Roles for Tumor Suppressor Gene Methylation on the Meningioma Grades and Outcome

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Abstract

Objective: Aberrant methylation of Cpg islands in promoter of human genes is known as an alternative mechanism of gene silencing that contributes to tumorigenesis in various human tumors. DNA methylation status of tumor suppressor genes (TSGs) is poorly understood in meningiomas, so we performed this study to determine the roles for DNA methylation on the meningioma grades and outcome.

Methods: We have examined the methylation status of 5 TSGs in 81 human meningiomas (59 Grade I, 15 Grade II, and 7 Grade III) by methylation specific polymerase chain reaction (PCR).

Results: Five TSGs showed different profile of methylation status: 46.9% for p16, 21.0% for p14, 42.0% for RB1, 7.4% for RASSF1A, and 42.0% for E-cadherin. DNA hypermethylation frequencies in p16, p14, RASSF1A, and E-cadherin were higher in Grade II than in Grade I and Grade III except RB1. There were no correlations of the methylation status of these TSGs with meningioma grades. Also, the methylation status of all TSGs was not associated with recurrence and survival.

Conclusion: Our results suggest that DNA methylation may be one of the important events in tumorigenesis of meningiomas and it may be a major molecular mechanism especially for Grade II meningiomas. The methylation status of TSGs is not useful to discriminate among histologic grades and outcome of meningiomas. Further molecular studies will be needed to predict the malignancy and biological behavior of meningiomas.

Key Words: Methylation, Tumor suppressor genes, Meningiomas, Grades, Outcome.

Introduction

Hypermethylation of CpG islands within the promoter region is one of the mechanisms by which tumor suppressor genes (TSGs) can be silenced, and regional hypermethylation of TSGs is an alternative or complementary mechanism to gene mutations causing gene inactivation which leads to functional impairment. DNA hypermethylation is thus now recognized as one of important mechanisms in tumorigenesis, and there are some examples of TSGs inactivated by aberrant promoter hypermethylation in human cancers.

Meningiomas are common primary brain tumors of the central nervous system (CNS), and account for 13~26% of intracranial tumors. They arise from the arachnoid layer of the meninges, and most of them are slow-growing tumors that correspond histologically to grade I in the World Health Organization (WHO) grading system of meningiomas. Approximately 10% of cases are classified as WHO grade II or as WHO...
grade III, exhibiting more aggressive clinical behavior and with a higher risk of recurrence than typical grade I meningiomas.

Increasing number of findings of hypermethylation on TSGs in many types of tumors in recent years indicate that DNA hypermethylation is an alternative mechanism for TSG inactivation in carcinogenesis. However, DNA methylation status of TSGs is poorly understood in meningiomas. We examined the methylation status of multiple TSGs in meningiomas to investigate the effect of methylation status of these target genes on meningioma grade and outcome.

**Materials and Methods**

1. **Tissue samples**

Randomly sampled 81 meningiomas (59 grade I, 15 grade II, and 7 grade III, formalin-fixed, paraffin embedded tissues) were analyzed. Histopathologic diagnosis of tumor was based on the 2000 WHO classification of tumors of the CNS.

2. **DNA extraction and methylation specific PCR (MSP)**

We performed MSP in meningiomas to detect methylation status of multiple TSGs such as *p16*, *p14*, *RB1*, *RASSF1A*, and *E-cadherin* which were known to be targets for hypermethylation in some tumors.

Tissues were digested in lysis buffer (10 mM Tris, pH 8.5; 10 mM EDTA; 0.5% SDS; and 100 mM NaCl) with proteinase K (20 mg/ml, Gibco BRL, USA). Genomic DNA was extracted with phenol chloroform and precipitated with ethanol. Extracted DNA was modified by sodium bisulfite to determine the methylation status of genes by MSP as previously described. PCR for *p16* gene was performed at 94°C for 5 minutes; 94°C for 1 min, 60°C for 30 sec, 72°C for 1 min for 28 cycles, followed by 72°C for 10 min. The other targets were amplified by 35 cycles, and annealing temperatures are as shown in Table 1. The primer sequences for detecting methylated or unmethylated *p16*, *p14*, *RB1*, *RASSF1A*, and *E-cadherin* genes are also shown in Table 2. The reaction mixture was in a 25 ml volume containing 50 ng of modified DNA, 0.2 mM dNTP, and 1 unit of Taq polymerase (Takara, Kyoto, Japan) in 1× PCR buffer (10 mM Tris, pH 8.3; 50 mM KCl; and 1.5 mM MgCl2). The PCR products were analyzed on a 2.5% agarose gel, stained with ethidium bromide, and visualized by UV illumination.

3. **DNA sequencing analysis**

The PCR products were purified using the JETSORB gel extraction kit (Genomed, Bad Oeynhausen, Germany), and...