HDAC9 in stress response of the heart.[8] To which extent these and other HDACs also serve redundant functions that can be substituted

for by other HDAC isoenzymes is largely unknown.

Colonic cancer is frequently associated with loss of a functional product of the *adenomatosis polyposis coli* (APC) tumor suppressor gene.[9,10] We recently showed that loss of APC function leads to c-Myc-dependently increased expression of HDAC2 in intestinal epithelial cells and tumors.[11] Overexpression of HDAC2 is specific since other class I enzymes HDAC1 and 3 were found unchanged. The majority of human colonic tumor samples as well as apparently normal mucosa and adenomas in APC deficient mice express elevated levels of HDAC2. This isoenzyme serves a specific role in prevention of apoptosis in colonic cancer cells since interference with elevated HDAC2 expression by siRNA in APC deficient HT-29 colonic cancer cells was sufficient to induce apoptosis. Furthermore, reconstitution of wildtype APC in these cells reduced expression of endogenous HDAC2 and induced apoptosis, the latter of which was prevented by maintaining high levels of HDAC2 expression from a transiently transfected vector. Inhibition of the enzymatic activity of class I HDACs and induction of HDAC2 degradation by treating APC^{min} mice with valproic acid (VPA) reduced number and sizes of intestinal adenomas which spontaneously develop in these mice.

For many cells and tumors exposed to HDAC inhibitors the induction of the $p21^{Cip/Waf}$ CDK inhibitor (CKI) and subsequent cell cycle arrest have been described.[3,12] Whereas HDAC inhibition appears to affect expression of a few percent of the transcriptome in different experimental systems[13] the induction of the p21 CKI appears to be one of the most prominent and most frequently observed effects. We therefore investigated whether HDAC2 plays a critical role in mediating p21 induction. HT-29 colonic cancer cells have been found to express increased levels of p21 mRNA upon HDAC inhibitor treatment in cDNA array analyses.[14,15]

We confirmed induction of p21 protein in HT-29 cells (Fig. 1A) by using valproic acid (VPA) as and inhibitor of HDACs which preferentially inhibits class I enzymes.[16,17] Since HDAC2 but not other isoenzymes such as HDAC1 or 3 are under negative control of the APC tumor suppressor[11] we tested regulation of p21 expression in APC-deficient HT29 cells in which expression of wildtype APC can be expressed by Zn^{2+}, HT29-APC cells.[18] Zn^{2+} exposure only poorly induced p21 expression in the HT29-APC and not in a control cell line which expressed β-galactosidase instead of APC (data not shown). In both transgenic cell lines exposure to an HDAC inhibitor induced p21 expression to a similar extent as in HT29 wildtype cells (data not shown). To test a specific role of HDAC2 we analyzed p21 protein expression upon knockdown of HDAC2 expression in HT-29 cells by siRNA transfection. Knockdown of HDAC2 was efficient and specific since other class I enzymes HDACs 1 and 3 were not affected (Fig. 1b). Expression of p21 was not substantially affected upon HDAC2 knockdown and induction by at most 50% was poor compared to the induction upon inhibition of HDACs by VPA.

Induction of p21 expression by the various manipulations in HT-29 cells lead only to moderate effects on cell cycle progression. A moderate decrease in proliferation was seen in wildtype HT-29 cells that had been treated for 48 hours with 1 mM VPA. The percentage of cells in the G_1 phase of the cell cycle compared to all nonapoptotic cells within the cell cycle increased moderately from 68% to 77% indicating a subtle delay in G_1 to S progression. Induction of wildtype APC in HT29-APC cells which lead to reduced expression of HDAC2 was rather associated with increased numbers of cells in S and G_2 phases of the cell cycle. This suggests that certainly under conditions of APC reconstitution and reduced expression of

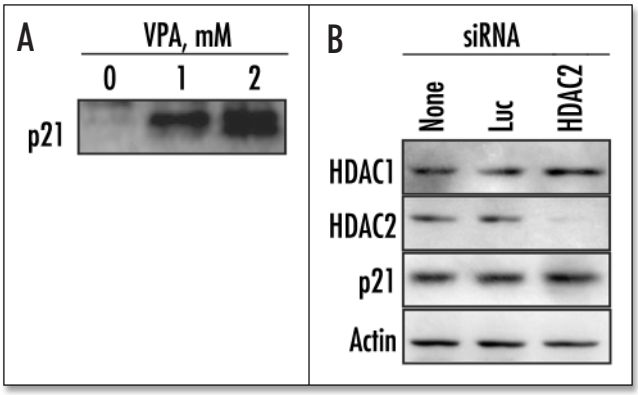

Figure 1. Expression of the $p21^{Cip1/Waf}$ cell cycle inhibitor in HT-29 cells upon modulation of HDAC activity and expression. (A) HT-29 cells were treated for 48 h with 1 or 2 mM of the HDAC inhibitor VPA. Expression of the p21 protein was determined by Westernblot analysis and equal loading of lanes was ensured by protein staining of a part of the gel. (B) Expression of HDAC2 was knocked down in HT-29 cells by transfection of an siRNA directed against HDAC2 or a nonrelated mRNA (Luc). Whole cell lysates were prepared and used for Western blot analysis after 48 h. Antibodies were from Santa Cruz Biotech: HDAC1 (sc-6298), HDAC2 (sc-9959), p21 (sc-471), and Actin (sc-1616).

HDAC2 cell cycle arrest is not the predominant response. We had described previously that knock-down of HDAC2 had no substantial effect on cell cycle progression 48 h after transfection of an siRNA directed against HDAC2.[11] At 72 h after siRNA transfection percentages of cells in G_1 were essentially unchanged with 72% or 71% in cultures transfected with a nonrelated (Luc) or HDAC2-specific siRNA. Taken together these data suggest that reduction of HDAC2 expression is not associated with increased expression of the p21 CKI or any signs of a G_1 delay in cell cycle progression. This is in contrast to the response observed upon interference with several HDACs by the use of, at best, moderately specific chemical inhibitors.

These findings show specific differences between cell cycle regulation and apoptosis with respect to dependency on individual HDAC isoenzymes. Whereas HDAC2 appears to play a limiting role in the prevention of apoptosis in colonic cancer cells this seems not to be the case for cell cycle arrest and induction of p21. Likewise, HDAC1 has been found to play a limiting role in repressing CKI expression during embryogenesis.[7] Whether HDAC2 also contributes to repression of CKI expression at least in some cell types can't be excluded. HDAC2, however, would not suffice to compensate for the lack of HDAC1 in HDAC1-deficient mouse embryos which die early presumably due to a proliferation defect. In tumor cells like HT-29 HDAC2 appears to be required at elevated expression levels to prevent apoptosis but not to permit uncontrolled cell cycle progression.

These and other studies in genetically defined model systems allow dissection of specific roles of individual HDACs. A long-term goal will be to develop HDAC inhibitory drugs that selectively interfere with those HDACs that according to evidences from the genetically engineered experimental systems can be expected to play critical roles in the development of cancer and might be particularly relevant targets for treatment strategies.

References

1. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000; 100:57-70.
2. Berger SL. Histone modifications in transcriptional regulation. Curr Opin Genet Dev 2002; 12:142-8.

3. Krämer OH, Göttlicher M, Heinzel T. Histone deacetylase as a therapeutic target. Trends Endocrinol Metab 2001; 12:294-300.
4. Melnick A, Licht JD. Histone deacetylases as therapeutic targets in hematologic malignancies. Curr Opin Hematol 2002; 9:322-32.
5. 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.
6. Verdin E, Dequiedt F, Kasler HG. Class II histone deacetylases: Versatile regulators. Trends Genet 2003; 19:286-93.
7. Lagger G, O'Carroll D, Rembold M, Khier H, Tischler J, Weitzer G, Schuettengruber B, Hauser C, Brunmeir R, Jenuwein T, Seiser C. Essential function of histone deacetylase 1 in proliferation control and CDK inhibitor repression. EMBO J 2002; 21:2672-81.
8. Zhang CL, McKinsey TA, Chang S, Antos CL, Hill JA, Olson EN. Class II histone deacetylases act as signal-responsive repressors of cardiac hypertrophy. Cell 2002; 110:479-88.
9. Fodde R. The multiple functions of tumour suppressors: It's all in APC. Nat Cell Biol 2003; 5:190-2.
10. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. Cell 1996; 87:159-70.
11. Zhu P, Martin E, Mengwasser J, Schlag P, Janssen KP, Göttlicher M. Induction of HDAC2 expression upon loss of APC in colorectal tumorigenesis. Cancer Cell 2004; 5:455-63.
12. Marks PA, Miller T, Richon VM. Histone deacetylases. Curr Opin Pharmacol 2003; 3:344-51.
13. Van Lint C, Emiliani S, Verdin E. The expression of a small fraction of cellular genes is changed in response to histone hyperacetylation. Gene Expr 1996; 5:245-53.
14. Archer SY, Meng S, Shei A, Hodin RA. $p21^{WAF1}$ is required for butyrate-mediated growth inhibition of human colon cancer cells. Proc Natl Acad Sci USA 1998; 95:6791-6.
15. Della Ragione F, Criniti V, Della Pietra V, Borriello A, Oliva A, Indaco S, Yamamoto T, Zappia V. Genes modulated by histone acetylation as new effectors of butyrate activity. FEBS Lett 2001; 499:199-204.
16. Gottlicher M, Minucci S, Zhu P, Kramer OH, Schimpf A, Giavara S, Sleeman JP, Lo Coco F, Nervi C, Pelicci PG, Heinzel T. Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. EMBO J 2001; 20:6969-78.
17. Phiel CJ, Zhang F, Huang EY, Guenthor MG, Lazar MH, Klein PS. Histone deacetylase is a direct target of of valproic acide, a potent anticonvulsant, mood stabilizer and teratogen. J Biol Chem 2001; 276:36734-41.
18. Morin PJ, Vogelstein B, Kinzler KW. Apoptosis and APC in colorectal tumorigenesis. Proc Natl Acad Sci USA 1996; 93:7950-4.