Title: MSI2 expression is decreased in grade II of gastric carcinoma

Author: Modjtaba Emadi-Baygi Parvaneh Nikpour Faezeh Mohammad-Hashem Mohamad Reza Maracy Shaghayegh Haghjooy-Javanmard

PII: S0344-0338(13)00202-1
DOI: http://dx.doi.org/10.1016/j.prp.2013.07.008
Reference: PRP 51078

To appear in:

Received date: 13-1-2013
Revised date: 15-7-2013
Accepted date: 29-7-2013

Please cite this article as: M. Emadi-Baygi, P. Nikpour, F. Mohammad-Hashem, M.R. Maracy, S. Haghjooy-Javanmard, MSI2 expression is decreased in grade II of gastric carcinoma, Pathology - Research and Practice (2013), http://dx.doi.org/10.1016/j.prp.2013.07.008

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
MSI2 expression is decreased in grade II of gastric carcinoma

Modjtaba Emadi-Baygi\textsuperscript{1,2}, Parvaneh Nikpour\textsuperscript{3,4, 5*}, Faezeh Mohammad-Hashem\textsuperscript{5}, Mohamad Reza Maracy\textsuperscript{6}, Shaghayegh Haghjooy-Javanmard\textsuperscript{7}

\textsuperscript{1} Department of Genetics, Faculty of Basic Sciences,\textsuperscript{2} Research Institute of Biotechnology, Shahrekord University, Shahrekord, Iran

\textsuperscript{3} Child Growth and Development Research Center,\textsuperscript{4} Pediatric Inherited Diseases Research Center,\textsuperscript{5} Division of Genetics, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

\textsuperscript{6} Department of Community Medicine, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

\textsuperscript{7} Applied Physiology Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

Keywords: MSI2, Gastric cancer, Gene expression

Running Title: MSI2 expression in gastric cancer

*Corresponding author:

Parvaneh Nikpour, PhD, Division of Genetics, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran, Tel: +98 311 7922423, Fax: +98 311 7753480

Email: pnikpour@med.mui.ac.ir
Summary

Gastric cancer is the second most frequent cause of cancer death worldwide. In Iran, gastric cancer is the first cause of national cancer-related mortality in men and the second one in women. In mammals, the Musashi family of RNA binding proteins comprises the Musashi1 and Musashi2 proteins, encoded by the MSI1 and MSI2 genes. Mammalian Musashi contributes to the self-renewal of various types of stem cells. Furthermore, there is mounting evidence that stem cells exist in many tissues. Due to this, Msi appears to be associated with tumorigenesis. In the present study, 30 paired gastric tissue samples were examined for MSI2 gene expression by quantitative real-time RT-PCR. Our results demonstrated that the relative expression of the gene did not significantly alter between tumoral and non-tumoral tissues and different tumor types; but there was a statistical difference between the MSI2 gene expression in different tumor grades, of note between grade I and grade II.
Introduction

Gastric cancer is a common disease worldwide and the second most frequent cause of cancer death, affecting about one million people per year [6]. The ratio of men to women is about 2:1. In Iran, gastric cancer is the first leading cause of national cancer-related mortality in men and the second one in women [15].

Post transcriptional regulation of pre-mRNA is one of the key aspects of regulating gene expression in eukaryotes. In mammals, the Musashi family of RNA binding proteins comprises the Musashi1 and Musashi2 proteins, encoded by the MSI1 and MSI2 genes [3, 16]. The Musashi (Msi) family of RNA-binding proteins is specified by two RNA recognition motifs (RRMs) [14]. Originally, Msi was found as an essential factor for asymmetric cell division in adult Drosophila external sensory organ [10]. In mammals, Msi activates Notch signaling pathway by translational repression of NUMB through binding to the 3′ untranslated region (UTR) of its mRNA [4].

Mammalian Msi contributes to the self-renewal of neural stem/progenitor cells. Furthermore, there is mounting evidence that stem cells exist in many tissues [4]. Therefore, Msi appears to be associated with tumorigenesis [14]. The Msi expression deregulates during tumorigenesis in different adult tissues [14], including glioblastoma [17], esophageal [17], colon [18], pulmonary [8], breast [7] and bladder [11] cancers. Located on 17q23 chromosome, Musashi2 is a highly conserved RNA binding protein [16]. Although its expression in the central nervous system is cell type-specific and developmentally regulated, Musashi2 is transcribed over the tissues ubiquitously [16]. It has been shown that MSI2 expression associates prominently with normal hematopoietic stem cells and the most immature fraction of leukemic cells [5]. Microarray analysis revealed a significant upregulation of MSI2 in patients during chronic myelogenous
leukemia (CML) progression [5]. Moreover, MSI2 was found in large amounts in acute myeloid leukemia (AML). To our knowledge, the expression of this gene has been mainly examined in hematopoietic malignancies, and there is no data on the expression of *MSI2* in gastric cancer. Therefore, we evaluated its expression in 30 paired tumoral and non-tumoral gastric tissue specimens using quantitative real-time RT-PCR (qRT-PCR). Our results demonstrated that *MSI2* expression decreased significantly in grade II of gastric cancer. However, there was not any significant association between *MSI2* expression and tumor types. Moreover, *MSI2* expression did not change significantly between tumoral and non-tumoral tissue specimens.

**Material and Methods**

**Subjects**

Thirty paired gastric tumoral and non-tumoral tissue specimens were examined for gene expression. The characteristics of the samples are described elsewhere [12]. The experimental procedures described herein were approved by the Ethics Committee of Isfahan University of Medical Sciences. The patients’ informed written consent forms were obtained as described previously [12].

**Gene expression analyses**

Total RNA was extracted from tumoral and non-tumoral tissues using Qiazol reagent (Qiagen, Hilden, Germany) followed by purification and elimination of any genomic DNA with RNeasy mini kit (Qiagen, Hilden, Germany). The quality of the extracted RNA was assessed by gel electrophoresis, and the concentration of the extracted RNA was determined by optical density at 260 nm. MMLV Reverse Transcriptase was used to synthesize cDNA (Fermentas, Vilnius,
Lithuania) with oligo-dT primers as described previously [9]. Gene expression quantitation was performed with the Maxima SYBR Green/ROX qPCR Master Mix (Fermentas, Vilnius, Lithuania) using specific primers for *MSI2* [11] and *TBP* [13] (internal control) mRNAs. All reactions were run on the Rotor-gene 6000 (Qiagen, Hilden, Germany). Thermal conditions of the PCR cycles were as follows: an initial denaturation step at 95°C for 10 min, followed by 45 amplification cycles consisting of denaturation at 95°C for 20 s, annealing at 55°C for 20 s and an extension at 72°C for 20 s. As further verification, the PCR products were run on 1.5% agarose gel. To calculate relative gene expression, the standard curve method was used.

**Statistical analyses**

All measurements were performed in triplicate for each independent preparation. Student’s t-test and ANOVA were used to analyze the statistical significance of the data. The SPSS software, version 16.0, was used for statistical analyses. A value of p <0.05 was considered as statistically significant.

**Results**

**Optimization of RT-PCR reaction**

Both conventional and quantitative real time RT-PCR reactions were optimized on a tumoral gastric tissue specimen using specific primers for human *MSI2*. A single band with the expected size was obtained with electrophoresis of the PCR product on agarose gel for the amplified *MSI2* (129 bp) fragment (Fig. 1a). Furthermore, a unique melting curve without primer dimers was obtained for the gene examined (Fig. 1b). A serial dilution of a cDNA synthesized from a tissue
specimen was used to optimize the quantitation. Of note, PCR amplification efficiency close to 100% was gained.

Expression of MSI2 gene in gastric tissue samples

Relative expression of MSI2 in 30 paired gastric tissue specimens was determined using the standard curve method in which the expression amount of MSI2 gene was divided into the expression amount of the TBP gene for each sample. The expression of MSI2 gene did not change significantly between tumoral and non-tumoral tissues (p value: 41×10⁻²) and different tumor types, i.e., intestinal versus diffuse gastric cancer (p value: 49×10⁻²) (Fig. 2a and 2b). However, there was a significant difference between the MSI2 gene expression in grade I versus grade II (p value: 48×10⁻³) (Fig. 2c).

Discussion

Using qRT-PCR, We evaluated and quantified the expression of MSI2 in a set of paired gastric cancer tissues. According to our results, the expression of MSI2 at the transcriptional level was changed significantly between grades I and II. However, the expression of the examined gene did not change significantly between tumoral and non-tumoral tissues and different tumor types.

Msi2 plays an important role in regulating the heamatopoietic stem cell pool as shown by gain and loss of function experiments. Furthermore, Msi2 is over-expressed in human myeloid leukemias, and its expression correlates with poor prognosis [1]. Moreover, Wuebben et al. recently showed that MSI2 is required for the self-renewal and pluripotency of embryonic stem cells [19]. To our knowledge, MSI2 expression was rarely evaluated in solid tumors, including carcinomas, except for pulmonary carcinomas [8] in which Moreira et al. determined the
expression of some putative stem cell markers, including MSI2 in the paraffin-embedded archival material of resected untreated pulmonary carcinomas by immunohistochemistry. Therefore, our results may provide a preliminary insight into the possible role of MSI2 in solid tumors as well. Taking into account that MSI2 plays an important role in regulating various types of stem cells, it is not uncommon to observe that there is not a significant difference between the gastric tumoral and non-tumoral tissues as stomach is a regenerating organ containing stem/progenitor cells [2].

In conclusion, this study shows that the \textit{MSI2} gene expression was decreased from grade I to grade II of gastric cancer. However, there was not a significant alteration in \textit{MSI2} gene expression between tumoral and non-tumoral tissues and different tumor types. To the best of our knowledge, there is no study investigating the role and biological importance of Musashi gene family in gastric cancer. Therefore, our results call for further investigation to precisely define the role of \textit{MSI2} in normal and pathological conditions of major human organs, including the stomach.
References:


Legends:

**Fig.1** Optimization of conventional and real-time qRT-PCR

a) Agarose gel electrophoresis of *MSI2* PCR product b) A unique melting curve without primer dimers indicating specific amplification of *MSI2* on real-time PCR

**Fig.2** Relative expression of *MSI2* in gastric tissue specimens as determined by qRT-PCR

Histograms comparing the relative expression of *MSI2* to *TBP* in a) tumoral and non-tumoral tissue samples b) different tumor types and c) different tumor grades. The values shown represent the mean ±SEM.