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Identification of *PRTFDC1* silencing and aberrant promoter methylation of *GPR150*, *ITGA8* and *HOXD11* in ovarian cancers

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Abstract

Methylated promoter CpG islands (CGIs) can be used to find novel tumor-suppressor genes and disease markers. In this study, to identify promoter CGIs aberrantly methylated in human ovarian cancers, we performed a genome-wide screening for differentially methylated DNA fragments using methylation-sensitive-representational difference analysis (MS-RDA). MS-RDA isolated 185 DNA fragments specifically methylated in an ovarian cancer cell line (ES-2), compared with a normal human ovarian surface epithelial cell line (HOSE6-3), and 33 of them were derived from putative promoter CGIs. Ten ovarian cancer cell lines were analyzed by methylation-specific PCR, and seven (*GPR150*, *LOC222171*, *PRTFDC1*, *LOC339210*, *ITGA8*, *C9orf64* and *HOXD11*) of the 33 CGIs were methylated in one or more of the cell lines. Their downstream genes were barely expressed in cell lines without unmethylated DNA molecules by quantitative reverse-transcription-PCR. Demethylation of methylated cell lines with 5-aza-2′-deoxycytidine restored expression of two genes (*PRTFDC1* and *C9orf64*). In primary ovarian cancers, CGIs of *GPR150* (in 4 of 15 cancers), *ITGA8* (2/15), *PRTFDC1* (1/15), and *HOXD11* (1/15) were methylated. Silencing of *PRTFDC1* was revealed here for the first time, and aberrant methylation of *GPR150*, *ITGA8* and *HOXD11* could be candidate tumor markers.
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Keywords: Ovarian cancer; CpG island; DNA methylation; Epigenetics

Introduction

Aberrant methylation of CpG islands (CGIs) in gene promoter regions is known to silence their downstream genes (Herman and Baylin, 2003; Jones, 2005). In ovarian cancers, gene silencing due to promoter methylation is reported for *CDKN2A/p16* (0–40%) (Kawauchi et al., 2004; Shih et al., 1997; Katsaros et al., 2004), *BRCA1* (15%) (Esteller et al., 2000; Baldwin et al., 2000), *hMLH1* (48%) (Geisler et al., 2003) and *RASSF1A* (10–40%) (Yoon et al., 2001; Agathangelou et al., 2001). In addition to analysis of known tumor-suppressor genes, genome-wide screenings for aberrantly methylated CGIs have been successfully used to identify novel tumor-suppressor genes in colorectal, hepatocellular, gastric and other cancers (Ushijima, 2005). Since genetic and epigenetic alterations known in ovarian cancers are still limited (Li and Karlan, 2001; Imura et al., 2006), genome-wide screening procedures are expected to be useful to identify novel tumor-suppressor genes in ovarian cancers.

Silencing of a specific gene and methylation profiles of a set of genes can also be used as clinical biomarkers to predict drug response, prognosis, and other clinically useful information (Laird, 2003; Miyamoto and Ushijima, 2005). In ovarian cancers, novel prognostic marker genes were successfully identified by use of a genome-wide screening of aberrant methylation (Wei et al., 2002, 2006). Furthermore, DNA methylation itself can be used to detect cancer cells or cancer-derived DNA, taking advantage of techniques that detect

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Table 1
List of primer sequences and PCR conditions for MSP

Genes	Methylation	Forward	Reverse	Annealing temperature (°C)	Length of PCR product (bp)	Number of PCR cycles
GPR150	M	5′-ATT CGT ATA GAT TTA GCG TC-3′	5′-AAT ATT AAA CGC CGA CG-3′	53	151	35
	UM	5′-GATTTAGTGTTGTTTTTTGT-3′	5′-AAAATATTAAACACCAACA-3′	49	144	33
LOC222171	M	5′-TAT AGA AAG CGT TTG TAA CGG C-3′	5′-GAA AAC GAA TCC ACA CCC G-3′	61	117	32
	UM	5′-TTA TAG AAA GTG TTT GTA ATG GT-3′	5′-AAA CAA ATC CAC ACC CA-3′	56	116	33
PRTFDC1	M	5′-GGG TTG TAC GCG ATT ATT C-3′	5′-AAC TAA ACC GCG AAA ACG-3′	58	129	32
	UM	5′-GGG GTT GTA TGT GAT TAT TT-3′	5′-AAC TAA ACC ACA AAA ACA CA-3′	54	130	33
LOC339210	M	5′-GGA GAT TTG CGT CGC GTC-3′	5′-CAA CGT CGT TCT CCT CCT ACG-3′	63	106	32
	UM	5′-TTT TTG GAG ATT TGT GTT GT-3′	5′-AAC ATC ATT CTC CTC CTA CA-3′	56	110	32
ITGA8	M	5′-GCG GGT GGG AGT AGA CGT C-3′	5′-CTA CCC AAA AAC GCG AAC CG-3′	61	138	32
	UM	5′-GGT GGG TGG GAG TAG ATG TT-3′	5′-CTA CCC AAA AAC ACA AAC CA-3′	58	139	33
C9orf64	M	5′-GGA GGT ATC GTC GTT TAT GTC-3′	5′-AAA CGC CTT CGA CAA CG-3′	60	119	32
	UM	5′-GAG GTA TTG TTG TTT ATG TT-3′	5′-AAA ACA CCT TCA ACA ACA-3′	55	119	32
HOXD11	M	5′-ATG CGT TTA GCG GTG ATA GC-3′	5′-AAA CGA CTC CTA ACG CCG-3′	56	143	32
	UM	5′-ATG TGT TTA GTG GTG ATA GT-3′	5′-AAA CAA CTC CTA ACA CCA-3′	55	143	32

aberrant methylation in a minor population of DNA molecules (Laird, 2003; Miyamoto and Ushijima, 2005; Abe et al., 2005). Successful detection of cancer cells has been reported in nipple aspirates, sputum, urine, feces, lymph nodes and other clinical materials. Since one of the causes for the high mortality of ovarian cancers lies in the difficulty in detecting them at early stages (Barnholtz-Sloan et al., 2003), identification of aberrantly methylated genes for marker development is important in ovarian cancers.

In this study, we performed a genome-wide screening for CGIs aberrantly methylated in ovarian cancers, which can lead to identification of novel tumor-suppressor genes and biomarkers. As a procedure for the screening, we adopted methylation-sensitive-representational difference analysis (MS-RDA) (Ushijima et al., 1997; Kaneda et al., 2003; Ushijima, 2005) that has been successfully used to identify aberrantly methylated CGIs in various human cancers (Takai et al., 2001; Kaneda et al., 2002; Hagihara et al., 2004; Miyamoto et al., 2003, 2005; Abe et al., 2005).

Materials and methods

Cell lines, primary tumor samples, and DNA/RNA extraction

OV-90 (serous), TOV-112D (endometrioid), ES-2 (clear cell) and TOV-21G (clear cell) were purchased from the American Type Culture Collection (Manassas, VA). MCAS (mucinous), RMUG-L (mucinous), RMG-I (clear cell), RTSG (poorly differentiated), TYK-nu (undifferentiated), and KURAMOCHI (undifferentiated) were provided by the Japanese Collection of Research Bioresources (Osaka, Japan). Human ovarian surface epithelial (HOSE6-3) cells, established by immortalizing normal human ovarian surface cells with human papilloma virus E6 and E7 (Tsao et al., 1995, 2001), were a

Table 2
List of primer sequences and PCR conditions for RT-PCR

Genes	Forward	Reverse	Annealing temperature (°C)	Length of PCR product (bp)
GPR150	5′-GCTGGCACCTGCAGGTCTA-3′	5′-CGCCACCAGACGGAGAGTA-3′	68	106
LOC222171	5′-GCCTCCGGGTCCTGTTAA-3′	5′-GTCTCCGGCCGTTCACTC-3′	71	107
PRTFDC1	5′-TGTGGTGGGATATGCCTTAGA-3′	5′-TCTGGGACTTTAGTGGTGAGAAT-3′	61	128
LOC339210	5′-GACAGAGAAGCAGGCCAAAC-3′	5′-CAGGTGGTGCATGTATTCCC-3′	72	103
ITGA8	5′-CTGTCAGGCGTTCAACC-3′	5′-CACCAAGACACTCGCTGTG-3′	72	121
C9orf64	5′-TTGGAGCCCTGAAATACTCTGAT-3′	5′-CAAAGCGAGCACCCTCTGAT-3′	67	106
HOXD11	5′-CGCTGTCCCTATACCAAGT-3′	5′-GCATCCGAGAGAGTTGAAGT-3′	69	100
GAPDH	5′-AGGTGAAGGTCGGAGTCA-3′	5′-GGTCATTGATGGCAACAA-3′	68	99

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