Letter to the Editor

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Association between *SOX17*, *Wif*-1 and *RASSF1A* promoter methylation status and response to chemotherapy in patients with metastatic gastric cancer

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To the Editor,

Gastric cancer is one of the most frequently diagnosed cancers worldwide [1]. In 2020, it is estimated that there will be more than 27,000 new cases and more than 11,000 patients will die from the disease, in the United States [2]. A global epigenetic marker is methylation of specific DNA bases. Several genes are generally hypomethylated in cancer patients, whereas tumor suppressor genes are hypermethylated in the vast majority of cases [3]. One of the most important events in carcinogenesis is the hypermethylation of CpG islands of the promoter region of these genes [4]. This procedure may affect the function of the aforementioned genes, involved in critical cell functions, such as DNA repair, cell cycle regulation, metabolism, cell–cell interactions, angiogenesis, apoptosis etc., leading to cancer development [5]. Methylation of the promoter region of tumor suppressor genes leads to inactivation of them, whereas unmethylated promoters are considered active, so the methylation patterns of cancer cells is translated into gene expression patterns. Cell-free DNA methylation may be used for the detection of treatment resistance in cancer patients. This resistance can be due to the reversal of existent or novel DNA changes induced by drugs. In many studies, DNA methylation patterns have been evaluated as predictors of treatment response in cancer patients, including breast and colon cancer patients. The relapse of the disease after chemotherapy is likely to be due, to some extent, to changes in the methylation pattern of DNA, which are induced by chemotherapy itself. On the other hand, the relapse of the disease may be due to methylation patterns already present in the tumor before the onset of any treatment, so that specific methylation patterns cause a more aggressive phenotype with a poor response to chemotherapy [6]. *SOX17* has a very important role in regulating the growth and function of progenitor cells located in bone marrow via suppression of the activation of wnt signaling pathway. It seems that methylation of this gene results in activation of the wnt signaling pathway [7]. *RASSF1A* gene is responsible for the production of RASSF1A protein which is involved in the regulation of microtubules, the maintenance of genomic stability, the modification of apoptosis, the mobility of cells, the regulation of cell cycle and the control of tumor infiltration [8, 9]. *Wif*-1 gene (wnt inhibitory factor-1) acts as an antagonist of the wnt signaling pathway and is frequently methylated in many cancer patients [10]. In

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the present study, we tried to analyze the promoter methylation status of tumor-suppressor genes *SOX-17*, *Wif*-1 and *RASSF1A*.

Our study material consisted of 105 blood samples obtained from gastric cancer patients suffering from metastatic disease. Additionally, 20 blood samples taken from healthy individuals were used as a control group. All of the patients were treated with the following chemotherapy regimen for advanced/metastatic gastric cancer: docetaxel 50–60 mg/m^2, oxaliplatin 80–85 mg/m^2, capecitabine 2,000 mg/m^2 Q3W (TEX regimen). CpG islands in *SOX17*, *Wif*-1 and *RASSF1A* genes were examined by real-time sybr-green–based methylation-specific PCR. Statistical analysis of the data was performed using the Statistical Package for the Social Sciences (SPSS), version 21.0 (IBM), IBM Corp., Released 2012, Armonk, NY.

In the present group, the promoter of *SOX17* gene was methylated in 61 out of 105 patients (58.1%), the promoter of the *Wif*-1 gene was methylated in 34 out of 105 patients (32.4%) and the promoter of the *RASSF1A* gene was methylated in 68 out of 105 patients (64.8%). In healthy control group, no promoters from the three genes were found to be methylated (0%). Regarding response to treatment, complete response (CR) was seen in 0 out of 105 patients (0.0%), partial response (PR) was seen in 30 out of 105 patients (28.6%), stable disease (SD) was seen in 29 out of 105 patients (27.6%) and progressive disease (PD) in 46 out of 105 patients (43.8%).

In our study, the methylation status of *SOX17* gene promoter was significantly associated with response to treatment, which was a combination of docetaxel, oxaliplatin and capecitabine. More specifically: (a) of 61 patients with a methylated *SOX-17* promoter, no one (0.0%) showed complete response (CR), 12 (19.7%) showed partial response (PR), 10 (16.4%) showed stable disease (SD) and 39 (63.9%) showed progressive disease (PD). (b) Of 44 patients with an unmethylated *SOX-17* promoter, 18 (40.9%) showed PR, 19 (43.2%) showed SD and 7 (15.9%) showed PD. These differences were significant (p=0.000). Totally, response and/or tumor stabilization (PR + SD) was observed in 59 out of the 105 cases (56.2%) examined in our study. We combined the patterns of response and stabilization (PR + SD), as good response and we observed that 22 (37.3%) of cases had the *SOX-17* gene promoter methylated and 37 (62.7%) of them had the gene promoter unmethylated (p=0.000).

An association between *Wif*-1 promoter methylation status and response to treatment was also observed. More specifically, 44 out of 59 patients (74.6%) who had stable disease and partial response jointly, had the gene promoter unmethylated, and this was statistically significant (p=0.002). Moreover, 27 out of 46 patients (58.7%) who had disease progression had the gene promoter of *Wif*-1 unmethylated, though not statistically significant (p=0.065). An association between *RASSF1A* promoter methylation status and response to treatment was also observed. More specifically, 35 out of 46 patients (76.1%) who had disease progression had the gene promoter of *RASSF1A* methylated, and this was statistically significant (p=0.025).

SOX17, *Wif*-1, and *RASSF1A* are frequently methylated genes in gastric cancer, during the evolution of carcinogenesis. These genes are major tumor suppressor genes, often methylated in gastric cancer. In the present study, we found that an unmethylated *SOX17* and *RASSF1A* gene promoters, correlates with good response to chemotherapy and the methylated ones, correlate with poor response to chemotherapy. It therefore seems that methylation of these genes is associated with an aggressive tumor phenotype, as the majority of patients in the study who carried these two methylated gene promoters, showed an early relapse of the disease despite the administration of the standard chemotherapeutic regimen (TEX). This finding indicates that methylation of the *SOX17* and *RASSF1A* gene promoters could potentially serve as predictive biomarkers for chemotherapy response in patients with metastatic gastric cancer, at least for those receiving the TEX regimen. Moreover, there was a tendency toward disease progression of the disease when the promoter of *Wif*-1 gene was unmethylated, in contrast to the results for the *SOX17* and *RASSF1A* genes. We don't have a clear explanation for this finding but these results are possibly indicative that *Wif*-1 gene may not play a crucial role at final disease stages in metastatic gastric cancer. It may be involved in carcinogenesis of the stomach at earlier stages of the disease. However, no safe considerations can be drawn, because of the relatively small size of the sample in our study. We consider these findings of interest, although more studies, with a larger number of patients, are needed to clarify whether the three genes studied could take a place as predictive biomarkers of response to chemotherapy in gastric cancer patients.

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