Aberrant promoter CpG methylation and its translational applications in breast cancer

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Abstract

Breast cancer is a complex disease driven by multiple factors including both genetic and epigenetic alterations. Recent studies revealed that abnormal gene expression induced by epigenetic changes, including aberrant promoter methylation and histone modification, plays a critical role in human breast carcinogenesis. Silencing of tumor suppressor genes (TSGs) by promoter CpG methylation facilitates cells growth and survival advantages and further results in tumor initiation and progression, thus directly contributing to breast tumorigenesis. Usually, aberrant promoter methylation of TSGs, which can be reversed by pharmacological reagents, occurs at the early stage of tumorigenesis and therefore may serve as a potential tumor marker for early diagnosis and therapeutic targeting of breast cancer. In this review, we summarize the epigenetic changes of multiple TSGs involved in breast pathogenesis and their potential clinical applications as tumor markers for early detection and treatment of breast cancer.

Key words Breast cancer, tumor suppressor gene, CpG, methylation, tumor marker

Breast cancer is the most prevalent tumor and a major cause of morbidity and mortality among women worldwide[1]. Over the past decades, there has been a significant increase in breast cancer incidence, with more than one million new cases each year[2]. Diagnosis of breast cancer at the early stage results in a high survival rate (~98%), whereas diagnosis at the advanced stage results in a significantly lower survival rate (~27%)[3]. Due to the lack of early detection methods for breast cancer, current therapies are necessary but not sufficient to improve the survival of women with breast cancer. Thus, identification of tumor markers for the early detection and therapeutic targeting of breast cancer is essential.

Although much progress has been made in understanding the biology of breast cancer, its etiology is still not very clear. Activation of oncogenes and inactivation of tumor suppressor genes (TSGs) synergistically contribute to the cancer progression. Early studies have demonstrated that genetic alterations, such as chromosomal translocations and point mutations, are responsible for TSG inactivation. Recently, accumulating evidence indicates that epigenetic alterations provide an alternative yet important mechanism for TSG silencing. Epigenetic modifications include DNA methylation and histone modifications, which cooperatively affect chromatin structure and genomic stability[4,5]. Epigenetic modifications play important roles in the regulation of cell cycle, apoptosis, signal transduction, and tumorigenesis[6].

A series of TSGs silenced by promoter CpG methylation have been identified in breast cancer, indicating aberrant methylation of TSGs as a key factor in breast cancer pathogenesis. In addition, breast cancer usually progresses gradually from a less aggressive, hormone-dependent to a highly invasive, hormone-
independent phenotype \cite{18-23}, implying that silencing of hormone-mediated TSGs occurs in breast cancer progression. Frequent TSG methylation in breast cancer makes it a potentially useful marker for disease diagnosis. Here, we summarize some recent studies on epigenetic alterations in breast cancer and further discuss their biological and clinical implications.

**Aberrant Promoter Methylation Silences Critical TSGs in Breast Carcinoma**

Although inactivating mutations of TSGs have been well documented in familial breast cancer, the incidence of mutations in sporadic breast cancer is rare. Yet, a large body of evidence has demonstrated that epigenetic aberration is a key player in silencing a variety of TSGs, which triggers breast tumor progression \cite{10-16}. Identification of an epigenetic gene profile for breast cancer will thus be helpful to elucidate the molecular mechanisms underlying breast cancer pathogenesis.

As shown in Table 1, a series of promoter-methylated key TSGs involved in breast tumorigenesis have been reported, including genes for cell cycle regulation (p16INK4a, p14ARF, p15, 14-3-3-\(\alpha\), cyclin D2, p57KIP2), DNA repair (glutathione S-transferase pi 1 (GSTP1), O-6-methylguanine-DNA methyltransferase (MGMT), breast cancer 1 (BRCA1), human mutL homolog 1 (hMLH1)), hormone and receptor-mediated cell signaling pathways [estrogen receptor alpha (ER\(\alpha\)), progesterone receptor (PR), retinoic acid receptor beta (RAR\(\beta\)), Ras association domain family member 1 (RASSF1A), spleen tyrosine kinase (SYK), TGF\(\beta\) receptor II (TGF\(\beta\) RII), high in normal 1 (HIN1), normal epithelial cell-specific 1 (NES1), suppressor of cytokine signaling 1 (SOCS1), secreted frizzled-related protein 1 (SFRP1), WNT inhibitory factor 1 (WIF1), apoptosis [adenomatous polyposis coli (APC), death-associated protein kinase (DAPK1), hypermethylated in cancer 1 (HIC1), homeobox A5 (HOXA5), TWIST homolog of drosophila (TWIST)], target of methylation-induced silencing (TMS1), cell adhesion and metastasis [cadherin 1 (CDH1), cadherin 13 (CDH13), APC, tissue inhibitor of metalloproteinases 3 (TIMP3)], angiogenesis [maspin, thrombospondin 1 (THBS1)], and other processes. These epigenetic changes may lead to chromosomal instability, accumulated mutations in critical cell signaling pathways, ultimately contributing to breast cancer progression. Our group has also identified a series of TSGs silenced or down-regulated by promoter CpG methylation in breast cancer, such as WIF1, phospholipase C delta 1 (PLCD1), CKLF-like MARVEL transmembrane domain containing 5 (CMTM5), CKLF-like MARVEL transmembrane domain containing 3 (CMTM3), opioid binding protein/cell adhesion molecule-like (OPCML), ubiquitin carboxyl-terminal esterase L1 (UCHL1), deleted in liver cancer 1 (DLC1), interferon regulatory factor 8 (IRF8), and dapper homolog 1 (DACT1), that are involved in breast tumor cell cycle control, apoptosis, and metastasis\cite{10-16}.

**Clinical Implications of Promoter Methylation in Breast Cancer**

Understanding the critical roles of promoter CpG methylation–mediated transcriptional repression/silencing in breast cancer led us to consider its potential clinical applications. Here, we further discuss the usage of DNA methylation markers for early cancer detection and prognosis prediction, as well as the application of demethylation drugs in breast cancer therapy.

**Aberrant promoter methylation as a marker for early detection and prognosis in breast cancer**

Women diagnosed with early-stage breast cancer have a better prognosis and require less severe treatment regimens than those diagnosed with advanced stage diseases. With the development of magnetic resonance imaging (MRI) and digital mammography, the accuracy of breast cancer detection has distinctly increased. However, current screening methods lack sensitivity and specificity \cite{17}, and new methods with higher sensitivity, specificity, and lower invasiveness are urgently required. Using tumor markers thus provides a valuable alternative approach.

In recent years, many studies demonstrated that aberrant DNA methylation has strong potential to serve as a novel tumor marker for early diagnosis and progression evaluation. First, aberrant promoter methylation of a series of TSGs is a common feature of malignancy; compared to gene mutations and copy number alternations, it occurs more frequently as an early event in tumorigenesis. Importantly, different types of tumors present distinctive promoter methylation profiles, which can improve specificity and sensitivity for tumor detection. Second, aberrant promoter methylation patterns can be detected even when they are embedded in an excess amount of normal DNA molecules. Third, techniques required for the detection of methylation patterns, such as methylation-specific PCR (MSR), MethyLight, and quantitative multiplex methylation-specific PCR (QM-MSP), are relatively simple, rapid, non-radioactive, and sensitive.

A number of cancer-specific genes have been found to be frequently methylated in breast cancer. These epigenetic biomarkers show promise for distinguishing between malignant and benign disease or normal tissue \cite{16-20}. Further, considering that no single methylated gene was detected in any breast cancer types, it is
| Gene name  | Chr location | Reported percentage of methylation in breast tumors | Reported percentage of methylation in breast cancer cell lines | Major functions                                                                 | Reference(s) |
|------------|--------------|--------------------------------------------------|-------------------------------------------------------------|---------------------------------------------------------------------------------|--------------|
| ATM        | 11q22–q23    | 78%                                              | –                                                           | DNA repair, cell cycle regulator                                               | [60]         |
| ABCB1      | 7q21.12      | –                                                | –                                                           | Multidrug resistance                                                           | [61,62]      |
| APC        | 5q21–q22     | 36%–44%                                         | 44%                                                         | Cell polarity and chromosome segregation                                         | [63,64]      |
| BECN1      | 17q21        | –                                                | –                                                           | Autophagy                                                                      | [65]         |
| BIN1       | 2q14         | 18%                                              | 100%                                                        | Apoptosis                                                                       | [66]         |
| BMP4       | 6p24–p23     | –                                                | –                                                           | Regulate TGFβ signaling pathway                                                | [3]          |
| BRCA1      | 17q12–21     | 51%                                              | –                                                           | Cell cycle regulator, DNA repair, transcription, regulation, apoptosis          | [67,68]      |
| CMTM5      | 14q11.2      | –                                                | 100%                                                        | Apoptosis                                                                       | [14]         |
| CMTM3      | 16q22.1      | 18%                                              | 44%                                                         | Apoptosis                                                                       | [10]         |
| C/EBPα     | 8p11.2–p11.1 | –                                                | –                                                           | Cellular proliferation, differentiation, metabolism, inflammatory response      | [69]         |
| CDKN1C     | 11p15.5      | –                                                | 85%                                                         | Cell cycle regulator                                                           | [70]         |
| CST6       | 11q13        | 48%–60%                                          | –                                                           | Inhibit cystein proteases activity                                              | [71,72]      |
| CDH2       | 18q22.1      | –                                                | –                                                           | Calcium-dependent adhesion                                                     | [62,73]      |
| CDH13      | 16q24.2–3    | 33%                                              | 35%                                                         | Cell adhesion, proliferation, metastasis                                         | [74]         |
| CDK2       | 1q25.2–q25.3 | 35%                                              | –                                                           | Cycloxygenase-2; inflammation, mitogenesis                                       | [75]         |
| CDK1       | 5q23.2       | –                                                | –                                                           | Unknown                                                                         | [76]         |
| CIDEA      | 18p11.2      | 53%                                              | 86%                                                         | Caspase-independent cell death                                                 | [77]         |
| Cyclin D2  | 12p          | 46%                                              | –                                                           | Cell cycle regulator                                                           | [78]         |
| DBC1       | 9q32–33      | 26%                                              | 33%                                                         | Unknown                                                                         | [77]         |
| DAPK       | 9q34.1       | –                                                | –                                                           | Apoptosis                                                                       | [79]         |
| DKK1       | 10q11.2      | 19%                                              | 27%                                                         | Wnt pathway inhibitor                                                          | [80]         |
| DKK3       | 11p15.2      | 61.30%                                           | 71%                                                         | Wnt pathway inhibitor                                                          | [81]         |
| Dlc1       | 8p22         | 36%                                              | 22%–33%                                                     | Signal transduction, cell adhesion                                              | [15,82]      |
| ER         | 6q25.1       | 49%                                              | –                                                           | Estrogen receptor                                                               | [83–85]      |
| E-cadherin | 16q22.1      | 48%                                              | 38%–55%                                                     | Cell adhesion, proliferation, metastasis                                        | [75,86]      |
| EMLIN2     | 18p11.3      | 44%                                              | 56%                                                         | Extracellular matrix glycoproteins                                             | [77]         |
| EDN3       | 20q13.2–q13.3| 70%                                              | 83%                                                         | Unknown                                                                         | [87]         |
| EPHA5      | 4q13.1       | 64%                                              | –                                                           | Unknown                                                                         | [88]         |
| FLJ25161   | 3p14.1       | 57%                                              | 75%                                                         | Unknown                                                                         | [89]         |
| FBXW7      | 4q31.3       | 50%                                              | –                                                           | Cell cycle control                                                             | [90]         |
| FBLN2      | 3p25.1       | 34%                                              | 56%                                                         | Cell motility and invasion                                                     | [77]         |
| FOXC1      | 6p25         | 50%                                              | –                                                           | Embryonic development                                                          | [62]         |
| GADD45a    | 1p31.2–p31.1 | 67%                                              | 67%                                                         | Growth arrest and DNA repair                                                   | [91]         |
| GSTP1      | 11q13        | 31%                                              | 44%                                                         | Glutathione transferase activity                                               | [92]         |
| HER4       | 2q34         | –                                                | –                                                           | Tyrosine kinase-type cell surface receptor                                      | [93]         |
| HIC1       | 17p13.3      | –                                                | –                                                           | Transcription Factor                                                           | [94]         |
| HIN1       | 5q35–qter    | 65%                                              | –                                                           | Cell communication, signal transduction                                         | [95,96]      |
| HMLH1      | 3p21.3       | 14%                                              | –                                                           | DNA repair                                                                      | [62]         |
| HOXD11     | 2q31.1       | 75%                                              | 100%                                                        | Transcription factor, morphogenesis                                            | [89]         |
| HOXA5      | 7p15–p14     | 80%                                              | –                                                           | Transcription regulation                                                       | [97]         |
| IGF2       | 11p15.5      | –                                                | –                                                           | Unknown                                                                         | [98]         |
| IRF8       | 16q24.1      | 36%                                              | 66%                                                         | Transcription factor                                                           | [16]         |
| KV1.3      | 1p13.3       | 42.30%                                           | –                                                           | Apoptosis                                                                       | [99]         |
| OPCML      | 11q25        | 91%                                              | 90%                                                         | Cell adhesion                                                                   | [11]         |
| PC0H10     | 4q28.3       | 43%                                              | 88%                                                         | Apoptosis, metastasis, invasion                                                | [89]         |
| P3H2       | 3q28         | 42%                                              | 46%                                                         | A family of collagen prolyl hydroxylases required for proper collagen biosynthesis, folding, and assembly | [100]        |

(To be continued)
necessary to use cancer-specific methylation marker panels to screen breast cancer\textsuperscript{[24]}. For example, a three-methylated-gene panel (Cyclin D2, RAR\textsubscript{B}, and TWIST) was successfully used to detect malignant breast cancer cells in ductal fluids\textsuperscript{[25]}. A four-methylated-gene panel (RASSF1A, TWIST, HIN1, and Cyclin D2) with a high level of sensitivity and specificity was used to detect malignant breast tissues\textsuperscript{[26]} as well. Compared to conventional ductal lavage cytology, a nine-methylated-gene panel (RASSF1A, TWIST, HIN1, Cyclin D2, RAR\textsubscript{B}, APC, BRCA1, BRCA2, and p16) was developed to double the sensitivity for breast cancer detection\textsuperscript{[27]}. Cancer-specific methylation can also be extended to assess risk and predict prognosis for breast cancer. For example, frequently methylated RASSF1A and APC, as well as methylated GSTP1 and SFRP1\textsuperscript{[28-29]}, were associated with poor outcome\textsuperscript{[28,30]}. Recently, subtype-specific methylation markers were used to evaluate optional targeted treatments in breast cancer\textsuperscript{[30]}. Using an array-based methylation assay, Holm et al.\textsuperscript{[31]} uncovered a subtype-specific methylation pattern for distinguishing different breast tumor phenotypes, which will provide more detailed information for predicting breast cancer prognosis.

Additionally, blood-based detection of cancer-specific methylated DNA in breast cancer has shown potential for early detection and prognostic prediction\textsuperscript{[32-35]}.

Radpour et al.\textsuperscript{[36]} found significant promoter methylation of seven genes (APC, bridging integrator 1 (BIN1), bone morphogenetic protein 6 (BMP6), BRCA1, cystin 6 (CST6), P16, and TIMP3) in serum and tumor tissues from patients with breast cancer by using

| Table 1, Summary of major tumor suppressor genes methylated in breast cancer (continued) |
|---------------------------------------------------------|
| Gene name | Chr location | Reported percentage of methylation in breast tumors | Reported percentage of methylation in breast cancer cell lines | Major functions | Reference(s) |
|-----------|--------------|-------------------------------------------------|-------------------------------------------------|----------------|--------------|
| P3H3      | 12q13        | 26%                                            | 31%                                            | A family of collagen prolyl hydroxylases required for proper collagen biosynthesis, folding, and assembly | [100]          |
| PLCD1     | 3p22–p21.3   | 52%                                            | 78%                                            | Phospholipase activity, cell cycle arrest, metastasis, invasion | [13]           |
| PCDHB6    | 5q31         | 62%                                            | 75%                                            | Cell adhesion | [89]          |
| PGR       | 11q22        | –                                              | –                                              | Progesterone receptor | [101]         |
| PPP2R2B   | 5q32         | 56%–65%                                       | –                                              | Cell growth and division control | [102]         |
| p16/CDKN2A| 9p21         | 0–67%                                         | 33%                                            | Cell cycle arrest | [103–108]      |
| PTEN      | 10q23.3      | 22%–76%                                       | –                                              | Cell cycle arrest, apoptosis, cell adhesion, migration | [62,109]       |
| RAR1      | 3p24         | –                                              | –                                              | Tumor suppressive activity | [110,111]      |
| RRM2      | 1p36.21      | –                                              | –                                              | Apoptosis, metastasis | [112]         |
| RASSF1A   | 3p21.3       | 62%                                            | 100%                                           | Cell cycle control, apoptosis, DNA repair | [113–115]      |
| SIM1      | 6q16.3–q21   | 75%                                            | 100%                                           | Unknown | [89]          |
| SALL1     | 16q12.1      | 63%                                            | 67%                                            | Unknown | [77]          |
| SFRP1     | 8p11–12      | 40%–75%                                       | 30%–100%                                      | Wnt antagonist | [80,116,117]  |
| SFRP2     | 4q31.3       | 77%                                            | 100%                                           | Wnt antagonist | [80]          |
| SFRP5     | 10q24.1      | 71%–73%                                       | 90%–91%                                       | Wnt antagonist | [80,118]      |
| SOX17     | 8q11.23      | 80.6%                                         | –                                              | Growth inhibition, cell cycle arrest | [119]         |
| SREC1     | 1p15         | 60%                                            | –                                              | Unknown | [120]         |
| SYK       | 9q22         | 32%                                            | 30%                                            | Growth inhibition, metastasis | [121]         |
| TIMP3     | 22q12.3      | 27%                                            | 29%                                            | Metastasis, invasion | [122]         |
| TWIST     | 7p21.2       | 5.6%–32%                                      | –                                              | Transcription regulator, EMT | [95,123]      |
| TMS1      | 16p11.2–12.1 | 41%                                            | 77%                                            | Apoptosis | [124]         |
| TGM2      | 20q12        | 44.4%                                         | 5.7%–73.1%                                    | Cell migration | [125]         |
| TRIP10    | 19p13.3      | –                                              | –                                              | Cell migration | [126]         |
| THRB      | 3p24.2       | 100%                                           | 40%                                            | Thyroid hormone receptor | [127]         |
| WIF1      | 12q14.3      | 67%                                            | 80%                                            | Wnt signaling inhibitor | [12]          |
| XT3       | 3p21.3       | 43%                                            | 75%                                            | Unknown | [89]          |
| H3–3–3    | 2p25.1       | 83%–96%                                       | 50%                                            | Cell cycle arrest, metastasis | [128,129]     |

, not available.
MALDI-TOF mass spectroscopy. Together, these studies show that cancer specific methylation changes in tumor tissues, plasma, and other fluids can be used as tumor markers for risk assessment and early diagnosis of breast cancer. More effective and simplified approaches for detecting methylated genes are being developed to increase the sensitivity and specificity of early detection and prognosis of breast cancer.[38-39].

Aberrant promoter methylation as therapeutic target for breast cancer

Unlike genetic changes in cancers, gene silencing due to DNA methylation changes can be reversed by pharmacological demethylation. Thus, reactivating epigenetically silenced cancer genes and restoring their tumor suppression functions provide new insight for cancer therapy. Moreover, targeting epigenetic alterations also provides alternative ways for breast cancer preventative care, novel anticancer therapeutics, and drug investigation.

DNA methyltransferase (DNMTs) inhibitors, 5-aza-cytidine (5-Aza-CR) and 5-aza-2'-deoxycytidine (5-Aza-dC), are the first discovered epigenetic drugs. These compounds act by incorporating into DNA in place of the natural base, cytosine, during DNA replication, leading to covalent trapping of DNMTs[39]. This causes the depletion of active DNMTs and demethylation of genomic DNA. These reagents have already been approved by the US Food and Drug Administration (FDA) for treatment of a myelodysplastic syndrome (MDS), malignant mesothelioma, preleukemic disease, breast cancer, nasopharyngeal carcinoma (NPC) and other diseases[40-43].

Recently, Zebularine, a novel DNMT inhibitor with low toxicity and high selectivity for tumor cells, was reported to reactivate key genes silenced in breast cancer cell lines even at low doses[44]. In addition, other demethylation approaches, including DNMT inhibition via siRNA, ribozymes, and antisense oligonucleotides, have also been proposed but are still in their infancy. Some of these agents have been demonstrated to be promisingly effective in cell culture systems, animal models, and even clinical trials, whereas having little effect on normal cells[45]. These agents include MG98, an antisense oligonucleotide to DNA methyltransferase 1[46,47], and RG108[48,49], a novel small molecule that binds to the catalytic site of DNA methyltransferases.

The combination of histone deacetylases inhibitors (HDACis), such as TSA and phenylbutyrate[50], with DNMT inhibitors is particularly valuable for cancer treatment. Furthermore, loss of estrogen receptor (ER) expression due to aberrant DNA methylation and histone modifications results in resistance to anti-estrogen therapy in breast cancer. Studies showed that combination of 5-azacytidine with TSA could induce re-expression of functional ER, thereby sensitizing ERα-negative breast cancer cells to tamoxifen therapy[51,52]. Combined with HDACis, trastuzumab (Herceptin®), a humanized monoclonal anti-HER2 antibody, produced a synergistic effect on cell growth repression and apoptosis induction in breast cancer cells[53,54].

Furthermore, combinations of epigenetic drug treatments with conventional chemotherapeutic reagents or natural dietary ingredients could potentially work synergistically to increase therapeutic effects. A preclinical study has shown that 5-Aza-dC in combination with docetaxel, an anti-mitotic chemotherapeutic regent, could produce synergistic anti-cancer effects on breast cancer lines[55]. 5-Aza-dC combined with either amsacrine oridarubicin also showed promising efficacy. The green tea polyphenol, (−)-epigallocatechin-3-gallate (EGCG), can cause the chromatin structural remodelling of ERα promoter by altering histone acetylation and methylation status, thereby resulting in ERα reactivation. Combined with TSA, EGCG reactivated multiple methylation-silenced TSGs by directly and indirectly inhibiting the enzymatic activities of DNMTs[56]. Dietary sulforaphane (SFN)[57], a histone deacetylase inhibitor, significantly inhibited the viability and proliferation of breast cancer cells but not normal cells in vitro.

Therefore, combined demethylation therapies may offer better therapeutic strategies for breast cancer. For example, clinical trials of trastuzumab combined with HDACi are in progress for locally advanced breast cancer[58], and a phase II breast cancer trial combining valproic acid (VPA) with FEC100 (5-fluorouracil, epirubicin, and cyclophosphamide) is ongoing[59].

Conclusions

In summary, substantial evidence demonstrates that epigenetic alterations, especially promoter CpG methylation of TSGs, play critical roles in breast tumorigenesis. Significant advances have been made for early detection biomarkers, risk assessment, prognostic prediction, and drug development in breast cancer. With the development of new epigenomic techniques and further investigations, a better perspective of the epigenetic profile of breast cancer will soon be revealed.

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References

[1] American cancer society’s breast cancer resource. Available at: http://www.cancer.org.

[2] Jemal A, Bray F, Center MM, et al. Global cancer statistics. CA Cancer J Clin. 2011;61:69–90.

[3] Radpour R, Barekat Z, Kohler C, et al. Hypermethylation of tumor suppressor genes involved in critical regulatory pathways for developing a blood-based test in breast cancer. PLoS One, 2011;6:e16080.

[4] Esteller M. Cancer epigenomics: DNA methylomes and histone-modification maps. Nat Rev Genet. 2007;8:286–298.

[5] Singal P, Ginder GD. DNA methylation. Blood. 1999;93:4059–4070.

[6] Widschwendter M, Jones PA. DNA methylation and breast carcinogenesis. Oncogene. 2002;21:5462–5482.

[7] Polyak K. Breast cancer: origins and evolution. J Clin Invest. 2007;117:3155–3163.

[8] Clarke R, Thompson EW, Leenova F, et al. Hormone resistance, resilience, and metastatic potential in breast cancer. Breast Cancer Res Treat. 1993;24:227–239.

[9] Parello P. Epigenetic signatures in breast cancer: clinical perspective. Breast Care (Basel). 2010;5:66–73.

[10] Wang Y, Li J, Cui Y, et al. CMTM3, located at the critical tumor suppressor locus 16q22.1, is silenced by CpG methylation in carcinomas and inhibits tumor cell growth through inducing apoptosis. Cancer Res. 2009;69:5194–5201.

[11] Cui Y, Ying Y, van Hasselt A, et al. OPCML is a broad tumor suppressor for multiple carcinomas and lymphomas with frequently epigenetic inactivation. PLoS One, 2008;3:e2990.

[12] Ai L, Tao Q, Zheng S, et al. Inactivation of Wnt inhibitory factor-1 (WIF1) expression by epigenetic silencing is a common event in breast cancer. Carcinogenesis, 2006;27:1341–1348.

[13] Xiang T, Li L, Fan Y, et al. PLCD1 is a functional tumor suppressor inducing G (2)/M arrest and frequently methylated in breast cancer. Cancer Biol Ther, 2010;10:520–527.

[14] Shao L, Cui Y, Li H, et al. CMTM5 exhibits tumor suppressor activities and is frequently silenced by methylation in carcinoma cell lines. Clin Cancer Res, 2007;13:5756–5762.

[15] Low JS, Tao Q, Ng KM, et al. A novel isoform of the 8p22 tumor suppressor gene DLG1 suppresses tumor growth and is frequently silenced in multiple common tumors. Oncogene, 2011;30:1923–1935.

[16] Lee KY, Geng H, Ng KM, et al. Epigenetic disruption of interferon-gamma response through silencing the tumor suppressor interferon regulatory factor 8 in nasopharyngeal, esophageal and multiple other carcinomas. Oncogene, 2008;27:5267–5276.

[17] Brekelmans CT, Seynaeve C, Bartels CC, et al. Effectiveness of breast cancer surveillance in BRCA1/2 gene mutation carriers and women with high familial risk. J Clin Oncol, 2001;19:924–930.

[18] Kiblata M, Balkouranidou I, Sotiropoulu G, et al. Methylation of cysteine-rich promoter is associated with unfavorable prognosis in operable breast cancer. Int J Cancer, 2009;125:2887–2892.

[19] Potapaov A, Hoffman AM, Godwin AK, et al. Promoter hypermethylation of the PALB2 susceptibility gene in inherited and sporadic breast and ovarian cancers. Cancer Res, 2008;68:998–1002.

[20] Karray-Chouayekh S, Trifa F, Khabir A, et al. Clinical significance of epigenetic inactivation of MLH1 and BRCA1 in Tunisian patients with invasive breast carcinoma. J Biomed Biotechnol, 2009, 2009:369129.

[21] Veeck J, Wild PJ, Fuchs T, et al. Prognostic relevance of Wnt-inhibitory factor-1 (WIF1) andDickkopf-3 (DKK3) promoter methylation in human breast cancer. BMC Cancer, 2009;9:217.

[22] Tian K, Wang Y, Huang Y, et al. Methylation of WTH3, a possible drug resistant gene, inhibits p53 regulated expression. BMC Cancer, 2008;8:327.

[23] Ko E, Park SE, Cho EY, et al. Cystatin M loss is associated with the losses of estrogen receptor, progesterone receptor, and HER4 in invasive breast cancer. Breast Cancer Res, 2010;12:R110.

[24] Brooks J, Cairns P, Zeleniuch-Jacquotte A. Promoter methylation and the detection of breast cancer. Cancer Causes Control, 2009;20:1539–1550.

[25] Evron E, Dooley WC, Umbricht CB, et al. Detection of breast cancer cells in ductal lavage fluid by methylation-specific PCR. Lancet, 2001;357:1336–1336.

[26] Fackler MJ, Malone K, Zhang Z, et al. Quantitative multiplex methylation-specific PCR assay for the detection of promoter hypermethylation in multiple genes in breast cancer. Cancer Res, 2004;64:4442–4452.

[27] Fackler MJ, Malone K, Zhang Z, et al. Quantitative multiplex methylation-specific PCR analysis doubles detection of tumor cells in breast ductal fluid. Clin Cancer Res, 2006;12:3306–3310.

[28] Veeck J, Niederaicher D, An H, et al. Aberrant methylation of the Wnt antagonist SFRP1 in breast cancer is associated with unfavourable prognosis. Oncogene, 2008;25:3479–3488.

[29] Arai T, Miyoshi Y, Kim SJ, et al. Association of GSTP1 CpG islands hypermethylation with poor prognosis in human breast cancers. Breast Cancer Res Treat, 2006;100:169–176.

[30] Yan PS, Perry MR, Laux DE, et al. CpG island arrays: an application toward deciphering epigenetic signatures of breast cancer. Clin Cancer Res, 2000;6:1432–1438.

[31] Holm K, Hegardt C, Staaaf J, et al. Molecular subtypes of breast cancer are associated with characteristic DNA methylation patterns. Breast Cancer Res, 2010;12:R36.

[32] Widschwendter M, Mienon U. Circulating methylated DNA: a new generation of tumor markers. Clin Cancer Res, 2006;12:7205–7206.

[33] Muller HM, Widschwendter A, Fieg H, et al. DNA methylation in serum of breast cancer patients: an independent prognostic marker. Cancer Res, 2003;63:7641–7645.

[34] Dulaimi E, Hillenck J, Ibanez de Caceres I, et al. Tumor suppressor gene promoter hypermethylation in serum of breast cancer patients. Clin Cancer Res, 2004;10:6189–6193.

[35] Martineze-Galan J, Torres B, Del Moral R, et al. Quantitative detection of methylated ER1 and 14-3-3-sigma gene promoters in serum as candidate biomarkers for diagnosis of breast cancer and evaluation of treatment efficacy. Cancer Biol Ther, 2008;7:956–965.

[36] Xue X, Teare MD, Holen I, et al. Optimizing the yield and utility of circulating cell-free DNA from plasma and serum. Chin Chim Acta, 2009;404:100–109.

[37] Steyerberg EP, van der Wall E, van Diest PJ, Oxytocin: bringing magic into nipple aspiration. Ann Oncol, 2007;18:1743–1744.

[38] Sun Z, Asmam YW, Kalari KR, et al. Integrated analysis of gene expression, CpG island methylation, and gene copy number in breast cancer cells by deep sequencing. PLoS One, 2011;6:e17490.

[39] Mack GS. Epigenetic cancer therapy makes headway. J Natl Cancer Inst, 2006;98:1443–1444.
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[40] Appleton K, Mackay HJ, Judson I, et al. Phase I and pharmacodynamic trial of the DNA methyltransferase inhibitor decitabine and carboplatin in solid tumors. J Clin Oncol, 2007;25:4603–4609.

[41] Samuels BL, Herndon JE, 2nd, Harmon DC, et al. Dihydro-5-aza-2’-cytidine and cisplatin in the treatment of malignant mesothelioma: a phase II study by the Cancer and Leukemia Group B. Cancer, 1998;82:1578–1584.

[42] Tao Q, Young LS, Woodman CB, et al. Epstein-Barr virus (EBV) and its associated human cancers—genetics, epigenetics, pathobiology and novel therapeutics. Front Biosci, 2006, 11:2672–2713.

[43] Issa JP, Kantarjian HM, Kirkpatrick P. Azacitidine. Nat Rev Drug Discov, 2005;4:275–276.

[44] Bell K, Sobolewski MD, Davidson NE. Effects of a novel DNA methyltransferase inhibitor zebularine on human breast cancer cells. Breast Cancer Res Treat, 2010;120:581–592.

[45] Yan L, Nass SJ, Smith D, et al. Specific inhibition of DNMT1 by antisense oligonucleotides induces re-expression of estrogen receptor-alpha (ER) in ER-negative human breast cancer cell lines. Cancer Biol Ther, 2003;2:552–556.

[46] Plummer R, Vidal L, Griffin M, et al. Phase I study of MG98, an olgosodeoxynucleotide antisense to human DNA methyltransferase 1, given as a 7-day infusion in patients with advanced solid tumors. Clin Cancer Res, 2009;15:3177 – 3183.

[47] Klisovic RB, Stock W, Cataland S, et al. A phase I biological study of MG98, an oligodeoxynucleotide antisense to DNA methyltransferase 1, in patients with high-risk myelodysplasia and acute myeloid leukemia. Clin Cancer Res, 2008;14:2444 – 2449.

[48] Brueckner B, Garcia Boy R, Siedlecki P, et al. Epigenetic reactivation of tumor suppressor genes by a novel small-molecule inhibitor of human DNA methyltransferases. Cancer Res, 2005;65:6305–6311.

[49] Zheng YG, Wu J, Chen Z, et al. Chemical regulation of epigenetic silencing of tumor-promoter CpG islands by a novel small-molecule inhibitor of human DNA methyltransferases. Cancer Res, 2005;65:6305–6311.

[50] Cameron EE, Bachman KE, Mychanchuk S, et al. Synergy of demethylation and histone deacetylation inhibition in the re-expression of genes silenced in cancer, Nat Genet, 1999;21: 103–107.

[51] Yang X, Phillips DL, Ferguson AT, et al. Synergistic activation of functional estrogen receptor (ER)-alpha by DNA methyltransferase inhibition and histone deacetylation inhibition in human ER-alpha-negative breast cancer cells. Cancer Res, 2001;61: 7025–7029.

[52] Jang ER, Lim SJ, Lee ES, et al. The histone deacetylase inhibitor trichostatin A sensitizes estrogen receptor alpha-negative breast cancer cells to tamoxifen. Oncogene, 2004;23:1724–1736.

[53] Bai P, Pranapat M, Swaby R, et al. Activity of suberoanilide hydroxamic acid against human breast cancer cells with amplification of her-2. Clin Cancer Res, 2005, 11:6382 – 6389.

[54] Fuino L, Bai P, Wittmann S, et al. Histone deacetylase inhibitor LAQ824 down-regulates Her-2 and sensitizes human breast cancer cells to trastuzumab, taxotere, gemcitabine, and etoposide. Mol Cancer Ther, 2003;2:971–984.

[55] Hurtubise A, Mompardier RL. Evaluation of antiosteoplastic action of 5-aza-2’-deoxycytidine (Dacogen) and decetaxel (Taxotere) on human breast, lung and prostate carcinoma cell lines. Anticancer Drugs, 2004;15:161 – 167.

[56] Li Y, Yuan YY, Meeran SM, et al. Synergistic epigenetic reactivation of estrogen receptor-alpha (ERalpha) by combined green tea polyphenol and histone deacetylase inhibitor in ERalpha-negative breast cancer cells. Mol Cancer, 2010;9:274.

[57] Meeran SM, Patel SN, Tofolol TO. Sulforaphane causes epigenetic repression of hTERT expression in human breast cancer cell lines. PLoS One, 2010;5:e11457.

[58] Ganesan A, Nolan L, Crabb SJ, et al. Epigenetic therapy: histone acetylation, DNA methylation and anti-cancer drug discovery. Curr Cancer Drug Targets, 2009;9:883–981.

[59] Munster P, Marchion D, Bicaku E, et al. Phase I trial of histone deacetylase inhibition by valproic acid followed by the topoisomerase II inhibitor epirubicin in advanced solid tumors: a clinical and translational study. J Clin Oncol, 2007;25:1979 – 1985.

[60] Vo QN, Kim WJ, Cvitanovic L, et al. The ATM gene is a target for epigenetic silencing in locally advanced breast cancer. Oncogene, 2004;23:9432–9437.

[61] David GL, Yergin J, Srinivasan S, Kumar A, et al. MDR1 promoter hypermethylation in MCF-7 human breast cancer cells: changes in chromatin structure induced by treatment with 5-Aza-cytidine. Cancer Biol Ther, 2004;3:540–548.

[62] Dejeux E, Ronneberg JA, Svolang H, et al. DNA methylation profiling in doxorubicin treated primary locally advanced breast tumors identifies novel genes associated with survival and treatment responses. Mol Cancer, 2010.

[63] Virmani AK, Rathi A, Sathyaranayana UG, et al. Aberrant methylation of the adenomatous polyposis coli (APC) gene promoter 1A in breast and lung carcinomas. Clin Cancer Res, 2001, 7:1998–2004.

[64] Jin Z, Tamura G, Tsuchiya T, et al. Adenomatous polyposis coli (APC) gene promoter hypermethylation in primary breast cancers. Br J Cancer, 2001,85:69–73.

[65] Li Z, Chen B, Wu Y, et al. Genetic and epigenetic silencing of the beclin 1 gene in sporadic breast tumors. BMC Cancer, 2010;10:98.

[66] Kuznetsova EB, Kekseva TV, Larin SS, et al. Methylation of the B1N1 gene promoter CpG island associated with breast and prostate cancer. J Carcinog, 2007;6:9.

[67] Tapia T, Smalley SV, Kohen P, et al. Promoter hypermethylation of BRCA1 correlates with absence of expression in hereditary breast cancer tumors. Epigenetics, 2008;3:157–163.

[68] Esteller M, Silva JM, Dominguez G, et al. Promoter hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors. J Natl Cancer Inst, 2000;92:564–569.

[69] Si J, Yu X, Zhang Y, et al. Myc interacts with Max and Mz1 to repress C/EBPdelta promoter activity and gene expression. Mol Cancer, 2010;9:92.

[70] Yang X, Karuturi RK, Sun F, et al. CDKN1C (p57) is a direct target of EZH2 and suppressed by multiple epigenetic mechanisms in breast cancer cells. PLoS One, 2009;4:e5011.

[71] Rivens V, Livasca CA, Boyle CE, et al. Methyltransferase-dependent silencing of CDH1 in primary human breast tumors and metastatic lesions. Exp Mol Pathol, 2007;83:188–197.

[72] Al I, Kim WJ, Kim TY, et al. Epigenetic silencing of the tumor suppressor cystatin M occurs during breast cancer progression. Cancer Res, 2006;66:7899–7909.

[73] Paredes J, Aitbergaria A, Oliveira JT, et al. P-cadherin overexpression is an indicator of clinical outcome in invasive breast carcinomas and is associated with CDH3 promoter hypomethylation. Clin Cancer Res, 2005;11:5869–5877.

[74] Toyooka KO, Toyooka S, Virmani AK, et al. Loss of expression and aberrant methylation of the CDH13 (H-cadherin) gene in breast and lung carcinomas. Cancer Res, 2001;61:4556–4560.

[75] Loo WT, Jin L, Cheung MN, et al. Epigenetic change in E-cadherin and COX-2 to predict chronic periodontitis. J Transl Med, 2010;8:110.

[76] Dietrich D, Kripin M, Dietrich J, et al. CDK1 promoter
methylating is a biomarker for outcome prediction of anthracycline treated, estrogen receptor-positive, lymph node-positive breast cancer patients. BMC Cancer, 2010, 10:247.

[77] Hill VK, Hesson LB, Dansranjav T, et al. Identification of 5 novel genes methylated in breast and other epithelial cancers. Mol Cancer, 2010, 9:51.

[78] Evron E, Umbricht CB, Korz D, et al. Loss of cyclin D2 expression in the majority of breast cancers is associated with promoter hypermethylation. Cancer Res, 2001, 61:2782 – 2787.

[79] Van der Auwerda I, Bovie C, Svensson C, et al. Quantitative methylation profiling in tumor and matched morphologically normal tissues from breast cancer patients. BMC Cancer, 2010, 10:97.

[80] Suzuki H, Toyota M, Carraway H, et al. Frequent epigenetic inactivation of Wnt antagonist genes in breast cancer. Br J Cancer, 2008, 98:1147 – 1156.

[81] Veeck J, Bektas N, Hartmann A, et al. Wnt signalling in human breast cancer: expression of the putative Wnt inhibitor Dickkopf-3 (DKK3) is frequently suppressed by promoter hypermethylation in mammmary tumours. Breast Cancer Res, 2008, 10:R82.

[82] Seng TJ, Low JS, Li H, et al. The major Ilp22 tumor suppressor DCL1 is frequently silenced by methylation in both endemic and sporadic nasopharyngeal, esophageal, and cervical carcinomas, and inhibits tumor cell colony formation. Oncogene, 2007, 26:934 – 944.

[83] Nass SJ, Herman JG, Gabrielson E, et al. Aberrant methylation of the estrogen receptor and E-cadherin 5’ CpG islands increases with malignant progression in human breast cancer. Cancer Res, 2000, 60:4346 – 4348.

[84] Ottaviano YL, Issa JP, Paré FF, et al. Methylation of the estrogen receptor gene CpG island marks loss of estrogen receptor expression in human breast cancer cells. Cancer Res, 1994, 54:2552 – 2555.

[85] Lapidus RG, Nass SJ, Butash KA, et al. Mapping of ER gene CpG island methylation-specific polymerase chain reaction. Cancer Res, 1998, 58:2515 – 2519.

[86] Graff JR, Herman JG, Lapidus RG, et al. E-cadherin expression is silenced by DNA hypermethylation in human breast and prostate carcinomas. Cancer Res, 1995, 55:5195 – 5199.

[87] Wiesmann F, Veeck J, Gaim O, et al. Frequent loss of endoelin-3 (EDN3) expression due to epigenetic inactivation in human breast cancer. Breast Cancer Res, 2009, 11:R34.

[88] Fu DY, Wang ZM, Wang BL, et al. Frequent epigenetic inactivation of the receptor tyrosine kinase EphA5 by promoter methylation in human breast cancer. Hum Pathol, 2010, 41:48 – 58.

[89] Miyamoto K, Fukutomi T, Akashi-Tanaka S, et al. Identification of 20 genes aberrantly methylated in human breast cancers. Int J Cancer, 2005, 116:407 – 414.

[90] Akhoondi S, Lindstrom L, Widschwendter M, et al. Inactivation of FBXW7/hCDC4-beta expression by promoter hypermethylation is associated with favorable prognosis in primary breast cancer. Breast Cancer Res, 2010, 12:R105.

[91] Wang W, Huper G, Guo Y, et al. Analysis of methylation-sensitive transcriptome identifies GADD45a as a frequently methylated gene in breast cancer. Oncogene, 2005, 24:2705 – 2714.

[92] Esteller M, Corn PG, Urena JM, et al. Inactivation of glutathione S-transferase P1 gene by promoter hypermethylation in human neoplasia. Cancer Res, 1998, 58:4515 – 4518.

[93] Das PM, Thor AD, Edgerton SM, et al. Reactivation of epigenetically silenced HER4/ERBB4 results in apoptosis of breast tumor cells. Oncogene, 2010, 29:5214 – 5219.

[94] Wales MM, Biel MA, El Deir Y, et al. p53 activates expression of HIC-1, a new candidate tumour suppressor gene on 17p13.3. Nat Med, 1995, 1:570 – 577.

[95] Fackler MJ, McVeigh M, Evron E, et al. DNA methylation of RASSF1A, HIN-1, RAR-beta, Cyclin D2 and Twist in in situ and invasive lobular breast carcinoma. Int J Cancer, 2003, 107:970 – 975.

[96] Krop IE, Sgri G, Porter DA, et al. HIN-1, a putative cytokine highly expressed in normal but not cancerous mammary epithelial cells. Proc Natl Acad Sci U S A, 2001, 98:9796 – 9801.

[97] Raman V, Martensen SA, Reisman D, et al. Compromised HOXAX5 function can limit p53 expression in human breast tumours. Nature, 2000, 405:974 – 978.

[98] Issa JP, Vertino AM, Boehm CD, et al. Switch from monoaetic to biallelic human IGF2 promoter methylation during aging and carcinogenesis. Proc Natl Acad Sci U S A, 1996, 93:11757 – 11762.

[99] Brevet M, Ducks F, Chatelain D, et al. Deregulation of 2 potassium channels in pancreas adenocarcinomas: implication of KV1.1 gene promoter methylation. Pancreas, 2009, 38:649 – 654.

[100] Shah R, Smith P, Purdie C, et al. The prolyl 3-hydroxylases P3H2 and P3H3 are novel targets for epigenetic silencing in breast cancer. Br J Cancer, 2009, 100:1687 – 1696.

[101] Lapidus RG, Ferguson AT, Ottaviano YL, et al. Methylation of estrogen and progesterone receptor gene 5’ CpG islands correlates with lack of estrogen and progesterone receptor gene expression in breast tumors. Clin Cancer Res, 1998, 2:805 – 810.

[102] Keen JC, Garrett-Mayer E, Pettit C, et al. Epigenetic regulation of protein phosphatase 2A (PP2A), lymphoactin (XCL1) and estrogen receptor alpha (ER) expression in human breast cancer cells. Cancer Biol Ther, 2004, 3:1304 – 1312.

[103] Herman JG, Merlo A, Mao L, et al. Inactivation of the CDKN2/p16/MTS1 gene is frequently associated with aberrant DNA methylation in all common human cancers. Cancer Res, 1995, 55:4525 – 4530.

[104] Suikerbuij KP, Fackler MJ, Sukumar S, et al. Methylation is less abundant in BRCA1-associated compared with sporadic breast cancer. Ann Oncol, 2008, 19:1870 – 1874.

[105] Esteller M, Fraga MF, Guo M, et al. DNA methylation patterns in hereditary human cancers mimic sporadic tumorigenesis. Hum Mol Genet, 2001, 10:3001 – 3007.

[106] Esteller M, Corn PG, Baylin SB, et al. A gene hypermethylation profile of human cancer. Cancer Res, 2001, 61:3225 – 3229.

[107] Lehmann U, Langer F, Feist H, et al. Quantitative assessment of promoter hypermethylation during breast cancer development. Am J Pathol, 2002, 160:605 – 612.

[108] Di Vinci A, Perrelli L, Baneli B, et al. p16 (INK4a) promoter methylation and protein expression in breast fibroadenoma and carcinoma. Int J Cancer, 2005, 114:414 – 421.

[109] Garcia JM, Silva J, Pena C, et al. Promoter methylation of the PTEN gene is a common molecular change in breast cancer. Genes Chromosomes Cancer, 2004, 41:117 – 124.

[110] Widschwendter M, Berger J, Hermann M, et al. Methylation and silencing of the retinoic acid receptor-beta2 gene in breast cancer. J Natl Cancer Inst, 2001, 93:826 – 832.

[111] Sircia SM, Ren M, Pili R, et al. Endogenous reactivation of the RARbeta2 tumor suppressor gene epigenetically silenced in breast cancer. Cancer Res, 2002, 62:2455 – 2461.

[112] Du Y, Carling T, Fang W, et al. Hypermethylation in human cancers of the RIZ1 tumor suppressor gene, a member of a histone/protein methyltransferase superfamily. Cancer Res, 2001, 61:8094 – 8098.

[113] Dannmann R, Yang G, Pfeifer GP. Hypermethylation of the cPgs island of Ras association domain family 1A (RASSF1A), a
putative tumor suppressor gene from the 3p21.3 locus, occurs in a large percentage of human breast cancers. Cancer Res, 2001,61:3105–3109.

[114] Yeo W, Wong WL, Wong N, et al. High frequency of promoter hypermethylation of RASSF1A in tumorous and non-tumourous tissue of breast cancer. Pathology, 2005,37: 125–130.

[115] Agathanggelou A, Cooper WN, Latif F. Role of the Ras-association domain family 1 tumor suppressor gene in human cancers. Cancer Res, 2005,65:3497–3506.

[116] Yang ZQ, Liu G, Bollig-Fischer A, et al. Methylation-associated silencing of SFRP1 with an 8p11-12 amplification inhibits canonical and non-canonical WNT pathways in breast cancers. Int J Cancer, 2009,125:1613–1621.

[117] Dahl E, Veeck J, An H, et al. Epigenetic inactivation of the WNT antagonist SFRP1 in breast cancer. Verh Dtsch Ges Pathol, 2005,89:169–177.

[118] Veeck J, Geister C, Noetzel E, et al. Epigenetic inactivation of the secreted frizzled-related protein-5 (SFRP5) gene in human breast cancer is associated with unfavorable prognosis. Carcinogenesis, 2008,29:991–998.

[119] Fu DY, Wang ZM, Li C, et al. Sox17, the canonical Wnt antagonist, is epigenetically inactivated by promoter methylation in human breast cancer. Breast Cancer Res Treat, 2010,119: 601–612.

[120] Xu XL, Wu LC, Du F, et al. Inactivation of human SRBC, located within the 11p15.5-p15.4 tumor suppressor region, in breast and lung cancers. Cancer Res, 2001,61:7943–7949.

[121] Yuan Y, Mendez R, Sahin A, et al. Hypermethylation leads to silencing of the SYK gene in human breast cancer. Cancer Res, 2001,61:5558–5561.

[122] Bachman KE, Herman JG, Corn PG, et al. Methylation-associated silencing of the tissue inhibitor of metalloproteinase-3 gene suggest a suppressor role in kidney, brain, and other human cancers. Cancer Res, 1999,59:798–802.

[123] Antill YC, Mitchell G, Johnson SA, et al. Gene methylation in breast ductal fluid from BRCA1 and BRCA2 mutation carriers. Cancer Epidemiol Biomarkers Prev, 2010,19:266–274.

[124] Conway KE, McConnell BB, Bowing CE, et al. TMS1, a novel proapoptotic caspase recruitment domain protein, is a target of methylation-induced gene silencing in human breast cancers. Cancer Res, 2000,60:6236–6242.

[125] Ai L, Kim WJ, Demircan B, et al. The transglutaminase 2 gene (TGM2), a potential molecular marker for chemotherapeutic drug sensitivity, is epigenetically silenced in breast cancer. Carcinogenesis, 2008,29:510–518.

[126] Hsu CC, Leu YW, Tseng MJ, et al. Functional characterization of Tripl10 in cancer cell growth and survival. J Biomed Sci, 2011,18:12.

[127] Li Z, Meng ZH, Chandrasekaran R, et al. Biallelic inactivation of the thyroid hormone receptor beta1 gene in early stage breast cancer. Cancer Res, 2002,62:1939–1943.

[128] Ferguson AT, Evron E, Umbricht CB, et al. High frequency of hypermethylation at the 14-3-3 sigma locus leads to gene silencing in breast cancer. Proc Natl Acad Sci U S A, 2000,97: 6049–6054.

[129] Umbricht CB, Evron E, Gabrielson E, et al. Hypermethylation of 14-3-3 sigma (stratifin) is an early event in breast cancer. Oncogene, 2001,20:3348–3353.