Original Paper

Association Between MGMT Promoter Methylation and Breast Cancer: a Meta-Analysis

Nairui An Yu Shi Peng Ye Zhongya Pan Xinghua Long

Department of Laboratory Medicine, Zhongnan Hospital of Wuhan University, Wuhan, China

Key Words

Mgmt • Breast Cancer • Methylation • Metastasis • Diagnosis

Abstract

Background/Aims: Numerous studies have suggested that the promoter methylation status of O6-methylguanine-DNA methyltransferase (MGMT) is significantly associated with breast cancer. However, these studies have not demonstrated consistent results. **Methods:** To obtain more accurate results for this possible association, we performed a meta-analysis-based study using the relevant data. A total of 14 articles were included in this meta-analysis. **Results:** Our study showed that the frequency of MGMT promoter methylation was significantly higher in patients with breast cancer than non-breast cancer subjects with an Odds Ratio (OR) of 4.47, a 95% Confidence Interval (CI) ranging between 1.95 - 10.25 and a P value of 0.0004. Moreover, MGMT methylation was significantly associated with the negative expression of the MGMT protein (OR = 4.65, 95%CI = 2.66 - 8.12, P < 0.00001), Oestrogen Receptor (ER)-negative tumours (OR = 1.79, 95%CI = 1.09 - 2.93, P = 0.02), postmenopausal status (OR = 1.84, 95%CI = 1.18 - 2.87, P = 0.007) and histological grade III tumours (OR = 2.49, 95%CI = 1.53 - 4.07, P = 0.0003) in breast cancer patients. However, breast cancer was not significantly correlated with lymph node metastasis (OR = 1.19, 95%CI = 0.83 - 1.70, P = 0.35), Progesterone Receptor (PR) status (OR = 1.08, 95%CI = 0.58 - 2.00, P = 0.81), Human epidermal growth factor receptor - 2 (HER-2/neu) status (OR = 1.01, 95%CI = 0.65 - 1.57, P = 0.97), P53 mutation (OR = 1.30, 95%CI = 0.76 - 2.21, P = 0.34) and age > 50 (OR = 1.07, 95%CI = 0.46 - 2.51, P = 0.88). **Conclusions:** Our study suggests that MGMT promoter methylation may be an early biomarker for the diagnosis of breast cancer.

© 2017 The Author(s)
Published by S. Karger AG, Basel

Introduction

Cancer is a type of malignant tumour that originates in the epithelial tissue. Cancer may be the synthetic result of acquired inheritance in somatic cells, the genome and epigenetic alterations in cancer cells [1]. Breast cancer is the most common type of cancer in women

and the primary cause of death among women worldwide [2-6]. The pathogenesis of breast cancer is still not clear; however, advances in molecular biology have led more researchers to study the epigenetics of breast cancer. Esteller et al. provided evidence to strongly support epigenetic mechanisms, including the methylation of cytosines in DNA, changes in histone and chromatin structure due to the covalent posttranslational modifications of histone proteins and alterations in the expression of microRNAs in the progression of breast tumourigenesis [7, 8]. The alterations in epigenetics, particularly in the methylation status of the CpG islands of DNA promoter regions, were considered as early events in cancer [9], which play crucial roles in tumour progression and may serve as biomarkers for cancer diagnosis [10-12]. DNA methylation in mammals occurs at carbon-5 of the cytosine residues of cytosine-phosphatidyl-guanosine (CpG) dinucleotides [12]. DNA methylation fulfils the essential requirements of epigenetic cyphers by stably regulating gene expression and maintaining the mechanism of these expression patterns through mitosis [13]. Mitosis can sufficiently account for the gene expression of these specific patterns from imprinting X-inactivation to germ cell-restricted gene expression observed in mature organisms [14]. Notably, increasing evidence suggests that the regulation of DNA methylation patterns involves microRNAs. Sun et al. found that miR-361-5p was significantly down regulated in hepatocellular carcinoma tissues due to epigenetic mechanisms involving DNA methylation [15]. A study by Teng et al. has shown that the transcriptional silencing of miR-29b in epithelial ovarian cancer cells is associated with the hypermethylation of CpG islands [16]. MGMT, located on chromosome 10q26, is a DNA repair gene that encodes the DNA-repair protein O⁶-alkylguanine (O⁶-AG) DNA alkyl transferase (AGT) [17], which transfers the alkyl group from the O6 position of guanine in DNA to a cysteine residue of its active site [4, 17-21]. If the alkyl group is not removed, the interchain cross-links formed by O6-alkyl guanine would inhibit DNA replication and cause the incorporation of incorrect base pairs [4]. Moreover, O6-methylguanine has the tendency to pair with thymine during replication [22] and trigger the conversion of G-to-A during base-pairing, causing mutations together with other factors. The MGMT protein restores guanine and inactivates itself during the process [4], providing to the cell a significant defence mechanism against tumourigenesis [23]. MGMT methylation has been reported to occur in one-third of all breast cancers, irrespective of the hormone receptors and Her-2 status [24]. Currently, the study of MGMT methylation is no longer confined to tumour tissues. Spitzwieser et al. tested the applicability of the MGMT promoter methylation as biomarkers in breast cancer tissues, tumour-adjacent and tumour-distant tissues [12], Taback et al. used bone marrow (BM) aspirates *and* paired serum samples [25] and Sharma et al. used the serum of breast cancer patients [22] to detect MGMT methylation. These studies concluded that the percentages of MGMT promoter methylation were different among tumour tissues, paired adjacent normal tissues and peripheral blood derived from the breasts cancers of healthy women. Recently, many researchers have studied the association between MGMT promoter methylation and breast cancer risk or its clinicopathological features. Perhaps, small samples or errors existed among different studies due to which the percentage of MGMT methylation as well as its association with clinicopathological features or breast cancer risk were not always consistent. To obtain more reliable and systematic results, we performed a meta-analysis to implicate MGMT gene promoter methylation as a risk factor for breast cancer and study its association with clinicopathological features.

Materials and Methods

Search Strategy

Two independent retrievers (Nairui An and Yu Shi) searched and obtained information from previously published studies. All articles contained in this meta-analysis are from PubMed, CNKI and EMBASE databases and date up to April 2017. The keywords used to carry out this search were: 'MGMT' or 'O6-methylguanine-DNA methyltransferase', 'DNA methylation' or 'methylation' or 'promoter methylation' and 'breast cancer' or 'breast tumour' or 'breast carcinoma'.

Inclusion criteria. (1) Studies related to MGMT gene promoter methylation and breast cancer risk or clinicopathological features. (2) Data used to calculate OR and 95% CI that could be obtained directly or indirectly. (3) The study included human female participants.

Exclusion Criteria. (1) The reviewed literature included review articles, case reports, abstracts and letters. (2) Cell lines. (3) Repetitive articles: notably, only the most recent and most complete articles were included. (4) Although there were complete cases of MGMT promoter methylation, the number of unmethylated controls were vague or absent.

Literature Screening and Data Extraction

The literature was strictly selected according to the inclusion and exclusion criteria. Nairui An, Peng Ye and Zhongya Pan extracted data independently from qualified articles, and any inconsistencies were resolved by consensus. A standard protocol was applied to extract data, which were retrieved from qualified studies including the surname of the first author, the year of publication, country, specimen type, methylation quantitative detection methods, the number of MGMT gene promoter methylation and unmethylation in the case group and the control group, as well as controls and cases characteristics.

Statistical Analysis

RevMan5.3 Software from Cochrane Collaboration and STATA 13.1 were used for statistical analysis. OR and its corresponding 95%CI were used for quantifying the association between MGMT gene promoter methylation and breast cancer risk or clinicopathological characteristics. The heterogeneity among the included studies was verified by the χ^2 test based on Q - test. P value ≥ 0.10 and $I^2 \leq 50\%$ indicated that the literature was homogeneous. The fixed effect model (Mantel-Haenszel) [26] was used for this meta-analysis. Similarly, $P < 0.10$ or $I^2 > 50\%$ indicated the existence of heterogeneity among literature, and the Random effect model (DerSimonian Laird) [27] was used. The I^2 value from the χ^2 test was used to represent the degree of heterogeneity. It is believed that there is no heterogeneity if I^2 is in the range from 0% to 25% while the range from 25% to 50% shows moderate heterogeneity. Moreover, the range from 50% to 75% indicates large heterogeneity and the range from 75% to 100% implies extremely large heterogeneity [28]. A sensitivity analysis was performed to observe the stability of the results by deleting one study at each time in our meta-analysis. We used funnel plots and Egger's linear regression to evaluate the publication bias of the included literature. If the funnel plot is visually symmetric and the P value from the Egger's test is greater than 0.05, there is no statistically apparent publication bias. All statistical tests were bilateral in our meta-analysis. The consolidated ORs were based on the Z-test, and a P value of less than 0.05 was considered to be statistically significant unless otherwise stated.

Results

Qualified Study Characteristics

According to the retrieval scheme of this study, we initially obtained 178 articles. Ultimately, 14 articles [4, 12, 22-25, 29-35] and Fu [2009] (unpublished data) met the inclusion and exclusion criteria of our meta-analysis. The flow chart of the specific screening process is shown in Fig. 1. Notably, one study [22] examined the methylation of MGMT promoter in breast cancer and normal tissues and paired serum and serum from healthy female participants. Because of different study materials, we considered them as two separate case-control studies. Therefore, 15 studies overall were involved in our meta-analysis. Ten studies [12, 22, 25, 29-32, 34, 35] and Fu [2009] (unpublished data) involving

Fig. 1. Flow diagram of the study selection process in this meta-analysis.

Table 1. Characteristics of studies included in the association of MGMT methylation and breast cancer risk in this meta-analysis. M: methylated; UM: unmethylated; MS-HRM: methylation-sensitive high-resolution melting; MSP: Methylation-specific PCR; qMSP: Quantitative methylation-specific PCR; BM: bone marrow

First author	Year	Country	Materials	Methods	Case M/UM	Control M/UM	Control characteristics
Zemlyakova[32]	2003	Russia	Tissue	Multiplex MSP	8/97	0/35	Five normal breast tissues and peripheral blood lymphocytes of 30 healthy subjects
TABACK [25]	2006	USA	Serum	qMSP	2/31	0/10	Peripheral blood from healthy females
Fu [unpublished data]	2009	China	Plasma	Multiplex nested- MSP	11/47	3/35	Plasma of Benign breast diseases
Sharma [22]	2010	India	Serum	MSP	26/74	0/30	Sera from healthy females
Alkam [31]	2013	Japan	Tissue	MSP	7/19	0/3	Normal breast tissues
Klajic [30]	2013	Norway	Tissue	pyrosequencing	24/170	0/6	Normal breast biopsies
Fu [35]	2014	China	Tissue	MSP	20/86	5/101	Paired normal breast tissues
Wu [34]	2015	China	Tissue	MSP	19/51	5/15	Benign breast disease
Asiaf [29]	2015	India	Tissue	MSP	51 /77	3/125	Adjacent matched normal breast tissues
Spitzwieser [12]	2015	Austria	Tissue	MS-HRM	11/6	1/3	Normal breast biopsies

Table 2. Characteristics of studies included in the meta-analysis for the association between MGMT methylation and clinicopathologic features of breast cancer. MS-HRM: methylation-sensitive high-resolution melting; MSP: Methylation-specific PCR; qMSP: Quantitative methylation-specific PCR; BM: bone marrow; BLBC: basal-like breast cancer; DCIS: ductal carcinoma in situ; IDC: Invasive ductal carcinoma

First author	Year	Country	Methods	Materials	Patients characteristics
Zemlyakova[32]	2003	Russia	Multiplex MS-PCR	Tissue	Sporadic breast cancer
Munot MD [4]	2006	UK	MSP	Tissue	Invasive breast tumours
TABACK [25]	2006	USA	q MSP	Serum	Early stage patients undergoing surgery for breast cancer
Fu [unpublished data]	2009	China	Multiplex nested- MSP	Plasma	Breast cancer patients (IDC, DCIS and others)
Sharma [22]	2010	India	MSP	Tissue	Untreated primary invasive ductal breast carcinoma patients
Sharma [22]	2010	India	MSP	Serum	Untreated primary invasive ductal breast carcinoma patients
Fumagalli [24]	2012	Italy	MSP	Tissue	Patients with triple-negative breast cancer
TSERGA [23]	2012	Greece	MS-HRM	Tissue	Patients with invasive breast cancer
Lu [33]	2012	China	MSP	Tissue	Invasive ductal breast carcinoma patients
Klajic [30]	2013	Norway	pyrosequencing	Tissue	Breast cancer patients with DCIS to invasive tumours stage I to IV
Alkam [31]	2013	Japan	MSP	Tissue	Locally advanced Japanese BLBC patients
Fu [35]	2014	China	MSP	Tissue	Breast cancer patients (Non-invasive and Invasive)
Spitzwieser [12]	2015	Austria	MS-HRM	Tissue	Breast cancer patients without a family history of breast cancer
Asiaf [29]	2015	India	MSP	Tissue	Invasive ductal breast carcinoma patients of Kashmir
Wu [34]	2015	China	MSP	Tissue	Female breast cancer patients (82.9% for invasive breast cancer)

Fig. 2. Forest plot for evaluating the association of MGMT promoter methylation with breast cancer risk. A random-effect model was used for the analysis.

Table 3. Stratification analysis for the association between MGMT promoter methylation and different subgroups of breast cancer

Subgroup category	Number of studies	Methylation			Heterogeneity		Publication bias	
		OR	P	95%CI	I²	P	P	P
Methods	MSP	5.81	0.01	[1.43,23.54]	76%	0.002		0.818
	Others	3.05	0.02	[1.15,8.08]	0%	0.95		0.855
Materials	Blood	3.78	0.05	[1.01,14.18]	14%	0.31		0.722
	Tissue	4.57	0.006	[1.56,13.43]	62%	0.02		0.885
	Total	4.47	0.0004	[1.95,10.25]	50%	0.03		0.979

837 cases and 380 controls elucidated the association between MGMT methylation and the breast cancer risk. Ten studies from 9 articles [4, 22-24, 29, 31, 33-35] analysed the association of MGMT promoter methylation with the clinicopathological features of breast cancer. The characteristics of these studies, included in the control and case groups, are shown in Table 1 and Table 2, respectively.

The Results of the Meta-analysis

Association of MGMT promoter methylation with breast cancer risk. Our results showed that the frequency of MGMT promoter methylation in the breast cancer groups was significantly higher than in the non-breast cancer groups (OR = 4.47, 95%CI = 1.95 - 10.25, P = 0.0004), as shown in Fig. 2. Because of the existence of heterogeneity (P = 0.03, I² = 50%) in our results, we performed a subgroup analysis based on the retrieved sample materials and methylation detection methods to find the potential sources of heterogeneity. The subsequent detailed results of the stratification analysis are summarized in Table 3. The consolidated OR using MSP was 5.81 (95%CI = 1.43 - 23.54, P = 0.01), which was significantly higher than the others (OR = 3.05, 95%CI = 1.15 - 8.08, P = 0.02) in the subgroup analysis, classified by methylation assay methods. Furthermore, the consolidated OR of the breast cancer patients compared with healthy women or benign female participants was 4.57 (95%CI = 1.56 - 13.43, P = 0.006) in tissues. The results of the subgroup analysis are shown in Table 3.

Association of MGMT promoter methylation with clinicopathological characteristics of breast cancer

Our results suggested that MGMT promoter methylation was significantly associated with the negative expression of the MGMT protein (OR = 4.65, 95%CI = 2.66 - 8.12, P<0.00001), postmenopausal status (OR = 1.84, 95%CI = 1.18 - 2.87, P = 0.007), ER-negative tumours (OR = 1.79, 95%CI = 1.09 - 2.93, P = 0.02) and histological grade III tumours (OR = 2.49, 95%CI = 1.53 - 4.07, P = 0.0003) in breast cancer, as shown in Figures 3, 4, 5 and 6. Similarly, as shown in Table 4, MGMT promoter methylation was not significantly associated

Fig. 3. Fixed effects OR with 95% CI for evaluating the association between MGMT promoter methylation and MGMT protein expression in breast cancer.

Fig. 4. Forest plot for evaluating the association of MGMT promoter methylation with menopausal status in breast cancer. A fixed-effect model was used for the analysis.

Fig. 5. Fixed effects OR with 95%CI for evaluating the association of MGMT promoter methylation with ER status in breast cancer.

with the P53 mutation (OR = 1.30, 95%CI = 0.76 - 2.21, P = 0.34), PR status (OR = 1.08, 95%CI = 0.58 - 2.00, P = 0.81), HER-2/neu status (OR = 1.01, 95%CI = 0.65 - 1.57, P = 0.97), lymph node metastasis (OR = 1.19 95%CI = 0.83 - 1.70, P = 0.35) and age > 50 (OR = 1.07, 95%CI = 0.46 - 2.51, P = 0.88).

Sensitivity analysis and publication bias assessment

We used Begg's funnel plot and Egger's linear regression test by STATA 13.1 software to evaluate the publication bias of the included studies. The funnel plot (Fig. 7) showed the basic symmetrical distribution of literature and the P value of Egger's linear regression test (p = 0.783, Table 3) was greater than 0.05, indicating that there was no publication bias in the study on the association of MGMT promoter methylation with the risk of breast cancer. The P values of Egger's linear regression test for the association between MGMT promoter methylation and clinicopathological features of breast cancer are shown in Table 4. Only the P value for the negative expression of the MGMT protein (P = 0.008) was less than 0.05, suggesting a small publication bias in the study. Moreover, the P values for Egger's linear regression test for subgroup analysis were all greater than 0.05 (Table 3), showing

Fig. 6. Fixed effects OR with 95%CI for evaluating the association of MGMT promoter methylation with the histological grade tumours in breast cancer.

Table 4. Analysis of the MGMT gene promoter methylation and clinicopathological characteristics of breast cancer

Clinical and pathological characteristics	Methylation			Heterogeneity		Publication bias
	OR	P	95%CI	I ²	P	P
P53 status	1.30	0.34	[0.76,2.21]	0%	0.54	0.669
ER Negative	1.79	0.02	[1.09,2.93]	27%	0.25	0.540
Postmenopausal	1.84	0.007	[1.18,2.87]	0%	0.99	0.522
HER -2/neu Negative	1.01	0.97	[0.65,1.57]	0%	0.48	0.352
Histological grade III	2.49	0.0003	[1.53,4.07]	21%	0.28	0.262
MGMT protein negative	4.65	<0.00001	[2.66,8.12]	38%	0.19	0.008
Age/year > 50	1.07	0.88	[0.46,2.51]	77%	0.007	0.913
PR Negative	1.08	0.81	[0.58,2.00]	59%	0.03	0.809
Lymph node metastasis	1.19	0.35	[0.83,1.70]	0%	0.54	0.713

the absence of a publication bias. Sensitivity analysis (Fig. 8) was conducted by ignoring individual studies to examine the change in the integration outcome. Sensitivity analysis in our meta-analysis showed that the results were stable with no significant changes in the omission of individual studies.

Discussion

Although the specific pathogenesis of breast cancer, a major malignant disease threatening women's health globally in recent years, is not yet clear, it is undeniable that abnormal DNA methylation is an important molecular mechanism in tumour formation. MGMT is located on the chromosome 10q26 and plays a key role in DNA repair. Our comprehensive survey included 10 studies with

Fig. 7. Begg's funnel plot seeking publication bias for the association of MGMT promoter methylation and breast cancer risk.

837 cases and 380 controls to explore the association between MGMT promoter methylation and the risk of breast cancer. Our results showed that the methylation of the MGMT promoter in the breast cancer group was 4.47 times higher than that in the non-breast cancer group, which further illustrated a statistically significant association. Moreover, our study verified that MGMT methylation was significantly related to the negative expression of the MGMT protein, ER-negative tumours, postmenopausal status and histological grade III tumours in breast cancer. Our research suggested that the methylation of the MGMT promoter may be a potential biomarker for the diagnosis of breast cancer. The abundance of genes in cancer tissues, abnormalities in the early stages of cancer formation, the relative stability of methylated biomarkers and the easily detectable changes in serum, sputum, or minimally invasive surgery facilitate the use of monitoring sequences containing DNA promoter hypermethylation of CpG islands as a diagnostic tool in cancer [36]. The methylation of the MGMT promoter could be a powerful, potential biomarker in the clinical diagnosis and prognosis of breast cancer.

Our study demonstrated a significantly strong association between the frequency of MGMT gene promoter methylation and the risk of breast cancer, indicating that the abnormal methylation of the MGMT promoter would elevate the risk of breast cancer. In the subgroup analysis grouped with sample materials, our data showed that the combined OR in tissues (OR = 4.57) was higher than in blood samples (OR = 3.78), indicating a higher risk of breast cancer in tissues. Notably, DNA can be easily extracted from serum or plasma with small trauma. It is more interesting to extract DNA from bone marrow aspirates [25]. Thus, these studies are innovative in the diagnosis of breast cancer. Furthermore, there were 5 studies in the MSP groups (OR = 5.81) and 5 studies in Others (OR = 3.05) in the subgroup analysis based on MGMT methylation detection methods. Accordingly, different methods of MGMT methylation detection may affect the early diagnosis of breast cancer, and it is important to select the appropriate methods that can be used to guide clinical practices.

In recent decades, the studies on the inverse relationship between the MGMT promoter methylation and MGMT protein negative expression have been increasing in epigenetics. Our findings were consistent with the results of Asiaf (rs = -0.285, $p < 0.05$) [29] and Sharma ($p = 0.002$, OR = 4.5, 95%CI = 1.7 - 12.0) [22] that the MGMT promoter methylation was significantly associated with the negative expression of the MGMT protein. Thus, MGMT promoter methylation was not associated with breast cancer patients who were over 50 years of age in our study. However, Tserga et al. believed that MGMT methylation was more often observed in older patients (Mann-Whitney U test, $p = 0.0222$) [23]. When we extracted the age data related to the MGMT promoter methylation, we classified the breast cancer patients > 50 years of age into the experimental group and ≤ 50 years old into the control group. Unexpectedly, patients ≥ 50 years old were divided into the same groups as in the two studies by Fu [35] and Fumagalli [24]. We speculated that the threshold of age caused this difference; thus, further studies are needed to demonstrate the association between MGMT methylation and age.

Fig. 8. Diagram for sensitivity analysis for evaluating the association of MGMT promoter methylation with breast cancer risk.

Our results showed that MGMT promoter methylation had an enhanced association with the ER-negative tumours, indicating that such breast tumours rarely benefit from traditional hormone therapy. However, MGMT methylation was not associated with the PR status or the Her2 and P53 status. Similarly, our comprehensive survey showed that the MGMT promoter methylation was not significantly associated with the lymph node metastasis in breast cancer. Interestingly, we found that the MGMT promoter methylation was significantly associated with the postmenopausal status, suggesting an increase in the risk of breast cancer in older women. Moreover, our meta-analysis showed that the association between the MGMT promoter methylation and the histological grade III tumours was more significant in the breast cancer group compared with the control group.

Although our study used a rational design and good procedure implementation, there were some limitations. First, this meta-analysis was a comprehensive investigation on the basis of case-control studies, which may have a selection bias of literature. Second, our results did not consistently show an association between the frequency of MGMT promoter methylation and the risk of breast cancer. We used subgroup analysis and regression analysis to determine the sources of heterogeneity in our results. Our results showed that sample materials and methylation detection methods were not significant factors in causing heterogeneity. However, from a professional perspective, the differences in methods and materials may cause heterogeneity among studies, pending further studies based on the exploration of large samples. We also did a sensitivity analysis by eliminating the studies one by one but did not identify the factors that could significantly affect heterogeneity. Third, to a certain extent, the absence of a clear definition of a control group affected the results of our study. Furthermore, since this study was an analysis based on the original data from relevant studies, it was not possible to correct the confounding factors in the original data such as age, race, and sex. Finally, the lack of original data from the existing research limited our further assessment of the potential association between MGMT methylation and breast cancer.

Conclusion

Our study suggested that the methylation of the MGMT promoter was significantly associated with the risk of breast cancer and several clinicopathological features. Compared with patients with breast cancer, the prevalence of MGMT methylation in healthy women or benign female patients was significantly lower. The prevalence of MGMT methylation was closely associated with the negative expression of the MGMT protein, ER-negative tumours, postmenopausal status and histological grade III tumours in breast cancer patients. The detection of MGMT methylation might provide a highly specific and sensitive molecular substitute for diagnosis and clinical management of breast cancer. However, studies based on large samples and well-defined control groups are necessary before MGMT is deemed a biomarker for the diagnosis and prognosis of breast cancer.

Acknowledgements

This work was supported by grants from National Natural Science Foundation of China (81272372 and 30873044). The Project-sponsored by SRF for ROCS, SEM.

Disclosure Statement

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

- Chin L, Andersen JN, Futreal PA: Cancer genomics: from discovery science to personalized medicine. *Nat Med* 2011;17:297-303.
- Deo S, Bhutani M, Shukla NK, Raina V, Rath GK, Purkayasth J: Randomized trial comparing neo - adjuvant versus adjuvant chemotherapy in operable locally advanced breast cancer (T4b N0 - 2 M0). *J Surg Oncol* 2003;84:192-197.
- Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghafoor A, Feuer EJ, Thun MJ: Cancer statistics, 2005. *CA Cancer J Clin* 2005;55:10-30.
- Munot K, Bell SM, Lane S, Horgan K, Hanby AM, Speirs V: Pattern of expression of genes linked to epigenetic silencing in human breast cancer. *Hum Pathol* 2006;37:989-999.
- Parkin DM, Bray F, Devesa S: Cancer burden in the year 2000. The global picture. *Eur J Cancer* 2001;37:4-66.
- Parkin DM, Bray F, Ferlay J, Pisani P: Global cancer statistics, 2002. *CA Cancer J Clin* 2005;55:74-108.
- Esteller M, Corn PG, Baylin SB, Herman JG: A gene hypermethylation profile of human cancer. *Cancer Res* 2001;61:3225-3229.
- Toh Y, Egashira A, Yamamoto M: Epigenetic alterations and their clinical implications in esophageal squamous cell carcinoma. *Gen Thorac Cardiovasc Surg* 2013;61:262-269.
- Gao Y, Feng B, Han S, Lu L, Chen Y, Chu X, Wang R, Chen L: MicroRNA-129 in Human Cancers: from Tumorigenesis to Clinical Treatment. *Cell Physiol Biochem* 2016;39:2186-2202.
- Jones PA, Baylin SB: The epigenomics of cancer. *Cell* 2007;128:683-692.
- Simó-Riudalbas L, Esteller M: Cancer genomics identifies disrupted epigenetic genes. *Hum Genet* 2014;133:713-725.
- Spitzwieser M, Holzweber E, Pfeiler G, Hacker S, Cichna-Markl M: Applicability of HIN-1, MGMT and RASSF1A promoter methylation as biomarkers for detecting field cancerization in breast cancer. *Breast Cancer Res* 2015;17:125.
- Bird A: DNA methylation patterns and epigenetic memory. *Genes Dev* 2002;16:6-21.
- Issa JP, Kantarjian HM: Targeting DNA methylation. *Clin Cancer Res* 2009;15:3938-3946.
- Sun J-J, Chen G-Y, Xie Z-T: MicroRNA-361-5p inhibits cancer cell growth by targeting CXCR6 in hepatocellular carcinoma. *Cell Physiol Biochem* 2016;38:777-785.
- Teng Y, Zuo X, Hou M, Zhang Y, Li C, Luo W, Li X: A Double-Negative Feedback Interaction between MicroRNA-29b and DNMT3A/3B Contributes to Ovarian Cancer Progression. *Cell Physiol Biochem* 2016;39:2341-2352.
- Gerson SL: MGMT: its role in cancer aetiology and cancer therapeutics. *Nat Rev Cancer* 2004;4:296-307.
- Olsson M, Lindahl T: Repair of alkylated DNA in *Escherichia coli*. Methyl group transfer from O6-methylguanine to a protein cysteine residue. *J Biol Chem* 1980;255:10569-10571.
- Daniels DS, Woo TT, Luu KX, Noll DM, Clarke ND, Pegg AE, Tainer JA: DNA binding and nucleotide flipping by the human DNA repair protein AGT. *Nat Struct Mol Biol* 2004;11:714-720.
- Pegg AE: Repair of O 6-alkylguanine by alkyltransferases. *Mutat Res Rev Mutat Res* 2000;462:83-100.
- Srivenugopal KS, Yuan X-H, Friedman HS, Ali-Osman F: Ubiquitination-dependent proteolysis of O 6-methylguanine-DNA methyltransferase in human and murine tumor cells following inactivation with O 6-benzylguanine or 1, 3-bis (2-chloroethyl)-1-nitrosourea. *Biochemistry* 1996;35:1328-1334.
- Sharma G, Mirza S, Parshad R, Srivastava A, Gupta SD, Pandya P, Ralhan R: Clinical significance of promoter hypermethylation of DNA repair genes in tumor and serum DNA in invasive ductal breast carcinoma patients. *Life Sci* 2010;87:83-91.
- Tserga A, Michalopoulos NV, Levidou G, Korkolopoulou P, Zografos G, Patsouris E, Saetta AA: Association of aberrant DNA methylation with clinicopathological features in breast cancer. *Oncol Rep* 2012;27:1630.
- Fumagalli C, Pruneri G, Possanzini P, Manzotti M, Barile M, Feroce I, Colleoni M, Bonanni B, Maisonneuve P, Radice P: Methylation of O6-methylguanine-DNA methyltransferase (MGMT) promoter gene in triple-negative breast cancer patients. *Breast Cancer Res Treat* 2012;134:131-137.
- Taback B, Giuliano AE, Lai R, Hansen N, Singer FR, Pantel K, Hoon DS: Epigenetic Analysis of Body Fluids and Tumor Tissues. *Ann N Y Acad Sci* 2006;1075:211-221.
- Higgins J, Thompson SG: Quantifying heterogeneity in a meta - analysis. *Stat Med* 2002;21:1539-1558.
- DerSimonian R, Laird N: Meta-analysis in clinical trials. *Control Clin Trials* 1986;7:177-188.

- 28 Higgins J, Thompson SG, Deeks JJ, Altman DG: Measuring inconsistency in meta-analyses [journal article as teaching resource, deposited by John Flynn]. *BMJ* 2003;327:557-560.
- 29 Asiaf A, Ahmad ST, Malik AA, Aziz SA, Rasool Z, Masood A, Zargar MA: Protein expression and methylation of MGMT, a DNA repair gene and their correlation with clinicopathological parameters in invasive ductal carcinoma of the breast. *Tumor Biol* 2015;36:6485-6496.
- 30 Klajic J, Fleischer T, Dejeux E, Edvardsen H, Warnberg F, Bukholm I, Lønning PE, Solvang H, Børresen-Dale A-L, Tost J: Quantitative DNA methylation analyses reveal stage dependent DNA methylation and association to clinico-pathological factors in breast tumors. *BMC Cancer* 2013;13:456.
- 31 Alkam Y, Mitomi H, Nakai K, Himuro T, Saito T, Takahashi M, Arakawa A, Yao T, Saito M: Protein expression and methylation of DNA repair genes hMLH1, hMSH2, MGMT and BRCA1 and their correlation with clinicopathological parameters and prognosis in basal - like breast cancer. *Histopathology* 2013;63:713-725.
- 32 Zemlyakova V, Zhevlova A, Strelnikov V, Lyubchenko L, Schabanov M, Vishnevskaya YV, Tretyakova V, Zaletayev D, Nemtsova M: Abnormal methylation of several tumor suppressor genes in sporadic breast cancer. *Mol Biol* 2003;37:591-597.
- 33 Lu C, Wu Y: Expression of MGMT Gene in Breast Cancer and Its Significance. *Mod Diagn Treat* 2012;23:886-887.
- 34 Wu L, Wang J: Study on the Correlation between Abnormal DNA Methylation and Clinical Features of Breast Cancer. *J Med Res* 2016;45:82-86.
- 35 Fu D, Wei J, Zhu Y, Tan H, Zhang J: Clinical significance of BRCA1, GSTP1 and MGMT gene methylation status in breast cancer. *China Oncol* 2014;24:487-492.
- 36 Laird PW: The power and the promise of DNA methylation markers. *Nat Rev Cancer* 2003;3:253-266.