Assessment of Oxidative Stress Genes SOD2 and SOD3 Polymorphisms Role in Human Colorectal Cancer

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ABSTRACT: Purpose: The aim of this study was to ascertain the oxidative stress genes SOD2 and SOD3 polymorphisms in patients with colorectal cancer and to assess the possible involvement of these polymorphisms that might increase the risk for patients to develop malignant intestinal tumors. Material and methods: A total number of 306 subjects were divided into two groups (109 colorectal cancer patients as the study group and 197 normal healthy individuals as the control group). We genotyped two polymorphisms, SOD2 – 201A>G (rs4880) and SOD3 – 896C>G (rs1799895), by allelic discrimination, with TaqMan RT-PCR specific probes. Results: No significant differences were found with either of the polymorphisms when comparing the association between them and an increased risk of developing colorectal tumors. Conclusion: In Romanian population, the risk of developing colorectal cancer is not increased by SOD2 and SOD3 polymorphisms.

KEYWORDS: colorectal cancer, polymorphism, genotype, SOD2, SOD3

Introduction

Colorectal carcinogenesis induction is a multi-stage process based on several molecular and cellular events that transform a normal cell phenotype into a malignant one [1]. It was revealed that chemicals play an important role in initiation, promotion and progression of carcinogenesis process [2, 3]. One of the mechanisms they use to participate in this multi-step process is oxidative stress induction through occurrence of an imbalance between reactive oxidative species (ROS) production and cellular antioxidant defense systems [4, 5]. Oxidative stress role in carcinogenesis is based on generation of DNA damages with mutagenic effects and modulation of cells redox potential that can modify gene expression pattern [1, 6, 7].

Chronic gastrointestinal disorders generates an excess of ROS shown by several studies attempted to find out how oxidative stress modulates molecular mechanisms involved in colorectal cancer [8-10]. It was demonstrated that an important role in the development of malignant colorectal tumors is played by the dysfunction of the enzymatic and non-enzymatic antioxidant defense systems [11-13]. The enzymatic antioxidant defense system includes enzymes like SOD, CAT, GSH-Px, and GSH involved in maintenance of a normal metabolism and healthy state of the human body [14-16]. Furthermore, it was revealed that the activity of these enzymes is altered in cancer subjects [17, 18].

Superoxide dismutases (SODs) family is involved in detoxification of superoxide free radicals through conversion of oxidative phosphorylation products (superoxides) to hydrogen peroxide and diatomic oxygen [19, 20]. SODs are expressed in many tissues and organs. In humans were identified three forms of superoxide dismutases: one located in cells cytoplasm – SOD1, another isoform in the mitochondria – SOD2 and an extracellular form – SOD3 [21, 22].

Single-nucleotide polymorphisms (SNPs) of the gene that encodes for SOD2 were identified to be related with an increased risk to develop colorectal malignant tumors [23]. It is the case of SOD2- 201 A>G polymorphism that causes an amino-acid substitution (Ala16Val). Thus, revealing that the A allele is linked with a high risk of colorectal cancer development [17, 24]. SOD3 polymorphisms were associated with an increased level of the enzyme in the serum but the studies conducted so far did not find any association with colorectal cancer risk [25, 26].

Material and methods

Patients and samples

A total number of 109 patients diagnosed with sporadic CRC and 197 healthy controls
were included in this study, following a standard diagnostic procedure at the Emergency County Hospital of Craiova, Romania. Matched controls were recruited based on a negative history of tumor or chronic inflammatory diseases. Demographic data, age, gender, clinical information (family history of CRC and personal history of chronic disorders) were also collected for each patient. Biological samples (peripheral whole blood) were obtained from both groups after they signed a written informed consent. The Ethics Committee of University of Medicine and Pharmacy of Craiova, Romania approved this study.

DNA extraction and genotyping

Peripheral blood leukocytes were used to isolate and purify DNA with the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA), following the manufacturer’s protocol. Samples genotyping was performed using predesigned TaqMan assays from Applied Biosystems (Foster City, CA, USA): C_8709053_10 (SOD2, -201A>G, rs4880) and C_2307506_10 (SOD3, -896C>G, rs1799895).

Genotyping was carried out in a 5-μL reaction volume using TaqMan probes fluorescently labeled with FAM or VIC and following the protocol recommended by the supplier (Applied Biosystems, Foster City, CA, USA).

Real Time PCR cycling conditions (Real Time ViiA7 - Applied Biosystem) for the denatured reactions were 95°C for 10 minutes, followed by 45 cycles of 92°C for 15 seconds and 60°C for 90 seconds annealing temperature. Interpretation of samples was done using ViiA™ 7 Software v1.0 with the Allelic Discrimination option.

Statistical analysis

The Hardy-Weinberg equilibrium was tested using Pearson's chi-squared test ($\chi^2$). Associations between genotypes and CRC were calculated as odds ratio (OR) with a 95% confidence interval (95%CI). In addition to the overall association analysis, the analysis was performed stratified by tumors histological grade (G1 – well-differentiated, G2 – moderate differentiated, G3 – poorly differentiated) to further assess the association between oxidative stress genotypes and CRC risk in each stratum. In all cases, the most common allele in Caucasian was used as reference. A value higher or equal to 0.05 was considered statistical insignificant.

Results

All 306 samples harvested from colorectal cancer patients during surgery and healthy controls were genotyped. Genotyping was performed in 109 CRC patients and 197 controls.

The average age of our subjects with colorectal cancer was 66 (± 11), while for the control group it was 60.3 (± 11.87). Out of the total 109 cases of colorectal cancer 28% (30 cases) had well differentiated tumors, 54% (60 cases) had moderate differentiated tumors, while the other 18% (19 cases) had poorly differentiated tumors.

Both polymorphisms we studied were in Hardy-Weinberg equilibrium for both colorectal cancer and healthy control groups.

### Table 1: Colorectal cancer risk associated with SOD2 – 201A>G genotype

| SOD2 – 201A>G | Colorectal Cancer | Control | OR(95%CI) | p |
|---------------|-------------------|---------|-----------|---|
| GG            | 37 (33.94%)       | 52 (26.40%) | Reference | |
| AG            | 45 (41.28%)       | 97 (49.24%) | 0.652 (0.376 – 1.130) | 0.128 |
| AA            | 27 (24.77%)       | 48 (24.37%) | 0.791 (0.420 – 1.488) | 0.466 |
| A allele Carriers | 99 (45.41%) | 193 (48.98%) | 0.866 (0.622 – 1.207) | 0.397 |

### Table 2: Colorectal cancer risk associated with SOD2, G1 tumor grade

| SOD2 – 201A>G | Well-differentiated tumors (G1) | Control | OR(95%CI) | p |
|---------------|---------------------------------|---------|-----------|---|
| GG            | 9 (30%)                         | 52 (26.40%) | Reference | |
| AG            | 13 (43.33%)                     | 97 (49.24%) | 0.774 (0.310 – 1.932) | 0.586 |
| AA            | 8 (26.67%)                      | 48 (24.37%) | 0.963 (0.344 – 2.697) | 0.943 |
| A allele Carriers | 29 (48.33%) | 193 (48.98%) | 0.974 (0.566 – 1.678) | 0.925 |
Genotype frequency for SOD2 – 201A>G polymorphism is shown in Table 1. Results reveal a p value of 0.397 with an OR value of 0.866 (95%CI: 0.622 – 1.207) that demonstrates no significant differences in the association between the presence of this polymorphism and increased risk for patients to develop colorectal cancer. Furthermore, on a stratified analyze by histological grade we have obtained no significant association for colorectal cancer (Table 1, 2, 3 and 4). SOD3 – 896 C>G polymorphism frequency is shown in Table 5. We did not find any statistical significant difference of this polymorphism and susceptibility to develop colorectal cancer.

Discussion

SOD2 is a member of superoxide dismutase (SOD) family involved in converting oxidative phosphorylation products (superoxides) to hydrogen peroxide and diatomic oxygen [27]. SOD2 gene is found on chromosome 6q25.3 [28]. SOD2 – 201A>G polymorphism is located in exon-2 of SOD2, with an amino-acid substitution (Ala16Val) that was associated with several pathologies like Parkinson’s disease, Alzheimer’s disease, breast cancer, colorectal cancer, or dilated cardiomyopathy [24, 29-33]. Several studies carried out between 2001 – 2014 tried to identify an association of SOD2 gene polymorphisms with malignant tumors [34-38]. The published results were inconsistent and contradictory. Thus, a study conducted by Funke et al it showed that there is no association between SOD2 – 201A>G polymorphism and susceptibility to colorectal cancer [37]. Another study conducted in the Czech Republic by Meplan et al on colorectal cancer revealed an association between SOD2 polymorphism - 201A>G and sporadic colorectal cancer incidence. Their results suggested that this polymorphism may represent a potential biomarker for assessing susceptibility to colorectal cancer [39].

In our study, we found no statistical association between SOD2 polymorphism - 201A>G presence or absence and risk in developing colorectal cancer. Furthermore we did a stratified analysis based on tumors histological grade (G1 – well-differentiated, G2 – moderate differentiated, G3 – poorly differentiated). Also the stratified analysis that we performed showed no statistically significant correlations;
results that could be associated with the risk of developing colorectal cancer.

SOD3 is another member of the SOD family the main defense mechanism against reactive oxygen species (ROS) [40]. It acts by eliminating the strong superoxide radical and producing H2O2 that can then be degraded by enzymes like CAT or GPXs [41]. It is localized exclusively in extracellular spaces and has a tetrameric structure [42]. SOD3 gene is found on chromosome 4p15.2 [28]. Increased expression of SOD3 protein in the white and brown adipose tissue and in the plasma of obese mice was associated in a recent study with gene polymorphisms presence [42]. There are no recent studies conducted on identification of association between this polymorphism and a high susceptibility to cancer.

In our study, the results did not show a statistical association between SOD3 – 896C>G polymorphism and susceptibility to colorectal cancer. Moreover, even the stratified analysis based on tumor grade revealed no statistically relevant data.

Conclusion

In conclusion, our study shows that the genotype frequency for SOD2 – 201A>G and SOD3 – 896C>G were not associated with an increased risk for colorectal cancer. Further extensive studies are needed on different ethnic groups in order to clarify the role of oxidative stress enzymes polymorphisms in colorectal carcinogenesis.

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All authors had equal contribution.

Conflict of interests

The authors declare that they have no conflict of interests.

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