Chemico-Biological Interactions xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

Epigenetic changes in therapy-related MDS/AML

Maria Teresa Voso*, Francesco D'Alò, Mariangela Greco, Emiliano Fabiani, Marianna Criscuolo, Giuseppe Migliara, Livio Pagano, Luana Fianchi, Francesco Guidi, Stefan Hohaus, Giuseppe Leone

Istituto di Ematologia, Università Cattolica Sacro Cuore, Largo A. Gemelli, 8, 00168Rome, Italy

ARTICLE INFO

Article history:
Available online xxx

Keywords:
t-MDS/AML
Epigenetics
Methylation
DNA damage

ABSTRACT

Therapy-related Myelodysplastic Syndromes/Acute Myeloid Leukemias (t-MDS/AML) are one of the most compelling long term adverse events occurring in cancer survivors treated with chemo-radiotherapy regimes. Beside several well-described genetic lesions, a growing amount of data suggests that abnormalities in DNA methylation profile contribute to multistep secondary leukemogenesis.

Two distinct alterations of normal DNA methylation patterns may occur in cancer: a global hypomethylation resulting in chromosomal instability and loss of genetic integrity, and promoter specific DNA hypermethylation which leads to silencing of tumor suppressor genes. Cytotoxic drugs and radiation have been shown to affect tissue DNA methylation profile. Radiation is able to induce a stable DNA hypomethylation in both target and bystander tissues. Gene promoter methylation is a common finding in t-MDS/AML and has been associated to a shorter latency period from the treatment of the primary tumor. Among the studied genes, p15 methylation correlated to monosomy/deletion of chromosome 7q, suggesting that it could be a relevant event in alkylating agent-induced leukemogenesis. We found frequent methylation of DAPK in the t-MDS/AML group, especially in patients with a previous lymphoproliferative disease. In patients studied for concurrent methylation of several promoters, t-MDS/AML were significantly more frequently hypermethylated in 2 or more promoter regions than de novo MDS or AML suggesting that promoter hypermethylation of genes involved in cell cycle control, apoptosis and DNA repair pathways is a frequent finding in t-MDS/AML and may contribute to secondary leukemogenesis. However, how the epigenetic machinery is disrupted after chemo/radiotherapy and during secondary carcinogenesis is still unknown, warranting further studies.

1. Introduction

In addition to well-known genetic changes, therapy-related MDS/AML are characterized by changes in the methylation profile which may play a pivotal role in leukemogenesis and are more common in *therapy-related* than de novo MDS/AML.

Epigenetic mechanisms such as DNA methylation, post-translational modifications of histone proteins and remodeling of nucleosomes affect chromatin structure and contribute to define heritable changes in gene expression. DNA methylation consists in the addiction of a methyl group on the number 5 carbon of the pyrimidine ring of cytosines within the context of the CpG dinucleotide. In general, CpGs are under-represented within the genome as a result of the increased frequency of spontaneous deamination of methyl-cytosines, but their density is particularly high in specific DNA regions referred to as CpG islands, that include promoter regions and repetitive DNA sequences, such as Alu repeats and endoparasitic elements [1]. Multiple mathematical algorithms have subsequently been proposed for the classification of CpG islands and one of the most commonly used set of criteria requires a minimum observed-expected CpG ratio of 0.65 and GC content greater than 55% over a distance of 500 bp. By this definition, the human genome contains nearly 38,000 CpG islands. A large fraction of these islands (37%) localize to the 5′ regulatory regions of genes with approximately 70% of known genes having a CpG island within −2 kb to +1 kb of their transcription start site [2].

CpG methylation is catalysed by a family of enzymes known as DNA methyltransferases (DNMTs) that (DNMT1, DNMT3A, and DNMT3B), which transfer a methyl group from S-adenosyl-L-methionine to cytosines in CpG dinucleotides. DNMT-1 is mainly a maintenance methylase that recognizes and methylates hemimethylated CpG dinucleotides during DNA replication allowing the propagation and conservation of DNA methylation patterns through the future generations. DNMT3A and -3B are mainly de novo methylase and methylate unmethylated CpG dinucleotides [1]. The methyl-cytosines established by the DNMTs serve as binding sites for the methyl-CpG binding domain (MBD) proteins, such

* Corresponding author. Tel.: +39 0630154180; fax: +39 0635503777.
E-mail address: mtvoso@rm.unicatt.it (M.T. Voso).

0009-2797/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.cbi.2009.10.013

as MeCP2, MBD1, MBD2, MBD3, MBD4 and Kaiso. Through interactions with histone deacetylases, histone methyltransferases, and ATPdependent chromatin remodeling enzymes, the MBDs translate methylated DNA into a compacted chromatin environment that is repressive for transcription [1].

Indeed DNA methylation is only part of a broader epigenetic code that dictates the transcriptional potential of genomic domains. DNA is wrapped around an octamer of histone proteins to form the nucleosome, the smallest unit of chromatin. The amino terminal tails of the histones protrude from the nucleosome body and are subject to considerable post-translational modifications including acetylation, methylation, phosphorylation, ubiquitination, and sumoylation. The constellation of specific modifications, referred to as the histone code, influences interactions with the DNA backbone, neighboring nucleosomes, and nonhistone chromatin proteins to mediate the assembly of a chromatin environment that is either permissive or repressive for transcription. In general, permissive regions exhibit an open chromatin structure marked by hyperacetylation of histones H3 and H4 and di- and tri-methylation of histone H3 at lysine 4 (H3K4me2/3). In contrast, repressed regions exhibit a compact chromatin structure that lacks H3/H4 acetylation and H3K4 methylation, and instead is enriched in the repressive modifications, di- and tri-methylation of H3K9 (H3K9me2/3), tri-methylation of H3K27 (H3K27me3), and tri-methylation of H4K20 (H4K20me3). Although the code is not yet fully deciphered, it is apparent that DNA methylation can both influence and be influenced by histone modifications [3].

2. Deregulation of epigenetic control in cancer

Two distinct alterations of normal DNA methylation patterns occur in cancer: global hypomethylation and gene-specific promoter hypermethylation. DNA hypomethylation may result in potentially harmful expression of inserted viral DNA and transposable elements, weak transcriptional repression of normally silent regions of the genome, chromosomal instability and loss of genetic integrity. On the other hand, DNA hypermethylation leads to silencing of tumor suppressor genes, including cell-cycle inhibitors, inducers of apoptosis, DNA repair genes, transcription factors, cell adhesion mediators, hormonal receptors and detoxifiers [1]. Thus, through the effects of both hypo- and hypermethylation, DNA methylation significantly affects the genomic landscape of cancer cells, potentially similar to coding region mutations. In the future, the use of whole genome sequencing may address the question of the frequency of mutations in coding regions and their relations to epigenetic changes with greater precision.

Like DNA methylation, the histone portion of the epigenome undergoes both widespread and gene-specific changes in cancer. Overall, cancer cells exhibit a global decrease in the levels of H4K20me2/3, H3K9me2, and H4 acetylation, particularly from the repetitive fraction of the genome, contributing to a global dysregulation of transcription in cancer cells [4].

There is an interdependent relationship between DNA methylation and histone modification. The unmethylated CpG islands of active genes are enriched in acetylated H3 and H4 and H3K4me2. In contrast, the CpG islands of genes that are aberrantly methylated in cancer cells are remodeled such that there is a shift from H3/H4 acetylation and H3K4 methylation, to H3K9me2/3 and or H3K27me3 [5]. This is achieved through the recruitment of MBDs, histone deacetylases (HDACs), histone methyltransferases, and H3K9me2/3 binding proteins (e.g., HP1), which lock the domain into a heterochromatin-like state that is mitotically heritable [3].

3. Epigenetic changes and DNA damage

It is still matter of discussion what causes DNA methylation changes during carcinogenesis. Several hypothesis and experimental models are under investigation. However some reports have so far suggested a possible link between DNA methylation and DNA damage.

Using an engineered experimental model, O'Hagan et al. demonstrated as normal repair of a DNA break can occasionally cause heritable silencing of a CpG island-containing promoter by recruitment of proteins involved in silencing [6]. In particular they induced a defined double strand break in an exogenous promoter construct of the E-cadherin CpG island, which is frequently aberrantly DNA hypermethylated in epithelial cancers. Following induction of a doublestrand DNA break, the SIRT1 histone deacetylase was recruited to the site along with components of PRC2, DNMT1, and DNMT3B. Although in most cells selected after the break, DNA repair occurs faithfully with preservation of activity of the promoter, a small percentage of the plated cells demonstrate induction of heritable silencing. The chromatin around the break site in such a silent clone was enriched for most of the above silent chromatin proteins and histone marks, and the region harbors the appearance of increasing DNA methylation in the CpG island of the promoter. The region was subsequently deacetylated at H4K16, methylated at H3K27, transcriptionally silenced, and in some cases DNA hypermethylated [6].

Consistent with these findings, several reports have shown as the exposure to DNA-damaging agents is able to affect the DNA methylation pattern. Levels of cytosine-DNA methyltransferase 1 (DNMT1) protein were shown to increase significantly during tobacco-derived carcinogen exposure of immortalized bronchial epithelial cell lines and were associated with the detection of promoter hypermethylation of 5–10 genes in each transformed cell line. Stable knockdown of DNMT1 reversed transformation and gene silencing. Moreover, stable knockdown of DNMT1 protein before carcinogen treatment prevented transformation and methylation of cadherin genes [7]. The importance of detecting DNA hypermethylation in individuals at risk of cancer development have been suggested by several studies in smokers. Promoter methylation in sputum specimens increases as the time to lung cancer diagnosis decreases. Moreover, the concomitant methylation of three or more among six selected genes in sputum samples is associated with a 6.5-fold increased risk for lung cancer and a sensitivity and specificity for predicting lung cancer of 64% in high risk smokers cohort [8,9].

The reactive oxygen species (ROS) associated with chronic inflammation is another source of DNA damage with the potential to affect DNA methylation as halogenated pyrimidines, one form of ROS-induced damage, mimic 5-methyl-cytosine and stimulate DNMT1-mediated CpG methylation in vitro and in vivo [10].

Cytotoxic drugs and radiation are well-known DNA-damaging agents commonly used for cancer therapy and associated to the development of therapy-related myeloid neoplasms. However, their effects on tissue DNA methylation are only partially known. So far, several studies have shown as radiation is able to induce a stable DNA hypomethylation in both target and bystander tissues, even though DNA damage resulting from initial genotoxic radiation insult was repaired, suggesting the importance of epigenetic mechanisms in the development of radiation-related pathologies [11,12]. On the other end, only few data have been so far reported about epigenetic consequences of exposure to chemotherapy agents. The environmental alkylating agent benzene, a widespread pollutant associated with AML risk, was shown to induce a reduction of LINE-1 and AluI methylation and an increase of p15 methylation on blood DNA samples from 78 gas station attendants and 77 traffic police officers, compared to 58 unexposed referents in Milan. This study

Please cite this article in press as: M.T. Voso, et al., Epigenetic changes in therapy-related MDS/AML, Chem. Biol. Interact. (2009), doi:10.1016/j.cbi.2009.10.013

provides a link between altered DNA methylation, reproducing the aberrant epigenetic patterns found in malignant cells, to low-level carcinogen [13].

4. Epigenetic changes in therapy-related MDS/AML

As reported above, an increasing amount of data suggests that DNA damage response is likely to induce changes in DNA methylation pattern in at least a subset of the affected cells and this may represent an early event in the development of therapy-related myeloid neoplasms.

However, although several studies have so far focused on epigenetic changes in MDS and AML, only few reports have described epigenetic abnormalities in therapy-related MDS-AML. Moreover, most of these studies used a single gene approach or described the methylation of a small panel of genes. Among the most common hypermethylated genes, the *p15* promoter was found methylated in 58–68% of t-MDS/AML patients, in particular in relation to monosomy/deletion of chromosome 7q [14,15]. Furthermore studying 17 t-MDS/AML patients, Au et al. reported that *p15* methylation was present in 3 of 6 patients who had marrow samples obtained up to 2 years prior to the diagnosis of t-MDS/AML [16]. Two of these patients had a previous B-cell lymphoma where *p15* has also been reported to be frequently methylated.

Studying the methylation status of thirteen genes in a cohort of t-AML patients, Uehara et al. [17] identified methylation of at least one gene in 55% of patients and the average time to the development of t-AML after the treatment of the primary tumor was significantly shorter in methylated than unmethylated patients (49.3 months vs. 133.2 months, $p = 0.044$).

Our research group studied the methylation pattern of 74 t-MDS/AML when compared to 106 de novo MDS and 208 de novo AML. Using a methylation-specific PCR, we studied the promoter methylation status of E-cadherin (CDH1), TBSP-1, COX-2, DAP-kinase 1 (DAPK1) and GSTP1, which have been shown to be involved in the malignant transformation of progenitor cells, interfering with angiogenesis, interaction with micro-environment, apoptosis and xenobiotic detoxification. Death associated protein (DAP)-kinase 1 is a 16 kDa calmodulin-dependent serine/threonine kinase that carries a death domain at its C-terminus, functioning as a positive mediator of apoptosis induced by interferon-gamma, tumour necrosis factor α, and activated Fas [18]. Furthermore it plays a role in metastatic processes, by inhibiting cell motility [19]. DAPK1 inactivation by promoter hypermethylation in solid tumors leads to apoptosis inhibition and to increased metastatic potential. In this line, it has been shown to be predictive of shorter disease-free and overall survival in lung and gastric cancer. As a biomarker, it is a part of high sensitivity markers for early breast cancer diagnosis [3]. In chronic lymphocytic leukemia, DAPK1 promoter hypermethylation is very frequent and may play a pathogenetic role, since genetic and epigenetic alteration have been shown to cooperate in familiar cases, where a mutation/polymorphism causing persistent downregulation of DAPK1 expression was also identified in the promoter region [20].

Confirming our previous data [21], DAPK1 was more frequently methylated in t-MDS/AML when compared to de novo MDS and AML, while methylation of CDH1 was similar in t-MDS/AML and AML. GSTP1, COX-2 and TBSP-1 hypermethylation were rare and were not characteristic of t-MDS/AML. In patients studied for concurrent methylation of several promoters, t-MDS/AML were significantly more frequently hypermethylated in 2 or more promoter regions than de novo MDS or AML [22].

These data are still incomplete and may not reflect the epigenome of therapy-related neoplasms. The application of recently developed genome-scale methylation screening technologies will contribute to clarify the impact of epigenetic changes in the pathogenesis of therapy-related myeloid leukemias and will possibly provide a tool to monitor cancer survivors for the risk of developing such an unfavourable long term complication of cancer treatment.

Conflict of interest statement

None.

Acknowledgements

This work has been supported by grants from AIRC (Associazione Italiana per la Ricerca sul Cancro) and M.I.U.R. (Ministero dell'Istruzione, dell'Università e della Ricerca Scientifica) and Fondazione Roma "Progetto cellule staminali, Una nuova frontiera nella ricerca biomedica".

References

[1] G. Leone, F. D'Alò, G. Zardo, M.T. Voso, C. Nervi, Epigenetic treatment of myelodysplastic syndromes and acute myeloid leukemias, Curr. Med. Chem. 15 (2008) 1274–1287.
[2] D. Takai, P.A. Jones, Comprehensive analysis of CpG islands in human chromosomes 21 and 22, Proc. Natl. Acad. Sci. U.S.A. 19 (2002) 3740–3745.
[3] M.T. McCabe, J.C. Brandes, P.M. Vertino, Cancer DNA methylation: molecular mechanisms and clinical implications, Clin. Cancer Res. 15 (2009) 3927–3937.
[4] M.F. Fraga, E. Ballestar, A. Villar-Garea, M. Boix-Chornet, J. Espada, G. Schotta, T. Bonaldi, C. Haydon, S. Ropero, K. Petrie, N.G. Iyer, A. Pérez-Rosado, E. Calvo, J.A. Lopez, A. Cano, M.J. Calasanz, D. Colomer, M.A. Piris, N. Ahn, A. Imhof, C. Caldas, T. Jenuwein, M. Esteller, Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer, Nat. Genet. 37 (2005) 391–400.
[5] T. Jenuwein, C.D. Allis, Translating the histone code, Science 10 (2001) 1074–1080.
[6] H.M. O'Hagan, H.P. Mohammad, S.B. Baylin, Double strand breaks can initiate gene silencing and SIRT1-dependent onset of DNA methylation in an exogenous promoter CpG island, PLoS Genet. 15 (2008) e1000155.
[7] L.A. Damiani, C.M. Yingling, S. Leng, P.E. Romo, J. Nakamura, S.A. Belinsky, Carcinogen-induced gene promoter hypermethylation is mediated by DNMT1 and causal for transformation of immortalized bronchial epithelial cells, Cancer Res. 68 (2008) 9005–9014.
[8] S.A. Belinsky, K.C. Liechty, F.D. Gentry, H.J. Wolf, J. Rogers, K. Vu, J. Haney, T.C. Kennedy, F.R. Hirsch, Y. Miller, W.A. Franklin, J.G. Herman, S.B. Baylin, P.A. Bunn, T. Byers, Promoter hypermethylation of multiple genes in sputum precedes lung cancer incidence in a high-risk cohort, Cancer Res. 66 (2006) 3338–3344.
[9] P.P. Anglim, T.A. Alonzo, I.A. Laird-Offringa, DNA methylation-based biomarkers for early detection of non-small cell lung cancer: an update, Mol. Cancer 7 (2008) 81.
[10] V.V. Lao, J.L. Herring, C.H. Kim, A. Darwanto, U. Soto, L.C. Sowers, Incorporation of 5-chlorocytosine into mammalian DNA results in heritable gene silencing and altered cytosine methylation patterns, Carcinogenesis 30 (2009) 886–893.
[11] I. Koturbash, I. Pogribny, O. Kovalchuk, Stable loss of global DNA methylation in the radiation-target tissue–a possible mechanism contributing to radiation carcinogenesis? Biochem. Biophys. Res. Commun. 337 (2005) 526–533.
[12] Y. Ilnytskyy, I. Koturbash, O. Kovalchuk, Radiation-induced bystander effects in vivo are epigenetically regulated in a tissue-specific manner, Environ. Mol. Mutagen. 50 (2009) 105–113.
[13] V. Bollati, A. Baccarelli, L. Hou, M. Bonzini, S. Fustinoni, D. Cavallo, H.M. Byun, J. Jiang, B. Marinelli, A.C. Pesatori, P.A. Bertazzi, A.S. Yang, Changes in DNA methylation patterns in subjects exposed to low-dose benzene, Cancer Res. 67 (2007) 876–880.
[14] H.D. Preisler, B. Li, H. Chen, L. Fisher, J. Nayini, A. Raza, S. Creech, P. Venugopal, P15INK4B gene methylation and expression in normal, myelodysplastic, and acute myelogenous leukemia cells and in the marrow cells of cured lymphoma patients, Leukemia 15 (2001) 1589–1595.
[15] D.H Christiansen, M.K. Andersen, J. Pedersen-Bjergaard, Methylation of p15INK4B is common, is associated with deletion of genes on chromosome arm 7q and predicts a poor prognosis in therapy-related myelodysplasia and acute myeloid leukemia, Leukemia 17 (2003) 1813–1819.
[16] W.Y. Au, A. Fung, C. Man, S.K. Ma, T.S. Wan, R. Liang, Y.L. Kwong, Aberrant p15 gene promoter methylation in therapy-related myelodysplastic syndrome and acute myeloid leukaemia: clinicopathological and karyotypic associations, Br. J. Haematol. 120 (2003) 1062–1065.
[17] E. Uehara, S. Takeuchi, T. Tasaka, Y. Matsuhashi, Y. Yang, M. Fujita, T. Tamura, M. Nagai, H.P. Koeffler, Aberrant methylation in promoter-associated CpG islands of multiple genes in therapy-related leukemia, Int. J. Oncol. 23 (2003) 693–696.
[18] O. Cohen, A. Kimchi, DAP-kinase: from functional gene cloning to establishment of its role in apoptosis and cancer, Cell Death Differ. 8 (2001) 6–15.

Please cite this article in press as: M.T. Voso, et al., Epigenetic changes in therapy-related MDS/AML, Chem. Biol. Interact. (2009), doi:10.1016/j.cbi.2009.10.013

[19] J.C. Kuo, W.J. Wang, C.C. Yao, P.R. Wu, R.H. Chen, The tumor suppressor DAPK inhibits cell motility by blocking the integrin-mediated polarity pathway, J. Cell Biol. 172 (2006) 619–631.
[20] A. Raval, S.M. Tanner, J.C. Byrd, E.B. Angerman, J.D. Perko, S.S. Chen, B. Hackanson, M.R. Grever, D.M. Lucas, J.J. Matkovic, T.S. Lin, T.J. Kipps, F. Murray, D. Weisenburger, W. Sanger, J. Lynch, P. Watson, M. Jansen, Y. Yoshinaga, R. Rosenquist, P.J. de Jong, P. Coggill, S. Beck, H. Lynch, A. de la Chapelle, C. Plass, Downregulation of death-associated protein kinase 1 (DAPK1) in chronic lymphocytic leukemia, Cell 129 (2007) 879–890.
[21] M.T. Voso, A. Scardocci, F. Guidi, G. Zini, A. Di Mario, L. Pagano, S. Hohaus, G. Leone, Aberrant methylation of DAP-kinase in therapy-related acute myeloid leukemia and myelodysplastic syndromes, Blood 103 (2004) 698–700.
[22] M. Greco, F. D'Alò, A. Scardocci, M. Criscuolo, E. Fabiani, F. Guidi, A. Di Ruscio, L. Ranalli, G. Migliara, L. Pagano, L. Fianchi, P. Chiusolo, S. Hohaus, G. Leone, M.T. Voso, Promoter methylation of DAPK1, E-cadherin and Thrombospondin-1 in de novo and therapy-related Myelodysplastic Syndromes/Acute Myeloid Leukemias, Ann. Oncol. submitted for publication.

Please cite this article in press as: M.T. Voso, et al., Epigenetic changes in therapy-related MDS/AML, Chem. Biol. Interact. (2009), doi:10.1016/j.cbi.2009.10.013