

RAS-mediated epigenetic inactivation of OPCML in oncogenic transformation of human ovarian surface epithelial cells

Fang C. Mei,* Travis W. Young,* Jinsong Liu,[†] and Xiaodong Cheng*¹

*Department of Pharmacology and Toxicology, Sealy Center for Cancer Cell Biology, School of Medicine, The University of Texas Medical Branch, Galveston, Texas, USA; and [†]Department of Pathology, The University of Texas, M. D. Anderson Cancer Center, Houston, Texas, USA



To read the full text of this article, go to <http://www.fasebj.org/cgi/doi/10.1096/fj.05-4586fje>; doi:10.1096/fj.05-4586fje

SPECIFIC AIMS

Opioid binding protein/cell adhesion molecule-like gene (OPCML), recently identified as a tumor suppressor gene, is often inactivated by allele loss and CpG island promoter methylation in epithelial ovarian cancer (EOC). Since elevated activation of the RAS signaling pathway, including overexpression of HER-2/neu and mutations of RAS and BRAF, is common in human ovarian carcinoma, we asked whether the expression of OPCML in RAS-transformed human ovarian surface epithelial cells is suppressed and whether RAS activation is directly responsible for the epigenetic inactivation of OPCML.

PRINCIPAL FINDINGS

1. Silencing of OPCML gene in human ovarian surface epithelial cells oncogenically transformed by RAS

The expression of OPCML in a genetically defined ovarian cancer cell line transformed by oncogenic HRAS^{V12} (T29H) was completely abolished when probed by RT-PCR using primers specific for the OPCML gene, while an expected 615 bp PCR products was observed in control non-transformed T29 cells (Fig. 1A). DNA sequence derived from sequencing analysis of the 615 bp PCR product matched perfectly with the predicted OPCML fragment covered by the PCR primers, confirming that the 615 bp RT-PCR product is specific for OPCML.

2. Inactivation of RAS signaling pathway rescues OPCML expression

To ensure that the silencing of OPCML expression observed in T29H cells is indeed mediated by oncogenic RAS, we examined the level of OPCML expression in T29H cells with oncogenic RAS^{V12} levels selectively suppressed by retrovirus-mediated siRNA. As

shown in Fig. 1B, while the expression level of OPCML was undetectable in T29H cells, stable expression of siRNA specific for HRAS^{V12} in T29H-H1 cells led to the re-expression of OPCML.

3. OPCML gene is highly methylated in T29H cells

Sequence analysis of the OPCML promoter revealed a GC-rich region spanning 900 bp approximate 500 bp upstream of the translational start site. To address whether the apparent inactivation of OPCML expression in RAS-transformed human ovarian epithelial cells is associated with DNA methylation, we probed the methylation status of OPCML promoters using methylation sensitive PCR. As shown in Fig. 2A, genomic DNA isolated from T29 cells failed to be amplified after HpaII digestion, indicating the CpG sites of the OPCML promoter within these fragments are not significantly methylated in T29 cells. In contrast, positive products with DNA fragments of the predicted sizes were obtained using treated genomic DNA from T29H cells as template. To further test whether the RAS oncogene is directly responsible for the methylation of OPCML promoter, we examined the methylation status of OPCML promoter in T29H-H1 cells, in which the HRAS^{V12} oncogene activity was specifically suppressed by a retrovirus-mediated siRNA vector. As shown in Fig. 2B, inhibition of oncogenic RAS^{V12} activity decreased the extent of OPCML promoter methylation in T29H-H1 cells to levels similar to that of T29 cells.

4. Modulation of DNMT activity by the RAS signaling pathway in T29H cells

To examine whether the RAS signaling pathway is involved in regulating DNMT activity in T29H cells, we

¹ Correspondence: Department of Pharmacology and Toxicology, The University of Texas Medical Branch, 301 University Blvd., Galveston, Texas 77555-1031, USA. E-mail: xcheng@utmb.edu

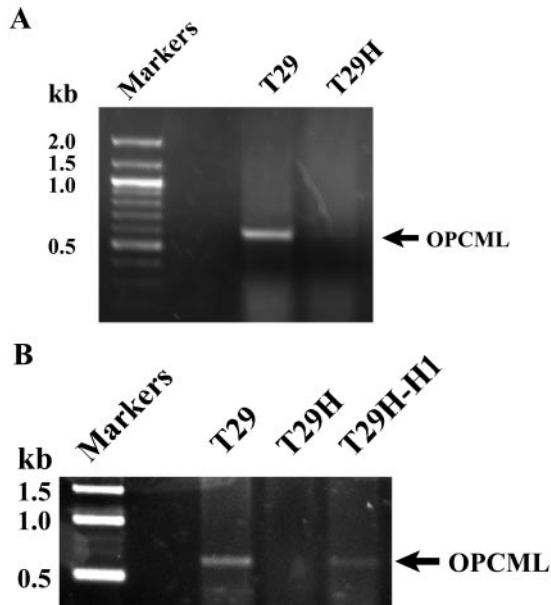


Figure 1. Regulation of OPCML expression by the HRAS^{V12} oncogene. *A)* Loss of OPCML expression in human ovarian surface epithelial cells transformed by oncogenic HRAS^{V12}. Expression of OPCML in T29 and T29H cells was analyzed using RT-PCR. Similar results were obtained from 6 independent experiments. *B)* Suppression of oncogenic RAS activation restored the expression of OPCML in RAS-transformed human ovarian epithelial cells. OPCML expression levels in T29, T29H, and T29H cells treated with H1 siRNA specific for oncogenic HRAS^{V12} were analyzed by RT-PCR. Similar results were obtained from 2 independent experiments.

measured levels of DNMT enzymatic activity of T29H cells in response to pharmacological inhibitors of the RAS signaling pathway. Although the nuclear DNMT activity of T29H cells was slightly higher than that of T29 cells, treatment of T29H cells with inhibitors U0126 and LY294002, which target RAS downstream effectors MEK and PI3K, respectively, resulted in significantly lower levels of DNMT activity than untreated controls. Blocking RAS activation using inhibitor FTI-277, which targets farnesyl transferase, also led to significant inhibition of DNMT activity.

5. DNA methylation is responsible for OPCML-silencing in T29H cells

To examine whether the observed CpG island methylation of OPCML promoter is responsible for the inactivation of the OPCML tumor suppressor gene in T29H cells, we treated T29H cells with 5'-aza-2'-deoxycytidine (AZA), a specific inhibitor of DNA methyltransferase. Southern blot analysis of OPCML RT-PCR products revealed robust and absent expressions of OPCML in T29 and T29H cells, respectively. Re-expression of OPCML and concomitant demethylation of the OPCML promoter were clearly evident after exposure of T29H cells to AZA.

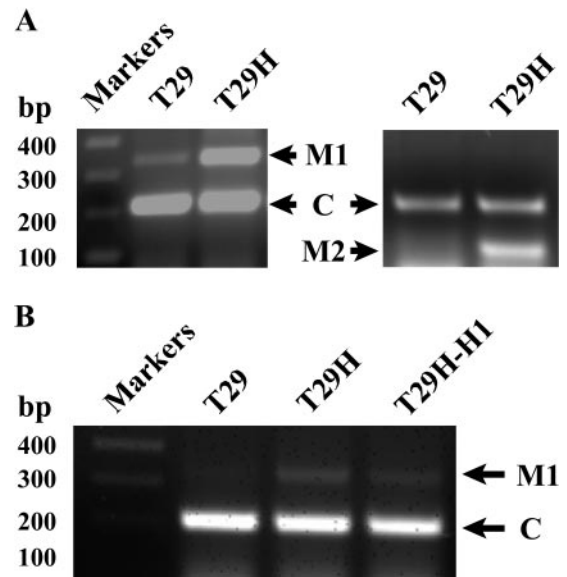


Figure 2. Regulation of OPCML promoter methylation by the HRAS^{V12} oncogene. *A)* Methylation of the OPCML promoter CpG dinucleotides in RAS-transformed human ovarian epithelial cells. *B)* Suppression of oncogenic RAS activation in T29H cells promoted the demethylation OPCML promoter. Methylation-sensitive PCR assay of genomic DNAs isolated from T29, T29H, and T29H-H1 cells was conducted. PCR products amplified using methylation-sensitive primers (M1 and M2) and control methylation-insensitive primers (C) were separated by agarose gel electrophoresis, and visualized by ethidium bromide staining. Similar results were obtained from 3 independent experiments.

CONCLUSIONS AND SIGNIFICANCE

We show here that expression of oncogenic RAS in immortalized human ovarian epithelial cells abolished the expression of OPCML, a recently identified tumor suppressor gene implicated in human EOC. Treatment of RAS-transformed human ovarian surface epithelial cells with retrovirus-mediated siRNA specific for oncogenic RAS^{V12} and AZA, a specific DNA methyltransferase inhibitor, restored OPCML expression. These

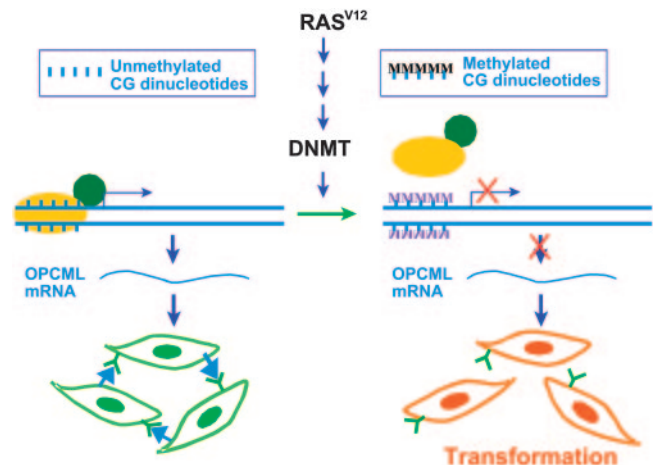


Figure 3. Schematic diagram.

data combined with our direct demonstration of promoter methylation using methylation-sensitive PCR suggest that oncogenic RAS-mediated gene silencing of the tumor suppressor gene OPCML in human ovarian surface epithelial cells involves CpG promoter hypermethylation. Therefore, epigenetic inactivation of OPCML by oncogenic RAS may represent an important mechanism of RAS-mediated oncogenic transformation of human ovarian surface

epithelial cells in EOC (**Fig. 3**). Consistent with this notion, our study shows that overall DNMT activity is modulated by the RAS signaling pathway in T29H cells. Further study to determine the molecular mechanism by which oncogenic RAS induces epigenetic inactivation of OPCML and other tumor growth inhibition genes in EOC will provide additional insights into understanding of ovarian cancer development and RAS-mediated tumorigenesis. EJ