Biochemical and Biophysical Research Communications 425 (2012) 290–296

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Clustered DNA methylation changes in polycomb target genes in early-stage liver cancer

Yao-Li Chen [a,b], Chih-Jan Ko [a,c], Ping-Yi Lin [a], Wan-Ling Chuang [a], Chia-Chen Hsu [d], Pei-Yi Chu [e], Mei-Yu Pai [d], Chun-Chun Chang [d], Ming-Han Kuo [d], Yi-Ru Chu [d], Chun-Hsin Tung [d], Tim H.-M. Huang [f], Yu-Wei Leu [d,*], Shu-Huei Hsiao [d,*]

[a] Transplant Medicine & Surgery Research Centre, Changhua Christian Hospital, Changhua, Taiwan
[b] School of Medicine, Chung Shan Medical University, Taichung, Taiwan
[c] Department of General Surgery, Changhua Christian Hospital, Changhua, Taiwan
[d] Human Epigenomics Center, Department of Life Science, Institute of Molecular Biology and Institute of Biomedical Science, National Chung Cheng University, Chiayi, Taiwan
[e] Department of Pathology, St. Martin De Porres Hospital, Chiayi, Taiwan
[f] Cancer Therapy and Research Center, Department of Molecular Medicine and Institute of Biotechnology, School of Medicine, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, USA

ARTICLE INFO

Article history:
Received 16 July 2012
Available online 25 July 2012

Keywords:
DNA methylation
Epigenetic
Liver cancer
Chromatin immunoprecipitation

ABSTRACT

Polycomb-group proteins mark specific chromatin conformations in embryonic and somatic stem cells that are critical for maintenance of their "stemness". These proteins also mark altered chromatin modifications identified in various cancers. In normal differentiated cells or advanced cancerous cells, these polycomb-associated loci are frequently associated with increased DNA methylation. It has thus been hypothesized that changes in DNA methylation status within polycomb-associated loci may dictate cell fate and that abnormal methylation within these loci may be associated with tumor development. To assess this, we examined the methylation states of four polycomb target loci – *Trip10*, *Casp8AP2*, *ENSA*, and *ZNF484* – in liver cancer. These four targets were selected because their methylation levels are increased during mesenchymal stem cell-to-liver differentiation. We found that these four loci were hypomethylated in most early-stage liver cancer specimens. For comparison, two non-polycomb tumor suppressor genes, *HIC1* and *RassF1A*, were also examined. Whereas the methylation level of *HIC1* did not differ significantly between normal and tumor samples, *RassF1A* was significantly hypermethylated in liver tumor samples. Unsupervised clustering analysis classified the methylation changes within polycomb and non-polycomb targets to be independent, indicating independent epigenetic evolution. Thus, pre-deposited polycomb marks within somatic stem cells may contribute to the determination of methylation changes during hepatic tumorigenesis.

1. Introduction

Numerous aberrant genetic and epigenetic mutations/modifications act in cooperation to create tumor variations among individuals and between different cancers. This complexity has also confounded the search for effective diagnostic biomarkers and therapeutic targets [1–3]. Instead of a function-dependent classification, statistical analyses followed by arbitrary cut-offs are often used to identify profiled loci where the expressional/epigenetic differences could significantly classify cancer diseases pathologically [4].

The effectiveness of using the most frequently found loci as cancer biomarkers was addressed by Wood et al. [1] Their study demonstrated that a series of minor (less frequent) expressional differences/mutations can be more relevant to tumorigenesis and have a better chance of predicting the development of cancer in a tissue-specific manner. Thus, better tracking of tumor evolution may require other ways to identify target loci.

Epigenetic changes within polycomb group-governed loci have been reported to be sufficient to change cell fate [5–7]. Similar polycomb-governed loci have been identified in embryonic stem cells (ES) [8], somatic stem cell-like mesenchymal stem cells (MSCs) [9], and cancer stem cell-like cells [8].

Abbreviations: MSC, mesenchymal stem cell; 5-Aza, 5-aza-2′-deoxycytidine; H3K27me3, histone 3 lysine 27 trimethylation.

* Corresponding authors. Address: Department of Life Science, Institute of Molecular Biology and Institute of Biomedical Science, National Chung Cheng University, Chiayi 621, Taiwan. Fax: +886 5 272 2871.
E-mail addresses: bioywl@ccu.edu.tw (Y.-W. Leu), bioshh@ccu.edu.tw (S.-H. Hsiao).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.07.084

Polycomb group proteins silence governed genes primarily through adding a third methyl group at lysine 27 of histone 3 (“H3K27me3”) [6,7,10]. Although the governed genes are silenced, enriched active histone markers, like acetyl groups, have also been identified within these loci, as has the co-existence of both marks, termed “bivalent” loci for these genes [5,6]. Loss of the maintenance of silencing within these loci is sufficient to differentiate the ES cells [11,12], and further, methylation of one of these bivalent loci, like *Trip10*, is sufficient to accelerate the neuronal differentiation of MSCs [9].

These data indicate that maintaining the silenced states within these bivalent loci is important for maintaining the stemness of different stem cells and that further epigenetic changes within these loci may steer cell fate [8,13]. If further epigenetic changes, such as additional DNA methylation, within certain bivalent loci are important for normal differentiation of the stem cells, then the seemingly contradictory removal of DNA methylation within these loci might represent a tractable epigenetic change for the transformed cell fate. If this is true, then abnormal methylation changes within these bivalent loci in primary tumors would be expected. Thus, we sought to identify such methylation changes within bivalent loci.

Specifically, we assessed methylation differences within four bivalent loci in 60 pairs of early-stage liver cancer samples and whether the methylation changes were reversed as during normal differentiation. *Trip10* (NM_004240), *Casp8AP2* (NM_001137667), *ENSA* (NM_207046), and *ZNF484* (NM_001007101) are four bivalent genes that we identified for which methylation was increased during *ex vivo* MSC-to-liver differentiation [9]. In comparison with the increased methylation within two non-polycomb group target and tumor suppressor loci, *HIC1* (NM_006497) and *RassF1A* (NM_170712) [9,14], we detected inversely decreased methylation within these four target loci.

Unsupervised clustering analysis [15] indicated that the methylation changes within the polycomb target loci belonged to the same class and were independent of that of the tumor suppressor genes. These results indicate that the clustered methylation changes within these loci may be associated with liver cancer development.

2. Materials and methods

2.1. Cell culture

MCF7 and HepG2 cells were grown in MEM medium, HT29 cells were grown in McCoy medium, and MDA-MB-231 cells were grown in Leibovitz’s L-15 medium. All cell culture media were supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, and 100 μg/mL penicillin/streptomycin. Cell passages were performed at 90% confluence. After treatment with trypsin, cells were allowed to adhere overnight at 37 °C in 95% O_2/5% CO_2, and non-adherent cells were washed out with medium changes. Thereafter, medium changes were performed twice weekly. All reagents were purchased from Invitrogen.

2.2. 5-Aza treatment

Cells were treated with 5 μM 5-Aza [16] or an equal final volume of DMSO (solvent) for 5 consecutive days.

2.3. Semi-quantitative MSP (qMSP)

qMSP was performed as described by Yan et al. [17]. Universal methylated DNAs (Millipore) were used as a positive control. *Col2A1* was used as a loading control and to amplify serially diluted (1/10, 1/100, 1/1000) control bisulfite-converted (Zymo) genomic DNAs and to generate a standard curve for quantitation with a Bio-Rad iQ5 real-time PCR machine and to normalize the amount of methylated DNA among test samples. The methylation percentage was calculated as (Intensity of Amplifications by *Target loci* MSP primer set) × 100/(Intensity of Amplifications by *Col2A1* MSP primer set) (%). qMSP was performed in a 25-μL reaction mixture containing 4 μL of template (bisulfite-treated DNA), 2 μL of primer pair (2.5 μM), 12.5 μL of 2× reaction buffer (SYBR Green real-time PCR Master Mix, Roche), and 6.5 μL of ddH_2O. The methylation fold change was then calculated using the methylation percentage obtained from: 5-Aza-treated, tumor parts (Fig. 3), or non-tumor (Fig. 4A and B), divided by the methylation percentage obtained from mock-treated, non-tumor, and tumor, respectively. The primers used for amplification are listed in Supplementary Table 1. To show that the MSP results were compatible with the bisulfite sequencing results, the physical maps of the overlapping regions between the two detections are provided in Supplementary Fig. 2.

2.4. Chromatin immunoprecipitation (ChIP) assays

ChIP-PCR [18,19] and semi-quantitative ChIP-PCR (qChIP-PCR) [20] were performed as described previously. Antibodies were purchased from Upstate. The enrichment fold was calculated using PCR amplification signals from ChIP pull-down with antibodies/PCR amplification signals from total input.

2.5. Semi-quantitative RT-PCR (qRT-PCR)

Total RNA isolation, first-strand cDNA synthesis, and detection of the transcripts were performed as described previously. Briefly, total RNA (2 μg) was reverse-transcribed using SuperScript II reverse transcriptase (Invitrogen). qRT-PCR was then performed using the SYBR Green I PCR kit (Toyobo) in an iQ5 Real-Time PCR instrument [18,21]. A serial dilution of *GADPH* (NM_002046) amplified cDNA was used to generate a standard curve and *GAPDH* was used as a loading control. The target gene specific primers are listed in Supplementary Table 1.

2.6. Statistics and clustering analysis

A paired Student *t*-test was used to compare methylation differences between normal versus tumor tissue. Cluster analysis was performed as described in Eisen et al. [15].

2.7. Human subjects

Human cancer tissue was collected following Institutional Review Board regulations mandated by the Changhua Christian Hospital, Taiwan. In total, 60 pairs of liver cancer samples were collected.

3. Results

3.1. Changed chromatin conformation in liver cancer cells

Trip10, *Casp8AP2*, *ENSA*, and *ZNF484* are four polycomb target genes that we identified previously with increased DNA methylation during *ex vivo* MSC-to-liver differentiation (Hsiao et al. [9] and Supplementary Fig. 1). In contrast, *HIC1* and *RassF1A* are not on the bivalent list and they are tumor suppressor genes. Their concurrent targeted DNA methylation was sufficient to transform MSCs [14]. To examine whether the epigenetic states within these loci were changed, resulting in loss of their maintenance during long-term tumor evolution, we determined the chromatin

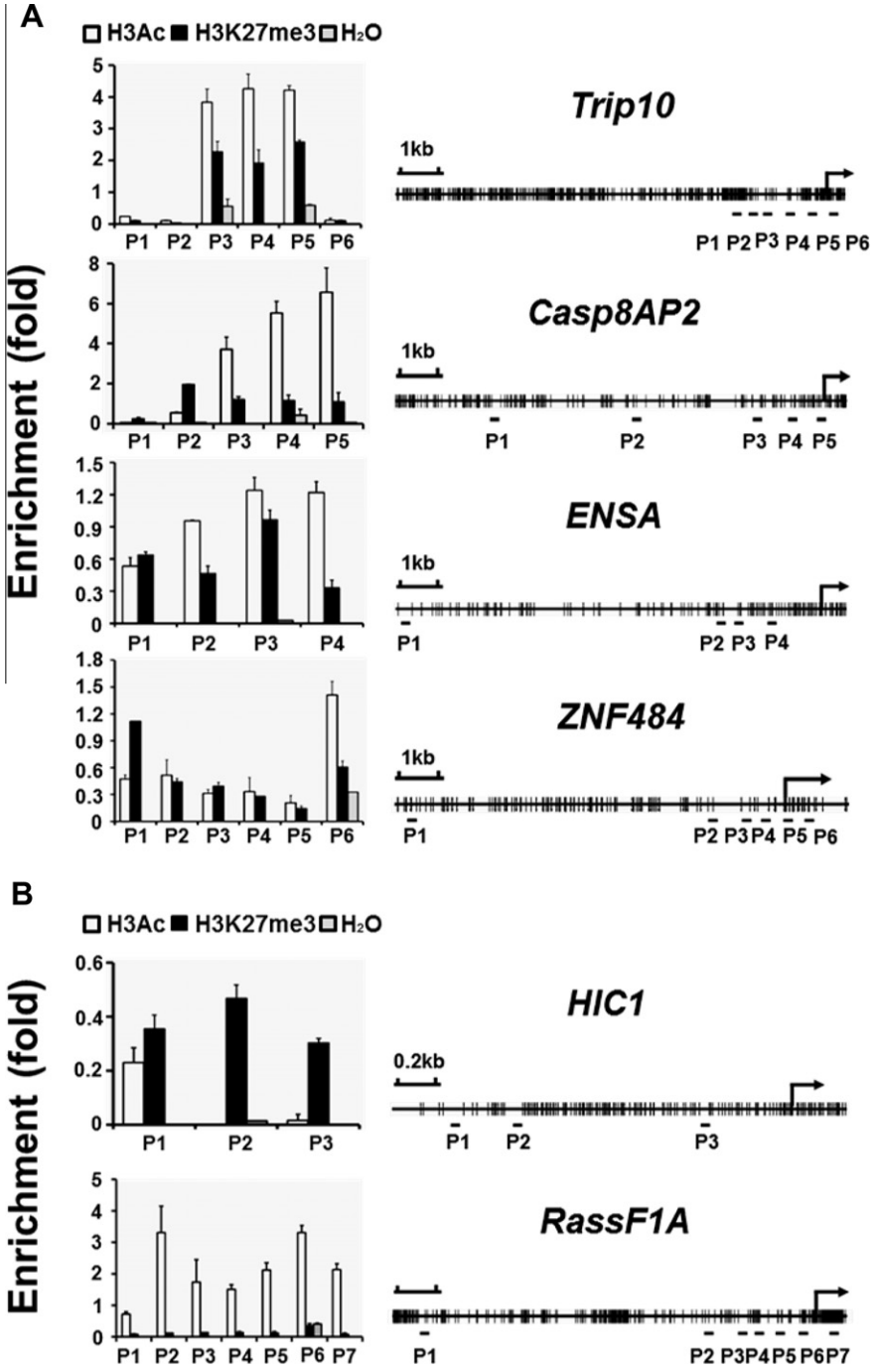

Fig. 1. Chromatin conformations within polycomb and non-polycomb targets in HepG2 liver cancer cells. (A) qChIP-PCR (left) detection of chromatin conformations within the designated polycomb target loci: *Trip10*, *Casp8AP2*, *ENSA*, and *ZNF484* (physical map, right panels). CpG locations are indicated as vertical bars in the promoter, and the arrows mark the location of the transcription start sites. Horizontal bars indicate the ChIP-PCR amplification/detection regions. Open histograms indicate the ChIP-PCR using an antibody against acetylated histone H3 (active); closed histograms indicated the ChIP-PCR detection using the H3K27me3 antibody. Both ChIP-PCRs detections were normalized with total inputs, and water amplifications were used as the specificity control for PCR reactions. (B) qChIP-PCR (left) detection of chromatin conformations within the designated non-polycomb target loci: *HIC1* and *RassF1A* (physical map, right panels).

conformation within polycomb and non-polycomb targets in a long-term cultured liver cancer cell line, HepG2. In Fig. 1A, close to the transcriptional start site (TSS), the chromatin conformations varied differently in these four polycomb target loci. As indicated in Fig. 1B, the chromatin conformation was markedly reversed in the non-polycomb targets *HIC1* (silenced) and *RassF1A* (active). Therefore, during long-term tumor development, the epigenetic states within polycomb and non-polycomb target loci varied.

3.2. DNA methylation silenced the corresponding genes

To assess whether DNA methylation within these loci was sufficient to silence the corresponding genes, we treated cancer cells including HepG2 (liver cancer), HT29 (colon cancer), and MCF7 and MDA-MB-231 (breast cancers), with the demethylating agent 5-Aza and examined whether gene expression could be restored after 5-Aza treatment. We found that if methylation was decreased

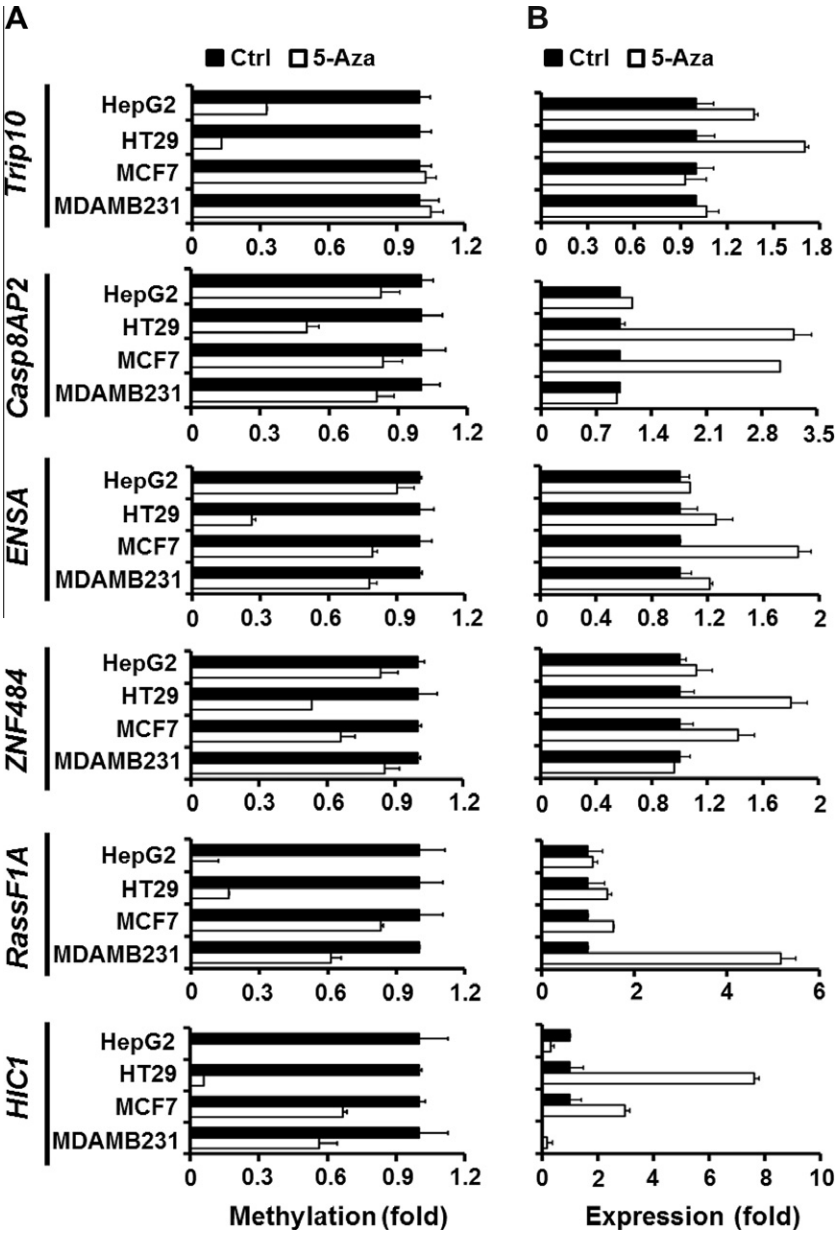

Fig. 2. Methylation silences polycomb and non-polycomb targets loci. The methylation and expression within the designated loci (polycomb targets: *Trip10, Casp8AP2*, *ENSA*, and *ZNF484,* and non-polycomb targets: *HIC1* and *RassF1A*) were detected by qMSP (A) and qRT-PCR (B) in 5-Aza- (open bars) or mock (DMSO, closed bars)-treated HepG2 (liver cancer), HT29 (colon cancer), MCF7, and MDA-MB-231 (MDAMB231, breast cancer) cells ($n = 4$ for each detection). Methylation and expression levels in 5-Aza-treated cells were normalized to the respective levels in the mock-treated control (designated as 1-fold).

within these loci (Fig. 2A), expression was increased (Fig. 2B). The methylation states of these loci were not increased or decreased simultaneously, suggesting that the tumoral epigenome chose different paths during long-term tumoral evolution, complicating the search for true biomarkers. Consistent with previous findings, DNA methylation within these loci dominantly suppressed the expression of the respective genes.

3.3. Polycomb target loci became hypomethylated in early-stage liver cancer

The methylation states of four bivalent loci within liver cancers and adjacent normal tissues were assessed by qMSP and compared (Fig. 3). In contrast to the increased methylation during the normal MSC-to-liver differentiation, the methylation states within these four loci were decreased significantly in the tumor tissue versus adjacent normal tissue ($n = 60$). Although the overall demethylation trend held up in early-stage liver cancers, some individual differences were observed within each locus. Following a rationale similar to that suggested by Wood et al. [1], instead of searching for single, most frequent epigenetic changes, the detection of methylation changes in more than one locus will better illustrate tumor evolution.

3.4. Clustered DNA methylation changes in polycomb target genes

HIC1 and *RassF1A* are two known tumor suppressor genes in ontology that were not listed as bivalent loci in a previous MSC profiling [9]. Genetic studies have indicated that loss of function in either gene is associated with tumorigenesis, and our previous report indicated that concurrent targeted DNA methylation of both

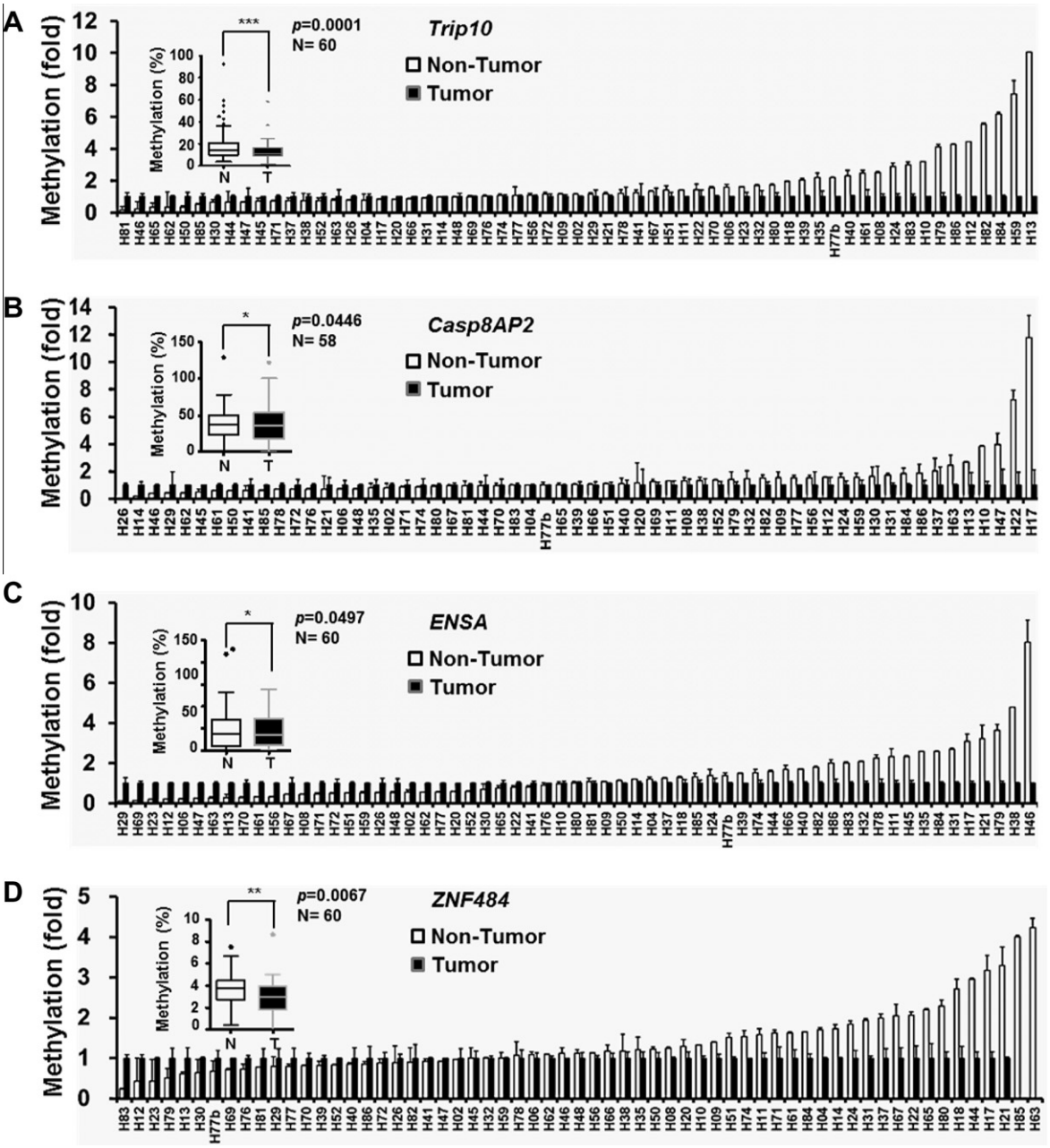

Fig. 3. Methylation differences within polycomb targets between non-tumor (open bars) and tumor tissue (closed bars) from 60 pairs of early-stage liver cancers. Tumor and non-tumor tissue methylation levels were detected in *Trip10* (A), *Casp8AP2* (B), *ENSA* (C), and *ZNF484* (D) by qMSP. The methylation level in tumor parts was designated as 1-fold, and then the levels from non-tumor parts were divided by the levels in tumor parts. Box plot comparisons are shown at the top left of each panel, and paired *t*-tests were performed to assess differences.

loci is sufficient to transform MSCs into cancer stem cell-like cells [14].

These data are consistent with the notion that a true tumor suppressor gene should not be bivalent. We detected the methylation states of both loci and found that in most patient samples, but not all of them, these loci were hypermethylated in early-stage liver cancers (Fig. 4A and B).

Unsupervised clustering analysis [15] revealed that the methylation patterns were increased primarily in tumor suppressor genes, but were decreased in polycomb targets (Fig. 4C). The clustered methylation pattern suggested that epigenetic modifications occurred within the loci based on their original epigenetic states. Also, a true tumor suppressor gene, in theory at least, would not be further silenced or methylated during normal differentiation and thus would not be expected to be a target of polycomb proteins. Therefore, time-dependent methylation changes during tumorigenesis in polycomb targets differ from the changes during normal differentiation as well as the changes with tumor suppressors.

4. Discussion

"Stemness" states have been reported to be more similar between ES cells and cancer cells than between cancer and normal differentiated cells [8]. This observation has been used to support the cancer stem cell theory. If this concept is true, then it would be expected that polycomb targets would be important not only for the determination of differentiation but that their aberrant methylation might also be associated with the initiation of

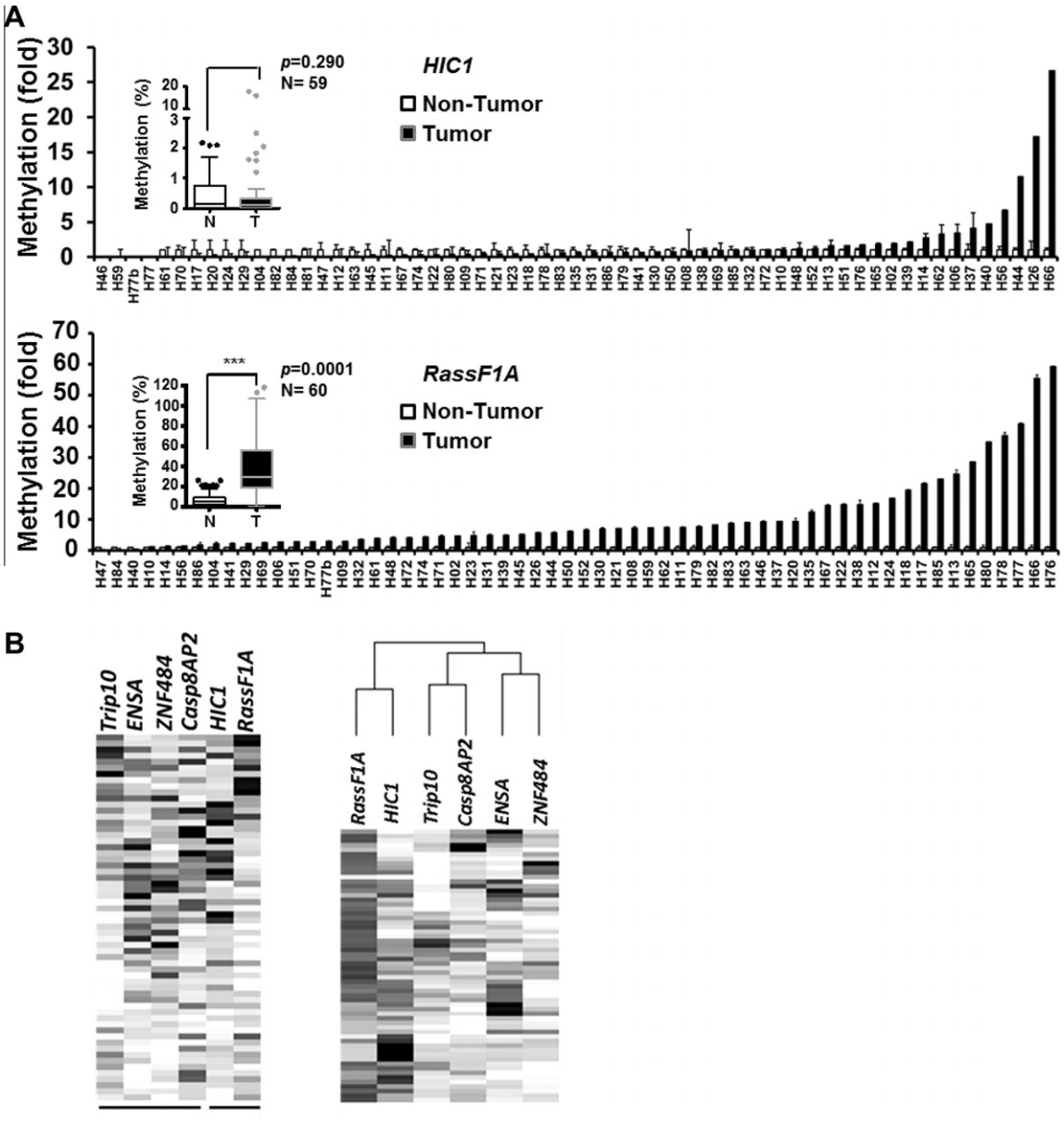

Fig. 4. Methylation differences within non-polycomb targets between non-tumor (open bars) and tumor tissue (closed bars) from 60 pairs of early-stage liver cancers and clustering analysis of methylation states in all loci. (A) Tumor and non-tumor tissue methylation levels were detected in *HIC1* (upper) and *RassF1A* (lower panel).The methylation level in non-tumor tissue was designated as 1-fold, and then the levels from non-tumor tissues were divided by the level from tumor tissues. Box plot comparisons are shown at the top left of each panel, and paired *t*-tests were used to assess differences. (B) Clustered methylation states within polycomb and non-polycomb target loci. Relevant methylation states (tumor vs. non-tumor) detected from 60 pairs of liver cancers in six loci were subjected to unsupervised cluster analysis (SOM left; hierarchical, right).

tumorigenesis. Because polycomb targets could be further up- or down-regulated to cause differentiation of a stem cell, a tumor suppressor gene that is necessary to keep the cell from tumorigenesis should not be bivalent. Also, because DNA methylation is a dominant silencing epigenetic modification, methylation changes within polycomb and non-polycomb tumor suppressor genes would be expected to be independent from each other. The clustered and independent methylation changes identified between the classes of loci are consistent with this idea.

Polycomb group target genes are critical in cell fate determination and their methylation affects their expression, shifting cell fates [6,8,13]. Profiling and tracking of epigenetic changes within loci known to control cell fate will add a functional aspect in searches for modeling components for tumor epigenomes other than using the most frequent gene(s). In this study, we identified classified methylation modifications in the early stages of liver cancers (Figs. 3 and 4), but the patterns will evolve further during long-term culture or in other types of cancers (Fig. 2). Therefore, the target loci deduced may be tissue-specific and the two grouped loci here may be suitable for tracking tumorigenesis at an early stage.

Researchers have realized that tracking expression changes within a single locus may not be sufficient to describe the tumorigenesis process. In addition, tracking of expression changes themselves is not only important for time-dependent modeling of tumorigenesis, but also for the identification of initial states, such as the epigenetic states in stem cells or tumor-initiating cells. Further expression changes are equally important for complete modeling. Our data suggest that sequential and clustered epigenetic changes occur as far back as the initial states in somatic

stem cells, and tracking of these further changes with functional implications will prevent these possibly important biomarkers from being missed due to their low frequency of occurrence.

Acknowledgments

Y.-L. Chen, P.-Y. Lin, and C.-J. Ko are supported by Changhua Christian Hospital, Taiwan. Y.-W. Leu, S.-H. Hsiao and P.-Y. Chu are supported by NSC 100-2321-B-750-001. Y.-W. Leu and S.-H. Hsiao are supported by NSC 100-2320-B-194-001 and the College of Science, National Chung Cheng University.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.07.084.

References

[1] L.D. Wood, D.W. Parsons, S. Jones, J. Lin, T. Sjoblom, R.J. Leary, D. Shen, S.M. Boca, T. Barber, J. Ptak, N. Silliman, S. Szabo, Z. Dezso, V. Ustyanksky, T. Nikolskaya, Y. Nikolsky, R. Karchin, P.A. Wilson, J.S. Kaminker, Z. Zhang, R. Croshaw, J. Willis, D. Dawson, M. Shipitsin, J.K. Willson, S. Sukumar, K. Polyak, B.H. Park, C.L. Pethiyagoda, P.V. Pant, D.G. Ballinger, A.B. Sparks, J. Hartigan, D.R. Smith, E. Suh, N. Papadopoulos, P. Buckhaults, S.D. Markowitz, G. Parmigiani, K.W. Kinzler, V.E. Velculescu, B. Vogelstein, The genomic landscapes of human breast and colorectal cancers, Science 318 (2007) 1108–1113.

[2] S.H. Hsiao, T.H. Huang, Y.W. Leu, Excavating relics of DNA methylation changes during the development of neoplasia, Semin. Cancer Biol. 19 (2009) 198–208.

[3] A.H. Ting, K.M. McGarvey, S.B. Baylin, The cancer epigenome – components and functional correlates, Genes Dev. 20 (2006) 3215–3231.

[4] Y.F. Leung, D. Cavalieri, Fundamentals of cDNA microarray data analysis, Trends Genet. 19 (2003) 649–659.

[5] M.E. Valk-Lingbeek, S.W. Bruggeman, M. van Lohuizen, Stem cells and cancer; the polycomb connection, Cell 118 (2004) 409–418.

[6] Y.B. Schwartz, V. Pirrotta, Polycomb complexes and epigenetic states, Curr. Opin. Cell Biol. 20 (2008) 266–273.

[7] M. Sauvageau, G. Sauvageau, Polycomb group proteins: multi-faceted regulators of somatic stem cells and cancer, Cell Stem Cell 7 (2010) 299–313.

[8] S.B. Baylin, Stem cells, cancer, and epigenetics, StemBook., Cambridge, MA, 2008.

[9] S.H. Hsiao, K.D. Lee, C.C. Hsu, M.J. Tseng, V.X. Jin, W.S. Sun, Y.C. Hung, K.T. Yeh, P.S. Yan, Y.Y. Lai, H.S. Sun, Y.J. Lu, Y.S. Chang, S.J. Tsai, T.H. Huang, Y.W. Leu, DNA methylation of the Trip10 promoter accelerates mesenchymal stem cell lineage determination, Biochem. Biophys. Res. Commun. 400 (2010) 305–312.

[10] P.H. Abbosh, J.S. Montgomery, J.A. Starkey, M. Novotny, E.G. Zuhowski, M.J. Egorin, A.P. Moseman, A. Golas, K.M. Brannon, C. Balch, T.H. Huang, K.P. Nephew, Dominant-negative histone H3 lysine 27 mutant derepresses silenced tumor suppressor genes and reverses the drug-resistant phenotype in cancer cells, Cancer Res. 66 (2006) 5582–5591.

[11] X. Shen, W. Kim, Y. Fujiwara, M.D. Simon, Y. Liu, M.R. Mysliwiec, G.C. Yuan, Y. Lee, S.H. Orkin, Jumonji modulates polycomb activity and self-renewal versus differentiation of stem cells, Cell 139 (2009) 1303–1314.

[12] N.L. Vastenhouw, Y. Zhang, I.G. Woods, F. Imam, A. Regev, X.S. Liu, J. Rinn, A.F. Schier, Chromatin signature of embryonic pluripotency is established during genome activation, Nature 464 (2010) 922–926.

[13] G.V. Glinsky, "Stemness" genomics law governs clinical behavior of human cancer: implications for decision making in disease management, J. Clin. Oncol. 26 (2008) 2846–2853.

[14] I.W. Teng, P.C. Hou, K.D. Lee, P.Y. Chu, K.T. Yeh, V.X. Jin, M.J. Tseng, S.J. Tsai, Y.S. Chang, C.S. Wu, H.S. Sun, K.D. Tsai, L.B. Jeng, K.P. Nephew, T.H. Huang, S.H. Hsiao, Y.W. Leu, Targeted methylation of two tumor suppressor genes is sufficient to transform mesenchymal stem cells into cancer stem/initiating cells, Cancer Res. 71 (2011) 4653–4663.

[15] M.B. Eisen, P.T. Spellman, P.O. Brown, D. Botstein, Cluster analysis and display of genome-wide expression patterns, Proc. Natl. Acad. Sci. USA 95 (1998) 14863–14868.

[16] C.M. Bender, M.M. Pao, P.A. Jones, Inhibition of DNA methylation by 5-aza-2′-deoxycytidine suppresses the growth of human tumor cell lines, Cancer Res. 58 (1998) 95–101.

[17] P.S. Yan, C. Venkataramu, A. Ibrahim, J.C. Liu, R.Z. Shen, N.M. Diaz, B. Centeno, F. Weber, Y.W. Leu, C.L. Shapiro, C. Eng, T.J. Yeatman, T.H. Huang, Mapping geographic zones of cancer risk with epigenetic biomarkers in normal breast tissue, Clin. Cancer Res. 12 (2006) 6626–6636.

[18] Y.W. Leu, P.S. Yan, M. Fan, V.X. Jin, J.C. Liu, E.M. Curran, W.V. Welshons, S.H. Wei, R.V. Davuluri, C. Plass, K.P. Nephew, T.H. Huang, Loss of estrogen receptor signaling triggers epigenetic silencing of downstream targets in breast cancer, Cancer Res. 64 (2004) 8184–8192.

[19] H. Shi, S.H. Wei, Y.W. Leu, F. Rahmatpanah, J.C. Liu, P.S. Yan, K.P. Nephew, T.H. Huang, Triple analysis of the cancer epigenome: an integrated microarray system for assessing gene expression, DNA methylation, and histone acetylations, Cancer Res. 63 (2003) 2164–2171.

[20] K.D. Lee, M.Y. Pai, C.C. Hsu, C.C. Chen, Y.L. Chen, P.Y. Chu, C.H. Lee, L.T. Chen, J.Y. Chang, T.H. Huang, S.H. Hsiao, Y.W. Leu, Targeted Casp8AP2 methylation increases drug resistance in mesenchymal stem cells and cancer cells, Biochem. Biophys. Res. Commun. 422 (2012) 578–585.

[21] Y.W. Leu, F. Rahmatpanah, H. Shi, S.H. Wei, J.C. Liu, P.S. Yan, T.H. Huang, Double RNA interference of DNMT3b and DNMT1 enhances DNA demethylation and gene reactivation, Cancer Res. 63 (2003) 6110–6115.