乳腺疾病病理学及基础研究专题
· 实验研究 ·

Aberrant methylation of Glutathione S-transferase P1 and E-cadherin in invasive ductal breast carcinoma and fibroadenoma

Wings Tjing Yung Loo, Mary Ngan Bing Cheung, Louis Wing Cheong Chow

【Abstract】 **Objective** To investigate the hypermethylation status of glutathione transferase P1(GSTP1) and E-cadherin (ECAD), TSGs (tumor suppressor genes) in our breast cancer samples and explore their correlation with clinicopathological features of corresponding cancer patients. **Methods** One hundred and thirty-six IDC (invasive ductal carcinoma) patients were recruited for analysis and 16 fibroadenoma patients acted as control. DNA extraction and methylation-specific PCR (MSP) were subsequently performed preceded by pathological examination. **Results** The percentage of hypermethylated GSTP1 in carcinoma and fibroadenoma groups was 34.92% and 15.79% respectively and the percentage of hypermethylated ECAD in carcinomas and fibroadenomas was 18.00% and 0.00% respectively. Carcinoma had the highest percentage of c-erbB2 overexpression being 54.55% among the clinicopathological parameters. **Conclusion** Hypermethylation patterns are frequent in IDC and seem to relate to c-erbB2 overexpression, and such epigenetic change should not be neglected in fibroadenoma. Tumor methylation status in cancer patients can be determined at early stage and it may be a reference for better treatment planning.

【Key words】 DNA hypermethylation; Methylation-specific PCR; Glutathione S-transferase P1 gene; E-cadherin; Breast invasive ductal carcinoma; Fibroadenoma

DNA methylation in the "CpG islands" of the promoter region of genes is a common process controlling gene expression in mammalian cells epigenetically and such process sometimes is necessary for normal growth and development[1]. X chromosome inactivation in females and imprinted genes are also examples of "CpG islands" methylation[2]. DNA methyl transferase I, IIIA and IIIB catalyzes these reactions[2-3]. DNA methyl transferase I is mainly involved in the maintenance of methylation status of genomes through DNA replication, whereas DNA methyl transferase IIIA

Authors' address: UNIMED Medical Institute, Hong Kong(Wings TY Loo, Mary NB Cheung, Louis WC Chow); Organisation for Oncology and Translational Research Hong Kong (Wings TY Loo, Louis WC Chow); School of Chinese Medicine, The University of Hong Kong (Wings TY Loo); Clinical Trial Centre, Li Ka Shing Faculty of Medicine, The University of Hong Kong (Louis WC Chow)

Corresponding author: Louis WC Chow, E-mail: lwcchow@hkucc.hk

and IIIB act principally for the de novo DNA methylation in the early stages of development [1].

Neoplastic cells display a variety of genomic alterations, among which those involving DNA methylation constitute the most spectacular epigenetic change. Such aberrant methylation and silencing of multiple genes include genes that regulate critical processes such as cell cycle control, DNA repair, and angiogenesis[3]. The cause of aberrant promoter methylation in neoplastic cells remains to be elucidated. In the case of cancer development, aberrant hypermethylation of tumor suppressor genes (TSGs) would transcriptionally silence their expression and increase the rate of genetic mutation[3-4]. Such alteration in gene expression patterns causes the change in many involved signaling pathways, and the patterns are different for different tumor types[5-11]. Regional DNA hypermethylation and silencing of TSGs in cancer has been the focus of attention in the last decade[3-4]. The biologic mechanisms implicated in the initiation and progression of human breast carcinoma is still poorly understood.

Fibroadenoma (FA) is one of the most common benign lesions of the breast and is considered a proliferative alteration of the epithelial and stromal components. Although this lesion is generally considered benign, several reports have demonstrated that a fibroadenoma increase the risk of developing breast cancer[12-13].

Glutathione S-transferase P1 (GSTP1) is a member of the glutathione-S-transferase (GST) superfamily that catalyses the conjugation of the peptide glutathione with electrophilic compounds including carcinogens, resulting in less toxic and more readily excreted metabolites[14-20]. Its corresponding gene, GSTP1, therefore is not considered as a DNA-repair gene, but rather, a DNA damage prevention gene[15]. Because of its non-specific detoxification function, epigenetic inactivation of GSTP1 might lead to the accumulation of dangerous compounds that covalently bind to DNA, forming apurinic stable adducts and perhaps mutations[15]. The pi-class GST has been associated with preneoplastic and neoplastic changes[16], it has also been discovered that the loss of GSTP1 expression in breast carcinomas is due to its promoter hypermethylation [16-22].

E-cadherin is one of the foci in the study of cancer progression and metastasis [23-26]. It is a calcium-dependent transmembrane glycoprotein, existing as dimmers, which mediates homophilic cell-cell adhesion and tissue homeostasis in normal epithelia[27]. Loss of E-cadherin expression is thought to facilitate tumor cell detachment from primary tumor[25-26]. Reduction or loss of expression of E-cadherin is associated with invasion, metastasis, and poor prognosis in several types of human malignancies[28-29], including breast cancer[30-31]. Loss of normal E-cadherin expression in invasive ductal carcinomas (IDC) is usually due to epigenetic mechanisms rather than genetic mutations [32].

The hypermethylation status of GSTP1 and ECAD was quantitatively assessed in this study using the methylation-specific PCR (MSP) method. Hypermethylation patterns in IDC were obtained and their relationship with clinicopathological features of the corresponding IDC patients was analysed. MSP was also performed on fibroadenoma cases using GSTP1 and ECAD methylated and unmethylated primers. Although studies regarding GSTP1[15-17] and ECAD[30-32] hypermethylation in breast cancer samples have been studied previously, a proper association between GSTP1 and ECAD hypermethylation and clinicopathological variables has not been reported.

This study aims to determine whether an association exists between GSTP1 and ECAD hypermethylated cases and the corresponding clinicopathological variables. If there is, we can then make use of GSTP1 and ECAD hypermethylation status to anticipate tumor characteristics in IDC. Furthermore, GSTP1 and ECAD hypermethylation in breast FA has not yet been studied, thus data obtained in this study can provide hints about the hypermethylation status of GSTP1 and ECAD in our pool of FA samples, and a comparison between the status in IDC and FA.

1 Materials and methods

1.1 Patients and samples

One hundred and thirty-six patients with breast invasive ductal carcinoma (IDC) and 16 patients with fibroadenoma (FA) were recruited for this study. In accordance with Bloom and Richardson Classification, breast

carcinomas of tumor differentiation grades I ($n=23$), II ($n=60$) and III ($n=53$) were analyzed. Written consent was obtained from all cancer patients. The tissues were collected in sterilized bottle containing 0.9% normal saline from the Queen Mary Hospital, The University of Hong Kong. Then the tissues were stored at −80 ℃ for further DNA extraction.

1.2 DNA extraction and methylation-specific PCR (MSP)

DNA was extracted by QLAamp DNA Mini Kit (QIAGEN, Canada). The extracted DNA was modified by CpG DNA Modification Kit (CHEMICON INTERNATIONAL, USA). Both the specific methylated and unmethylated primers for each gene were used for PCR. The sense and antisense primers for the methylated GSTP1 sequences were 5′-TTCGGGGTGTAGCGGTCGTC-3′ and 5′- GCCCCAATACTAAATCACG ACG-3′. The sense and antisense primers for the unmethylated GSTP1 sequence were 5′-GATGTTTGGGGTGTAGTGGTTGTT-3′ and 5′-CCA CCCCAATACTAAATCACAACA -3′. The sense and antisense primers for the methylated ECAD sequences were 5′-TTAGGTTAGAGGGTTATCG CGT-3′ and 5′-TAACTAAAAATTCACCTACCGAC-3′. The sense and antisense primers for the unmethylated ECAD sequence were 5′- TAATTT TAGGTTAGAGGGTTATTGT -3′ and 5′- CACAACCAATCAACAACACA-3′.

The PCR mixture consisted of 1×PCR buffer [20 mmol/L Tris-HCl (pH 8.4), 50 mmol/L KCl], 1.5 mmol/L $MgCl_2$, 0.2 mmol/L dNTPs, 40 pmol sense and antisense primers, and 0.75 units of Taq DNA polymerase. Initial denaturation at 94 ℃ for 5 min was followed by 50 cycles of denaturation at 94 ℃ for 30 sec, annealing at 55 ℃ for both methylated and unmethylated sequences for 30 sec and extension at 72 ℃ for 30 sec, and a final extension at 72 ℃ for 10 min. GSTP1 product sizes of methylation and unmethylation were 91 bp and 97 bp respectively. ECAD product sizes of methylation and unmethylation were 115 bp and 97 bp respectively. The PCR products were analyzed on 2% agarose gel stained with ethidium bromide and they were electrophoresed against a 50bp ladder (Invitrogen, USA). The images were captured under ultra-violet light.

1.3 Immunocytochemical studies

The primary monoclonal antibodies (Dako Corporation, CA, USA) of estrogen receptor (ER), progesterone receptor (PR) and c-erbB2 were immunocytochemically tested on tissue samples. This was performed by using a labeled streptavidin biotin (LSAB) complex kit (Dako Corporation, CA, USA). The paraffin sections were de-waxed and treated with 3% H_2O_2 in methanol for 10 mins. Antigen retrieval was performed in 0.01 mol/L citrate buffer at pH 6.0 and microwaved prior to being cooled down at room temperature. The slides preceded by treatment with 10% horse serum for 30 minutes to block nonspecific antibody binding sites, then incubated with primary antibodies in a ratio of 1∶200 at 4 ℃ overnight. After incubation, the sections were washed extensively with PBS and then treated with biotinylated link and streptavidin-HRP. Staining was performed using 2% DAB substrate-chromogen solution. Cells were counterstained with hematoxylin and mounted in Permount.

The intensity of the stain was graded as negative (−), weak positive [(+), less than 50%)], or strong positive[(++) to (+++), 50% or above], depending on the thickness and darkness of the DAB precipitate[33].

1.4 Statistical analysis

Spearman correlation and Pearson Chi-square test were performed for statistical analysis using SPSS 15.0 (SPSS Inc., USA). Percentages of the patients' clinicopathological parameters including high tumor grade (grade Ⅱ or above), lymph node(LN) and lymphovascular permeation(LVP) positive, and the overexpression of estrogen receptor(ER), progesterone receptor(PR) and c-erbB2 within both methylated and unmethylated groups for GSTP1 and ECAD were calculated.

2 Results

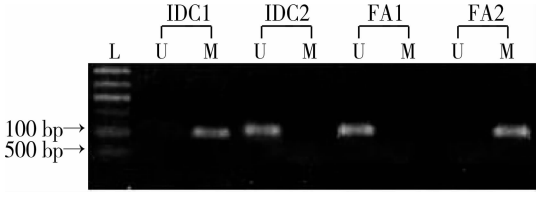
2.1 Pathological results of patients

Patients' tissues were stained for ER (45.16% overexpressed, (++) or above), PR (56.77% overexpressed, (++) or above) and c-erbB2 (52.26% overexpressed. (++) or above); the LN positive (57.42%) and

LVP positive (62.58%), and the tumor grades (I: 35.48%, II: 36.13%, III: 28.39%) were also identified. The correlation between LN and LVP is significant (Spearman's rho=0.4682, $P<0.0001$).

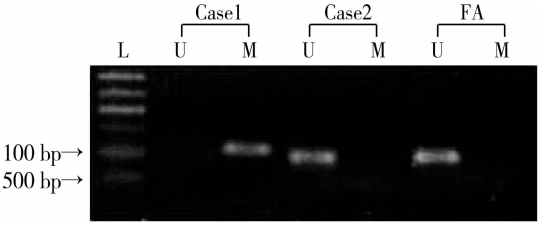
2.2 Correlation of methylation and immunohistochemical studies of GSTP**1** and ECAD

Figure 1 indicates the hypermethylation status of four MSP products of GSTP1. Figure 2 indicates the hypermethylation status of three MSP products of ECAD, two IDC cases and one fibroadenoma case. The rest of the samples demonstrated similar results.



IDC: invasive ductal carcinoma; FA: fibroadenoma; L: DNA ladder (50 bp); M: methylated sequence; U: unmethylated sequence.

Figure **1** Detection of GSTP1 hypermethylation by MSP in tumors of patients with invasive ductal breast carcinoma or benign fibroadenoma.



IDC: invasive ductal carcinoma; FA: fibroadenoma; L: DNA ladder (50 bp); M: methylated sequence; U: unmethylated sequence.

Figure **2** Detection of E-cadherin gene hypermethylation by MSP in tumors of patients with invasive ductal breast carcinoma or benign fibroadenoma.

Hypermethylated GSTP1 cases and the percentages of the patients' clinicopathological parameters including lymph node (LN) and lymphovascular permeation (LVP) positive, and estrogen receptor (ER), progesterone receptor (PR) and c-erbB2 overexpressed are detailed in Table 1. The percentages for ECAD are detailed in Table 2. Only the percentages of positive and overexpressed clinicopathological parameters are shown, the negative and under-expressed ones

are omitted. Methylation frequency of GSTP1 in the breast cancer samples was 34.92% (Table 1), and 15.79% for the fibroadenoma cases (data not shown). Methylation frequency of ECAD in the breast cancer samples was 18.00% (Table 2), and all the fibroadenoma samples were unmethylated.

Table 1 Comparison of hypermethylated and unmethylated GSTP1 of IDC cases with clinicohistopathological status (%)

Methylation status of GSTP1	High Grade	LN(+)	LVP(+)	ER overexpression	PR overexpression	c-erbB2 overexpression
M 34.92	45.45	31.82	36.36	40.91	27.27	54.55[a]
U 65.08	56.10	34.15	26.83	41.46	36.58	41.46

M: hypermethylation; U: un-methylation; High grade: tumor differentiation grade II or above; Overexpression: (++) or above; LN(+): lymph node positive; LVP(+): lymphovascular permeation positive;

a: $P<0.05$, Pearson χ^2 test shows the significant difference from the unmethylated, Odds ratio: 2.394 (95% *CI*: 1.348—4.253).

Table 2 Comparison of hypermethylated and unmethylated ECAD of IDC cases with clinicohistopathological status (%)

Methylation status of ECAD	High Grade	LN(+)	LVP(+)	ER overexpression	PR overexpression	c-erbB2 overexpression
M 18.00	41.67	29.17	29.17	41.67	33.33	58.33
U 82.00	74.05	58.22	59.04	50.84	51.66	54.94

M: hypermethylation; U: un-methylation; High grade: tumor differentiation grade II or above; Overexpression: (++) or above; LN(+): lymph node positive; LVP(+): lymphovascular permeation positive

Thirty percent (41/136) of IDC samples were positive for both ER and PR and 14% (19/136) of carcinomas were positive for one of the receptors. As much as 46 out of 136 (34%) breast carcinomas were positive for c-erbB2 expression. The epigenetic alterations detected were not significantly associated with the other prognostic factors, the histological differentiation grade, hormone receptors and c-erbB2 expression.

3 Discussion

The growing number of tumor suppressor and other cancer genes reported to be hypermethylated with associated transcriptional silencing provides an opportunity for the examination of the pattern of epigenetic alteration in breast cancer[3-4]. In an attempt to better understand the epigenetic events that lead to breast cancer development and progression, we have examined the methylation status of GSTP1 and ECAD in invasive breast ductal carcinoma lesions. Methylation frequency of GSTP1 gene is 34.92% (Table 1), which is similar to former findings[15-16], although our

results demonstrated a higher percentage[17].

Hypermethylation as with other mechanisms of inactivation of suppressor gens, deletion and point mutation, can be found in different types of cancer. GSTP1 is frequently hypermethylated in breast cancer[16] and prostate cancer[18] but infrequently methylated in other cancer types[6, 16]. As there are many researchers claiming that GSTP1 hypermethylation could be a prognostic marker for certain types of cancers including breast cancer[19-20], and provided that such claim is getting more support from other studies[6, 15-16], the association between hypermethylation of GSTP1 and clinicopathological parameters become necessary to anticipate the future characteristics of the corresponding tumor type. This optimizes future treatment planning by physicians.

Because our sample size was small, statistical significant correlation between the methylated and unmethylated cases and the clinicopathological parameters could not be found. Nonetheless, we calculated the percentages of the positive or overexpressed parameters within the hypermethylated cases, which were compared with the unmethylated ones (Table 1).

LVP positive and c-erbB2 overexpression were 36.36% and 54.55% respectively in the GSTP1 methylated cases. Both figures were higher than the unmethylated counterparts by at least 10%, with the figure for c-erbB2 overexpression being the highest amongst the variables. These significant contrasts have made LVP positive and c-erbB2 overexpression highly suspicious to be related to GSTP1 methylation. Unfortunately, there has not been any data published concerning such relationship, and the mechanisms of the possible relationship are waiting to be elucidated by further studies.

Both GSTP1 methylated and unmethylated cases have similar percentages on ER overexpression, with methylated cases being 40.91%. ER overexpression is definitely ER positive, but in a previous study on breast cancer cell lines[21], all the ER positive cell lines were hypermethylated in GSTP1 whereas the unmethylated ones were all ER negative. Our results, therefore, demonstrated a difference between real human breast cancer tissue and breast cancer cell lines. This means that GSTP1 hypermethylation would not always occur concurrently with ER

overexpression in human IDC samples.

Studies had shown that women with fibroadenoma (FA) have a significant higher risk of developing a breast cancer[12-13]. GSTP1 hypermethylation was also detected in some FA cases (15.79%), proportionally smaller than that in the IDC ones, which is consistent with a former study[22]. Using candidate gene approach in hypermethylation detection can further identify the risk of a particular benign tumor-bearing patient. If most TSGs tested are methylated in the FA samples, then the corresponding patients can be regarded as of higher risk in developing cancer subsequently, so more frequent check-ups can be provided for such patients.

E-cadherin plays a role in maintaining the normal differentiated state of the mammary gland epithelium[34-35]. Loss of its expression has been repeatedly associated with increased invasive and metastatic potential, and decreased patient survival[28-31]. ECAD repression in IDC has proven to be attributable to epigenetic mechanisms rather than mutations[32, 36]. ECAD hypermethylation frequency was 18% (24/136) in this study, quite similar to our former finding, 26.1% (6/23)[37].

Lymphovascular permeation is a predictor of lymphogenous spread and metastases in regional lymph nodes[38]. Tumor cells may penetrate to adjacent lymphatics that form concomitantly with blood capillaries or, hypothetically, malignant cells may pass from blood stream into the lymphatic via lymphaticovenous junctions[39-40]. Loss of E-cadherin proper function is thought to facilitate tumor cell detachment from primary tumor[25-26], which makes it a tumor suppressor that attenuates malignant behavior[29,41]. But interestingly, lymphovascular permeation positive (LVP-positive) and lymph node positive (LN-positive) patients both exhibited high-intensity expression of E-cadherin in LN and LVP sites by immunohistochemical studies, and E-cadherin has proven to play an important role in tumor development and growth within the lymphatics[42]. In our findings, 29.17% hypermethylated ECAD cases were LN-positive, same as the figure obtained for LVP-positive (Table 2). Our data also showed strong correlation between LN and LVP, but ECAD did not seem to be associated with either of them. This illustrates that a much greater

proportion of LN and LVP positive cases were expressing ECAD (ECAD unmethylated) in the primary site of the tumor (breast). Loss of E-cadherin expression due to promoter hypermethylation has long been claimed for tumor metastasis[28-31], but the migration of cancer cells from the primary site (breast) to lymph node seems not to be related to loss of E-cadherin expression. Such observation needs to be followed by further studies due to the lack of investigation of the relationship among LN, LVP and E-cadherin.

A previous study by D'souza *et al*[43] had shown transcriptionally by nuclear run-on assays that overexpression of c-erbB2 down-regulates E-cadherin expression. Their analysis of E-cadherin expression in the c-erbB2 transfectants by Western blotting also showed a clear decrease in E-cadherin protein levels. Acting as an epigenetic follow-up to the above study, we checked our hypermethylated ECAD cases in relation to c-erbB2 overexpression. The results showed that in those cases where ECAD protein is expressed, there is a percentage up to 58.33% of c-erbB2 overexpression (Table 2). From the above interpretation, hypermethylation of ECAD maybe related to the overexpression of c-erbB2 either directly or indirectly. Currently, there are only a few investigations involved in the initiation of hypermethylation mechanisms of tumor suppressor genes, hence further molecular follow-up maybe required to answer the above question. Such research may yield significant implications to a deeper understanding of DNA regional hypermethylation.

Breast fibroadenoma is considered a lesion of biphasic origin, showing proliferative defects without evidences of malignant evolution[12-13]. Therefore, the molecular comparison of benign and malignant lesions might provide important information on the role of ECAD in breast cancer. In our pool of benign fibroadenoma (FA) samples, none of them showed ECAD hypermethylation, it is quite a contrast to the IDC cases (18% methylated). Some degree of TSGs hypermethylation (p16 and GSTP1) has been shown in breast benign lesions in previous studies[44,17]. In protein expression studies, there were only slight disturbances in the expression of E-cadherin in the luminal epithelium of hyperplastic tissues[45], comparing to 11 out of 14 of the breast carcinoma cases. Although TSGs hypermethylation is

thought to play a part in the transformation from FA to carcinomas, ECAD hypermethylation seemed not to be the case in our observation. Perhaps it is due to its correlation with the later stage of tumor development (metastasis), making the promoter hypermethylation of ECAD less significant in FA.

The association of GSTP1 and ECAD methylation with lymphovascular permeation positive and c-erbB2 receptor overexpression may enable us to further understand the relationship between GSTP1 and ECAD hypermethylation and IDC tumor characteristics. Such information can act as a direction guide to future studies on the mechanistic level, which may, in turn, enable physicians to make a better treatment combination plan. Furthermore, we observed that the hypermethylation of GSTP1 and ECAD in FA was much less significant when compared to IDC. If tumors' methylation status is determined in early stages, it may potentially be a reference to the planning of neoadjuvant treatment.

References

[1] Wolffe AP, Matzke MA. Epigenetics: regulation through repression. Science (Wash. DC), 1999, 286: 481-486.

[2] Paulsen M, Ferguson-Smith AC. DNA methylation in genomic imprinting, development, and disease. J Pathol, 2001, 195: 97-110.

[3] Jones PA, Laird PW. Cancer epigenetics comes of age. Nat Genet, 1999, 21: 163-167.

[4] Baylin SB, Herman JG, Graff JR, et al. Alterations in DNA methylation: a fundamental aspect of neoplasia. Adv Cancer Res, 1998, 72: 141-196.

[5] Melki JR, Vincent PC, Clark SJ. Concurrent DNA hypermethylation of multiple genes in acute myeloid leukemia. Cancer Res, 1999, 59: 3730-3740.

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

[7] Ueki T, Toyota M, Sohn T, et al. Hypermethylation of multiple genes in pancreatic adenocarcinoma. Cancer Res, 2000, 60: 1835-1839.

[8] Zochbauer-Muller S, Fong KM, Virmani AK, et al. Aberrant promoter methylation of multiple genes in nonsmall cell lung cancers. Cancer Res, 2001, 61: 249-255.

[9] Dong SM, Kim HS, Rha SH, et al. Promoter hypermethylation of multiple genes in carcinoma of the uterine cervix. Clin Cancer Res, 2001, 7: 1982-1986.

[10] Maruyama R, Toyooka S, Toyooka KO, et al. Aberrant promoter methylation profile of bladder cancer and its relationship to histopathological features. Cancer Res, 2001, 61: 8659-8663.

[11] Kwong J, Lo KW, To KF, et al. Promoter hypermethylation of multiple genes in nasopharyngeal carcinoma. Clin Cancer Res, 2002, 8: 131-137.

[12] Wang J, Costantino JP, Tan-Chiu E, et al. Lower-category benign breast disease and the risk of invasive breast cancer. J Natl Cancer Inst, 2004, 96: 616-20.

[13] Hartmann LC, Sellers TA, Frost MH, et al. Benign breast disease and the risk of breast cancer. N Engl J Med, 2005, 353: 229-37.

[14] Shen H, Kauvar L, Tew KD. Importance of glutathione and associated enzymes in drug response. Oncol Res, 1997, 9: 295-302.

[15] Esteller M. Epigenetic lesions causing genetic lesions in human cancer: promoter hypermethylation of DNA repair genes. Eur J Cancer, 2000, 36: 2294-2300.

[16] Meiers I, Shanks JH, Bostwick DG. Glutathione S-transferase pi (GSTP1) hypermethylation in prostate cancer: review 2007. Pathology, 2007, 39: 299-304.

[17] Parrella P, Poeta ML, Gallo AP, et al. Nonrandom distribution of aberrant promoter methylation of cancer-related genes in sporadic breast tumors. Clin Cancer Res, 2004, 10: 5349-5354.

[18] Lin X, Asgari K, Putzi MJ, et al. Reversal of GSTP1 CpG island hypermethylation and reactivation of pi-class glutathione S-transferase (GSTP1) expression in human prostate cancer cells by treatment with procainamide. Cancer Res, 2001, 61: 8611-8616.

[19] San-Miguel J, García-Sanz R, López-Pérez R. Analysis of methylation pattern in multiple myeloma. Acta Haematol, 2005, 114: 23-26.

[20] Wong IH, Ng MH, Huang DP, et al. Aberrant p15 promoter methylation in adult and childhood acute leukemias of nearly all morphologic subtypes: potential prognostic implications. Blood, 2000, 95: 1942-1949.

[21] Jhaveri MS, Morrow CS. Methylation-mediated regulation of the glutathione S-transferase P1 gene in human breast cancer cells. Gene, 1998, 210: 1-7.

[22] Jeronimo C, Costa I, Martins MC, et al. Detection of gene promoter hypermethylation in fine needle washings from breast lesions. Clin Cancer Res, 2003, 9: 3413-3417.

[23] Xie X, Wang CY, Cao YX, et al. Expression pattern of epithelial cell adhesion molecule on normal and malignant colon tissues. World J Gastroenterol, 2005, 11: 344-347.

[24] Parisini E, Higgins JM, Liu JH, et al. The crystal structure of human E-cadherin domains 1 and 2, and comparison with other cadherins in the context of adhesion mechanism. J Mol Biol, 2007, 373: 401-411.

[25] Kokenyesi R, Murray KP, et al. Benshushan A, Invasion of interstitial matrix by a novel cell line from primary peritoneal carcinosarcoma, and by established ovarian carcinoma cell lines: role of cell-matrix adhesion molecules, proteinases, and E-cadherin expression. Gynecol Oncol, 2003, 89: 60-72.

[26] Zhang H, Stephens LC, Kumar R. Metastasis tumor antigen family proteins during breast cancer progression and metastasis in a reliable mouse model for human breast cancer. Clin Cancer Res, 2006, 12: 1479-1486.

[27] Christofori G, Semb H. The role of the cell-adhesion molecule Ecadherin as a tumour-suppressor gene. Trends Biochem Sci, 1999, 24: 73-76.

[28] Shimoyama Y, Hirohashi S. Expression of E- and P-cadherin in gastric carcinomas. Cancer Res, 1991, 51: 2185-2192.

[29] van Oort IM, Tomita K, van Bokhoven A, et al. The prognostic value of E-cadherin and the cadherin-associated molecules alpha-, beta-, gamma-catenin and p120ctn in prostate cancer specific survival: a long-term follow-up study. Prostate, 2007, 67: 1432-1438.

[30] Vos CB, Cleton-Jansen AM, Berx G, et al. E-cadherin inactivation in lobular carcinoma in situ of the breast: an early event in tumorigenesis. Br J Cancer, 1997, 76: 1131-1133.

[31] Turashvili G, Bouchalova K, Bouchal J, et al. Expression of E-cadherin and c-erbB-2/HER-2/neu oncoprotein in high-grade breast cancer. Cesk Patol, 2007, 43: 87-92.

[32] Tomlinson JS, Alpaugh ML, Barsky SH. An intact overexpressed E-cadherin/alpha, beta-catenin axis characterizes the lymphovascular emboli of inflammatory breast carcinoma. Cancer Res, 2001, 61: 5231-5241.

[33] Suzuki T, Nakata T, Miki Y, et al. Estrogen sulfotransferase and steroid sulfatase in human breast carcinoma.

Cancer Res, 2003, 63: 2762-2770.

[34] Bracke ME, Van Roy FM, Mareel MM. The E-cadherin/catenin complex in invasion and metastasis. Curr Top Microbiol Immunol, 1996, 213: 123-161.

[35] Blaschuk OW, Munro SB, Farookhi R. E-cadherin, estrogens and cancer: is there a connection? Can J Oncol, 1994, 4: 291-301.

[36] Graff JR, Gabrielson E, Fujii H, et al. Methylation patterns of the E-cadherin 5-CpG island are unstable and reflect the dynamic, heterogeneous loss of E-cadherin expression during metastatic progression. J Biol Chem, 2000, 275: 2727-2732.

[37] Hu XC, Loo WT, Chow LW. E-cadherin promoter methylation can regulate its expression in invasive ductal breast cancer tissue in Chinese woman. Life Sci, 2002, 71: 1397-1404.

[38] Valkovic T, Dobrila F, Melato M, et al. Correlation between vascular endothelial growth factor, angiogenesis, and tumor-associated macrophages in invasive ductal breast carcinoma. Virchows Arch, 2002, 440: 583-588.

[39] Abe R. Angiogenesis in tumor growth and metastasis. Curr Pharm Des, 2008, 14: 3779.

[40] Mercurio AM, Bachelder RE, Rabinovitz I, et al. The metastatic odyssey: the integrin connection. Surg Oncol Clin N Am, 2001, 10: 313-328.

[41] Vleminckx K, Vakaet L Jr, Mareel M, et al. Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role. Cell, 1991, 66: 107-119.

[42] Gupta A, Deshpande CG, Badve S. Role of E-cadherins in development of lymphatic tumor emboli. Cancer, 2003, 97: 2341-2347.

[43] D'souza, Tayler-Papadimitriou J. Overexpression of ERBB2 in human mammary epithelial cells signals inhibition of transcription of the E-CD gene. Proct Natl Acad Sci USA, 1994, 91: 7202-7206.

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

[45] Dias Pereira P, Gartner F. Expression of E-cadherin in normal, hyperplastic and neoplastic feline mammary tissue. Vet Rec, 2003, 153: 297-302.

(Received: June 20, 2010)

(Edited by: Zhao Bin)

Loo WTY, Cheung MNB, Chow LWC. Aberrant methylation of Glutathione S-transferase P1 and E-cadherin in invasive ductal breast carcinoma and fibroadenoma [J/CD]. 中华乳腺病杂志: 电子版, 2010, 4(5): 508-520.