ANTICANCER RESEARCH *35*: 4569-4574 (2015)

Review

The Role of Methylation in Breast Cancer Susceptibility and Treatment

MARIE-CHRISTINE POULIOT, YVAN LABRIE, CAROLINE DIORIO and FRANCINE DUROCHER

CHU de Quebec Research Centre, Laval University, Quebec, Canada

Abstract. *DNA methylation is a critical mechanism of epigenetic modification involved in gene expression programming, that can promote the development of several cancers, including breast cancer. The methylation of CpG islands by DNA methyltransferases is reversible and has been shown to modify the transcriptional activity of key proliferation genes or transcription factors involved in suppression or promotion of cell growth. Indeed, aberrant methylation found in gene promoters is a hallmark of cancer that could be used as non-intrusive biomarker in body fluids such as blood and plasma for early detection of breast cancer. Many biomarker genes have been evaluated for breast cancer detection. However, in the absence of a unique biomarker having the sufficient specificity and sensitivity, a panel of multiple genes should be used. Treatments targeting aberrant methylation by DNA methyltransferase inhibitors, which trigger re-expression of silenced genes, are now available and allow for better treatment efficiency.*

Carcinogenesis is a multi-stage process consisting of initiation, promotion, progression and malignant conversion phases. Thus, a series of several mutations or alterations to certain classes of genes is usually required before the transformation of a normal cell into a cancer cell (1). With the addition of alterations, cancer severity increases until invasion of cancer cells and ultimately death in the absence of treatment. These modifications can occur at the genetic and epigenetic levels. For instance, the introduction of a mutation in a tumor suppressor gene (TSG) is a characteristic that predisposes cells to accumulation of DNA damage and therefore mutations in other genes. In the absence of proper DNA repair, the cell is more prone to becoming cancerous. It is also recognized that epigenetic abnormalities in the expression of key genes, including TSGs, play an important role in general carcinogenesis and particularly in breast cancer development (2-4). An epigenetic alteration such as aberrant DNA methylation does not involve changes in DNA sequence but is a covalent chemical modification of DNA which has a great effect on whole-gene expression. Thus, this altered gene expression leads to many accumulated changes, paving the way for tumorigenesis (5).

This article is freely accessible online.

Correspondence to: Francine Durocher, 2705 Laurier Boulevard, Room R4720 Québec, Québec, Canada, G1V 4G2. Tel: +1 418 525-4444 ext 48508, Fax: +1 418-654-2298, e-mail: francine.durocher@crchudequebec.ulaval.ca

Key Words: DNA methylation, breast cancer, CpG islands, biomarkers, treatment, review.

DNA Methyltransferases and Methylation of CpG islands

DNA methylation is a reversible mechanism that occurs most commonly with an addition of a methyl group in the fifth position of the pyrimidine ring of cytosine on CpG sites within the genome. Human DNA methylation is introduced into the sequence of nucleotides by enzymes of the DNA cytosine methyltransferases family including DNMT1, DNMT3A, DNMT3B and DNMT3L (6).

The DNMT1 protein methylates newly-synthesized strands prior to chromatin packaging and is localized to the replication foci during the S-phase. This highly expressed enzyme is mainly responsible for maintenance of the methylation pattern during replication (7). An increased expression of DNMT1 is observed in many types of cancers and this overexpression is associated with cellular transformation, while reduced DNMT1 expression levels seem to be associated with a protective effect (8, 9).

The DNMT3A and -B proteins allow *de novo* DNA methylation activity *in vitro*, without distinction between unmethylated and hemi-methylated DNA (10). In particular, the expression of DNMT3B is elevated in several human cancer types, while its suppression results in tumor cell apoptosis (11, 12). Indeed, inactivation of the TSG Ras association domain

family 1 isoform A (*RASSF1A*), through promoter hypermethylation triggered by up-regulation of DNMT3B, is a common event in numerous cancers or tumor types (13). Several studies suggest that DNMT3B plays a predominant role over DNMT3A and DNMT1 in breast tumorigenesis, given its overexpression in breast cancer tissues compared to DNMT1 and -3A (14). Although DNMT3L has no catalytic activity on its own, this enzyme promotes the activity of DNMT3A and -B by increasing their binding capacity for the methyl group donor, S-adenosyl-L-methionine (15).

In mammalian tissues, DNA methylation occurs in CpG dinucleotides, and clustering of these elements in the 5' regulatory regions of approximately 60% of genes are referred to as CpG islands (16). Unusual methylation of these CpG islands may lead to silencing of certain genes involved in key proliferation and apoptosis pathways such as TSGs, DNA repair and hormone receptor genes, as well as genes that inhibit angiogenesis (17). Two different DNA methylation mechanisms lead to transcriptional gene repression: CpG dinucleotide methylation inhibits the binding of transcriptional factors to their promoter regulatory elements; and methylated cytosines enhance the recruitment of methylated binding domain proteins, which inhibit binding of transcription factors through inactive chromatin configuration state around the genes (18). On the other hand, ten-eleven translocation (TET) proteins such as TET2 can remove DNA methyl marks and trigger re-expression of silenced genes. Of interest, *TET2* has been shown to be mutated and therefore inactivated in many types of cancer (19).

In addition, growing evidence suggests that methylation of regions located upstream of CpG islands, called CpG island shores, as well as intragenic sequences, are also important for regulation of gene expression and can be involved in disease development and progression (20, 21). Generally in normal cells, gene promoters are unmethylated, while gene bodies and intergenic regions are methylated.

DNA Methylation as Biomarker for Breast Cancer Detection

Magnetic resonance imaging and digital mammography represent the current screening diagnostic methods largely used for breast cancer detection. However, these techniques lack sensitivity and specificity and are considered invasive methods (22). As an alternative approach, assessment of tumor biomarkers could be used as a non-invasive and valuable method for early breast cancer detection. Indeed, high-sensitivity and specificity biomarkers can be detected in accessible tissues or body fluids, thus rendering the detection test easier and cost-efficient. Because DNA methylation pattern alterations are one of the earliest modifications occurring in the process of cancer development, they can be helpful for early detection (23).

Analysis of DNA methylation can be assessed through different methods including bisulfite conversion. This can be performed in combination with real-time methylation-specific PCR and specific primers to amplify CpG islands of a panel of selected genes (2). A genome-wide approach with high-throughput sequencing can also be performed using bisulfite-converted DNA from different non-invasive biological sources such as whole blood, serum and plasma.

Using a genome-wide approach and blood samples from breast cancer cases and controls, several promising epigenetic markers for early detection have been identified. For instance, Yang *et al*. discovered that hyaluronoglucosaminidase 2 (*HYAL2*) CpG islands were significantly hypomethylated in peripheral blood from breast cancer cases compared to controls (24). Given that the HYLA2 locus is hypermethylated in malignant breast cancer tissue, this strongly suggests that *HYAL2* hypomethylation in blood represents an early peripheral cancer biomarker that does not originate from circulating tumor DNA. They concluded that the *HYAL2* methylation level in blood has a power to distinguish very early breast cancer cases from controls with 64% sensitivity and 90% specificity. Another similar study performed on plasma of affected and unaffected women identified hypermethylation status of the kinesin family member 1A (*KIF1A*) promoter in breast cancer cases. Thus, they conclude that assessment of *KIF1A* promoter methylation level in plasma could also represent an early breast cancer biomarker (2). Furthermore, twin studies of DNA methylation revealed that docking protein 7 (*DOK7*) promoter was hypermethylated in blood of affected patients compared to their twin. This hypermethylation was apparent several years before the diagnosis, suggesting that alteration of *DOK7* is an early event in tumorigenesis. Therefore, *DOK7* promoter methylation level could potentially represent a biomarker for early detection of breast cancer (25).

DNA methylation status within and outside of CpG islands of a certain number of genes have also been studied, and some of them have been linked to breast cancer risk. In the study conducted by Kloten *et al*., the methylation status of a panel of TSGs (secreted frizzled-related protein (*SFRP1, SFRP2, SFRP5*), inter-alpha-trypsin inhibitor heavy chain family, member 5 (*ITIH5*), WNT inhibitory factor 1 (*WIF1*), dickkopf WNT signaling pathway inhibitor 3 (*DKK3*) and *RASSF1A*) was evaluated in circulating cell-free DNA (26). *DKK3* and *ITIH5* CpG islands were found unmethylated in women with benign disease or without breast cancer, and significantly hypermethylated in affected women. They hypothesized that promoter methylation of *DKK3* and *ITIH5* in blood could be used as a biomarker mainly in patients with dense breast tissue, while *RASSF1A* CpG islands methylation did not represent a good biomarker given its low rate of detection in healthy women. Brennan *et al*. studied DNA methylation status located outside of CpG island

clusters of ATM serine/threonine kinase (*ATM*) and long interspersed nuclear element 1 (*LINE1*) repetitive elements in a large cohort of women with and without breast cancer using white blood cells (27). They observed higher methylation of the *ATM*mvp2a locus in breast cancer cases compared to controls, and concluded that that region could be used as a biomarker for breast cancer risk. They also found that the association of *ATM* methylation with breast cancer risk was more reliable in younger women and that this biomarker remains stable over time for at least six years.

Global methylation profile in leukocyte DNA has also been assessed, and Kuchiba *et al*. found that the level of global methylation was lower in breast cancer cases than controls. In addition to gene hypermethylation, global hypomethylation was also associated with an increased risk of cancer (28). Indeed, hypomethylation leads to genomic instability, reactivation of transposable elements, and loss of imprinting, all of which promoting cancer development (29).

Breast Cancer Treatment Through Regulation of Methylation

Early-stage detection (stages I and II) and treatment of breast cancer improve the 5-year survival rate (>93%) in comparison to a late-stage diagnosis for patients with metastatic cancer (stage IV: 22%) (30). Numerous TSGs (*e.g*. DNA repair, apoptosis, hormone receptor, cell cycle and transcription factor genes) have been identified as being differentially methylated in breast cancer and may be good therapeutic targets; breast cancer treatment through regulation of proteins involved in methylation processes is also promising.

Potential breast cancer treatments include regulation of methylation activity that can be achieved using DNA methyltransferase inhibitors. A lower DNA methyltransferase activity inhibits tumor growth through an increased expression of silenced genes such as TSGs, estrogen receptor alpha, E-cadherin and SFRPs. Cytidine analogs such as decitabine (5-aza-2'-deoxycytidine) and 5-azacytidine act as DNMT inhibitors and thus can reactivate expression of key genes through depletion of DNMT1 (32). Both these analogs were approved by the U.S. Food Drug Administration for the treatment of myelodysplastic syndrome. These DNMT inhibitor residues are incorporated into DNA during the S-phase of the replication process and establish irreversible bonds with DNA methyltransferase enzymes to prevent their action (33). A large range of efficacy was reported in breast cancer studies aiming to assess the *in vivo* action of these compounds on solid breast tumors (34). However, in addition to their weak stability and lack of specificity for cancer cells, these drugs are rapidly inactivated by the action of cytidine deaminase. Therefore, these drugs have serious limitations for treatment of advanced solid tumors, including breast cancer. These unfavorable characteristics led to the development of new DNMT inhibitors namely zebularine, SGI-110 and NPEOC-DAC, which are more selective for cancer cells and show higher resistance to deamination.

Zebularine has a potent inhibitory effect on both DNMTs and cytidine deaminase. This drug has been shown to inhibit cell growth of mammary tumors and triggers necrosis and apoptosis of early-onset tumor cells in transgenic mice that develop mammary tumors (35). Zebularine in combination with decitabine was shown to exert a significant inhibitory effect on cell proliferation and colony formation in MDA-MB-231 breast cancer cell line through induction of estrogen receptor alpha and progesterone receptor mRNA expression (36). Although zebularine initially demonstrated promising effects associated with its higher selectivity for cancer cells, its toxicity renders this molecule less attractive for breast cancer treatment.

SGI-110 is a modified dinucleotide showing enhanced resistance to cytidine deaminase and increased half-life compared to decitabine and 5-azacytidine (37). This short oligonucleotide could be envisaged to ensure effective delivery of the nucleotide drug and protection from deamination. NPEOC-DAC is a metabolic precursor of the decitabine molecule and exerts dose-dependent repressive effects on DNA methylation (38).

Several other natural compounds containing specific molecules such as anthocyanines and polyphenols have also been shown to possess anti-DNMT activity [for review see (6)]. In addition, knockdown of *DNMT1* achieved using small interference RNA led to promising results in HCT116 colon cancer cells (39).

Given that side effects of DNMT inhibition include the concomitant activation of both TSGs and proto-oncogenes, some studies assessed combinations of cytidine analogs with chemotherapeutic agents, immunotherapy or knockdown of specific genes such as methyl-CpG binding domain protein 2 (*MBD2*) and lysine (K)-specific demethylase 1B (*KDM1B*/*LSD2*).

Vijayaraghavalu *et al*. noted a significant enhancement of doxorubicin treatment efficiency in MCF-7, MDA-MB-231 and BT-459 cells when combined with decitadine (40). This dual treatment triggered cell cycle phase arrest for over 90% of cells and restored sensitivity to doxorubicin through up-regulation of *p21* proto-oncogene expression to overcome drug resistance of these breast cancer cells. In another study, Wrangle *et al*. assessed the sequential combination of 5-azacytidine and immunotherapy. Their results revealed that a combined epigenetic therapy using DNA methyltransferase inhibitor and programmed death pathway blockade produced a synergistic anti-tumorigenic response in patients with non-small cell lung cancer (41).

MBD2 has been shown to silence methylated genes on one hand, and on the other, to be involved in activation of gene

expression through its ability to interact with gene promoters. A recent study reported that a treatment combining methyltransferase inhibitor (5-azacytidine) administration and suppression of *MBD2* expression using RNA interference technology led to activation of apoptosis and reduced cell growth, as well as inactivation of invasion and metastatic processes in breast cancer cells (42). They concluded that depletion of *MBD2* antagonizes the activation effect on proto-oncogenes resulting from hypomethylation treatment. The same strategy including DNA methyltransferase inhibition and *LSD2* knockdown was also efficient for cell growth inhibition in MDA-MB-231 and MCF-7 breast cancer cells. This treatment enhanced expression of epigenetically silenced genes such as those encoding progesterone receptor and estrogen receptor alpha (43).

Conclusion

In the last decade, several gene expression signatures have been established for characterization and subtyping of breast tumors. However, methylation is now considered as a major player involved in gene expression regulation and unlike RNA transcription profiles, which represent snapshots of the transcription activity at a specific time, DNA methylation signature constitutes a more stable and long-lasting marker of the molecular state and cancer predisposition of a cell.

Although numerous models of metastatic processes have been proposed, recent insights suggest that the metastatic capacity of breast tumors is an inherent feature of the genetic background of the host. Before such a metastatic process, epigenetic change is believed to occur early in the process of breast carcinogenesis, and the methylation profile has been demonstrated to be representative, to a certain extent, of the genetic background of individuals.

Hence, all these observations support the notion that assessment of the methylation profile in body fluids could represent a promising clinical avenue for pinpointing women predisposed to or affected with breast cancer. Given that circulating cell-free DNA in blood plasma contains tumor-specific mutations and DNA methylation patterns associated with the disease, the identification of new precursor biomarkers of potential cancer predisposition or aggressiveness in such DNA would be a huge advance in predictive medicine for targeting women at high risk of developing breast cancer. Non-invasive testing such as blood-borne screening is a more convenient and cost-efficient methodology compared to mammography and magnetic resonance imaging. However, mainly due to the limited number of affected and matched control DNA samples included in studied cohorts, no specific methylation biomarker has been yet validated for clinical use. In the next years, the broader availability of blood samples combined with epigenome-wide studies could definitely contribute to the establishment of a panel of breast cancer biomarkers in order to improve breast cancer detection and diagnostic of women.

Although the action and effect of a few DNMT inhibitors have been reported as being of benefit in some types of cancers, further studies regarding the *in vivo* action of de-methylating agents in solid tumors is highly warranted. The effect of DNMT inhibitors requires cell division and proliferation, and therefore repeated treatment is necessary to enhance their efficiency (44). In addition, several factors including toxicity, lack of specificity and low stability, as well as the simultaneous activation of proto-oncogenes, promote the development of new inhibitors. However, combination of these molecules with other treatments such as chemotherapeutic agents and RNA interference has been demonstrated to yield promising results.

In summary, epigenomics combined with a larger access to non-invasive biological material will be crucial over the increased understanding of the biology of breast cancer and development of personalized therapy to provide a better outlook for patients with breast cancer.

References

1 Weinstein IB, Begemann M, Zhou P, Han EK, Sgambato A, Doki Y, Arber N, Ciaparrone M and Yamamoto H: Disorders in cell circuitry associated with multistage carcinogenesis: exploitable targets for cancer prevention and therapy. Clin Cancer Res *3*: 2696-2702, 1997.

2 Guerrero-Preston R, Hadar T, Ostrow KL, Soudry E, Echenique M, Ili-Gangas C, Pérez G, Perez J, Brebi-Mieville P, Deschamps J, Morales L, Bayona M, Sidransky D and Matta J: Differential promoter methylation of kinesin family member 1a in plasma is associated with breast cancer and DNA repair capacity. Oncol Rep *32*: 505-512, 2014.

3 Kanwal R and Gupta S: Epigenetic modifications in cancer. Clin Genet *81*: 303-311, 2012.

4 Kanwal R and Gupta S: Epigenetics and cancer. J Appl Physiol (1985) *109*: 598-605, 2010.

5 Santos-Rebouças CB and Pimentel MMG: Implication of abnormal epigenetic patterns for human diseases. Eur J Hum Genet *15*: 10-17, 2007.

6 Subramaniam D, Thombre R, Dhar A and Anant S: DNA methyltransferases: a novel target for prevention and therapy. Front Oncol *4*: 80, 2014.

7 Kar S, Deb M, Sengupta D, Shilpi A, Parbin S, Torrisani J, Pradhan S and Patra SK: An insight into the various regulatory mechanisms modulating human DNA methyltransferase 1 stability and function. Epigenetics *7*: 994-1007, 2012.

8 Wu J, Issa J-P, Herman J, Bassett DE, Nelkin BD and Baylin SB: Expression of an exogenous eukaryotic DNA methyltransferase gene induces transformation of NIH 3T3 cells. Proc Natl Acad Sci USA *90*: 8891-8895, 1993.

9 Belinsky SA, Nikula KJ, Baylin SB and Issa J-P: Increased cytosine DNA-methyltransferase activity is target-cell-specific and an early event in lung cancer. Proc Natl Acad Sci USA *93*: 4045-4050, 1996.

10 Okano M, Bell DW, Haber DA and Li E: DNA methyltransferases Dnmt3a and Dnmt3b are essential for *de novo* methylation and mammalian development. Cell *99*: 247-257, 1999.
11 Beaulieu N, Morin S, Chute IC, Robert M-F, Nguyen H and MacLeod AR: An essential role for DNA methyltransferase DNMT3B in cancer cell survival. J Biol Chem *277*: 28176-28181, 2002.
12 Robertson KD, Uzvolgyi E, Liang G, Talmadge C, Sumegi J, Gonzales FA and Jones PA: The human DNA methyltransferases (DNMTs) 1, 3a and 3b: coordinate mRNA expression in normal tissues and overexpression in tumors. Nucleic Acids Res *27*: 2291-2298, 1999.
13 Palakurthy RK, Wajapeyee N, Santra MK, Gazin C, Lin L, Gobeil S and Green MR: Epigenetic silencing of the *RASSF1A* tumor suppressor gene through HOXB3-mediated induction of *DNMT3B* expression. Mol Cell *36*: 219-230, 2009.
14 Girault I, Tozlu S, Lidereau R and Bièche I: Expression analysis of DNA methyltransferases 1, 3A, and 3B in sporadic breast carcinomas. Clin Cancer Res *9*: 4415-4422, 2003.
15 Kareta MS, Botello ZM, Ennis JJ, Chou C and Chédin F: Reconstitution and mechanism of the stimulation of *de novo* methylation by human DNMT3L. J Biol Chem *281*: 25893-25902, 2006.
16 Craig JM and Bickmore WA: The distribution of CpG islands in mammalian chromosomes. Nat Genet *7*: 376-382, 1994.
17 Jovanovic J, Rønneberg JA, Tost J and Kristensen V: The epigenetics of breast cancer. Mol Oncol *4*: 242-254, 2010.
18 Nan X, Campoy FJ and Bird A: MeCP2 is a transcriptional repressor with abundant binding sites in genomic chromatin. Cell *88*: 471-481, 1997.
19 Yang H, Liu Y, Bai F, Zhang J-Y, Ma S-H, Liu J, Xu Z-D, Zhu H-G, Ling Z-Q, Ye D, Guan K-L and Xiong Y: Tumor development is associated with decrease of *TET* gene expression and 5-methylcytosine hydroxylation. Oncogene *32*: 663-669, 2013.
20 Flanagan JM, Munoz-Alegre M, Henderson S, Tang T, Sun P, Johnson N, Fletcher O, Santos Silva Dos I, Peto J, Boshoff C, Narod S and Petronis A: Gene-body hypermethylation of *ATM* in peripheral blood DNA of bilateral breast cancer patients. Hum Mol Genet *18*: 1332-1342, 2009.
21 Choufani S, Shapiro JS, Susiarjo M, Butcher DT, Grafodatskaya D, Lou Y, Ferreira JC, Pinto D, Scherer SW, Shaffer LG, Coullin P, Caniggia I, Beyene J, Slim R, Bartolomei MS and Weksberg R: A novel approach identifies new differentially methylated regions (DMRs) associated with imprinted genes. Genome Res *21*: 465-476, 2011.
22 Brekelmans CT, Seynaeve C, Bartels CC, Tilanus-Linthorst MM, Meijers-Heijboer EJ, Crepin CM, van Geel AA, Menke M, Verhoog LC, van den Ouweland A, Obdeijn IM and Klijn JG: Effectiveness of breast cancer surveillance in *BRCA1/2* gene mutation carriers and women with high familial risk. J Clin Oncol *19*: 924-930, 2001.
23 Moulton T, Crenshaw T, Hao Y, Moosikasuwan J, Lin N, Dembitzer F, Hensle T, Weiss L, McMorrow L, Loew T, Kraus W, Gerald W and Tycko B: Epigenetic lesions at the H19 locus in Wilms' tumour patients. Nat Genet *7*: 440-447, 1994.
24 Yang R, Pfütze K, Zucknick M, Sutter C, Wappenschmidt B, Marme F, Qu B, Cuk K, Engel C, Schott S, Schneeweiss A, Brenner H, Claus R, Plass C, Bugert P, Hoth M, Sohn C, Schmutzler R, Bartram CR and Burwinkel B: DNA methylation array analyses identified breast cancer associated HYAL2 methylation in peripheral blood. Int J Cancer, *136*: 1845-1855, 2015.
25 Heyn H, Carmona FJ, Gomez A, Ferreira HJ, Bell JT, Sayols S, Ward K, Stefansson OA, Moran S, Sandoval J, Eyfjord JE, Spector TD and Esteller M: DNA methylation profiling in breast cancer discordant identical twins identifies DOK7 as novel epigenetic biomarker. Carcinogenesis *34*: 102-108, 2013.
26 Kloten V, Becker B, Winner K, Schrauder MG, Fasching PA, Anzeneder T, Veeck J, Hartmann A, Knüchel R and Dahl E: Promoter hypermethylation of the tumor-suppressor genes ITIH5, DKK3, and RASSF1A as novel biomarkers for blood-based breast cancer screening. Breast Cancer Research *15*: R4, 2013.
27 Brennan K, Garcia-Closas M, Orr N, Fletcher O, Jones M, Ashworth A, Swerdlow A, Thorne H, Riboli E, Vineis P, Dorronsoro M, Clavel-Chapelon F, Panico S, Onland-Moret NC, Trichopoulos D, Kaaks R, Khaw K-T, Brown R and Flanagan JM: Intragenic ATM methylation in peripheral blood DNA as a biomarker of breast cancer risk. Cancer Res *72*: 2304-2313, 2012.
28 Kuchiba A, Iwasaki M, Ono H, Kasuga Y, Yokoyama S, Onuma H, Nishimura H, Kusama R, Tsugane S and Yoshida T: Global methylation levels in peripheral blood leukocyte DNA by LUMA and breast cancer: a case-control study in Japanese women. Br J Cancer *110*: 2765-2771, 2014.
29 Esteller M: Epigenetic gene silencing in cancer: the DNA hypermethylome. Hum Mol Genet 16 Spec No *1*: R50-9, 2007.
30 American Cancer Society: Breast cancer survival rates by stage. http://wwwcancerorg/cancer/breastcancer/detailedguide/breast-cancer-survival-by-stage (last accessed November 14, 2014).
31 Xiang T-X, Yuan Y, Li L-L, Wang Z-H, Dan L-Y, Chen Y, Ren G-S and Tao Q: Aberrant promoter CpG methylation and its translational applications in breast cancer. Chin J Cancer *32*: 12-20, 2013.
32 Santi DV, Garrett CE and Barr PJ: On the mechanism of inhibition of DNA-cytosine methyltransferases by cytosine analogs. Cell *33*: 9-10, 1983.
33 Creusot F, Acs G and Christman JK: Inhibition of DNA methyltransferase and induction of Friend erythroleukemia cell differentiation by 5-azacytidine and 5-aza-2'-deoxycytidine. J Biol Chem *257*: 2041-2048, 1982.
34 Appleton K, Mackay HJ, Judson I, Plumb JA, McCormick C, Strathdee G, Lee C, Barrett S, Reade S, Jadayel D, Tang A, Bellenger K, Mackay L, Setanoians A, Schätzlein A, Twelves C, Kaye SB and Brown R: Phase I and pharmacodynamic trial of the DNA methyltransferase inhibitor decitabine and carboplatin in solid tumors. J Clin Oncol *25*: 4603-4609, 2007.
35 Chen M, Shabashvili D, Nawab A, Yang SX, Dyer LM, Brown KD, Hollingshead M, Hunter KW, Kaye FJ, Hochwald SN, Marquez VE, Steeg P and Zajac-Kaye M: DNA methyltransferase inhibitor, zebularine, delays tumor growth and induces apoptosis in a genetically engineered mouse model of breast cancer. Mol Cancer Ther *11*: 370-382, 2012.
36 Billam M, Sobolewski MD and Davidson NE: Effects of a novel DNA methyltransferase inhibitor zebularine on human breast cancer cells. Breast Cancer Res Treat *120*: 581-592, 2010.
37 Yoo CB, Jeong S, Egger G, Liang G, Phiasivongsa P, Tang C, Redkar S and Jones PA: Delivery of 5-aza-2'-deoxycytidine to cells using oligodeoxynucleotides. Cancer Res *67*: 6400-6408, 2007.

38 Byun H-M, Choi SH, Laird PW, Trinh B, Siddiqui MA, Marquez VE and Yang AS: 2'-Deoxy-N4-[2-(4-nitrophenyl)ethoxycarbonyl]-5-azacytidine: A novel inhibitor of DNA methyltransferase that requires activation by human carboxylesterase 1. Cancer Lett *266*: 238-248, 2008.

39 Morita R, Hirohashi Y, Suzuki H, Takahashi A, Tamura Y, Kanaseki T, Asanuma H, Inoda S, Kondo T, Hashino S, Hasegawa T, Tokino T, Toyota M, Asaka M, Torigoe T and Sato N: DNA methyltransferase 1 is essential for initiation of the colon cancers. Exp Mol Pathol *94*: 322-329, 2013.

40 Vijayaraghavalu S, Dermawan JK, Venugopalan C and Labhasetwar V: Highly synergistic effect of sequential treatment with epigenetic and anticancer drugs to overcome drug resistance in breast cancer cells is mediated *via* activation of p21 gene expression leading to G_2/M cycle arrest. Mol Pharm *10*: 337-352, 2013.

41 Wrangle J, Wang W, Koch A, Easwaran H, Mohammad HP, Pan X, Vendetti F, Vancriekinge W, Demeyer T, Du Z, Parsana P, Rodgers K, Yen R-W, Zahnow CA, Taube JM, Brahmer JR, Tykodi SS, Easton K, Carvajal RD, Jones PA, Laird PW, Weisenberger DJ, Tsai S, Juergens RA, Topalian SL, Rudin CM, Brock MV, Pardoll D and Baylin SB: Alterations of immune response of non-small cell lung cancer with azacytidine. Oncotarget *4*: 2067-2079, 2013.

42 Cheishvili D, Chik F, Li CC, Bhattacharyya B, Suderman M, Arakelian A, Hallett M, Rabbani SA and Szyf M: Synergistic effects of combined DNA methyltransferase inhibition and MBD2 depletion on breast cancer cells; MBD2 depletion blocks 5-aza-2'-deoxycytidine triggered invasiveness. Carcinogenesis: *35*: 2436-2446, 2014.

43 Katz TA, Vasilatos SN, Harrington E, Oesterreich S, Davidson NE and Huang Y: Inhibition of histone demethylase, LSD2 (KDM1B), attenuates DNA methylation and increases sensitivity to DNMT inhibitor-induced apoptosis in breast cancer cells. Breast Cancer Res Treat *146*: 99-108, 2014.

44 Nie J, Liu L, Li X and Han W: Decitabine, a new star in epigenetic therapy: the clinical application and biological mechanism in solid tumors. Cancer Lett *354*: 12-20, 2014.

Received April 23, 2015
Revised May 25, 2015
Accepted May 27, 2015