

## Methylation in the p53 promoter in epithelial ovarian cancer

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### Abstract

**Objective** Ovarian cancer is a leading cause of death from gynecologic tumors, however, the molecular and especially epigenetic events underlying this transformation are poorly understood. Promoter methylation status of tumor suppressor genes may be associated with transcriptional silencing and tumor progression. It has been shown that methylation of CpG dinucleotides located in the promoter region of p53 is associated with low expression levels of this gene. The aim of this study was to investigate promoter methylation of p53 gene in ovarian cancer by comparison with normal ovarian tissue.

**Methods** To search for promoter methylation of p53 gene we used methylation-specific PCR (MSP) to compare the methylation status of 66 tissue samples of ovarian cancer with 37 control samples.

**Results** In our study methylation specific PCR revealed p53 promoter methylation in 34 of 66 (51.5 %) of specimens with ovarian cancer.

**Conclusion** These results indicate that methylation in p53 promoter region may play an important role in

carcinogenesis of ovarian cancer and could potentially be used in screening of ovarian cancer, and may have implications for future chemotherapy based on epigenetic changes.

**Keywords** p53 · Promoter methylation · Ovarian cancer

### Introduction

Ovarian cancer is a leading cause of death from gynecologic tumors due to its aggressive nature and the fact that the majority of patients are diagnosed in advanced stages of the disease. Survival is high in women with stage-I ovarian cancer, who have 5-year survival rate of over than 90 % [1], but only 25 % of woman with advanced ovarian cancer are alive at 5 years from diagnosis. 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.

The role of epigenetics in cancer is undisputed. 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 [2].

Aberrant methylation of multiple CpG islands is a frequent event in epithelial ovarian cancer. CpG islands hypermethylation of tumor suppressor genes such as BRCA1 [3], RASSF1A [4], MLH1 [5], ARH1 [6] and OPMLC [7], among others, is a known event in ovarian tumorigenesis. However, the importance of epigenetic changes in tumor suppressor genes in ovarian cancer remains largely unknown, and it is possible that more genes will be identified as frequently inactivated through DNA methylation and involved in the pathogenesis of ovarian cancer.

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Protein p53 is a 53-kD nuclear phosphoprotein (393 amino acids), the product of a 20-Kb gene localized on the short arm of human chromosome 17, at position 17p13.1. The p53 gene has 11 exons, of which the first exon (213 bp), located 8–10 Kb away from the second exon, is noncoding [8]. The p53 promoter region has been sequenced and basal promoter activity localized to an 85 bp region (nucleotide 760–844) that is indispensable for full promoter activity [9] and the p53 promoter has putative binding sites for transcriptional factors.

This protein is the principal mediator of cell-cycle arrest, senescence and apoptosis in response to a broad array of cellular damage. Its multiple functions include regulation of gene transcription, induction of G1/S arrest and promotion of apoptosis.

The p53 is the most frequently mutated gene in ovarian cancer (50–70 %) [10], and therefore, it has been extensively investigated. However, its methylation status in ovarian cancer has not been reported. Methylation of the p53 promoter region in several human neoplasms has been reported [11–14]. The purpose of this study was to examine the promoter methylation status of p53 in ovarian cancer and correlate our findings with available clinicopathologic variables.

## Materials and methods

Formalin-fixed, paraffin-embedded tissue samples of ovarian adenocarcinomas and normal ovarian tissue were obtained from 103 women treated at the Department of Obstetrics and Gynecology, University Hospital Hradec Kralove, Czech Republic: 66 patients with ovarian cancer, and 37 patients with normal ovary. The samples of normal ovary were obtained from patients surgically treated for a non-malignant diagnosis (such as descent of uterus with adnexectomy, uterine myomas, etc.). The paraffin blocks were retrieved from the archive of the Fingert Department of Pathology, University Hospital Hradec Kralove. All slides were reviewed by an experienced pathologist and the carcinomas classified according to the current WHO classification of tumors of the female genital organs [15]. The study was approved by the Ethics Committee of University Hospital Hradec Kralove.

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

### p53 promoter methylation (MSP)

DNA methylation patterns in the CpG islands of the promoter region of the p53 gene were determined by MSP [16]. Sodium bisulfite modification was performed using the CpGenome DNA modification Kit (Chemicon

International, Temecula, CA) according to the manufacturer's protocol with minor modifications. Briefly, 1  $\mu$ g 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 (550  $\mu$ l) 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, followed by ethanol precipitation. DNA was re-suspended in elution buffer and stored at –80 °C.

Primer sequences have been reported previously [12]. 5'-TTGGTAGGTGGATTATTTGTTT-3' (sense) and 5'-CC AATCCAAAAAACATATCAC-3' (antisense) for unmethylated reaction (PCR product 247 bp), and 5'-TTCCG GTAGCGGATTATTTG-3' (sense) and 5'-AAATATCC CCGAAACCCAAC-3' (antisense) for methylated reaction (PCR product 193 bp). PCR was carried out in a 25- $\mu$ l mixture, containing 10 $\times$  Takara buffer (2.5  $\mu$ l), dNTPs 2.5 mM solution Takara (2.0  $\mu$ l), primers (1  $\mu$ l each 10 pmol/ $\mu$ l solution), polymerase Taq HS Takara 5U/ $\mu$ l (0.3  $\mu$ l) (Takara Bio Europe S.A.S, France), water and 2  $\mu$ l of bisulfite-modified DNA in a Veriti thermocycler (Applied Biosystems, CA). The cycling conditions consisted of an initial denaturation at 95 °C for 7 min, 40 cycles of denaturing at 95 °C for 45 s, annealing at 59 °C for 45 s, and extension at 72 °C for 60 s, followed by final extension for 5 min at 72 °C.

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

Amplified products were electrophoresed on 2 % agarose gels, and visualized under ultraviolet light after staining with ethidium bromide.

### Statistical analysis

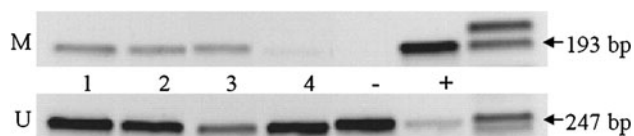
Proportions were compared by two-tailed Fisher's exact test. Associations with *p* value <0.05 were considered to be significant.

## Results

### MSP (Fig. 1)

In the present study, we used the MSP to analyse samples from 66 patients with ovarian cancer and 37 control samples. MSP revealed statistically significant higher promoter methylation (*p* = 0.04) of p53 gene in ovarian cancer patients than in the control group. Promoter of p53 gene was methylated in 34 of 66 (51.5 %) of ovarian cancer and surprisingly in 11 of 37 (29.7 %) of the control group.

The mean (median) age at the time of diagnosis was 54 years (range 21–79 years) in the carcinoma group and



**Fig. 1** Methylation-specific PCR of the p53 promoter region in tumor samples. *Plus symbol* universally methylated positive control DNA, *minus symbol* universally unmethylated negative control DNA. The presence of a visible PCR product in the *lane U* indicates the presence of unmethylated p53 genes, the presence of product in the *lane M* indicates presence of methylated p53 genes. Sample no. 1, 2, 3 have partial methylated promoter region of p53 gene and sample no. 4 has unmethylated promoter region of p53 gene

57 years (range 40–84) in the control group. The methylation results from the ovarian cancer specimens were compared with clinicopathological characteristics including age, histological type, tumor stage, histological grade (Table 1). No significant correlation between p53 methylation and any of these parameters was observed for the ovarian-cancer patients ( $p > 0.05$ ).

## Discussion

The biological features of ovarian cancer are determined by the underlying molecular alterations of the tumor cells, including the inactivation of tumor suppressor genes as well as mutations and deletions. It is now clear that *de novo* promoter methylation is a common mechanism for

**Table 1** Clinicopathological characteristics versus methylation of p53

| Characteristic   | Number of samples | % of samples | P53 methylation (% of samples) |
|------------------|-------------------|--------------|--------------------------------|
| Age              |                   |              |                                |
| ≤45 years        | 10                | 15.2         | 40                             |
| >45 years        | 56                | 84.8         | 53.6                           |
| Stage            |                   |              |                                |
| I. + II.         | 21                | 31.8         | 52.4                           |
| III. + IV.       | 45                | 68.2         | 51.1                           |
| Histology        |                   |              |                                |
| Serous           | 45                | 68.2         | 53.3                           |
| Endometrioid     | 15                | 22.7         | 40                             |
| Clear cell       | 2                 | 3            | 100                            |
| Mucinous         | 4                 | 6.1          | 50                             |
| Grade            |                   |              |                                |
| 1                | 11                | 16.7         | 45.5                           |
| 2                | 16                | 24.2         | 43.8                           |
| 3                | 37                | 56.1         | 56.8                           |
| Dedifferentiated | 2                 | 3            | 50                             |

inactivation of tumor suppressor genes. The promoter methylation status has been reported in several human neoplasms. The purpose of this study was to investigate promoter methylation of p53 gene in 66 ovarian cancer and 37 control samples.

As an important tumor suppressor gene, p53 methylation has been detected in variety of malignancies. For example Kang et al. showed p53 promoter methylation in 3 of 19 (16 %) breast carcinomas with wild-type p53. Amatya et al. showed p53 promoter methylation in over than 60 % human gliomas. Agirre et al. observed p53 promoter methylation in 8 of 25 cases (32 %) of acute lymphoblastic leukemia. Pogribny and James showed that hepatocellular carcinomas with wild type p53 exhibit p53 promoter methylation.

In the present study, we found p53 promoter methylation in 34 of 66 (51.5 %) of ovarian cancer, while the frequency of p53 methylation in non-malignant samples was 11 of 37 (29.7 %). The presence of p53 promoter methylation in non-malignant samples can be due to composition of the control group, most of selected samples were uterine myomas without increased mitotic activity. There is the statistically significant difference in p53 methylation frequency between ovarian cancer and non-malignant group, suggesting that methylation in the promoter region of p53 gene may play an important role in triggering the transformation to malignant tumors.

Promoter methylation in p53 gene in ovarian cancer may be associated with tumor stage, grade and histological type. However, we did not find any statistically significant correlation between p53 methylation status and the clinicopathological characteristics of the ovarian cancer patients. The incidence of p53 promoter methylation increases slightly in high-grade tumors (grade3) and the incidence of p53 promoter methylation also increases (100 %) in tumors with clear cell histology; however, the numbers of samples in this subgroup is very small ( $n = 2$ ) to draw any conclusions. Therefore, a study with a larger number of samples is necessary to determine whether p53 methylation is associated with histology of ovarian tumors.

In conclusion, our study showed that there is significant difference in promoter methylation of p53 gene between ovarian cancer and control samples, suggesting the importance of this gene in ovarian carcinogenesis. This finding could have implications for an ovarian cancer screening program and therapeutic strategies, especially chemotherapy based on epigenetic changes.

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**Conflict of interest** The authors declare that there are no conflicts of interest.

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