Determination of Gene Expression and Serum Levels of MnSOD and GPX1 in Colorectal Cancer

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Abstract. *Background/Aim: Oxidative stress plays a role on the development of colorectal cancer. Manganese superoxide dismutase (MnSOD) and glutathione peroxidase 1 (GPX1) are crucial in regulating oxidative balance and its stabilization. Possible mechanisms of action of these enzymes in various types of cancers require further investigation. We aimed to determine expression levels of these genes and their effects on protein levels in serum of patients with colorectal cancer. Materials and Methods: Expression levels of genes were determined using Real Time-Polymerase chain reaction in 35 patients with colorectal cancer. We used enzyme-linked immunosorbent assay to determine MnSOD and GPX1 levels. Results: We found significant differences in GPX1 expression between tumor and normal tissues, with a 2-fold decrease in tumor tissues ($p<0.05$). However, although no significant difference was found between the expression of MnSOD gene in tumor and that in normal tissues, there was a 1.13-fold change in expression. We observed no relationship between expressions of either gene and their levels in serum. Conclusion: The GPX1 gene may play a critical role in the development of colorectal cancer.*

Colon cancer is the third most common malignancy and is the fourth leading cause of death worldwide (1-3). Numerous factors may play a role in the development of colon cancer. The risk factors of colon carcinogenesis comprise susceptibility to mutation; red meat consumption; insufficient vitamin and mineral intake; and genetic predispositions such as hereditary non-polyposis colorectal cancer and familial adenomatous polyposis (4-6). Cancer cells are exposed to a higher level of oxidative stress compared to normal cells. Cancer cells have to defend themselves against reactive oxygen radicals to survive (7). Reactive oxygen radical-mediated oxidative damage to DNA, lipid, and protein play a role in the pathogenesis of various diseases, including cancer (8). It is suggested that reactive oxygen species (ROS) cause an increase in cell proliferation that results in tumor formation, and they are also considered to act in the induction and progression stages of colon cancer (9, 10). In addition to this, oxidative stress has been reported to be critical in the formation of colon cancer (11). The damage caused by continuously generated free radicals in living organisms is removed by antioxidant defense systems. There exists a balance between the rate of free radical production and elimination of the free radical-mediated damage by the antioxidant defense system. Disruption in this balance causes severe damage to tissues and organs. Therefore, the mechanisms underlying the elimination of the damage by antioxidant mechanisms are crucial (12-18). Endogenous intracellular antioxidant enzymes provide the primary defense against oxidative stress. The primary endogenous antioxidant defense enzymes, manganese superoxide dismutase (MnSOD), catalase, and glutathione peroxidase 1 (GPX1) are together responsible for protection against the damaging effects of free radicals. MnSOD detoxifies reactive oxygen radicals to hydrogen peroxide, and then catalase and GPX1 enzymes catalyze its decomposition into water and oxygen (18).

Although increased levels of MnSOD expression are detected in certain cancer types, including ovarian cancer, many studies report that *MnSOD* gene expression levels are low in malignant tumors. In addition, variable expression of *GPX1* gene has been found in different types of tumors (19-22). Increased levels of SOD and GPX expression have been reported in tumor tissues compared to surrounding normal tissues of patients with colon cancer (23).

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Further investigation is warranted to identify the mechanisms of action of these two enzymes in various cancer types because *MnSOD* and *GPX1* are critical antioxidant enzymes functioning in defense against oxidative damage. In this study, we aimed to determine expression levels of MnSOD and GPX1 genes and the effects on their plasma levels in patients with colorectal cancer.

Materials and Methods

Patient selection. Thirty-five patients with colorectal cancer (mean age=63.57±10.74 years, 10 women and 25 men) were enrolled in the study after obtaining their informed consent. The patients were selected from the Surgery Clinic of Istanbul Research and Education Hospital. Questionnaires, medical records, and pathological reports were received to confirm the diagnosis and cancer status. The patients received a standard questionnaire regarding the diagnosis time, family history, treatments and other medical issues. The tissue samples were collected before any chemotherepeutic or radiation therapy treatment had been started. Pathological staging information on all colorectal cancer diagnoses were confirmed by manual review of the pathology reports and clinical charts. Nodal status was categorized as no regional lymph nodes affected (N0) or at least one nodal metastasis (Table I).

Determination of MnSOD and GPX1 expression. Cancer and non-cancerous tissue samples obtained during surgical operation with a pathologist's assistance were stored. Total RNA was isolated from tissues using the TRIzol method. In brief, tissue was homogenized in TRIzol reagent (Ambion RNA Life technologies, Carlsbad, California, USA) using homogenizer. Tissue lysates were transferred to a polypropylene round-bottom tube and then incubated for 5 min at room temperature. RNA was extracted in the aqueous phase from TRIzol and chloroform mixture, and precipitated from the aqueous phase by mixing with isopropyl alcohol. RNA was stored in kit elution buffer. Total RNA was then treated with DNAase1 (Ambion purelink RNA mini kit Life Technologies, Carlsbad, California, USA) before processing further. Oligo DT (1 μg) and dNTPs (1 μl) were added to the total RNA sample (500 μg) and incubated at 65˚C for 5 min. Real-time PCR analysis was performed Stratagene Mx3005p (Agilent Technologies, Santa Clara, CA,USA) using universal TaqMan master mix. PCR primers and universal TaqMan probes for *MnSOD, GPX1* and *β-actin* as a housekeeping gene quantification were selected using primer software programs (Life Technologies, Applied Biosystem, TaqMan® Gene Expression Assay, Foster City, California, USA). The thermal cycling conditions to cDNA quantification assays were established in accordance with the Stratagene RT Detection System. Analysis of relative gene expression data was performed according to the threshold cycle (CT) method.

Serum levels of MnSOD and GPX1. MnSOD and GPX1 levels were determined by ELISA (USCN Life Science Inc. Wuhan, Huwei, PRC). Double serum samples were used for each patients and mean serum levels were assessed as actual values. Serum samples were recruited before chemotheraphy from patients.

Statistical analysis. Statistical analyses were performed using SPSS version 11.0 for Windows (SPSS Inc., Chicago, IL, USA). Differences in the fold changes of the tissues samples for GPX1 and MnSOD expressions were analyzed using the Mann–Whitney *U*-test.

Results

We found significant differences in GPX1 expression between tumor and normal tissues, with a 2-fold decrease in tumor tissues (p=0.045). Although no statistically significant difference was found, a considerable change (1.13-fold decrease in tumor tissues; p>0.05) in the expression of MnSOD between tumor and normal tissue was detected (Table II). Moreover, we observed no relationship between expressions of either gene and serum levels of the respective protein when we compared patients who had increased expression of genes to patients who had lower expression of genes (p>0.05) (Table III).

Discussion

Cancer incidence and cancer-related mortality rates have increased rapidly in recent years. Genetic factors, such as mutations in the structure of tumor-suppressor genes and oncogenes, and environmental factors play important roles in the development of cancer (24). Colon cancer is the third most common type of cancer in the world. 4 Approximately 1.4 million (746,000 male and 614,000 female) new cases of colorectal cancer were diagnosed in 2012 (25). Colon cancer is caused by the cumulative impact of genetic predisposition and environmental factors (26). The damage caused by continuously-generated free radicals in living organisms is removed by antioxidant defense systems. There is a balance between the rate of free radical production and elimination of the fee radical-mediated damage by the antioxidant defense system. Disruption in this balance causes severe damage to tissues and organs. Therefore, the mechanisms underlying the elimination of the damage by antioxidant mechanisms are crucial (12-18). Endogenous intracellular antioxidant enzymes provide the primary defense against oxidative stress. MnSOD, and GPX1 are together responsible for the protection against the damaging effects of free radicals (18).

Reactive oxygen radical-mediated oxidative damage to DNA, lipid, and protein plays a role in the pathogenesis of various diseases, which includes cancer (8). Aberrant gene expression or malfunction of the antioxidant enzymes leads to increased levels of ROS in cancer. MnSOD, a primary antioxidant enzyme in the mitochondria, plays a role in the maintainance of cellular redox homeostasis. Low levels of MnSOD expression have been reported in over 80 different virus- or chemical-induced neoplasms in humans and rodents (19, 20, 27). Many studies have suggested that overexpression of MnSOD suppressed tumor growth in a variety of cell lines. In addition, overexpression of MnSOD

Table I. *Patients' characteristics of study group.*

	n(%)
Tumor location	
Left Colon	2 (5.8)
Right Colon	8 (22.8)
Transverse Colon	4 (11.4)
Cecum	2 (5.8)
Rectum	5 (14.2)
Sigmoid colon	14 (40)
T Stage	
T1	2 (5.8)
T2	2 (5.8)
T3	18 (51.3)
T4	13 (37.1)
Lymph node involvement	
N0	12 (34.3)
N1	16 (45.7)
N2	7 (20)
Existence of distant metastases	
Yes	15 (42.9)
No	20 (57.1)
Differentiation	
Good	7 (21)
Medium	18 (51)
Poor	10 (28)

Table II. *Fold-change in the expression of GPX1 and MnSOD genes in tumor relative to normal tissues.*

Gene	Relative expression	95% CI	*p*-Value	Fold change
GPX1	0.496	(0.19-0.80)	0.045*	−2.015
MnSOD	0.880	(0.43-1.33)	0.384	−1.135

Table III. *Serum levels of GPX1 and MnSOD according to difference gene expression.*

	Gene expression level		*p*-Value
	Decreased (n=17)	Increased (n=18)	
Serum level			
MnSOD (ng/ml)	1.22±0.70	1.21±0.68	0.944
GPX1 (pg/ml)	129.17±40.11 (n=12)	168.70±140.92 (n=23)	0.351

n: Number of patients; data are the mean±SD.

was reported to suppress the growth of malignant human breast cancer, glioma, and mouse epidermal cells (28-31).

In the present study, we examined expression levels of *MnSOD* and *GPX1* genes in tumor and normal tissues of 35 patients with colon cancer using qRT-PCR. Our study showed that *MnSOD* gene was expressed approximately 1.13-times less in tumor tissue than in normal tissue. Although the results of other studies in the literature are in line with the results of our study, no statistically significant ($p>0.05$) relationship could be established between the expression of MnSOD in tumor and normal tissue. The low levels of MnSOD in some cancer types are associated with aberrant gene expression rather than deletion. Mutations in the proximal promoter region of the *MnSOD* gene lead to decreased MnSOD expression in colon cancer cell lines (32,33). Some studies showed that MnSOD activity is low in several tumor types; however, the fact that there is considerably higher activity of MnSOD in cells of colon, lung, stomach and esophageal cancer compared to normal tissues has been confirmed by a number of other research groups (1, 32-38).

GPX1 is the cytosolic form of GPX and it is the first and one of the most well-defined selenoproteins. This selenium-dependent enzyme is responsible for the detoxification of hydrogen peroxide and lipid peroxide (39). Variable expression levels of *GPX1* gene between different tumor types is documented in the literature (22). Aceto *et al.* reported that GPX enzyme activity is low in testicular tissue of individuals with prostate cancer (40). Similarly, another study noted reduced activity of GPX activity in human hepatoma (41).

The result of our study showed that *GPX1* gene expression was statistically significantly different (two-fold lower than normal tissue; $p<0.05$) between normal and tumor tissue. In contrast with these results, some studies showed increased GPX activity in the tumor tissue (*e.g.* breast cancer) compared with normal cells (42, 43). Oberley *et al.* examined the relationship between MnSOD and GPX and established that the overexpression of *CuZnSOD* and *MnSOD* genes inhibited the growth of human glioma cells; however, they suggest that the effect could depend on the intracellular GPX activity (33). On the contrary, there are some studies that reported overexpression of SOD and GPX together, which led to the rapid growth of tumor cells (44, 45). The findings of our study showed that there was no statistically significant difference between the gene expression profile of *GPX1* and histopathologic data when we examined parameters such as the presence of distant metastases, perineural invasion, tumor grade, and the presence of differentiation.

Unfortunately, we observed no meaningful relationship between expressions of both genes and serum levels.

As a result, the literature exhibits some contradictory results between different studies that examined *MnSOD* and

GPX1 gene expression. This contradiction could be due to the use of different populations, various methods for determining levels of the enzymes, or distinct cancer tissues with different tumor grades. Our study is the first to investigate expression levels of *MnSOD* and *GPX1* genes together in patients with colorectal cancer in a Turkish population. Although there are a wide range of data available in the literature regarding the investigation of the relationship between various cancer types and *MnSOD* and *GPX1* gene expression profiles, there exist few studies that correlate these expression profiles of both *MnSOD* and *GPX1* genes in colorectal cancer. The results of this study could provide clues for further investigation of colorectal cancer.

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