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Original article

Analysis of O6-methylguanine-DNA methyltransferase methylation status in sporadic colon polyps

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ABSTRACT

Background/aim: The aim of our study was to check how MGMT methylation status together with known factors influenced the risk of colon cancer development.

Materials and methods: We examined patients with colon polyps. Information concerning gender, age, lifestyle, diet, anthropometry and medical information, including cancer and family history of cancer, was analyzed. Polymorphism variety of MGMT gene was investigated in another study. Genetic analysis for MGMT methylation assessment was performed for polyp tissue samples from 143 patients.

Results: Positive methylation MGMT status was found in 55 patients. There was no correlation between gender and MGMT methylation status ($p = 0.43$). We did not find correlation between patients younger and older than 60 ($p = 0.87$). There was no correlation between smoking and MGMT methylation status ($p = 0.36$). We did not find correlation between BMI and MGMT methylation status ($p = 0.86$). We did not find correlation between MGMT methylation status and colon cancer in familial history ($p = 0.45$).

Conclusion: Our study showed no correlations between methylation status of MGMT polymorphisms and clinical features like age, gender, polyp localization, smoking status, or obesity. It has been shown previously that MGMT methylation status may show nonspecific methylation in colon polyps. Gene methylation status in adenoma tissues has also been associated by other authors with the adenoma's size, histology, and degree of atypia. In our study, we evaluated the gene methylation status in colon polyps and found no association with adenoma characteristics. The present study showed no correlation for MGMT methylation in polyps in different regions of colon.

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1. Background

MGMT promoter methylation is presented in approximately 20–30% of colorectal cancers and in many of them it coexists with or in some cases is independent of CIMP status. Transcriptional inactivation by promoter CpG island methylation tumor suppressor genes is an important mechanism in human carcinogenesis.[1–3] Epigenetic suppressor gene silencing has commonly been involved in all types of human tumors.[4–6] The inactivating of tumor suppressor genes may affect many important cellular processes, such as the cell cycle, the TP53, the WNT signaling pathways, DNA repair, apoptosis pathways and the metastasizing process.[1–3] The human MGMT gene is located on chromosome 10q26 and consists of 5 exons. MGMT is a DNA repair protein that removes adduct from 0^6 –G in DNA. The same effect is observed in many types of human tumors where the lack of MGMT expression results in higher frequency of mutations in genes critical for carcinogenesis, such as K-ras2 and p53.[7–9] Mutations in MGMT have rarely been found, and it has been suggested that MGMT inactivation is primarily manifested through hypermethylation-induced silencing of its promoter in human cancers, including those of the colon.[10–12]

We explored these potential biomarkers using methylation specific PCR (MSP) assay approach to measure the methylation status of MGMT gene in colon polyps. Then we examined the MGMT methylation status and correlations between clinical features like age, gender, polyp localization, smoking status, obesity and sporadic polyps characteristics.

2. Aim

The aim of the study was to establish the role of methylation status in colon polyps.

3. Materials and methods

The cases were recruited from colonoscopy department of the Greater Poland Cancer Centre between the years 2004–2008. Patients were chosen randomly; there was only one criterion for inclusion into the study: having a colon polyp. Volunteers were outpatients with no known gastrointestinal pathology who had undergone colonoscopy as a diagnostic procedure, typically to investigate nonspecific symptoms, such as abnormal bowel habit or unexplained rectal bleeding. Ethical approval for the project was received from the Institutional Review Board at Poznan University of Medical Sciences (local Research Ethics Committee, project reference 965/08) and the consent was obtained in advance of the expected date of endoscopy. Information concerning gender, age, lifestyle, diet, anthropometry and medical information, including cancer and family history of cancer, was obtained from a questionnaire. Experimental biopsies were collected from the endoscopy patients in the endoscopy department, immediately after procedure. All colon polyps were divided into two parts, one underwent histopathological estimation and the other one served as material for DNA extraction. All polyps were examined twice by two independent pathologists. Polymorphism variety of MGMT gene was investigated in another study.

3.1. Genetic analysis

Methylation-specific PCR. Bisulfite modification: DNA (1 μg) in volume of 50 μl was denatured by NaOH to final concentration of 0.2 M for 10 min at 37 °C. Thirty microliters of 10 nM hydroquinone and 520 μl of 3 M sodium bisulfite at pH 5 were mixed and added to the sample and incubated at 50 °C for 16 h. Modified DNA was purified using the Wizard DNA purification resin according to the manufacturer protocol (Promega) and eluted into 50 μl of water. Modification was completed by NaOH (final concentration of 0.3 M) incubation for 5 min at room temperature, followed by ethanol precipitation. DNA was resuspended in water and before PCR reaction, concentration of DNA was measured.

MGMT amplification: The following sets of primers were used for amplification: UM F: 5′-TTTGTGTTTTGATGTTTGTAGGTTTTTGT; UM R: 5′-AACTCCACACTCTTCCAAAAACAAAACA; M F: 5′-TTTCGACGTTCGTAGGTTTTCGC and M R: 5′-GCACTCTTCCGAAAACGAAACG.

PCR-SSCP analysis was performed for polyp tissue samples from 143 patients according to the MSP method. We used TaqPol (Applied Biosystem) polymerase, and primer sequences of *MGMT* for the unmethylated reaction F were: 5′-TTTGTGTTTTGATGTTTGTAGGTTTTTGT-3′ (upper primer) and R: 5′-AACTCCACACTCTTCCAAAAACAAAACA-3′ (lower primer) and for the methylated reaction F: 5′-TTTCGACGTTCGTAGGTTTTCGC-3′ (upper primer) and R: 5′-GCACTCTTCCGAAAACGAAACG-3′ (lower primer). Each PCR mixture contained a buffer (10×), $MGCl_2$ (2 nM), dNTP (300 μM), F (50 pM), R (50 pM), TaqPol (5 U), and DNA matrix (200 ng). Volume of reaction was 10 μl. The annealing temperature was 59 °C.

3.2. Statistical analysis

The Pearson's chi-square (χ^2) and Fisher's exact tests were used to test the differences in genotype and allele (respectively) distribution between patients and control subjects. Statistical analyses were performed using Statistica v.7.1 software (Statsoft, USA). For polymorphisms containing less than 5 observations per cell, the Fisher–Freeman–Halton exact test was performed using StatsDirect statistical software v.2.6.2. Logistic regression was employed to calculate odds ratios (OR) and 95% confidence intervals (95%CI) and used to calculate interactions. Odds ratios were calculated using a demonstration version of GraphPad InStat 3.

4. Results

Our study group consisted of 74 males (51.75%) and 69 females (48.25%). The median age in our group was 60.32. The main aim of our study was to check how MGMT methylation status together with known factors influenced the risk of colon cancer development. Positive methylation MGMT status was found in 55 patients. There was no correlation between gender and MGMT methylation status ($p = 0.43$). Patients were divided