RESEARCH COMMUNICATION

Deregulation of *MTDH* Gene Expression in Gastric Cancer

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Abstract

Aim: Gastric cancer is the third most frequent cause of cancer mortality worldwide. In Iran, it is one of the leading causes at the national level. Localized at chromosome 8q22, the human *MTDH* gene has been reported to be over-expressed in a spectrum of malignancies. However, since there is a lack of data concerning with expression in gastric cancer at the transcriptional level, in this study we evaluated *MTDH* expression in Iranian cases. Methods: Totally, thirty paired gastric samples were examined by quantitative real-time RT-PCR. Results: Although the mRNA expression was significantly elevated in 46.6% of the examined tumor tissues; its expression was low in others (36.6%). Moreover, there was only a marginal statistical difference between the *MTDH* gene expression of all tumor specimens compared to their paired non-tumor ones and no statistically significant association with the grades and types of the tumors. Conclusion: Taken together, our results demonstrated that expression of *MTDH* at the transcriptional level may be increased in gastric cancer tissue samples but with considerable heterogeneity. Due to this, it may have the potential to be used as a target for diagnostic/therapeutic purposes only in a subset of patients.

Keywords: Gastric cancer - gene expression - *MTDH - AEG-1*

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Introduction

Gastric cancer is the third most frequent cause of cancer mortality in the world (Keeney et al., 2006). Although its incidence varies between different geographical areas of Iran (Malekzadeh et al., 2009), it is the leading cause of cancer-related mortality in Iran (The National Cancer Registry, 2008). The main environmental factors of gastric cancer in Iran are high *H.pylori* prevalence, high dietary concentration of salt and smoking (Malekzadeh et al., 2009). Moreover, conventional therapeutic methods do not provide any survival benefit for the majority of Iranian patients with gastric cancer because it is being diagnosed in advanced stages of the disease (Malekzadeh et al., 2009).

Localized at chromosome 8q22, the human *MTDH* gene has been reported to be amplified in a number of malignancies such as malignant glioma (Kim et al., 1995), hepatocellular carcinoma (HCC) (Poon et al., 2006), and breast cancer (Wang et al., 2005). Intriguingly, expression analysis in cell lines and in patient-derived tumor samples revealed that *MTDH* significantly elevated in breast carcinoma (Li et al., 2008), HCC (Yoo et al., 2009), neuroblastoma (Lee et al., 2009), and malignant glioma (Liu et al., 2010) cell lines and tumor samples compared with their normal counterparts. Furthermore, it has been reported that *MTDH* plays important roles in multiple biological processes in the course of tumorigenesis via modulating of multiple signalling pathways (Lee et al., 2006; Lee et al., 2008; Sarkar et al., 2008; Li et al., 2009). Activation of NF-κB (Emdad et al., 2006), PI3K/AKT (Lee et al., 2006), MAP kinase and Wnt/β-catenin (Yoo et al., 2009) signal tarnsduction pathways by *MTDH* induces specific phenotypes including cell survival, anti-apoptosis, angiogenesis, migration and invasion in various cell types (Sarkar et al., 2009). More recently, Li et al have shown that over-expression of *MTDH* leads to increased acquisition of cancer stem cell markers in breast cancer cells (Li et al., 2011). However, there are some controversial issues about the potential localization of *MTDH* and its function. It has been reported that *MTDH* could localize at different cellular parts including tight junctions, perinuclear region, endoplasmic reticulum, nucleus, nucleolus, cytoplasm and cell surface in different cell types (Sarkar et al., 2009). The relationship between localization of *MTDH* and its function is not clear. While in breast cancer (Li et al., 2008), HCC (Yoo et al., 2009) and melanoma (Sarkar et al., 2009) increased *MTDH* nuclear staining is associated with advanced disease, in prostate cancer (Thirkettle et al., 2009), decreased nuclear staining is related to poor prognosis.

Due to the over-expression of *MTDH* in various cancer types (Sarkar et al., 2009) including gastric cancer (Jian-bo et al., 2010), as mainly shown by immunohistochemistry, and its crucial role(s) in tumorigenesis and the lack of data concerning with the expression of *MTDH* in gastric cancer at transcriptional level, we evaluated its expression in 30 paired tumoral and non-tumoral gastric tissue samples

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by using quantitative Real-Time RT-PCR (qRT-PCR). Our results demonstrated that the expression of *MTDH* at transcriptional level was increased in gastric cancer tissue samples (p value: 0.05). However, there was no statistically significant association between the *MTDH* gene expression and the grades and types of the tumors.

Materials and Methods

Subjects

Thirty paired gastric tumoral and non-tumoral tissue samples were obtained from Iran Tumoral Bank (Tehran, Iran) and examined for gene expression, of which 30 were non-tumoral and 30 were tumoral specimens of gastric from the same patients (Table 1). Prior to participation, the patients' consents were obtained in written form by Iran Tumoral Bank. All experimental procedures on human samples were approved by the Ethics Committee of Isfahan University of Medical Sciences in which the experiments were done or in accord with the Helsinki Declaration.

Gene expression analyses

Total RNA was isolated from powdered tissues using Qiazol reagent (Qiagen, Hilden, Germany) and purified with RNeasy Mini kit (Qiagen, Hilden, Germany). On-column DNase treatment was performed by using DNase set (Qiagen, Hilden, Germany) to eliminate genomic DNA. The quality of the extracted RNAs was verified by agarose gel electrophoresis, and the concentration of the RNAs was assessed by optical density at 260 nm. cDNA synthesis was done by using MMLV Reverse Transcriptase (Fermentas, Vilnius, Lithuania) with oligo-dT primers as described previously (Mowla et al., 2005). qRT-PCR for *MTDH* and *TBP* (Nikpour et al., 2009) transcripts was performed using specific primers (Table 2) with the Maxima SYBR Green/ROX qPCR Master Mix (Fermentas, Vilnius, Lithuania) and run on the Rotor-gene 6000 (Qiagen, Hilden, Germany). The cycling conditions for PCR included an initial denaturation step at 95°C for 10 min, followed by 45 amplification cycles consisting of denaturation at 95°C for 20 s, 55°C for 20 s and an extension at 72°C for 20 s. The PCR products were analyzed by agarose gel electrophoresis, stained with ethidium bromide, and visualized under the ultraviolet light. Relative gene expression was calculated using the standard curve method.

Statistical analyses

All measurements were done in triplicates for each independent preparation and the results were statistically analysed using Student`s t-test and ANOVA. The SPSS software, version 16.0, was used for statistical analyses and the p value less than 0.05 was considered as statistically significant.

Results

Optimization of RT-PCR reaction

Prior to quantitation, optimization procedures were performed for both conventional and real-time RT-PCR reactions on cancerous gastric tissue samples using specific primers for human *MTDH* and *TBP* genes. Electrophoresis of the PCR products on agarose gels demonstrated single bands with the expected sizes for the amplified *MTDH* (124 bp) and *TBP* (128 bp) segments (data not shown). Analysis of gene expression using real-time PCR showed a unique melting curve without primer dimers for each of the examined genes (data not shown), which was further confirmed by agarose gel separation and staining.

Expression of MTDH gene in gastric tissue specimens

After optimization, expression of *MTDH* and *TBP* genes was determined using qRT-PCR in the 30 tumor and 30 non-tumor tissues of stomach. Relative expression of target gene was determined by dividing its expression amount to that of the *TBP* gene. The results of real-time qRT-PCR experiments demonstrated that while the relative expression of the gene was significantly high in some examined tumor tissues (46.6%) (p value: 7×10^{-3}); its expression was low in some others (36.6%) (p value: 1×10^{-4}) in comparison to their paired non-tumor tissues (Figure1a and b). Furthermore, in remaining 16.6% of the samples the expression of the gene did not change. Moreover, there was a marginal statistical difference between the *MTDH* gene expression of all tumor specimens compared to their paired non-tumor ones (p value: 5×10^{-2}) (Figure 1c). However, there was no statistically significant association between the *MTDH* gene expression and the grades and types of the tumors.

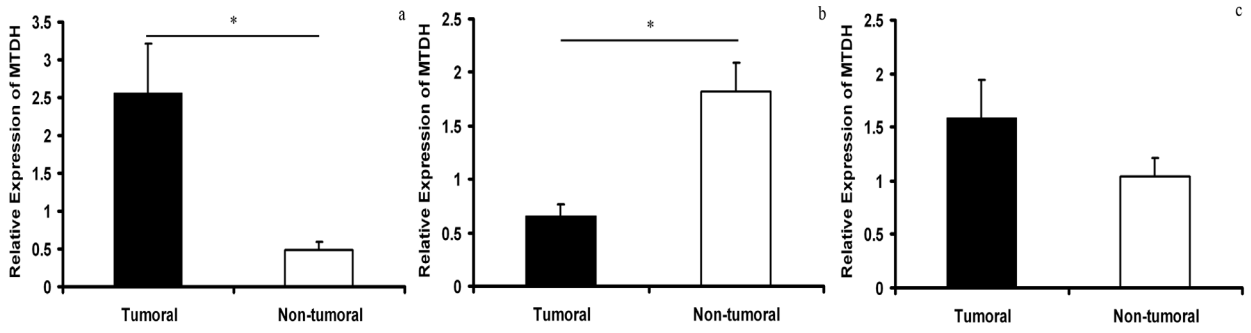

Figure 1. Relative Expression of *MTDH* in Tissue Samples of Gastric. Charts comparing the relative gene expression of *MTDH* as determined by quantitative real-time RT-PCR in a) a subset of paired tissue samples in which *MTDH* relative expression was higher in tumoral ones b) a subset of paired tissue samples in which *MTDH* relative expression was higher in non-tumoral ones c) pooled tissue samples. Values shown represent the mean ± SEM. Statistical significant differences have been shown by an asterisk.

Table 1. A Brief Description of Patients with Gastric Cancer

Characteristics		Values†
Mean age, y		65. 4 ± 13.54
Age range, y		33-83
Sex:	Male	18 (60)
	Female	12 (40)
Tumor types:	Diffused	15 (50)
	Intestinal	15 (50)
Tumor grades:	I	10 (33.3)
	II	8 (26.7)
	III	12 (40)

† Values in parentheses are percents.

Table 2. Sequences of the PCR Primer Sets

Gene	Designation	Primer sequence	Size of the amplicon
MTDH	For	5'- AGCCACCAGAGATTGACAAG-3'	124 bp
	Rev	5'- CAGGCTCCTTCATCAACTTC-3'	
TBP†	For	5'-CAACAGCCTGCCACCTTAC-3'	128 bp
	Rev	5'-CACAGCCTATTCAGAACACCA-3'	

†Previously designed by Nikpour et al. (2009).

Discussion

To our knowledge, this is the first report that evaluates and quantifies the expression of *MTDH* in gastric cancer using real-time qRT-PCR. Our results demonstrated that the expression of *MTDH* at transcriptional level was increased in gastric cancer tissue samples (p value: 0.05) and showed a trend toward statistical significance. Moreover there was a heterogeneity in the expression of *MTDH* in gastric cancer, while over-expressed in some specimens, it under-expressed in the others. However, the differences in the gene expression found in the different tumor types and grades were not statistically significant.

Until now, over-expression of *MTDH* has been documented in different types of tumoral samples, including breast (Emdad et al., 2007), prostate (Kikuno et al., 2007), gastric (Jian-bo et al., 2010) and colorectal cancer (Song et al., 2010), mainly by immunohistochemistry (Sarkar et al., 2009). In breast (Li et al., 2008) and gastric cancer (Jian-bo et al., 2010), esophageal squamous cell carcinoma (Yu et al., 2009), non-small cell lung cancer (NSCLC) (Song et al., 2009) and hepatocellular carcinoma (Yoo et al., 2009), the level of upregulation correlates with clinical progression and poor prognosis of patients. Taken together, our results, in part, are in accord with Jian-Bo et al (2010) and the others as we also demonstrated that *MTDH* expression increased in human gastric cancer. However, we did not find any significant correlation between *MTDH* over-expression and tumor types and grades. These discrepancies may be due to allele-specific gene expression differences in humans (Buckland, 2004).

In literature, there is only one report showing that low levels of *MTDH* also occur in pathological conditions (Santarpia et al., 2011). As demonstrated by Santarpia et al in metastatic NSCLC, patients with low levels of both *BRCA1* and *AEG-1* showed significant improved progression-free survival (PFS) compared to those with high levels of both genes. Moreover, as presented in ONCOMINE (http://www.oncomine.org), there are two reports showing that *MTDH* under-expressed in gastric cancer (Chen et al., 2003; Ooi et al., 2009). Thus, the present report might provide some preliminary insight into the extent of *MTDH* expression in gastric cancer. All together, our results call for further investigations to elucidate the precise molecular mechanisms by which *MTDH* contribute to the pathogenesis of gastric cancer.

In conclusion, this report shows that the expression of *MTDH* at transcriptional level was increased in gastric cancer tissue samples and showed a trend toward statistical significance. However, there was heterogeneity in the expression of *MTDH* in gastric cancer. Due to this, it may have the potential to be used as a target for therapeutic interventions in a subset of patients. On the whole, further investigation should be done to clearly define the function of *MTDH* in malignancies including gastric cancer.

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