

# Importance of promoter methylation of GATA4 gene in epithelial ovarian cancer

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**Aims.** Ovarian cancer is the most lethal gynecological malignancy, with typically late diagnosis. Altered DNA methylation of tumor suppressor gene promoters probably plays a relevant role in ovarian carcinogenesis and frequently occurs as an early event in the development of different types of cancer including ovarian carcinoma. GATA4 methylation has been reported in a variety of human cancers. The aim of this study was to investigate promoter methylation of the GATA4 gene in ovarian cancer by comparison with that in normal ovarian tissue.

**Methods.** To search for promoter methylation of the GATA4 gene we used MSP (methylation-specific PCR) to compare the methylation status in 67 tissue samples of ovarian cancer with that in 40 control samples.

**Results.** In our study, methylation-specific PCR revealed GATA4 promoter methylation in 21 of 67 specimens with ovarian cancer (31.3%), and in none of the control ovarian tissue samples.

**Conclusion.** These results confirm that methylation in the GATA4 promoter region could play an important role in ovarian carcinogenesis, and show new loci which are highly methylated only in ovarian cancer samples and which are associated predominantly with the endometrioid type of ovarian carcinoma.

**Key words:** methylation, GATA4, ovarian cancer, epigenetics

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## INTRODUCTION

Ovarian cancer is the leading cause of death from gynecologic tumors due to its aggressive nature and the fact that the majority of patients are diagnosed in the advanced stages of the disease. It has generally been assumed that if ovarian cancer could be diagnosed at an early stage, this would result in a significant improvement in survival<sup>1</sup>. The etiology of ovarian cancer is still not clear. Based on epidemiology studies, the hypothesis has been proposed that repeated wound repair after ovulation may contribute to the ovarian tumorigenesis processes<sup>2</sup>.

Both genetic and epigenetic changes contribute to malignant transformation and progression. Commonly occurring epigenetic events include DNA methylation, the addition of a methyl group at the 5'-carbon of cytosine in CpG context, mediated by DNA methyltransferases (DNMTs). Aberrant methylation of normally unmethylated CpG islands, located in the 5' promoter region of genes, has been associated with transcriptional inactivation of several genes in human cancer, and can serve as an alternative to mutational inactivation<sup>3</sup>. It has been suggested that DNA methylation profiles may be useful biomarkers and may also serve as a potential target for development of antimethylation therapeutic strategies<sup>4</sup>.

Transcription factors of the GATA family are essential regulators of the specification and differentiation of

numerous tissues<sup>5</sup>. Six distinct vertebrate GATA proteins have been characterized and classified into two subfamilies based on their structural and expression patterns. GATA1, GATA2 and GATA3 are important in the development and differentiation of the hematopoietic cell lineage, while GATA4, GATA5 and GATA6 are expressed in various mesoderm- and endoderm-derived tissues such as heart, liver, lung, gonad and gut, where they play critical roles in regulating tissue-specific gene expression<sup>6,7</sup>.

It has been suggested that GATA4 and GATA5 tend to mark fully-differentiated epithelial cells, while GATA6 is expressed in the immature proliferating cells in the intestinal crypts. This would implicate GATA4 and GATA5 as potential tumor suppressors and GATA6 as a potential oncogene<sup>5</sup>.

Not only genetic changes but also epigenetic changes, such as methylation of CpG islands in the gene promoter, play important roles in the loss of GATA gene function. Frequent GATA4 and GATA5 methylation has been reported in primary gastrointestinal, lung and also ovarian cancers<sup>8-10</sup>. GATA is an important large family of transcription factors, and hence in our study we focused on methylation of the GATA4 transcription factor.

The aim of this study was to confirm the importance of GATA4 methylation in ovarian cancer and to find new loci which are methylated only in ovarian cancer samples.

## MATERIALS AND METHODS

Formalin-fixed and paraffin-embedded tissue samples of ovarian adenocarcinomas and normal ovarian tissue were obtained from 107 women treated at the Department of Obstetrics and Gynecology, University Hospital Hradec Kralove, Czech Republic: 67 patients with ovarian cancer, and 40 patients with normal ovary. The samples of normal ovary were obtained from patients treated surgically for a non-malignant diagnosis (such as descent of uterus with adnexectomy, uterine leiomyomas, etc.). The paraffin blocks were retrieved from the archive of the Fingerland Department of Pathology, University Hospital Hradec Kralove. All slides were reviewed by an experienced pathologist and the carcinomas were classified according to the current WHO classification of tumors of the female genital organs<sup>11</sup>. The study was approved by the Ethics Committee of Faculty Hospital Hradec Kralove.

DNA was extracted from formalin-fixed, paraffin-embedded samples using a Qiagen (Hilden, Germany) DNA extraction kit.

### GATA4 MSP (Methylation-specific PCR)

DNA methylation patterns in the CpG islands of the promoter region of the GATA4 gene were determined by methylation-specific PCR (MSP) (ref.<sup>12</sup>). Sodium bisulfite modification was performed using the CpGenome DNA modification Kit (Chemicon International, Temecula, CA) according to the manufacture's protocol, with minor modifications. Briefly, 1 µg isolated DNA was denatured with NaOH (final concentration, 0.2 M) for 10 min at 50 °C. Freshly-prepared sodium bisulfite solution at pH 5.0 (550 µL) was added and incubated at 50 °C for 17-19 h. The modified DNA was purified, treated with NaOH (final concentration 0.3 M) for 5 min at room temperature, and then precipitated with ethanol. The DNA was re-suspended in elution buffer and stored at -80 °C.

**Table 1.** Clinicopathological characteristics vs. methylation of GATA4.

Characteristic		Number of samples	% of samples	GATA4 methylation (% of samples)
Age	≤ 45 years	9	13.4	33.3
	> 45 years	58	86.6	31
Stage	I. + II.	23	34.3	43.5
	III. + IV.	44	65.7	25
Histology	serous	47	70.1	19.1
	endometrioid	13	19.4	69.2
	clear cell	2	3	50
	mucinous	5	7.5	40
Grade	1	12	17.9	33.3
	2	15	22.4	53.3
	3	38	56.7	23.7
	dedifferentiated	2	3	0

Primer sequences were designed using MethPrimer. 5'-GGTTAGTTAGTGTTTTAGGGTTGA-3' (left) and 5'-AACAAAAACAAAAAACTCCAAA-3' (right) for unmethylated reaction (PCR product 230 bp), and 5'-GTTAGTTAGCGTTTATAGGGTTCGA-3' (left) and 5'-CAAAAACGAAAAAACTCCGAA-3' (right) for methylated reaction (PCR product 228 bp). PCR was carried out in a 25-µL mixture containing 10x Takara buffer (2.5 µL), dNTPs 2.5 mM solution Takara (2.0 µL), primers (1 µL each 10 pmol/µL solution), polymerase Taq HS Takara 5U/µL (0.3 µL) (Takara Bio Europe S.A.S, France), water and 2 µL of bisulfite-modified DNA in a Veriti Thermocycler (Applied Biosystems, CA). The cycling condition consisted of an initial denaturation at 95 °C for 5 min, 40 cycles of denaturing at 95 °C for 45 s, annealing at 53.7 °C for 35 s, and extension at 72 °C for 35 s, followed by final extension for 5 min at 72 °C.

CpG universal methylated and unmethylated DNA (Chemicon International, Temecula, CA) were similarly treated with bisulfite and were used as controls.

Amplified products were separated by electrophoresis on 2% agarose gels and visualized under ultraviolet light after staining with ethidium bromide.

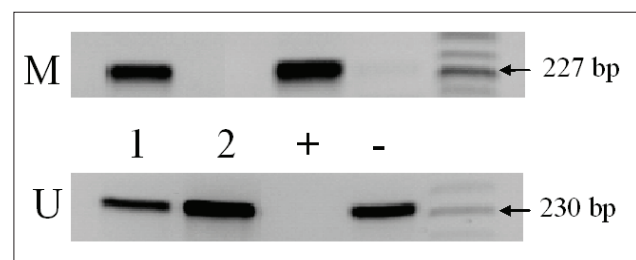
### Statistical analysis

Proportions were compared by two-tailed Fisher's exact test. Associations with p-values <0.05 were considered significant.

## RESULTS

### MSP (Methylation-specific PCR)

In the present study we used MSP to analyze samples from 67 patients with ovarian cancer and 40 control samples (Fig. 1). The median age at the time of diagnosis was 54 years (range 21-79 years) in the carcinoma group and 57.5 years (range 40-84 years) in the control group. MSP revealed statistically significant higher promoter methylation ( $P<0.001$ ) of the GATA4 gene in ovarian cancer pa-



**Fig. 1.** Methylation-specific PCR of the GATA4 promoter region in tumor samples. (+ universally methylated positive control DNA, - universally unmethylated negative control DNA). The presence of visible PCR product in the lane marked U indicates the presence of unmethylated GATA4 gene, the presence of product in the lane marked M indicates presence of methylated GATA4 gene. Sample No. 1 has partial methylated analyzed CpG loci of GATA4 gene and sample No. 2 has unmethylated analyzed CpG loci of GATA4 gene.

tients than in the control group. The promoter of GATA4 gene was methylated in 21 of 67 in the ovarian cancer group (31.3%), and in none of the control group.

The methylation results from the ovarian cancer specimens were compared against clinicopathological characteristics, including age of the patient, tumor type, tumor stage and tumor grade (Table 1). GATA4 showed statistically significant higher methylation in the endometrioid tumor type compared with the serous histological type of ovarian carcinoma.

## DISCUSSION

GATA factors are implicated in gene expression and cellular differentiation. Loss of GATA4 expression has been frequently observed in ovarian cancer<sup>13-15</sup>. Expression was found to be lost in most serous carcinomas but retained in the majority of mucinous ovarian carcinomas<sup>14</sup>. Cai et al.<sup>15</sup> showed absent GATA4 expression in high percentages of serous, clear-cell and endometrioid ovarian carcinomas. On the other hand, McEachin et al.<sup>16</sup> found that the majority of ovarian surface epithelial carcinomas retained GATA4 expression. These findings suggest that GATA4 transcription factor plays an important role in ovarian carcinogenesis and seems to influence the histological type of ovarian cancer.

Methylation-mediated downregulation of tumor suppressor genes has been described in cancer development and progression. Epigenetic silencing of the GATA4 gene has been reported in numerous human cancers<sup>8-10</sup>. The purpose of this study was to investigate promoter methylation of the GATA4 gene in 67 ovarian cancer and 40 control samples. In the present study, we found GATA4 promoter methylation in 21 of 67 in the ovarian cancer group (31.3%). There was a statistically significant difference in GATA4 methylation between the ovarian cancer group and the non-malignant group, suggesting that the methylation in the analyzed CpG loci of the GATA4 gene may play an important role in triggering the transformation to malignant tumors. This finding correlates well with the research of Wakana et al.<sup>10</sup>. Although these authors analyzed the methylation status of the GATA4 gene in different loci, they also found frequent methylation of the GATA4 gene in ovarian cancer cell lines and in 15 ovarian cancer patients. Based on these findings we can say that GATA4 methylation plays an important role in ovarian carcinogenesis, especially in the endometrioid type. Since methylated DNA has been detected in the body fluids of ovarian cancer patients, for example in plasma, and the level correlated reasonably well with methylation levels in tumor tissue<sup>17</sup>, detection of GATA4 methylation could be used in future screening for ovarian cancer.

Methylation patterns are often associated with pathological features of ovarian cancer<sup>18</sup>. Methylation in the GATA4 gene in ovarian cancer may be associated with tumor stage, grade and histological type. Ours is the first study to show significantly higher methylation in the GATA4 gene in the endometrioid type compared with the serous type of ovarian cancer (69.2% vs. 19.1%). The in-

cidence of GATA4 methylation increases slightly in early stage tumors compared to late stage ones (43.5% vs. 25%); however, the number of samples in the subgroups was too small to reveal any statistical significance and draw any conclusions. Grade 2 tumor showed higher methylation in the GATA4 gene than in other grades. A study with a larger number of samples is necessary to determine whether GATA4 methylation is associated with stage and grade of ovarian tumors.

Integrated genomic analyses of ovarian carcinoma showed four promoter methylation ovarian cancer subtypes that were significantly associated with differences in age, BRCA inactivation events and survival. The differences between ovarian cancer subtypes probably reflect a combination of etiological and lineage effects, and may present an opportunity to improve ovarian cancer outcomes through subtype-stratified care<sup>19</sup>. Our study showed promoter methylation in 31.3% of ovarian carcinomas, predominantly in the endometrioid type and hence we presume that this subset of samples could constitute some form of ovarian cancer subtype.

In conclusion, our study showed that there is a significant difference in promoter methylation of the GATA4 gene between ovarian cancer and control samples, suggesting the importance of this gene in ovarian carcinogenesis.

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## CONFLICT OF INTEREST STATEMENT

**Author's conflict of interest disclosure:** *None declared.*

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