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Artículo Original

Modulation of MGMT mRNA in rectal adenocarcinoma: a preliminary study

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Abstract

Preoperative chemoradiotherapy is usually applied in rectal cancer therapy. O6-methylguanine-DNA methyltransferase (MGMT), an enzyme implicated in DNA repair, has been related to chemoradiotherapy resistance and proposed as a prognosis biomarker. This study analyzes the modulation of mRNA MGMT expression levels in rectal cancer treatment. Tissue samples from rectal cancer patients were obtained before and after chemoradiotherapy. Quantitative-PCR was used to determine MGMT RNA levels which were correlated with clinical data. No significant differences in MGMT mRNA values were observed among samples before and after chemoradiotherapy. Thus, our preliminary study indicated that MGMT mRNA modulation was not a biomarker candidate to rectal cancer.

Keywords: rectal adenocarcinoma, MGMT, treatment response, 5-fluorouracil, radiotherapy

Introducción

Preoperative chemoradiotherapy (CRT) including capecitabine or 5-fluorouracil (5-FU) is usually used in rectal cancer stage II or III followed by adjuvant chemotherapy (1). However no molecular biomarkers for prognosis and/or treatment response of CRC have been detected (2,3). O6-methylguanine-DNA methyltransferase (MGMT) eliminates methyl groups in O6-guanine position and is able to repair DNA of tumor cells after cytotoxic exposition avoiding apoptosis (4, 5). In fact, low MGMT methylation levels have been detected in normal colon normal mucosa (6) while high MGMT methylation levels appeared in precursor lesions of CRC (7). Loss of MGMT expression has been detected in CRC in a greater proportion in patients with high microsatellite instability (8). In others tumors such as glioblastoma, the cytotoxic drug ineffectiveness and drug and radiotherapy resistance have been correlated with MGMT methylation (9). However, there are few data on the role of MGMT expression on the colorectal carcinogenesis (10, 11). In this preliminary study, MGMT RNA expression was evaluated in rectal cancer patients before

and after treatment in order to determine the possible modulation and role as a prognostic marker.

Material and Methods

Clinical history and tissue samples

Written informed consent was obtained from twenty-nine rectal adenocarcinoma patients (stage II-III) (Biomedical Investigation Ethic Committee; Servicio Andaluz de Salud). Before treatment, complete clinical history and examination (colonoscopy and biopsy, chest X-ray, abdominopelvic scan and/or endorectal ultrasound, and magnetic resonance image) were carried out. All patients were treated with pelvic radiotherapy (RT) (46-50 Gy in 2 Gy fractions) and intravenous 5-FU or capecitabine (825 or 225 mg/m2, respectively) during RT treatment. Total mesorectal excision 6 weeks after CRT was carried out. Finally, patients received four 5-day cycles of intravenous 5-FU (500 mg/m2) every 21 days or 4 cycles of 1250 mg/m2 capecitabine every 12 h for 14 days. Samples of the tumor mass were obtained before CRT (preCRT sample) and after CRT (postCRT sample) and embedded in OCT. Normal surrounding tissue samples were also obtained from each patient.

RNA extraction and quantitative real-time RT-PCR

Total RNA was obtained from preCRT, posCRT, and normal surrounding OCT-embedded tissue samples. Mechanical homogenization was done by syringe in TRIzol® solution. RNA purification was performed by RNAeasy Mini Kit (Qiagen, MD) and RNA integrity number (RIN) was determined using an Agilent BioAnalyzer 2100 with the RNA 6000 Nano Assay (Agilent Technologies, Las Rozas, Spain). A NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, NC) was used for RNA quantification. Finally, 200 ng RNA were reverse-transcribed into cDNA using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Real-time PCR (qPCR) was carried out in triplicate in 96-well plates using a 7500 Fast-Real time PCR machine (Applied Biosystems). Each well contained 20 ng of cDNA in 20 µL of TaqMan Universal PCR Mastermix (Applied Biosystems). Each sample was analyzed using TaqMan®Gene Expression Assays (Applied Biosystems) for MGMT (Hs01037698_m1). GADPH (catalog number: 4333764F) and a cDNA pool from normal surrounding tissue was used as endogenous and calibrator samples, respectively. Median Ct results were calculated to obtain ΔΔCt values. The default settings of the 7500 v.2.0.6 software were used. Patient samples were clustered in two groups: ≥2-fold and <2-fold for each gene. cDNA serial dilutions were performed to evaluate the efficiency of qPCR. All standard curves were linear in the analyzed range with an acceptable correlation coefficient (R2).

Statistical analysis

SPSS version 15.0 (IBM, Chicago, IL) was used for the data analyses. Student's t-test was also used to determine the significance of differences in means. $P<0.05$ was considered significant.

Results and Discussion

Rectal adenocarcinoma patients (stage II-III) showed a mean age of 64.43±12.24 years (range, 33-83 yrs). The gender distribution was 75.9% male (22/29) and 24.1% female (7/29) female. The majority of tumors were well- or moderately-differentiated (93.1%).. The median follow up was 20.53±9.07 months. No patient died due to the rectal cancer and disease recurrence was observed in 13.8% (4/29); the mean DFS was 18.62±9.11 (range, 5-37 months) (Table 1). These patients were used to carry out a preliminary analysis of the MGMT mRNA expression before and after treatment. Previous results in CRC patients in relation to MGMT (methylation and expression) have been contradictory. In relation to methylation, Nagasaka et al. (12) and Nilsson et al. (13)

founded a lower risk of recurrence in 5-FU-treated CRC patients with methylated versus unmethylated MGMT promoter and Shima et al. (14) concluded that neither MGMT promoter methylation nor loss of MGMT expression is a useful prognostic biomarker in this disease. In another study, Sinha et al. (15) observed that MGMT methylation was associated with stage III in sporadic CRC cases. Furthermore, the methylation of the MGMT promoter regions was associated with down-regulation of their mRNA expression (16). .Thus, the role of MGMT promoter methylation status as an early biomarker of CRC has not yet been established (17, 18, 19). On the other hand, in relation to MGMT expression, several studies showed contradictory results. Murakami et al. (20) found that the anti-tumor action of 5-FU was enhanced in CRC cell lines with decreased expression of MGMT but not RNA levels, suggesting that MGMT depletion is a post-translational consequence of 5-FU treatment. In this context, our preliminary studies confirm no significant changes in MGMT mRNA of the samples of CRC patients before and after treatment. Table 2 exhibits the mRNA MGMT findings. No significant differences in MGMT ΔΔCt values were observed among normal (2.00±1.03-fold), preCRT (2.00±1.56-fold) and postCRT (2.54±2.03-fold) specimens. These results may be related withy the theory that silencing of MGMT expression is a multifactorial complex process (21). Future studies will be necessary to correlate MGMT protein expression and mRNA. Despite the limited number of the patients, preliminary results showed a non-modulation of MGMT mRNA before and after CRC treatment indicating that this is not a promising prognostic biomarker of the disease.

	All patients (n = 29)
Age	
=50 yrs	27 (93.1%)
<50 yrs	2 (6.9%)
Sex	
Male	22 (75.9%)
Female	7 (24.1%)
Tumor stage	
II	10 (34.5%)
III	19 (65.5%)
Lymph node metastasis	
Yes	19 (65.5%)
No	10 (34.5%)
Recurrence	
Yes	4 (13.8%)
No	25 (86.2%)
Tumor differentiation grade	
Well-moderately	27 (93.1%)
Poorly	2 (6.9%)

Table 1. Rectal cancer patient characteristics

Variables	MGMT: n (%)	NA
RNA expression: normal sample		
<2-fold	8 (34.8)	6
=2-fold	15 (65.2)	
RNA expression: preCRT sample		
<2-fold	8 (34.8)	6
=2-fold	15 (65.2)	
RNA expression: postCRT sample		
<2-fold	9 (39.1)	6
=2-fold	14 (60.9)	

NA; not available; normal; normal surrounding tumor tissue.

Table 2. MGMT mRNA expression in rectal cancer patients

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