DNMT3A/3B overexpression might be correlated with poor patient survival, hypermethylation and low expression of *ESR1/PGR* in endometrioid carcinoma: an analysis of The Cancer Genome Atlas

Dan He[1], Xiao Wang[2], Yan Zhang[3], Jian Zhao[3], Rui Han[1], Ying Dong[1]

[1]Department of Pathology, Peking University First Hospital, Beijing 100034, China;
[2]Institute of Systems Biomedicine, School of Basic Medical Sciences, Peking University Health Science Center, Beijing 100191, China;
[3]Department of Gynecology and Obstetrics, Peking University First Hospital, Beijing 100034, China.

Abstract

Background: DNA methylation is involved in numerous biologic events and associates with transcriptional gene silencing, playing an important role in the pathogenesis of endometrial cancer. *ESR1/PGR* frequently undergoes *de novo* methylation and loss expression in a wide variety of tumors, including breast, colon, lung, and brain tumors. However, the mechanisms underlying estrogen and progesterone receptors (ER/PR) loss in endometrial cancer have not been studied extensively. The aims of this study were to determine the expression of DNA (cytosine-5)-methyltransferase 3A/3B (DNMT3A/3B) in endometrial cancer to investigate whether the methylation catalyzed by DNMT3A/3B contributes to low ER/PR expression.

Methods: The clinicopathologic information and RNA-Seq expression data of *DNMT3A/3B* of 544 endometrial cancers were derived from The Cancer Genome Atlas (TCGA) uterine cancer cohort in May 2018. RNA-Seq level of *DNMT3A/3B* was compared between these clinicopathologic factors with *t*-test or one-way analysis of variance.

Results: *DNMT3A/3B* was overexpressed in endometrioid carcinoma (EEC) and was even higher in non-endometrioid carcinoma (NEEC) (*DNMT3A*, EEC *vs.* NEEC: 37.6% *vs.* 69.9%, $t=-7.440$, $P<0.001$; *DNMT3B*, EEC *vs.* NEEC: 42.4% *vs.* 72.8%, $t=-6.897$, $P<0.001$). In EEC, *DNMT3A* overexpression was significantly correlated with the hypermethylation and low expression of the *ESR1* and *PGR* ($P<0.05$). The same trend was observed in the *DNMT3B* overexpression subgroup. In the *ESR1/PGR* low-expression subgroups, as much as 83.1% of *ESR1* and 59.5% of *PGR* were hypermethylated, which was significantly greater than the *ESR1/PGR* high-expression subgroups (31.3% and 11.9%, respectively). However, the above phenomena were absent in NEEC, while *DNMT3A/3B* overexpression, *ESR1/PGR* hypermethylation, and low ER/PR expression occurred much more often. In univariate analysis, *DNMT3A/3B* overexpressions were significantly correlated with worse prognosis. In multivariate analysis, only *DNMT3A* was an independent predictor of disease-free survival ($P<0.05$).

Conclusions: *DNMT3A/3B* expression increases progressively from EEC to NEEC and is correlated with poor survival. The mechanisms underlying low ER/PR expression might be distinct in EEC *vs.* NEEC. In EEC, methylation related to *DNMT3A/3B* overexpression might play a major role in ER/PR downregulation.

Keywords: DNA (cytosine-5)-methyltransferase 3A/3B; estrogens receptor; Progesterone receptor; Endometrial carcinoma; The Cancer Genome Atlas

Introduction

Endometrial carcinoma is the most common malignancy of the female genital tract. Histologically, it has long been categorized into two subtypes.[1] Type I tumors (approximately 80%) are endometrioid carcinomas (EECs) with estrogen and progesterone receptors (ERs and PRs) and usually, have a favorable prognosis. However, type II tumors (10–20%), the non-endometrioid carcinomas (NEEC), are not associated with estrogen excess and usually have a poor prognosis. However, approximately 20% of the cases do not fit within this dualistic model; some EECs are aggressive and have poor clinical outcomes.[2] In fact, endometrial carcinoma is a clinically heterogeneous disease, and it is now well recognized that this heterogeneity may be the result of various underlying molecular alterations.

The DNA methylation is involved in numerous biologic events and it concerns approximately 70% to 80% of CpGs in mammalian DNA and associated with

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Correspondence to: Dr. Ying Dong, Department of Pathology, Peking University First Hospital, No. 8 Xishiku St., Xicheng Dist., Beijing 100034, China
E-Mail: dongying_999@163.com

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transcriptional gene silencing when it occurs in promoters.[3] Studies have indicated that the silencing of the tumor suppressor genes by DNA methylation plays an important role in the pathogenesis of endometrial cancer. Methylation changes to the genome are catalyzed by DNA methyltransferases (DNMT). DNMT3A/3B is *de novo* methyltransferases with high activity on unmethylated substrates, while DNA methylation is maintained by DNMT1 after the methylation pattern has been established.[4,5] In recent years, our knowledge on the roles of DNMT in DNA methylation has increased substantially. The *ESR1/PGR* frequently undergoes *de novo* methylation in a wide variety of tumors, including breast, colon, lung, and brain tumors.[6-9] However, the mechanisms underlying the loss of expression in endometrial cancer have not been studied extensively. Previously, using immunohistochemistry, we found that DNMT3B protein expression was negatively correlated with ER and PR expression in EEC in a small cohort.[10]

The Cancer Genome Atlas (TCGA) provides a wealth of information concerning DNA (somatic mutation, copy number alteration [CNA], and methylation), RNA (transcript level), and particular proteins from thousands of tumor- and case-matched normal tissues.[11] Therefore, in the present study, we collected data from the TCGA database and analyzed the associations between DNMT3A/3B mRNA expression and the methylation and expression patterns of the *ESR1/PGR* in endometrial carcinomas. We hypothesized that methylation catalyzed by DNMT3A/3B might take part in the *ESR1/PGR* downregulation in endometrial carcinoma and might possess prognostic utility.

Methods

Study time and design

The TCGA data acquisition Level 3 RNA-Seq, protein RPPA (reverse-phase protein array) and clinical data for uterine cancer patients were obtained from the TCGA data portal (http://cancergenome.nih.gov/). Detailed information concerning the data processing, quality control, and normalization is available on the TCGA open access download directories (https://tcga-data.nci.nih.gov/tcga/tcgaDownload.jsp). RSEM (RAN-Seq by Expectation-Maximization) expression values were $\log_2$ transformed for statistical analysis.

The TCGA adopted the illumina infinium human methylation 450 (HM450) bead array to assay over 480K CpG sites, and about 99% of *RefSeq* genes and 96% of CpG islands from UCSC database are included. DNA methylation data were provided as beta values calculated using the formula M/(M+U+100), with M and U representing fully methylated and fully unmethylated intensities, respectively.[12] Mean value was used to stratify these biomarkers as high expression or low expression, hypermethylation or hypomethylation.

The CNA non-linear data were provided as putative copy number variation using GISTIC 2.0 with −2, −1, 0, 1, and 2 representing homozygous deletion, hemizygous deletion, neutral or no change, gain, and high-level amplification, respectively. Mutation data were provided in a mutation annotation manner derived from whole-exome sequencing.

Statistical analysis

Data were analyzed using SPSS19 (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov method was used to test the normal distribution of measurement data. An unpaired two-sample *t*-test was performed to compare mRNA or protein expression values. The Chi-squared test or Fisher's exact test were performed to compare categorical variables. A Bonferroni correction was applied for multiple comparisons. Bivariate correlation analysis was performed to investigate the relationship between gene expression values with Pearson's method for data following a normal distribution and Spearman's method for data with an abnormal distribution. Survival analysis was performed using Kaplan-Meier curves, with *P*-values calculated by the log-rank test. A Cox-proportional hazard regression model was used to perform multivariate analysis. All tests were two-sided, and a *P*-value <0.05 was considered statistically significant.

Results

Clinical and pathologic characteristics

A total of 544 endometrial cancers with both clinical and gene expression data were obtained from the TCGA database. The clinicopathologic features of all of the patients are summarized in Table 1. A total of 408 EEC (75.0%) and 136 NEEC (25.0%, 114 serous carcinomas and 22 mixed carcinomas) were included in this cohort. The median age of the patients was 64 years (range, 31–90 years). A total of 72.9% of the patients were White, 20.7% were African American, and 3.9% were Asian. The ER and PR methylation data were obtained for 430 cases, and 246 cases had mutation data. CNA data were available for 537 cases. Follow-up information was provided for 542 (99.6%) patients (406 EEC, 136 NEEC), and the median follow-up was 29.0 months (range, 0.1–225.3 months).

DNMT3A/3B is overexpressed in EEC and NEEC and correlated with poor patient survival

The *DNMT3A/3B* was overexpressed in EEC and was even higher in NEEC (*DNMT3A*, EEC *vs.* NEEC: 37.6% *vs.* 69.9%, $t=-7.440$, $P<0.001$; *DNMT3B*, EEC *vs.* NEEC: 42.4% *vs.* 72.8%, $t=-6.897$, $P<0.001$). Similarly, the expression levels of *DNMT3A* and *DNMT3B* increased in EEC ($\log_2$RSEM $=9.94\pm0.55$ and 8.07 ± 1.01) and became even higher in NEEC ($\log_2$RSEM $=10.49\pm0.81$ and 8.79 ± 1.16) compared with normal control tissues ($\log_2$RSEM $=8.85\pm0.60$ and 5.66 ± 0.69), and all the differences reached significance (all $P<0.001$) [Figure 1A]. Among the *DNMT3A* overexpressed subgroup, as many as 60.5% (150/248) of cases also had *DNMT3B* overexpression, and the correlation between these two biomarkers was statistically significant ($R=0.265$, $P<0.0001$; Figure 1B).

Table 1: Clinicopathologic features of 544 endometrial carcinomas

Variables	Values
Age (years)	64 (31–90)
Premenopausal	35 (7.0)
Perimenopausal	17 (3.4)
Postmenopausal	445 (89.6)
Race	
White	373 (72.9)
Black or African-American	106 (20.7)
Asian	20 (3.9)
Other	13 (2.6)
Histology	
EEC	408 (75.0)
NEEC	136 (25.0)
Grade	
1	97 (23.8)
2	118 (28.9)
3	193 (47.3)
Myometrial invasion	
Superficial (<50%)	260 (55.3)
Deep (≥50%)	210 (44.7)
Lymph node metastasis	
No	291 (77.8)
Yes	84 (22.2)
Stage	
I	340 (62.5)
II	52 (9.6)
III	123 (22.6)
IV	29 (5.3)

Data are presented as median (range) or *n* (%). EEC: Endometrial endometrioid carcinoma; NEEC: Non-endometrial endometrioid carcinoma.

In addition, higher *DNMT3A/3B* expression was associated with poor clinicopathologic variables, including tumor type, tumor grade, lymph node metastasis, and advanced stage with statistical significance [Table 2]. Survival analyses demonstrated reduced overall survival and disease-free survival for patients with *DNMT3A* overexpression ($P=0.002$ and $P=0.001$) [Figure 1C]. Likewise, *DNMT3B* overexpression indicated poor overall survival and reduced disease-free months, but the latter was on the borderline of significance ($P=0.026$ and $P=0.065$) [Figure 1D]. The combined *DNMT3A* and *DNMT3B* overexpression was significantly correlated with poor patient survival [Figure 1E]. However, in the multivariate analysis, only *DNMT3A* remained as an independent prognostic factor ($P=0.013$; Table 3).

DNMT3A/3B overexpression was correlated with hypermethylation and reduced expression of the ESR1 and PGR in EEC

Studies have indicated that the *ESR1/PGR* frequently undergoes *de novo* methylation and lost expression in a wide variety of tumors, including breast, colon, and lung cancer.[7,9] However, the mechanisms underlying their loss of expression in endometrial cancer have not been studied extensively. Therefore, we next analyzed the relationship between the expression of *DNMT3A/3B* and the methylation and expression of the *ESR1/PGR* in EEC and NEEC to assess whether methylation catalyzed by *DNMT3A/3B* contributes to the low ER/PR expression in endometrial cancer.

In the current study, the mean value was used to stratify *ESR1* or *PGR* (genes encoding ER-α and PR, respectively) as hypermethylation or hypomethylation. The mean value of *ESR1* and *PGR* methylation was 0.68 and 0.36, respectively. Using these criteria, in EEC, 41.1% of *ESR1* and 24.8% of *PGR* were hypermethylated, and in NEEC, the rate increased drastically up to 93.3% and 70.6%, respectively [Table 4].

Further analyses revealed that in EEC *DNMT3A* overexpression was significantly correlated with the hypermethylation of the *ESR1* and *PGR* ($P<0.001$) and its low expression, both at the transcript and protein level ($P<0.05$) [Table 5]. The hypermethylation (52.1% and 35.5%) and reduced expression (29.4%, 37.7%) of ER/PR occurred more frequently in tumors with *DNMT3A* overexpression than in those without *DNMT3A* overexpression (35.8%, 18.4%; 14.1%, 16.9%). The same trend was observed in the *DNMT3B* overexpression subgroup, and almost all the associations approached statistical significance, except for the correlation between *DNMT3B* overexpression and *ESR1* mRNA downregulation [Figure 2A and 2B].

However, the above phenomena were not present in NEEC. The *DNMT3A/3B* expression had no relationship to either *ESR1/PGR* methylation or expression status; however, *DNMT3A/3B* overexpression, *ESR1/PGR* hypermethylation, and ER/PR low expression occurred more often in NEEC than that in EEC [Table 5].

Hypermethylation was the dominant mechanism resulting in low ESR1/PGR expression in EEC

We found that DNA methyltransferase 3A/3B overexpression was associated with hypermethylation and reduced ER/PR expression in EEC. In addition, limited studies have reported that the downregulation of the estrogen and progesterone receptor in endometrial carcinoma was associated with the methylation of these two genes. For these reasons, we next explored the relationship between ER/PR expression and methylation, CNA and mutation status of these two genes to investigate whether and to what extent methylation contributes to the reduced ER/PR expression in endometrial cancer.

The reduced ER/PR expression occurred in approximately 20% of EEC cases (also stratified by mean value). Among these tumors with low ER/PR expression, as much as 83.1% (54/65) of *ESR1* and 59.5% (50/84) of *PGR* were hypermethylated, while only 7.6% (6/79) of *ESR1* and 13.1% (13/99) of *PGR* was deleted. Both hypermethylation and copy number deletion were significantly correlated with reduced ER/PR expression ($P<0.05$) [Table 4]. Obviously, hypermethylation played a major part in the low expression of the *ESR1* and *PGR*, while copy number deletion played a relatively minor role. The minority of EEC cases had mutations in the *ESR1* or *PGR*; however, it

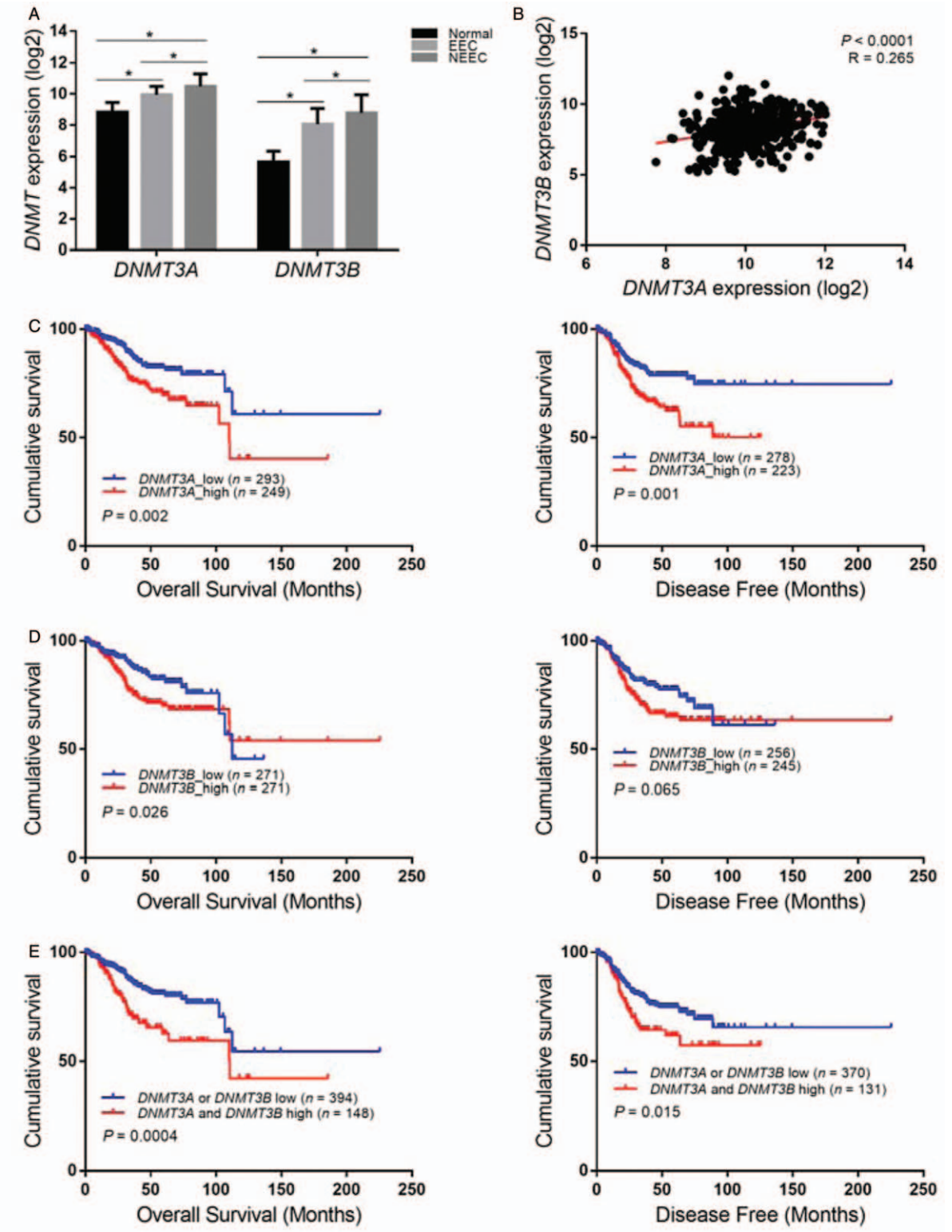

Figure 1: *DNMT3A/3B* overexpressed in endometrial cancers and indicated a poor prognosis. (A) *DNMT3A/3B* overexpressed in EEC and was much higher in NEEC compared with normal controls. *$P < 0.001$ *vs.* normal controls or EEC. (B) The expression of *DNMT3A* and *3B* was significantly positively correlated. (C and D) *DNMT3A* or *DNMT3B* overexpression correlated with poor survival and the combined two markers also implied unfavorable prognosis (e). *DNMT3A/3B*: DNA (cytosine-5)-methyltransferase 3A/3B; EEC: Endometrial endometrioid carcinoma; NEEC: Non-endometrial endometrioid carcinoma.

Table 2: Comparison of clinicopathologic features and expression levels of *DNMT3A/3B* in 544 endometrial cancers

Parameters	mRNA RSEM (log_2) *DNMT3A*	Statistics	*P*	*DNMT3B*	Statistics	*P*	Statistics	*P*
Age		0.931*	0.395		2.412*	0.091		
Pre-menopausal	9.96±0.44			7.90±1.12			10.090†	<0.001
Peri-menopausal	9.97±0.59			8.03±1.25			5.793†	<0.001
Post-menopausal	10.10±0.69			8.30±1.10			29.186†	<0.001
Race		0.800*	0.602		0.163*	0.959		
White	10.09±0.68			8.27±1.06			27.863†	<0.001
Black or African-American	10.09±0.72			8.19±1.17			14.198†	<0.001
Asian	9.89±0.45			8.29±1.10			5.999†	<0.001
Other	10.00±0.41			8.25±1.11			4.937†	<0.001
Histology		−7.440†	<0.001		−6.897†	<0.001		
EEC	9.94±0.55			8.07±1.01			32.757†	<0.001
NEEC	10.49±0.81			8.79±1.16			14.089†	<0.001
Grade		−2.226†	0.027		−4.358†	<0.001		
1 or 2	9.88±0.52			7.87±0.93			27.637†	<0.001
3	10.00±0.57			8.30±1.05			19.777†	<0.001
Myometrial invasion		−1.748†	0.081		−1.667†	0.096		
Superficial (<50%)	10.01±0.64			8.12±1.10			23.894†	<0.001
Deep (≥50%)	10.12±0.69			8.29±1.06			20.891†	<0.001
Lymph node metastasis		−3.089†	<0.001		−1.728†	0.025		
No	10.02±0.65			8.22±1.10			23.184†	<0.001
Yes	10.34±0.70			8.53±1.09			13.018†	<0.001
Stage		−3.785†	<0.001		−2.653†	0.008		
I or II	10.01±0.64			8.17±1.08			28.933†	<0.001
III or IV	10.25±0.70			8.45±1.11			16.952†	<0.001

Data are presented as mean±standard deviation. *DNMT3A/3B*: DNA (cytosine-5)-methyltransferase 3A/3B; EEC: Endometrial endometrioid carcinoma; NEEC: Non-endometrial endometrioid carcinoma; RSEM: RNA-Seq by Expectation maximization; SD: Standard deviation. *F values. †t values.

Table 3: Univariate and multivariate analyses for disease free survival

Variables	Hazard ratio	95% CI	*P*
Univariate analysis			
DNMT3A expression (high *vs.* low)	1.940	1.276–2.949	0.002
DNMT3B expression (high *vs.* low)	1.602	1.053–2.438	0.028
ESR1 expression (high *vs.* low)	0.419	0.278–0.632	< 0.001
PGR expression (high *vs.* low)	0.363	0.239–0.553	< 0.001
Age (continuous)	1.035	1.015–1.055	0.001
BMI (continuous)	0.991	0.969–1.014	0.453
Stage (III or IV *vs.* I or II)	3.889	2.573–5.877	< 0.001
Grade (3 *vs.* 1 or 2)	2.492	1.375–4.514	0.003
Histology (NEEC *vs.* EEC)	2.829	1.873–4.273	< 0.001
Myometrial invasion (deep *vs.* superficial)	2.869	1.780–4.624	< 0.001
Lymph node metastasis (yes *vs.* no)	3.673	2.247–6.004	< 0.001
Multivariate analysis			
DNMT3A expression (high *vs.* low)	2.171	1.180–3.995	0.013
Stage (III or IV *vs.* I or II)	2.604	1.405–4.827	0.002

BMI: Body mass index; CI: Confidence interval; *DNMT3A/3B*: DNA (cytosine-5)-methyltransferase 3A/3B; *ESR1*: Estrogen receptor 1; *PGR*: Progesterone receptor.

seemed that these mutations had little effect on their expression ($P > 0.05$).

In NEEC, approximately 70% to 80% of tumors had reduced ER or PR expression. Hypermethylation and copy number deletion of *ESR1* and *PGR* were frequently observed among these tumors (96.3%, 79.6%; 31.3%, and 63.0%, respectively) and were even higher compared with EEC (83.1%, 59.5%; 7.6%, and 13.1%, respectively). However, the mechanism behind low ER or PR expression seemed to be different. The low PR expression was still significantly correlated with the methylation of the *PGR*

Table 4: Associations between methylation, copy number alteration, and mutation of *ESR1*, *PGR*, and their expression status

Parameters	EEC						NEEC					
	ESR1			*PGR*			*ESR1*			*PGR*		
	Low	High	Total	Low	High	Total	Low	High	Total	Low	High	Total
Methylation			311			311			119			119
Hypomethylation	11 (16.9)	169 (68.7)	180 (57.9)	34 (40.5)	200 (88.1)	234 (75.2)	3 (3.7)	5 (13.5)	8 (6.7)	19 (20.4)	16 (61.5)	35 (29.4)
Hypermethylation	54 (83.1)	77 (31.3)	131 (42.1)	50 (59.5)	27 (11.9)	77 (24.8)	79 (96.3)	32 (86.5)	111 (93.3)	74 (79.6)	10 (38.5)	84 (70.6)
χ^2		56.536			74.664			–			16.539	
P		<0.001			<0.001			0.106			<0.001	
Copy number alteration			401			401			135			135
Deletion	6 (7.6)	5 (1.6)	11 (2.7)	13 (13.1)	18 (6.0)	31 (7.7)	30 (31.3)	5 (12.8)	35 (25.9)	68 (63.0)	20 (74.1)	88 (65.2)
No deletion	73 (92.4)	317 (98.4)	390 (97.3)	86 (86.9)	284 (94.0)	370 (92.3)	66 (68.7)	34 (87.2)	100 (74.1)	40 (37.0)	7 (25.9)	47 (34.8)
χ^2		–			5.375			4.905			1.175	
P		0.010			0.020			0.027			0.278	
Mutation			198			198			48			48
No	23 (92.0)	164 (94.8)	187 (94.4)	29 (93.5)	157 (94.0)	186 (93.9)	37 (77.1)	11 (22.9)	48 (100.0)	42 (87.5)	6 (12.5)	48 (100.0)
Yes	2 (8.0)	9 (5.2)	11 (5.6)	2 (6.5)	10 (6.0)	12 (6.1)	0	0	0	0	0	0
χ^2		–			–							
P		0.633			1.000							

The data were shown as *n* or *n* (%). EEC: Endometrial endometrioid carcinoma; *ESR1*: Estrogen receptor 1; NEEC: Non-endometrial endometrioid carcinoma; *PGR*: Progesterone receptor.

($P < 0.001$) but this association was lost in the low ER expression subgroup ($P = 0.106$). By contrast, deletion of the *ESR1* was more effective in causing ER low expression. In the low ER expression subgroup, 31.3% of cases showed a deletion in *ESR1*, which was significantly higher than that in the ER high-expression subgroup (12.8%; $P = 0.027$). No mutation of *ESR1* or *PGR* was found in NEEC [Table 4].

Combined DNMT3A/3B overexpression and ESR1/PGR methylation or expression were correlated with poor survival

In univariate analysis, *DNMT3A/3B* overexpression, *ESR1/PGR* hypermethylation, and low expression alone or in combination were correlated with poor survival in the whole cohort [Figures 1C, 3 and 4]. However, as mentioned above, in multivariate analysis, only *DNMT3A* was an independent prognostic factor of disease-free survival [Table 3].

Discussion

Taking advantage of the large-scale cancer data sets of TCGA, for the first time, we examined the *DNMT3A/3B* mRNA expression in 544 endometrial carcinomas. Our data set represents the largest series of endometrial cancer cases assessed for *DNMT3A/3B* alterations. We found that *DNMT3A/3B* mRNA was overexpressed progressively from EEC to NEEC compared with normal controls and was significantly correlated with a poor prognosis. The results of this study were not completely consistent with the previous study. Previously, using immunohistochemistry, we found in a small cohort that DNMT3B overexpression was more often associated with EEC than NEEC and was significantly correlated with high tumor grade.[10] Two other groups also observed that DNMT3B expression was higher in EEC than in NEEC.[13] In addition, Xiong *et al*[14] reported no differences of DNMT3A expression among normal endometrium, EEC, and serous endometrial carcinoma. Together, these discrepancies could be explained by two reasons. One might be due to the limited sample size and different testing methods or score criteria used by different studies. The other might be because the DNMT expression at the transcript level was not completely parallel with the protein level.[13] However, regarding the prognostic value, our findings from the present study were in line with others in that poorly differentiated endometrioid cells expressed higher *DNMT3B* and high *DNMT3A* or *DNMT3B* expression implied a poor prognosis.[14-17] In addition, in the present study, we found that *DNMT3A* and *DNMT3B* overexpression coexisted in most cases and the combination of these two biomarkers correlated well with poor prognosis. A study has shown that the structures and functions of DNMT3A and DNMT3B are very similar.[3] Thus, our findings were consistent with others that showed that *DNMT3A* and *DNMT3B* may cooperate and function synergistically in endometrial cancer.

More interestingly, our data indicated that methylation catalyzed by DNMT3A/3B might be the major mechanism resulting in ER/PR downregulation in EEC. First, our results

Table 5: Correlation between *DNMT3A/3B* expression and the methylation and expression status of *ESR1/PGR*

	DNMT3A			Expression			*DNMT3B*			Expression		
	EEC			NEEC			EEC			NEEC		
Parameters	Low	High	Total	Low	High	Total	Low	High	Total	Low	High	Total
ESR1												
Hypomethylation	122 (64.2)	58 (47.9)	180 (57.9)	1 (2.7)	7 (8.5)	8 (6.7)	114 (62.6)	66 (51.2)	180 (57.9)	2 (5.9)	6 (7.1)	8 (6.7)
Hypermethylation	68 (35.8)	63 (52.1)	131 (42.1)	36 (97.3)	75 (91.5)	111 (93.3)	68 (37.4)	63 (48.8)	131 (42.1)	32 (94.1)	79 (92.9)	111 (93.3)
χ^2		8.033			–			4.077			–	
P		**0.005**			0.432			**0.043**			1.000	
PGR												
Hypomethylation	155 (81.6)	78 (64.5)	233 (74.9)	13 (35.1)	22 (26.8)	35 (29.4)	145 (79.7)	89 (69.0)	234 (75.2)	10 (29.4)	25 (29.4)	35 (29.4)
Hypermethylation	35 (18.4)	43 (35.5)	78 (25.1)	24 (64.9)	60 (73.2)	84 (70.6)	37 (20.3)	40 (31.0)	77 (24.8)	24 (70.6)	60 (70.6)	84 (70.6)
χ^2		11.526			0.847			4.621			0.000	
P		0.001			0.357			**0.032**			1.000	
ESR1												
Low	36 (14.1)	45 (29.4)	81 (19.9)	27 (65.9)	70 (73.7)	97 (71.3)	43 (18.3)	38 (22.0)	81 (19.9)	29 (78.4)	68 (68.7)	97 (71.3)
High	219 (85.9)	108 (70.6)	327 (80.1)	14 (34.1)	25 (26.3)	39 (28.7)	192 (81.7)	135 (78.0)	327 (80.1)	8 (21.6)	31 (31.3)	39 (28.7)
χ^2		14.057			0.859			0.842			1.237	
P		**<0.001**			0.354			0.359			0.266	
PGR												
Low	43 (16.9)	58 (37.7)	101 (24.8)	34 (82.9)	75 (78.9)	109 (80.1)	46 (19.6)	55 (31.8)	101 (24.8)	31 (83.8)	78 (78.8)	109 (80.1)
High	211 (83.1)	96 (62.3)	307 (75.2)	7 (17.1)	20 (21.1)	27 (19.9)	189 (80.4)	118 (68.2)	307 (75.2)	6 (16.2)	21 (21.2)	27 (19.9)
χ^2		22.125			0.285			7.985			0.422	
P		**<0.001**			0.593			**0.005**			0.516	
ER-α												
Low	55 (27.1)	61 (48.8)	116 (35.4)	26 (78.8)	51 (66.2)	77 (70.0)	53 (28.5)	63 (44.4)	116 (35.4)	22 (71.0)	55 (69.6)	77 (70.0)
High	148 (72.9)	64 (51.2)	212 (64.6)	7 (21.2)	26 (33.8)	33 (30.0)	133 (71.5)	79 (55.6)	212 (64.6)	9 (29.0)	24 (30.4)	33 (30.0)
χ^2		15.946			1.734			8.874			0.019	
P		**<0.001**			0.188			**0.003**			0.890	
PR												
Low	105 (51.7)	86 (68.8)	191 (58.2)	31 (93.9)	63 (81.8)	94 (85.5)	98 (52.7)	93 (65.5)	191 (58.2)	27 (87.1)	67 (84.8)	94 (85.5)
High	98 (48.3)	39 (31.2)	137 (41.8)	2 (6.1)	14 (18.2)	16 (14.5)	88 (47.3)	49 (34.5)	137 (41.8)	4 (12.9)	12 (15.2)	16 (14.5)
χ^2		9.275			–			5.428			–	
P		**0.002**			0.141			**0.020**			1.000	

The data were shown as *n* (%). BMI: Body mass index; *DNMT3A/3B*: DNA (cytosine-5)-methyltransferase 3A/3B; EEC: Endometrial endometrioid carcinoma; ER-α: Estrogen receptor-α; ESR1: Estrogen receptor 1; NEEC: Non-endometrial endometrioid carcinoma; PGR: Progesterone receptor; PR: Progesterone receptor (PR is encoded by a single *PGR* gene).

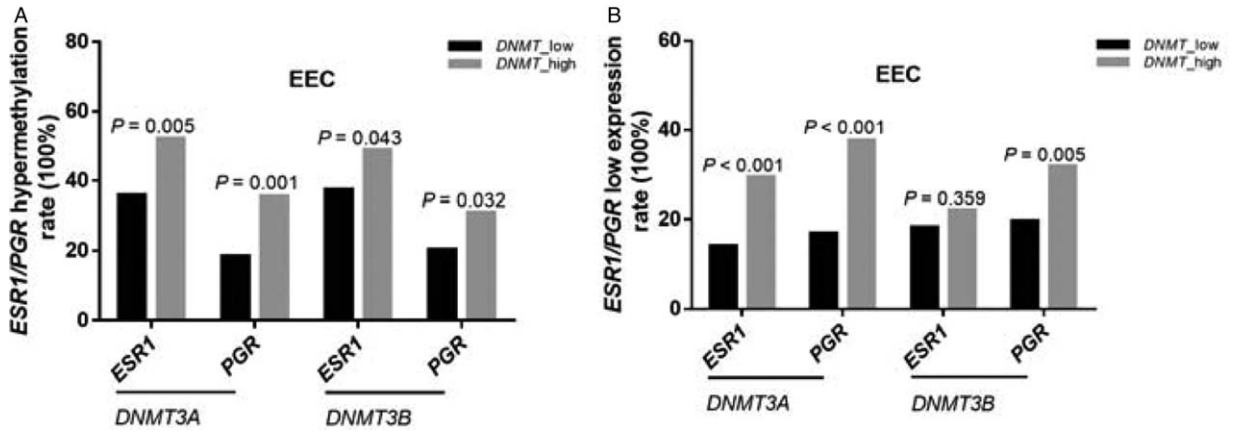

Figure 2: Hypermethylation (A) and reduced expression (B) of *ESR1/PGR* occurred more frequently in tumors with *DNMT3A/3B* overexpression compared with tumors without *DNMT3A/3B* overexpression in EEC; however, the phenomena were not present in NEEC. *DNMT3A/3B*: DNA (cytosine-5)-methyltransferase 3A/3B; EEC: Endometrial endometrioid carcinoma; *ESR1*: Estrogen receptor 1; NEEC: Non-endometrial endometrioid carcinoma; *PGR*: Progesterone receptor.

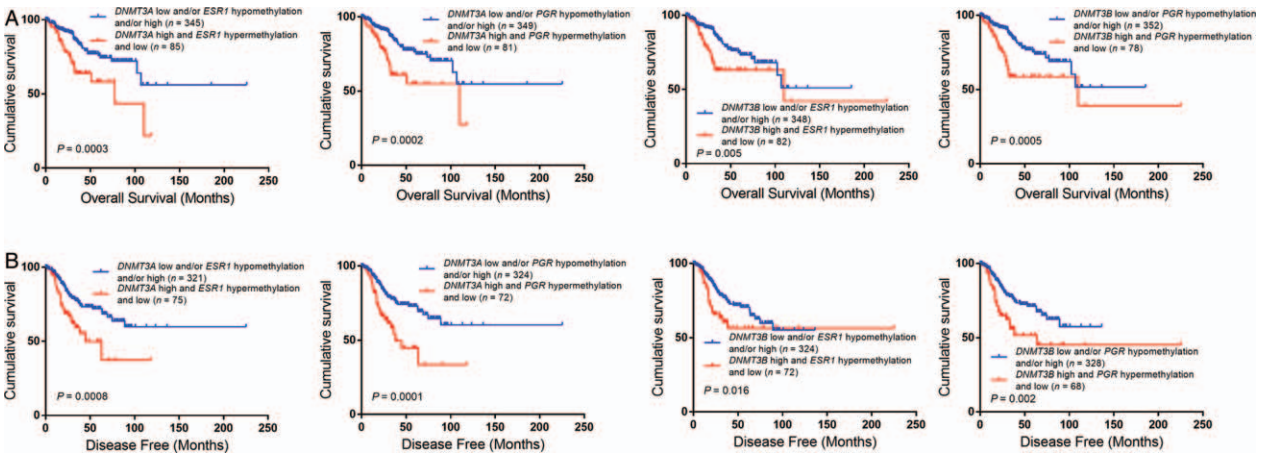

Figure 3: Hypermethylation or low expression of *ESR1/PGR* was correlated with poor survival. *DNMT3A/3B*: DNA (cytosine-5)-methyltransferase 3A/3B; EEC: Endometrial endometrioid carcinoma; *ESR1*: Estrogen receptor 1; NEEC: Non-endometrial endometrioid carcinoma; *PGR*: Progesterone receptor.

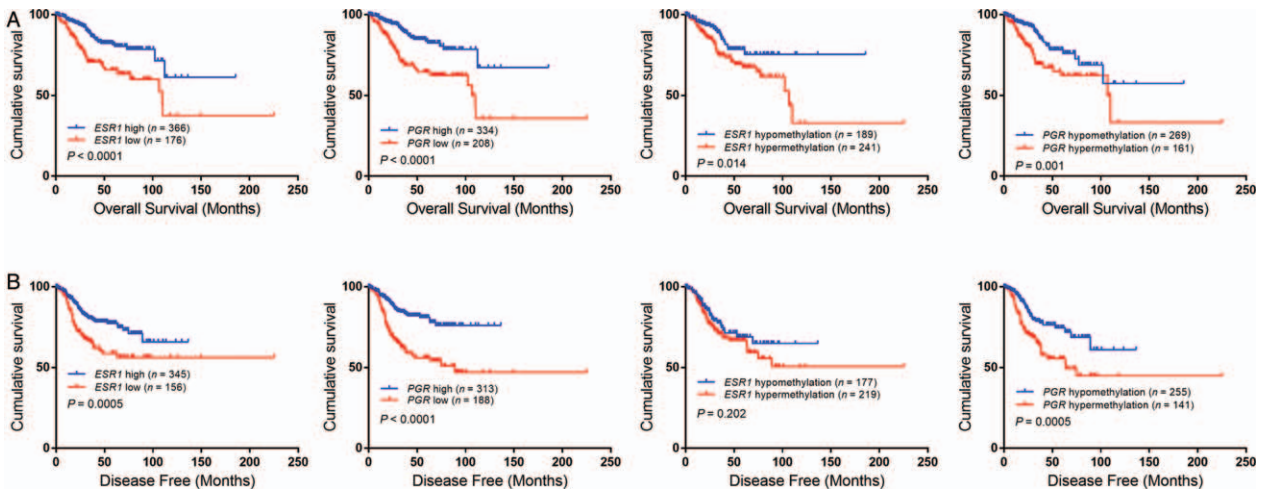

Figure 4: Combination of *DNMT3A/3B* overexpression with *ESR1/PGR* low expression and/or hypermethylation indicated poor prognosis in endometrial cancers. *DNMT3A/3B*: DNA (cytosine-5)-methyltransferase 3A/3B; *ESR1*: Estrogen receptor 1; *PGR*: Progesterone receptor.

support that *ESR1/PGR* silencing occurred primarily at the epigenetic level. Among the ER and PR low expression subgroups, 83.1% of *ESR1* and 59.5% of *PGR* were hypermethylated with statistical significance, with a minority of cases showing deletion (7.6% for *ESR1*, 13.1% for *PGR*) or mutation (8.0% for *ESR1*, 6.5% for *PGR*). The former was also significantly correlated with ER/PR downregulation, whereas mutations of *ESR1* and *PGR* seemed to have little effect. Second, in EEC, *DNMT3A* overexpression was significantly associated with *ESR1* and *PGR* hypermethylation and their low expression. The low ER/PR expression (29.4%, 37.7%) and hypermethylation (52.1%, 35.5%) occurred more frequently in the *DNMT3A* overexpression subgroup compared with the *DNMT3A* normal expression subgroup (14.1%, 16.9%; 35.8%, and 18.4%, respectively). Similar phenomena were observed in the *DNMT3B* overexpression subgroups. Third, the combined *DNMT3A/3B* and ER/PR status or these biomarkers alone were all correlated with shorter survival, and *DNMT3A* was an independent prognostic factor in multivariate analysis.

To date, studies concerning the basis of ER/PR downregulation in endometrial cancer are not extensive; however, a link between *ESR1/PGR* methylation and expression loss in breast carcinoma has been established.[6,18] Early studies reported that the *ESR1/PGR* was *de novo* methylated in some ER/PR-negative endometrial cancers,[19-22] while the other two groups found that the *ESR1* was highly refractory to *de novo* methylation.[23,24] Considering that TCGA adopted the HM450 bead assay to assess over 480K CpG sites covering approximately 96% of CpG islands from the UCSC database, the findings of the present study are robust and support that in EEC methylation-associated transcriptional silencing may account for the great majority of cases of ER/PR low expression, and this may be related to *DNMT3A/3B* overexpression. A recent study indicated that DNMT could be specifically targeted on particular loci and take part in specific *de novo* methylation.[3] From this point of view, our findings were consistent with this notion to some extent.

In addition, our data also demonstrated that the mechanism underlying ER or PR low expression in NEEC was distinct from that in EEC. In NEEC, the *ESR1* deletion might play a more important role in the low expression of *ESR1* compared with hypermethylation. NEEC showed many more *ESR1* deletions (25.9%) than EEC (2.7%), and *ESR1* deletion occurred more often in the ER low expression subgroup (31.3%) than in the ER high-expression subgroup (12.8%); the difference showed statistical significance. Furthermore, although *DNMT3A/3B* overexpression (69.9%, 72.8%), hypermethylation (93.3%) and low expression (68.9%) of ER the gene occurred more often in NEEC than those in EEC, their associations were not statistically significant. *ESR1* was consistently hypermethylated in the ER low expression subgroup (96.3%) as well as in the ER high-expression subgroup (86.5%). Together, our findings suggest that low ER expression in NEEC might be related to multiple aberrations, and the contribution of methylation catalyzed by DNMT3A/3B could be shielded by other more dominant factors.

For low PR expression in NEEC, our data support that methylation still exerted great effects, while CNA and mutation had little influence. In the low PR expression subgroup, the hypermethylation rate of *PGR* (79.6%) was significantly higher than in the PR high-expression subgroup (59.5%). However, such methylation was not correlated with *DNMT3A/3B* overexpression. Recently, one study indicated that PR expression is downregulated at four different levels.[25] In well-differentiated endometrial carcinomas, ligand-induced receptor activation and downregulation are intact. miRNAs mediate the fine tuning of the PR level. As differentiation is lost, PR silencing is mainly at an epigenetic level. Initially, recruitment of the polycomb repressor complex 2 to the PR promoter suppresses transcription. Subsequently, DNA methylation prevents PR expression. Together, all these findings might imply that although methylation is the primary mechanism of PR downregulation in EEC as well as in NEEC, other regulators may be different between endometrial cancer subtypes.

A major limitation of this study is that we used the RNA-Seq expression count of DNMTs from the TCGA to perform the analysis of EC samples without available corresponding protein data to confirm the result; thus, our findings should be interpreted with caution. In addition, data regarding lymphovascular space invasion, recurrence sites, and treatment modalities were not recorded in the TCGA data set, thus limiting the clinical outcome analysis of patients with EC. Nonetheless, the RNA-Seq and its large-scale, uniform accuracy, robust methodology, and high throughput nature, strengthen this study to some extent.

In summary, our findings suggest that *DNMT3A/3B* overexpression was associated with poor survival in endometrial cancer. *DNMT3A/3B* might function synergistically. The mechanisms underlying low ER/PR expression may be distinct in EEC *vs.* NEEC. In EEC, methylation induced by *DNMT3A/3B* overexpression might play a major role in ER/PR downregulation. Thus, advances in the understanding of the molecular mechanisms of endometrial carcinomas will facilitate the development of novel anticancer therapeutic strategies.

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Conflicts of interest

None.

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