Short Communication

MiR-320a downregulation is associated with imatinib resistance in gastrointestinal stromal tumors

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Introduction

Gastrointestinal stromal tumor (GIST) is one of the most common mesenchymal tumors of the gastrointestinal tract. Though imatinib improves the outcome, drug resistance remains the major problem for extending patient survival. Genetic mutation of the drug targets is the known mechanism for imatinib resistance. However, it cannot explain all of the phenomena of imatinib resistance, and numerous additional mechanisms have been proposed to account for imatinib resistance in various model systems. In this study, we applied the SYBR-green quantitative polymerase chain reaction-based array approach to screen the differentially expressed miRNAs between primary GIST patients and imatinib-resistant patients. The selected candidate miRNAs were validated in a cohort of 12 GIST patients. We found that low expression of miR-320a was correlated with short time to imatinib resistance, and proposed the potential mechanism of miR-320a for imatinib resistance.

Materials and Methods

Patients and specimens

Frozen tissue specimens of three GIST patients who did not receive imatinib and four GIST patients who developed imatinib resistance after at least half a year of imatinib therapy, were utilized for microarray analysis. Frozen tissue specimens of 16 GIST patients who did not receive imatinib and 12 GIST patients who developed imatinib resistance after at least half a year of imatinib therapy, were used to test the miRNAs. Formalin-fixed paraffin-embedded tissues of 12 GIST patients were obtained from Zhongshan Hospital, Fudan University (Shanghai, China). All of these patients had undergone surgery and then received imatinib. When the patients developed drug resistance, they also underwent surgical resection of their resistant tumors. Both the pre-imatinib and imatinib-resistant tissues were included in this study. Clinical information was obtained from the GIST
database, including extent of disease at the start of each TKI treatment, and duration of imatinib therapy before resistance. This study was approved by the Institution Review Board of Zhongshan Hospital, Fudan University.

Microarray analysis
Microarray-based miRNA expression analysis was conducted according to the manufacturer’s instructions (Agilent Technologies, Santa Clara, USA). Briefly, 100 ng of total RNA from frozen GIST tissues was labeled by using miRNA Labeling Reagent (Agilent Technologies), and then the labeled RNA was hybridized with a human miRNA Microarray kit (V3; Agilent Technologies), which covers 955 human miRNAs. The microarray data were analyzed by using Agilent Scan Control software and Agilent Feature Extraction software version 9.5.3 (Agilent Technologies).

Quantitative reverse transcription-polymerase chain reaction
MiR-320a expression was analyzed by using TaqMan microRNA Assays (Applied Biosystems, Foster City, USA). Briefly, 5 ng of total RNA was reverse transcribed by using specific stem-loop reverse transcription (RT) primers, then amplified and detected by using polymerase chain reaction (PCR) with specific primers and TaqMan probes. The PCR reaction was run in triplicate by using the 7500 Fast Real-Time PCR System (Applied Biosystems), and SDS v1.4 software (Applied Biosystems) was used for comparative ΔCt analysis. U6 snRNA (RNU6B; Applied Biosystems) was used as an endogenous control.

Statistical analysis
Quantitative RT-PCR (qRT-PCR) data were analyzed by the Student’s t-test and P value < 0.05 were considered statistically significant. Patients’ disease-free survival (DFS) rates were calculated by using the Kaplan–Meier method, and statistically significant differences in DFS were identified by using the log-rank test.

Results
Microarray analysis of primary and imatinib-resistant GISTs
To identify the upregulated and downregulated genes in the imatinib-resistant GISTs, microarray analyses were performed by using RNA extracted from three GIST patients without imatinib therapy and four GIST patients with imatinib-resistant GIST. Among the 955 genes analyzed, 13 genes were differentially expressed between primary and imatinib-resistant GIST by at least 2 folds (P < 0.05). Compared with the primary GISTs, the expression levels of five genes (hsa-miR-15a, hsa-miR-16, hsa-miR-195, hsa-miR-335, and hsa-miR-151-5p) were upregulated and the levels of eight genes (hsa-miR-1280, hsa-miR-140-5p, hsa-miR-320a, hsa-miR-135b, hsa-miR-664*, hsa-miR-483-5p, hsa-miR-140-3p, and hsa-miR-574-3p) were downregulated in imatinib-resistant GISTs (Fig. 1 and Supplementary Table S1).

qRT-PCR analysis of miRNA
To confirm the microarray data, qRT-PCR was performed by using cDNA synthesized from the RNA used in the microarray analysis as the template. We performed qRT-PCR analyses on 13 selected miRNAs in a cohort of 28 clinical samples. Of the 13 genes whose expression is different between primary and imatinib-resistant GISTs, the expression of miR-320a was found to be lower in imatinib-resistant GISTs (P = 0.018). Its expression level was consistent with the results of the microarray analysis. However, the expression levels of other miRNAs were significantly different from the results of microarray analysis (Supplementary Fig. S1). Hence, we considered that miR-320a may be involved in the process of imatinib resistance.

qRT-PCR analysis of miR-320a
We examined the miR-320a expression level by qRT-PCR in 12 cases of GISTs, and confirmed that miR-320a expression tended to be lower in imatinib-resistant samples (Fig. 2). These data suggested that the downregulation of miR-320a in GISTs could be responsible for the tumor acquiring drug-resistant potential, resulting in imatinib resistance.
Association of miR-320a expression in GISTs with time to imatinib resistance

By using Kaplan–Meier survival plots and log-rank analyses, we evaluated the association of each individual miRNA expression with time to imatinib resistance (TTR). We found significant associations with TTR for miR-320a. Patients with low expression of miR-320a (Fig. 3) were found to have significantly shorter TTR ($P = 0.005$). The medial TTP of low and high expression of miR-320a was about 11 and 33 months, respectively.

Discussion

Imatinib mesylate is the first approved rationally designed inhibitor of specific protein tyrosine kinases. Although the response rates of imatinib-treated patient were very high [7], disease progression after a certain period of imatinib therapy may occur [8]. Genetic mutation of the drug targets are the known mechanisms for this observed drug resistance [9]. However, numerous additional mechanisms have been proposed to account for imatinib resistance in various model systems [10–12].

Previous researches revealed that miRNAs participate in the process of cancer drug resistance. One of the studies indicated that miRNA play a role in the development of chemosensitivity and chemoresistance in different cancers [13]. MiR-320a is also downregulated, which can suppress tumor cell proliferation and migration in several cancer types, including colon cancer [14] and hepatocellular cancer [15]. Another study indicated that miR-320a may be related to chemoradiosensitivity of colorectal cancer cells [16]. In this study, we found that miR-320a was downregulated in imatinib-resistant GISTs and low expression of miR-320a was found to be associated with short TTR. This confirmed that miR-320a was involved in the process of imatinib resistance. MiR-320a has many target genes. Some of them were involved in signal pathways, such as Wnt pathway, IGF pathway, and so on. These pathways are related to cell apoptosis. Therefore, downregulation of miR-320a may suppress apoptosis of GIST cells, which contributes to imatinib resistance. Transient receptor potential channel 5 (TRPC5) is one of the target genes of miR-320a and it can induce the change of ABCB1 that is one of the drug-resistant genes [17]. Therefore, miR-320a can contribute to imatinib resistance theoretically. However, the significant role of miR-320a and its targets in the process of imatinib resistance remains to be further investigated. We need to further assess the combined treatment effects of miR-320a and imatinib on imatinib-resistant GISTs to determine whether miR-320a can severe as a novel target for GISTs treatment or not.

In conclusion, we reported that miR-320a is associated with imatinib resistance of GIST patients. These findings may provide important insights into understanding the process of imatinib resistance.

Supplementary Data

Supplementary data are available at ABBBS online.
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**References**