Promoter methylation of Wilms' tumor gene on the X-chromosome in gastric cancer

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Abstract: Objective To investigate the changes in methylation levels of the promoters of the tumor suppressor gene Wilms' tumor gene on the X-chromosome (WTX) and its possible role in gastric cancer. Methods WTX promoter methylation levels were detected in 20 pairs of specimens of gastric cancer and matched normal tissues and in 3 gastric cancer cell lines (MGC803, SCG7901, and BGC823) using the Sequenom MassARRAY quantitative analysis system. The gastric cancer cell line BGC823 was treated with 5-aza-2'-deoxycytidine (5-aza-dC) for demethylation and the changes in the level of WTX promoter methylation were investigated. Results WTX promoter methylation levels were very low and showed no significant differences among normal gastric tissues, gastric cancer tissues and the 3 gastric cancer cell lines. In BGC823 cells, treatment with 5-aza-dC did not obviously affect the promoter methylation levels of WTX. Conclusion High methylation levels of WTX promoters are rare in gastric cancer.

Key words: Wilms' tumor gene; X-chromosome; gastric cancer; promoter methylation.

INTRODUCTION

Gastric cancer is one of the most common human cancers and the second leading cause of cancer-related human death worldwide. Several factors have been known to contribute to the tumorigenesis of gastric cancer, including H. pylori infection, smoking and gastric ulcer, but the exact mechanisms of the tumorigenesis still remain unknown.

In 2007, a novel tumor suppressor gene, Wilms' tumor gene on the X-chromosome (WTX), was first identified in Wilms' tumor ¹. WTX belongs to the FAM123 gene family. This family includes 3 members, and the other two are FAM123A and FAM123C. The roles this gene family plays in signal transduction, cell behaviors and human diseases are poorly understood. It was reported that WTX protein could negatively regulate Wnt/β-catenin signaling by forming a complex with AXIN1, β-catenin, APC and β-TrCP2 to result in the degradation of β-catenin. WTX can also influence antioxidant response and cellular differentiation. Studies have shown that aberrant expression of WTX is implicated not only in Wilms' tumor but also in osteopathia striata with cranial sclerosis. So far the functional roles of WTX in gastric cancer have not been reported.

In general, three reasons account for the inactivation of tumor suppressor genes, namely gene mutation, promoter region methylation, and aberrant microRNA regulation. The condition of DNA methylation is one of the most important aspects in exploring the functions and actions of genes. DNA methylation is essential for regulating mammalian tissue development and suppressing gene activity by changing the structure of the chromatin. Aberrant DNA methylation is associated with gene silencing in cancer and plays an important role in tumorigenesis. As an important mechanism for the inactivation of tumor suppressor genes and tumor-related genes, promoter CpG island hypermethylation is found in virtually all human cancer tissue types. To explore the functions of WTX, we detected the promoter methylation levels of WTX in gastric cancer tissues and gastric cancer cell lines.

MATERIALS AND METHODS

Cell lines and tissue specimens

Gastric cancer cell lines, MGC803, SCG7901, and BGC823, were obtained from the American Type Culture Collection (ATCC). All the cell lines were cultured in RPMI 1640 (Hyclone, Logan, Utah, USA)
supplemented with 5% fetal bovine serum (FBS) (Gibco-BRL, Invitrogen, Paisley, UK) in a humidified incubator in 5% CO₂ at 37 °C. Twenty pairs of surgical specimens of human gastric cancer tissues and matched normal tissues were provided by the Tumor Tissue Bank of Nanfang Hospital. The gastric cancer tissues included 3 stages and were not classified in the study. The tissue specimens were frozen in liquid nitrogen immediately after surgery. All the gastric cancer cases were pathologically confirmed, and the normal tissues were taken at least 5 cm away from the cancer lesions. The Tumor Tissue Bank of Nanfang Hospital possesses a comprehensive set of clinicopathological data, including age, gender, size of primary tumor, tumor differentiation, lymph node metastasis and clinical stage. This study was approved by the ethics committee of Southern Medical University.

WTX promoter methylation detection in tissue samples and cell lines

DNA was extracted from 20 pairs of gastric cancer and normal tissues and 3 gastric cancer cell lines (MGC803, SCG7901, and BGC823) using a QIAamp DNA mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The genomic DNA (300 ng) was treated with sodium bisulfite to convert unmethylated cytosines to uracil using the EpiTect bisulfite kit (Qiagen, Germany). Sequenom MassARRAY quantitative methylation analysis (Sequenom, CA) was used to detect WTX gene promoter methylation. Using three pairs of primers, PCR was performed to analyze the three candidate methylation regions of WTX. The promoters include WTX1: 5'-aggaagagagGGTAGGATTGGGGGAACTCTAATTCTCCAAATTTAACCCTCCTT-3' (F), 5'-cagtaatacgactatagggagaggtcctaatcctaatacctcccaaaatattataaccttgcctt-3' (R) covering 2044-2340 bp of WTX gene promoter sequences; WTX2: 5'-aggaagagagGGTAGGATTGGGGAACTCTAATTCTCCAAATTTAACCCTCCTT-3' (F), 5'-cagtaatacgactatagggagaggtcctaatcctaatacctcccaaaatattataaccttgcctt-3' (R) for 1352-1598 bp. The culture medium was changed every day to maintain a stable concentration of 5-aza-dC. The culture medium was changed every day to maintain a stable concentration of 5-aza-dC. The culture medium was changed every day to maintain a stable concentration of 5-aza-dC. The culture medium was changed every day to maintain a stable concentration of 5-aza-dC. The culture medium was changed every day to maintain a stable concentration of 5-aza-dC.

RESULTS

WTX promoter methylation in gastric cancer, normal tissues and cell lines

DNA promoter Sequenom MassARRAY quantitative methylation analysis was used for analyzing the methylation levels in 3 putative DNA-methylation regions of WTX gene. For all of the 3 putative DNA-methylation regions, 9 segments were amplified by 3 methylation promoters, and the quantitative results were reported. The data revealed very low methylation levels of WTX promoters (no more than 30%) in the 20 pairs of cancer and matched normal tissues. No significant differences were found in the methylation levels of WTX promoters between normal and cancer tissues and the cell lines (Fig.1 and Fig.2).

Effect of 5-aza-dC on WTX methylation in gastric cancer cell lines

To verify WTX methylation in gastric cancer, we compared the differences of DNA promoter methylation levels between 5-aza-dC-treated and untreated gastric cancer BGC823 cells. The result did not show any obvious changes of the methylation levels of WTX gene promoters in BGC823 cells in response to 5-aza-dC treatment (Fig.2).

DISCUSSION

WTX is a novel tumor suppressor gene in Wilms' tumor[19], but its functions in gastric cancer still remains unknown. Abnormal promoter CpG island methylation is often associated with a transcriptional block and loss of the relevant protein, which is one of the main reasons for gene silencing[20]. Sequenom MassARRAY quantitative methylation analysis allows the comparison of methylation profiles between different samples, and is currently the most accurate method for analyzing gene methylation conditions. To explore the potential changes of WTX gene in gastric cancers, we analyzed the promoter methylation conditions of WTX. Our data showed that WTX promoter methylation levels were very low and comparable among normal gastric tissues, gastric cancer tissues and the 3 gastric cancer cell lines. To further verify the results, we treated gastric cancer BGC823 cells with two doses of 5-aza-dC for DNA demethylation and analyzed the changes in WTX promoter methylation, and the results showed that 5-aza-dC treatment did not obviously reduce the methylation levels of WTX promoters. These data suggest that high methylation levels of WTX promoters are rare in gastric cancer, and WTX gene promoter methylation was approved by the ethics committee of Southern Medical University.
Gastrointestinal cancer.

Methylation levels do not show significant changes in gastric cancer.

**REFERENCES**


胃癌中新抑癌基因 WTX 启动子区域甲基化水平的检测

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摘要: 目的 探讨抑癌基因 WTX 启动子区域甲基化水平及其在胃癌中的作用。方法 运用 MassARRAY 定量分析系统分析 20 例胃癌及配对正常组织和 3 种胃癌细胞株 (MGC803, SCG7901 和 BGC823) 中 WTX 基因启动子区域甲基化水平。应用 5-杂氮-2'-脱氧胞苷 (5-aza-dC) 对胃癌细胞 BGC823 进行去甲基化处理, 分析其对 WTX 基因启动子区域甲基化水平的影响。结果 在胃癌及其配对正常组织和胃癌细胞株中, WTX 基因启动子区域甲基化水平普遍较低, 并且 3 组之间无统计学差异。用 5-aza-dC处理后并不能改变胃癌细胞株中 WTX 基因启动子区域的甲基化水平。结论 胃癌中基本不存在 WTX 基因启动子区域高甲基化状态。

关键词: 抑癌基因; WTX; 胃癌; 甲基化

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