Histone deacetylation is directly involved in desilencing the expression of the catalytic subunit of telomerase in normal lung fibroblast

N.K. Mukhopadhyay ^{a,b}, G.J. Gordon ^a, G. Maulik ^c, G. Doerre ^a, Brian C.S. Liu ^b, R. Bueno ^a, D.J. Sugarbaker ^a, M.T. Jaklitsch ^{a*}

^a Division of Thoracic Surgery, Brigham and Women's Hospital, Harvard Medical School, Massachusetts, USA

^b Division of Surgery/Urology, Brigham and Women's Hospital, Harvard Medical School, Massachusetts, USA

^c Division of Adult Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts, USA

Received: June 9, 2005; Accepted: August 17, 2005

Abstract

The regulation of telomerase expression in normal cells is poorly understood. Moreover, the molecular mechanism underlying tumor-specific expression of telomerase remains unclear. We investigated the link between histone deacetylation and telomerase activity in normal lung and lung tumor cells. Four non-small-cell lung cancer (NSCLC) lines and one normal lung fibroblast line were tested for telomerase activity with or without Trichostatin A (TSA). The telomerase activity and the expression of telomerase associated components were determined by TRAP assay, RT-PCR analysis and Western blot analysis. All 4 NSCLC cell lines exposed to 1 μ M TSA for 24h had no change in telomerase activity or hTERT mRNA level. Telomerase activity was very low in normal lung fibroblasts (mrc-9) until exposed to 1 μ M TSA for 24h, at which time telomerase activity was readily detectable, with concomitant upregulation of hTERT mRNA (10-fold). The level of other telomerase associated components (hTER and TP1) were unaltered. Furthermore, 1 μ M TSA exposure for 24h did not alter the level of c-Myc or p21 mRNA. Immunodetection revealed that hTERT protein expression increased (~6 fold) compared to c-Myc, p21, or gelsolin. The effect of TSA on hTERT expression is independent of DNA methylation as judged by 5-azacytidine (5aza) treatment. TSA effect on mrc-9 cells is unaltered even in the presence of 200 μ g/ml cyclohexamide, suggesting a direct inhibition of histone deacetylation. Collectively, our study indicates that inhibition of histone deacetylation selectively regulates the transcriptional derepression of telomerase catalytic subunit in normal lung fibroblast cells compared to lung tumor cells.

Keywords: histone deacetylation • h-TERT • gene silencing • telomerase

Introduction

Telomerase integrity is required for the chromosomal replication and stability [1]. Telomeric

sequences are progressively lost with cell division due to the inability of DNA polymerase to completely replicate the ends of chromosome. One mechanism to overcome this loss is the activation of telomerase, an RNA dependent DNA polymerase that directs the *de novo* synthesis of telomeric repeats at chromosome end [1–2]. Telomerase is active during embryonic development, in adult

* Correspondence to: Nishit K. MUKHOPADHYAY, Division of Thoracic Surgery, Brigham and Women's Hospital, 75 Francis Street, Boston MA 02115, USA. Tel.: 617-732-5690 Fax: 617-730-2898 E-mail: mtjaklitsch@partners.org

germline tissues, and essentially in all malignancies but not in most normal somatic cells indicating that telomerase function is required for tumor growth [3–4]. Recent studies also reported that telomerase activity is also present in highly proliferative somatic cell types, such as activated lymphocytes [5].

Human telomerase core enzyme consists of two essential elements: the RNA subunit (hTER), which acts as a template for addition of new telomeric repeats [6], and the catalytic subunit (hTERT) with reverse transcriptase activity [4, 7]. A telomerase associated protein called TP1 has also been recognized in most of the tissues but its function remains unclear [8]. Many studies have found that hTERT is preferentially expressed in malignant tumor, but not expressed in normal tissues. It has also been found that the expression of hTERT parallels that of telomerase activity [9–11]. Furthermore, telomerase activity in telomerase negative cells can be restored by ectopic expression of hTERT [12–13]. These findings suggest that hTERT is a rate-limiting determinant of telomerase enzyme activity.

Modifications of chromatin structure are of fundamental importance in gene regulation and histone acetyltransferase (HAT) and deacetylase (HDAC) are known to interact with components of the transcription machinery, causing promoter specific alteration of chromatin. Recently it was demonstrated that several transcription factors, such as Mad, can repress transcription by recruiting HDACs to a specific promoter [14–16]. In addition, HDAC₁ has been shown to mediate transcriptional repression *via* a Sp1 binding site [17]. Given that the core promoter of hTERT contains E boxes that bind MAD as well as multiple Sp1 sites, the possibility is suggested that histone acetylation is involved in transcriptional regulation of hTERT. Recent reports indicated that telomerase activity of some normal cells is activated by histone deacetylase inhibitor treatment [18]. Moreover, suppression of hTER expression by treating with sodium butyrate (NaB) and TSA has also been reported in prostate Cancer Cells [19].

The expression and regulation of telomerase expression in normal cells is still one of the least well-understood issues in telomerase research today. Recently, we observed that normal lung fibroblast cells compared to lung tumor cells are fairly resistant to TSA, demonstrating a therapeutic

window [20]. Here, we show that TSA can induce hTERT expression in normal lung cells compared to NSCLC cells and expression of hTERT is directly correlated to histone acetylation in normal cells.

Materials and methods

Cell culture

The human non-small-cell lung cancer lines used in these studies (Calu-1, NCI-520, NCI-H23 and NCI-H441) are available through American Type Culture Collection (ATCC) (www.atcc.org). The normal lung fibroblast line mrc-9 was a kind gift from Dr. LanBo Chen of the Dana Farber Cancer Institute (Boston, MA), but is also available from ATCC. All the above cell lines were grown in either Dulbecco's Modified Eagle's Medium (DMEM) or RPMI supplemented with 10% heat-inactivated fetal bovine serum as described previously [21, 22]. Cells were passaged for not more than eight generations and then replaced from frozen stocks of earlier passaged cells.

TRAP assay

Telomerase repeat amplification protocol (TRAP) assay was performed using a TRAPeze[®] telomerase Detection Kit (Integrin Company) according to the manufacturer's protocol with some modifications. Briefly, a master mix containing all of the reagents outlined below was made for a final volume of 50 μ l. The reagents included 5 μ l of 10x TRAP buffer, 1 μ l of TS primer, 1 μ l of TRAP primer mix, 1 μ l of 50x dNTP mix, 1 μ l of Taq polymerase (5 units/ μ l) and RNAase and DNAase free distilled water to a final volume of 48 μ l. 2 μ l of cell extracts in CHAPS lysis buffer (2 μ g/2 l) was added to each tube containing the reagent mix. 2 μ l of CHAPS lysis buffer and 1 μ l of TSR8 (supplied with TRAP kit) were used as negative and positive control respectively. All the tubes were incubated at room temperature for 30 minutes before the PCR was performed with the two steps PCR protocols (30 seconds at 94°C and 30 seconds at 59°C) for 30 cycles. The PCR products (one half) were electrophoresed on a 10% polyacrylamide gel and visualized with SYBR[®] Green I Nucleic Acid Gel Stain (Molecular Probes, Inc. Eugene, OR) as described in the manufacturer's protocol. The stained bands were visualized and

photographed using a UV light source (300nm transilluminator) with a Gold gel stain photographic filter (S-7569). We also used the internal standards to compare the relative telomerase activity.

RT-PCR analysis

Total RNA was extracted from all the cell lines with Trizol (Life Technologies, Inc) according to the manufacturer's protocol and incubated with Dnase I prior to c-DNA synthesis. Briefly, a 80µl reaction containing 2µg of total RNA, 16µl of 10mM MgCl₂, 8µl of 10x RT-PCR buffer, 32µl of 2.5mM dNTP mix, and 1µl each of RNase inhibitor, reverse transcriptase (all from Taq-Man RT kit) and random hexamer (Invitrogen) was subjected to PCR reaction (10 min. at 25°C, 30 min. at 48°C and 5 min. at 95°C). One fourth of the RT-PCR product was subjected to PCR amplification with the primers (5'-GTTTGCTC-TAGAATGAACGGTGAAG-3' and 5'-TCTAACCC-TAACTGAGAAGGGCGTAG-3' for hTR; 5'-CGGAA-GAGTGTCTGGAGCAA-3' and 5'-GGAT-GAAGGGGAGTCTGGA-3' for hTERT; 5'-TCAAGC-CAAACCTGAATCTGA-3' and 5'-CCCCGAGT-GAATCTTTCTACGC-3' for TP1; 5'-TCATCAC-CATTGGCAATGAG-3' and 5'-CACTGTGTTGGCG-TACAGGT-3' for β-actin; 5'-CCTCTTCGGCCCGGTG-GAC-3' and 5'-CCGTTTTTCGACCCTGAGAG-3' for p21; 5'-AAGTCCTGCGCCTGCGCCTCGCAA-3' and 5'-GCTGTGGCCTCCAGCAGA-3' for c-Myc; 5'-CAGCAGCAGTAGTGCTCATAGCTC-3' and 5'-GCCATATGGCTGGAAAGGTAGG -3' for gelsolin) for the various telomerase subunits (hTR, hTERT, TP1), β-Actin, p21.c-myc and gelsolin following the protocols: 94°C for 2 min (one cycle), 94°C for 45 min, 60°C for 1 min. 72°C for one and half minutes (30 cycles) and 72°C for 7 min (one cycle). The PCR products were electrophoresed in 2% agarose gel and visualized with SYBR Green as described before.

Western blot analysis

Immunodetection of hTERT, p21 (Transduction Laboratories), c-Myc (Oncogene Science) and gelsolin protein were performed as follows: Whole cell extracts (80µg protein) of TSA treated or untreated mrc-9 cells were resolved by 10% SDS polyacrylamide gel electrophoresis and the proteins were Western transferred to a nitrocellulose membrane. The membranes were first

probed with primary antibody against specific protein followed by a horseradish peroxidase conjugated 2nd-ary antibody. Following extensive washing, immunoreactive bands were visualized by ECL (ECL reagent, New England Nuclear). For the detection of acetylation of histone H4, the TSA and 5-aza treated and untreated cells were lysed directly in six well plates and separated the protein in a 15% SDS-PAGE.

Statistical analysis

Quantitation of the Western blots was accompanied by using a densitometric-based analysis performed on scanned fluorograms using Scion Image Beta 4.02 software from Scion Corporation (Frederick, Maryland, info@scioncorp.com).

Results

Telomerase activity in TSA treated tumor cells

To investigate whether histone deacetylation is involved in the activation or inhibition of telomerase activity in tumor cells, all the telomerase positive NSCLC lines were treated for 24h with 1µM TSA, a specific and stable inhibitor of histone deacetylation. As indicated in Fig. 1A, TSA was unable to influence any telomerase activity in NSCLC cells and all four NSCLC lines showed detectable amount of basal telomerase activity.

The levels of hTERT mRNA were measured in all untreated and TSA treated NSCLC cells by RT-PCR analysis (Fig. 1B). The hTERT mRNA was readily detectable in all tumor cell lines tested and did not alter after TSA treatment. It is likely that in the lung tumor cells the maximum level of transcriptional activity for the hTERT gene has already been reached. β-actin mRNA level was also unaltered which was used as a loading control.

TSA induced telomerase activity in normal lung cells

Telomerase activity was either very low or undetectable in normal lung cell line mrc-9. But when

Fig. 1 Telomerase activity and expression of hTERT in NSCLC cells. A. TSA treated (+) and DMSO (vehicle) treated (-) NSCLC cells were subjected to TRAP assay using 2 μ g of DNA in CHAPS lysis buffer as described in Material and Methods. Telomerase activity was visualized by SYBER-Green staining of DNA ladders. Internal standard was indicated by arrow. B. Expression of hTERT was detected by RT-PCR analysis of RNA treated (+) and untreated (-) cells using specific primers as discussed before. β -actin was detected as loading control.

these cells were treated with 1 μ M of TSA for 24h, telomerase activity was readily detectable (Fig. 2A). As indicated in Fig. 2B, the hTERT mRNA was undetectable in mrc-9 cells but treatment with TSA upregulated the h-TERT mRNA (10 fold) in this cell line (indicated by arrows). The level of other telomerase associated components namely hTER, the RNA subunit of telomerase; TP1, the telomerase associated protein, and β -actin, the loading control, were unaltered (Fig. 2B). Our results indicated that inhibiting deacetylase activity by TSA can reverse telomerase repression in normal lung cells and hTERT was the discriminatory factor of telomerase activity in mrc-9 cells.

Enhancement of telomerase activity in normal cells is specific to hTERT expression

To determine whether the upregulation of telomerase activity after TSA treatment of normal cells is due to the induced expression of hTERT, we analyzed the expression of mRNA and protein of some components that can modulate telomerase activity. We investigated whether TSA induced hTERT expression was mediated through modification of p21 and c-Myc which has been reported to regulate hTERT transcription and telomerase activity. Treatment of 1 μ M TSA for 24h did not alter significantly the level of c-Myc or p21 mRNA expression as compared to hTERT mRNA (10 fold; Fig 3A). The expression of gelsolin

Fig. 2 Telomerase activity and expression of telomerase associated components in mrc-9 cells. A telomerase activity of normal lung fibroblast cells after 1 μ M of TSA treatment for 24h. Control indicates the assay with CHAPS lysis buffer alone. Telomerase activity was visualized after TSA treatment (+) but not with DMSO treatment (-). B RT-PCR analysis of telomerase components in DMSO (-) and TSA (+) treated cells using specific primers as discussed in Materials and Methods.

Fig. 3 RT-PCR and Western blot analysis of telomerase regulatory components. MRC-9 cells were treated with either DMSO or 1 μ M of TSA for 24h and either the expression of RNA or expression of proteins were detected. A RNA (2 μ g) from TSA treated (+) or DMSO treated (-) cells were subjected to RT-PCR analysis with specific primers as described in Materials and Methods. β -actin was used as loading control. B Expression of proteins was determined by immunoblotting with specific antibodies after Western transfer of 80 μ g of whole cell lysate following 1 μ M TSA treatment for 24h.

mRNA, a frequently activated molecule after TSA treatment and a marker of apoptosis and the housekeeping gene β -actin were also unaltered. Immunodetection analysis of proteins also revealed that hTERT protein expression altered significantly (~6 fold) compared to c-Myc (no detectable band), p21 (~2.5 fold), or gelsolin (no change, Fig. 3B). Longer exposure of hTERT blot shows a band at control cells (data not shown).

Effect of TSA on hTERT expression is due to histone acetylation not DNA methylation.

Like TSA, the DNA methylated agent 5-azacytidine (5aza) can affect expression of many genes. To determine what effects 5aza had on hTERT expression either alone or in combination with TSA, we treated the normal lung fibroblast cells with 5 μ M of 5aza and 1 μ M of TSA for different time intervals. As indicated in the Fig. 4, 72h treatment of 5 μ M 5aza alone did not induce any hTERT or gelsolin RNA expression. When mrc-9 cells were treated with 5aza plus TSA for different time periods, the expression of gelsolin was still unaltered but it did alter the level of hTERT mRNA significantly. The hyperacetylation of histone H4 protein was also detected following TSA treatment during that period. Treatment with 5aza in combination with TSA did not change significantly the level of acetylated H4. The above results suggest strong specificity of hTERT expression related to TSA treatment.

Fig. 4 Effect of 5aza alone or in combination with TSA on RNA and protein expression in normal lung fibroblast. Mrc-9 cells were treated with 5 μ M of 5aza for 72h with a fresh addition after 48h. When needed, TSA (1 μ M) was added to the culture medium either for 24h or 48h. A RT-PCR analysis of gelsolin, h-TERT and β -actin with specific primers as described before. Positive (+) sign indicates the presence of 5 μ M of 5aza for 72h. Numbers above each lane indicate the duration (h) of TSA treatment. B. Detection of acetylated H4 protein expression with and without TSA and 5aza treatment. In practice, the treated and untreated cells were lysed in triple detergent lysis buffer and the whole cell extract (80 μ g protein) were transferred to a 15% SDS-PAGE followed by Western blotting with acetylated H4 antibody.

Fig. 5 TSA induced hTERT activation is a direct effect. Mrc-9 cells were incubated with 1 mM TSA overnight in the absence (-) or presence (+) of 200 μ M of cyclohexamide (CHX). RNA (4 μ g) from treated or untreated cells was subjected to RT-PCR analysis for hTERT and β -actin message as discussed earlier in Methods and Materials.

Effect of protein synthesis inhibitor on TSA induced hTERT expression

Having demonstrated that TSA induced hTERT expression in normal lung fibroblast cells is fairly specific to histone acetylation, we sought to strengthen the findings to ask whether the activation of hTERT in this cells is a direct effect of TSA treatment. Mrc-9 cells were incubated with 1 μ M of TSA in the presence or absence of a potent protein synthesis inhibitor, cyclohexamide (CHX), at a concentrations of 200 μ g/ml. Analysis of hTERT mRNA revealed that the presence of the protein synthesis inhibitor did not affect the level of mRNA synthesis during that periods. The rapid induction of hTERT mRNA in presence of TSA is independent of new protein synthesis as indicated in the Fig. 5. The level of hTERT mRNA rather increased slightly in presence of CHX. These findings clearly demonstrated that the TSA induced activation of hTERT gene is a direct effect of inhibition of histone deacetylation by TSA.

Discussion

The present study demonstrated clearly that a histone deacetylase inhibitor, TSA, activates telom-

erase activity in normal lung fibroblast cells through the induction of telomerase catalytic subunit, hTERT. But when telomerase positive NSCLC cells were examined by TSA, a nonsignificant increase in telomerase activity was observed suggesting that action of TSA is specific to normal cells. In contrary to these observations, a recent report indicated that hTERT expression and telomerase activity are decreased in prostate cancer cells after TSA treatment [19]. Moreover, the increase in hTERT expression following TSA treatment has also been reported in other cancer cell lines including Ha1-IM, SiHa and Hela cells [23–24]. These findings suggest that telomerase repression by inhibiting deacetylase activity and deacetylation dependent degree of repression may persist in immortal tumor cells. The level of hTERT mRNA in all TSA treated NSCLC cells are unaltered as determined by RT-PCR analysis suggesting that either TSA is ineffective in NSCLC cells to induce hTERT activity or level of hTERT message are already in saturated level beyond the effect of TSA.

On the other hand, TSA up-regulates the hTERT mRNA and telomerase activity in mrc-9 cells although this observation can not rule out the possibility of the enhance stability of hTERT mRNA by TSA treatment. We have also shown that hTERT is the discriminatory factor for telomerase enzyme activity in mrc-9 cells.

Increase in p21 mRNA and decrease in c-Myc mRNA have been reported following HDACI treatment [25]. Over expression of p21 suppresses hTERT expression and subsequent telomerase activity in various cancer cells including breast [26] and head and neck tumors [27]. On the other hand, hTERT promoter contains numerous MYC binding regions and presence of MYC protein is an important factor in activating telomerase through hTERT transcription in neoplasm including prostate cancer [19, 28–29]. Considering the results of our unpublished observations and this report, we can argue that overexpression of p21 in NSCLC cells by TSA have no influence on telomerase activity and independent of hTERT mRNA induction. Moreover, our RT-PCR result and immunoblot analysis revealed that the expression of p21 or c-Myc mRNA or protein were not altered significantly when hTERT mRNA and protein expression were increased. It is also evident from our results that unaltered level of gelsolin in mrc-9 cells after TSA treatment may not

be related to the upregulation of hTERT or telomerase activity.

Acetylation/deacetylation process is not the only mechanism of up and down regulations of genes. Changes in methylation status are associated with the altered expression of both oncogenes and tumor suppressor genes [28]. Recent reports have provided strong correlation between DNA methylation and histone deacetylation [31]. Recently, two groups provided a substantive clue that a methyl CpG binding protein MeCP2, forms a complex with histone deacetylases (HDAC) that alters chromatin architecture and gene regulation [32–33]. It was also reported that TSA acts synergistically with 5aza-2'-deoxycytidine (DAC) to restore mRNA expression of several methylated tumor suppressor genes. Moreover, in addition to the functional link noted between histone deacetylation and DNA methylation, several reports recently indicated a direct physical link with major DNA methyl transferase enzyme, Dnmt 1 [34]. On the basis of our experimental results we can say that in normal lung fibroblast line mrc-9, we did not see any effect of DNA methylation on TSA induced activation of hTERT mRNA or protein and also 5aza did not have any antiproliferative effect on mrc-9 cells (data not shown). Our data also suggests that DNA methylation does not have any influence on histone acetylation status of histone H4 in mrc-9 cells.

In summary, our data indicates that hTERT is the discriminatory factor of telomerase activity in normal lung cells compared to NSCLC cells and the induction of hTERT expression in this cell line is directly correlated to histone acetylation. Whether the induction of hTERT by TSA is cell cycle regulated or whether it is regulated by any other proteins other than p21 or c-Myc are currently under investigation.

Acknowledgements

The authors would like to thank Debra H Cole and Amy YM Wong for secretarial support and Angeline S. Ferdinand for critical reading. This work was partially supported by grants from the Lowe Center for Thoracic Oncology of the Dana-Farber Cancer Institute and the Brigham and Women's Physician Organization

References

1. **Harley CB, Futcher AB, Greider CW.** Telomeres shorten during ageing of human fibroblast. *Nature* 1990; 345: 458–60.
2. **Greider CW.** Telomere length regulation. *Annu Rev Biochem.* 1996; 65: 337–65.
3. **Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, Coviello GM, Wright WE, Weinrich SL, Shay JW.** Specific association of human telomerase activity with immortal cells and cancer. *Science* 1994; 266: 2011–5.
4. **Oulton R, Harrington L.** Telomeres, telomerase, and cancer: life on the edge of genomic stability. *Curr Opin Oncology* 2000; 12: 74–81.
5. **Hyman K, Hirai Y, Kyoizumi S, Akiyama M, Hiyama E, Piatyszek MA, et al.** Activation of telomerase in human lymphocytes and hematopoietic progenitor cells. *J Immunol.* 1995; 155: 3711–5.
6. **Lingner J, Cech TR.** Telomerase and chromosome end maintenance. *Curr Opin Genet Dev.* 1998; 8: 226–32.
7. **Liu JP.** Studies of the molecular mechanisms in the regulation of telomerase activity. *FASEB J.* 1999; 13: 2091–100.
8. **Harrington L, McPhail T, Mar V, Zhou W, Oulton R, Bass MB., Arruda I, Robinson MO.** A mammalian telomerase-associated protein. *Science* 1997; 275: 973–7.
9. **Meyerson M, Counter CM, Eaton EN, Ellisen LW, Steiner P, Caddle SD, Ziaugra L, Beijersbergen RL, Davidoff MJ, Liu Q, Bacchelti S, Haber DA, Weinberg RA.** hEST2, the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization. *Cell* 1997; 90: 785–95.
10. **Nakamura TM, Morin GB, Chapman KB, Weinrich SL, Andrews WH, Lingner J, Harley CB, Cech TR.** Telomerase catalytic subunit homologs from fission yeast and human. *Science* 1997; 277: 955–9.
11. **Takakura M, Kyo S, Kanaya T, Tanaka M, Inoue M.** Expression of human telomerase subunits and correlation with telomerase activity in cervical cancer. *Cancer Res.* 1998; 58: 1558–61.
12. **Bodnar AG, Ouellette M, Frolkis M, Holt SE., Chiu CP, Morin GB, Harley CB, Shay JW, Lichtsteiner S, Wright WE.** Extension of life-span by introduction of telomerase into normal human cells. *Science* 1998; 279: 349–52.
13. **Hahn WC, Stewart SA, Brooks MW, York SG, Eaton E, Kurachi A, Beijersbergen RL, Knoll JH, Meyerson, M, Weinberg RA.** Inhibition of telomerase limits the growth of human cancer cells. *Nat Med.* 1999; 10: 1164–70.
14. **Hassig CA, Fleischer TC, Billin AN, Schreiber SL, Ayer DE.** Histone deacetylase activity is required for full transcriptional repression by mSin3A. *Cell* 1997; 89: 341–7.
15. **Heinzer T, Lavinsky RM, Mullen TM, Sonderstrom M, Laherty CD, Torchia J, Yang WM, Brard G, Ngo D, Davie JR, et al.** A complex containing N-CoR, msin3 and histone deacetylase mediates transcriptional repression. *Nature* 1997; 387: 43–8.

16. **Laherty CC, Yang WM, Sun JM, Davie JR, Seto E, Eisenman RN.** Histone deacetylases associated with the mSin3 corepressor mediate Mad transcriptional repressor. *Cell* 1997; 89: 349–56.
17. **Doetzlhofer A, Rotheneder H, Lagner G, Koranda M, Kurtev V, Brosch G, Wintersberger ER, Seiser C.** Histone deacetylase 1 can repress transcription by binding to Sp1. *Mol Cell Biol.* 1999; 19: 5504–11.
18. **Takakura M, Kyo S, Sowa Y, Wang Z, Yatabe N, Maida Y, Tanaka M, Inoue M.** Telomerase activation by histone deacetylase inhibitor in normal cells. *Nucleic Acids Res.* 2001; 29: 3006–11.
19. **Suenaga M, Soda H, Oka M, Yamaguchi A, Nakatomi K, Shiozawa K, Kawabata S, Kasai T, Yamada Y, Kamihira S, Chuwa T, Kohno S.** Histone deacetylase inhibitors suppress telomerase reverse transcriptase mRNA expression in prostate cancer cells. *Cancer Cell Biol.* 2002; 97: 621–5.
20. **Mukhopadhyay NK, Weisberg E, Gilchrist D, Bueno R, Sugarbaker DJ, Jaklitsch MT.** Effectiveness of Trichostatin A as a potential candidate for anticancer therapy in non-small-cell lung cancer. *Ann Thorac Surgery* 2005.
21. **Mukhopadhyay NK, Gilchrist D, Gordon GJ, Chen CJ, Bueno R, Lu ML, Salgia R, Sugarbaker DJ, Jaklitsch MT.** Integrin dependent protein tyrosine phosphorylation is a key regulatory event in collagen IV mediated adhesion and proliferation of human lung cell line, Calu-1. *Ann Thorac Surgery* 2004; 78: 450–7.
22. **Mukhopadhyay NK, Gordon GJ, Chen CJ, Bueno R, Sugarbaker DJ, Jaklitsch MT.** Activation of Focal adhesion kinase in human lung cancer cells involves multiple and potentially parallel signaling events. *J Cell Mol Med.* 2005; 9: 387–97.
23. **Cong Y-S, Bacchetti S.** Histone deacetylation is involved in the transcriptional repression of hTERT in normal human cells. *J Biol Chem.* 2000; 275: 35665–8.
24. **Hou M, Wang XB, Popov N, Zhang A, Zhao X, Zhou R, Zetterberg A, Bjorkholm M, Henriksson M, Gruber A, Xu D.** The histone deacetylase inhibitor Trichostatin A derepresses the telomerase reverse transcriptase (hTERT) gene in human cells. *Exp Cell Res.* 2002; 274: 25–34.
25. **Weidle UH, Grossmann A.** Inhibition of histone deacetylases: a new strategy to target epigenetic modifications for anticancer treatment. *Anticancer Res.* 2000; 20: 1471–86.
26. **Wang Z, Kyo S, Takakura M, et al.** Progesterone regulates human telomerase reverse transcriptase gene expression via activation of mitogen-activated protein kinase signaling pathway. *Cancer Res.* 2000; 60: 5367–81.
27. **Henderson YC, Breau RL, Liu T-J, Clayman GL.** Telomerase activity in head and neck tumors after introduction of wild-type p53, p21, p16, and E2F-1 genes by means of recombinant adenovirus. *Head Neck* 2000; 22: 347–54.
28. **Kyo S, Takamura M, Kanaya T, Taira T, Kanaya T, Itoh H, Yutsudo M, Ariga H, Inoue M.** Sp1 cooperates with c-Myc to activate transcription of human telomerase reverse transcriptase (hTERT) gene. *Nucleic Acids Res.* 2000; 28: 669–77.
29. **Wu KJ, Grandori C, Amacker M, Simon-Vermont N, Polack A, Lingner J, Dalla-Favera R.** Direct activation of TERT transcription by c-MYC. *Nat Genet.* 1999; 21: 220–4.
30. **Mielnicki LM, Ying AM, Head KL, Asch HL, Asch BB.** Epigenetic regulation of gelsolin expression in human breast cancer cells. *Exp Cell Res.* 1999; 249: 161–76.
31. **Razin A.** CpG methylation, chromatin structure and gene silencing-A three way connection. *EMBO J.* 1998; 17: 4905–8.
32. **Jones PL, Veenstra GJ, Wade PA, Vermaak D, Kass S, U, Landsberger N, Strouboulis J, Wolffe AP.** Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. *Nat Genet.* 1998; 19: 187–91.
33. **Nan X, Ng HH, Johnson CA, Laherty CD, Turner BM, Eisenman RN, Bird A.** Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature* 1998; 393: 386–9.
34. **Fuks F, Burgers WA, Brehm A, Hughes-Davies L, Kouzarides T.** DNA methyltransferase Dnmt 1 associates with histone deacetylase activity. *Nat Genet.* 2000; 24: 88–91.