ORIGINAL RESEARCH

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Transcript Level of MicroRNA Processing Elements in Gastric Cancer

Milad Asadi[1,2] · Dariush Shanehbandi[2] · Venus Zafari[3] · Vahid Khaze[2] · Mohammad Hosein Somi[1] ·
Shahriar Hashemzadeh[4,5]

Abstract

Background Aberrant expression of microRNAs (miRNAs) has been implicated in the etiopathogenesis and development of various cancers. Drosha and Dicer are the main components of the miRNA biosynthesis machine. Another enzyme, DGCR8, is the assistant of Drosha in the processing complex. Here, we tried to evaluate the mRNA transcript level of Drosha, Dicer, and DGCR8 genes in involved tissues from patients with gastric cancer.

Methods Fifty tumoral and their marginal tissues, as the control group, were obtained from patients with gastric cancer. After RNA extraction from tissues and cDNA synthesis, quantification of mRNA expression of Drosha, Dicer, and DGCR8 was conducted using SYBR Green master mix and real-time PCR.

Results It was observed that mRNA expression levels of Drosha, Dicer, and DGCR8 were significantly upregulated in tumoral tissues compared with marginal tissues. Upregulation of these genes was not correlated with clinical manifestations of the patients.

Conclusions Upregulation of Drosha, Dicer, and DGCR8 plays a role in the development of cancer, probably through dysregulated the expression level of miRNAs.

Keywords Drosha · Dicer · DGCR8 · Gastric cancer · MicroRNA

Introduction

Gastric cancer is one of the most common cancers throughout the world, which is ranked as the second cause of cancer-related death [1]. Like other cancers, gene expression patterns are changed in gastric cancer. In fact, there are mainly two types of genes, which are involved in cancer development, some with increased level of expression known as oncogenes while some with decreased expression level known as tumor suppressor genes [2].

MicroRNAs (miRNAs) are non-coding RNAs with 18–24 nucleotides, which are important in genes expression regulation and due to their roles in cell biology, they have a great role in cancer development [3]. About 1000 miRNAs are encoded in human genome and each of them regulates expression level of about 200 genes; hence, approximately 30% of all human genes are regulated by these small molecules [4]. With regard to their expression level and function, miRNAs may act like tumor suppressor genes or oncogenes [5]. All in all, cancer pathogenesis is related to mRNA-miRNA interaction and their expression levels [6]. Expression of miRNAs is regulated and processed by a complicated biological system. At the first step, they are transcribed by RNA polymerase 2 in nucleus and this immature molecule is known as primary miRNA (pri-miRNA) [7]. At the second step, an endonuclease 3 known as Drosha, which is linked with a nuclear protein known as DiGeorge syndrome critical region gene 8 (DGCR8 or Pasha), cutes and processes the pri-miRNA to pre-miRNA in cell nucleus which is about 75–90 nucleotide long. After processing by Drosha, the pre-miRNA transported to cytoplasm via exportin-5 where another endonuclease 3 known

✉ Shahriar Hashemzadeh
  shahriar_90@yahoo.com

1 Research Centers for Liver and Gastrointestinal Disease, Tabriz University of Medical Sciences, Tabriz, Iran

2 Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

3 Tuberculosis and Pulmonary Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

4 Hematology and Oncology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

5 Department of General and Vascular Surgery, Imam Reza Educational Hospital, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

as Dicer cutes and converts pre-miRNA to mature and active microRNA [8–10].

As a result, Dicer, Drosha, and DGCR8 have an important role in the activation and expression profile of microRNAs. Previous studies have disclosed the dysregulation of microRNA in human cancers, such as gastric cancer [5, 11–17]. However, there is a gap in studying the expression level of these three genes, as most important part of microRNA maturing machinery, and their probable role in miRNA expression levels dysregulation. Herein, in this study, we evaluated the microRNA processing machinery system elements Drosha, Dicer, and DGCR8 in tumoral tissues from gastric cancer patients in order to fill this gap.

Material and Methods

Patients and Sampling

Fifty tumoral and their marginal tissues, as the normal control group, were collected from gastric cancer patients who had referred to Imam Reza Hospital of Tabriz University of Medical Sciences during 2012–2016. To get a pure sample population, all the patients were native to East Azerbaijan, North West of Iran. Through sample gathering, patients who had undergone chemotherapy and radiation therapy were excluded. All samples were collected during surgery and then transferred into RNAase inhibitor solation (Qiagen, Cat No. 76104) and stored in − 80 °C till RNA extraction. The clinical data of the patients were gathered and summarized in Table 1. The Human Research Ethics Committees from the Tabriz University of Medical Sciences approved the

protocol of this study and written informed consent was taken by all patients.

RNA Extraction

Total RNA was extracted from tumoral and marginal tissues by Tripure isolation reagent (Roche, Cat No. 11667165001) according to the manufacturer's protocol. Quantity of RNA samples was determined by Nanodrop and quality was examined by gel electrophoreses on 1% agarose. Afterwards, RNA samples were stored in − 80 °C till cDNA synthesis.

Complementary DNA Syntheses and Real-time PCR Quantification

In this study, 2 step real time PCR was used for quantitative measurement of target genes expression level. We applied TAKARA complementary DNA (cDNA) syntheses kit (TAKARA, Cat No. 6130) to synthesize cDNA. After that, quantitative real-time PCR was conducted by SYBR green PCR master mix (TAKARA, Cat No. RR820W) and specific primer set for each gene (Table 2). Primers were adopted from the studies by Sand et al. to evaluate Drosha, Dicer, DGCR8, and RNA induced silencing complex (RISC) components in epithelial skin cancer [18, 19]. For normalizing, the expression level of target genes GAPDH (housekeeping gene) expression level was used. At the end, the average of duplicated Ct values was measured and the relative expression level of target genes was determined by comparative C_t method [20].

Statistical Analysis

Statistical analysis was performed using the Graph Pad Prism 6 (Graph Pad Software Inc. San Diego, CA, USA). Kolmogorov-Smirnov's normality test was applied for evaluating normality of data. Independent sample t test was conducted to compare target gene expression level between cancerous tissues and their paired marginal tissues. Pearson's correlation test was performed for evaluating the correlation between expression of target genes and patient's clinical parameters. All results were expressed as mean ± standard deviation (SD). Statistical significance level for all P value was less than 0.05.

Results

Analysis of mRNA expression level of Dicer, Drosha, and DGCR8 in tumoral tissues from gastric cancer patients and comparison of those with marginal tissues indicated that mRNA expression levels of Drosha (fold change = 2.8, P = 0.0023), Dicer (fold change = 2.1, P = 0112), and DGCR8

Table 1 Clinicopathological data of gastric cancer patients

Feature	Group	Number of subjects
Sex	Male	31
	Female	19
Age	> 62	23
	< 62	27
Nodal status	pN0	12
	pN1–3	38
Distant metastasis	pM0	42
	pM1	8
Tumor grade	G1	3
	G2	13
	G3	34
Smoking	Yes	29
	No	21
Alcohol consumption	Yes	11
	No	39

Table 2 Primer sequences and their specifications used in quantification real-time PCR

Target	Sequence	Amplicon size (bp)	Temp (°C)
Drosha-F	5′-CATGTCACAGAATGTCGTTCCA-3′	115	58.4
Drosha-R	5′-GGGTGAAGCAGCCTCAGATTT-3′		59.8
Dicer-F	5′-TTAACCTTTTGGTGTTTGATGAGTGT-3′	94	58.5
Dicer-R	5′-GGACATGATGGACAA TTTTCACA-3′		57.1
DGCR8-F	5′-GCAAGATGCACCCACAAAGA-3′	93	57.3
DGCR8-R	5′-TTGAGGACACGCTGCATGTAC-3′		59.8
GAPDH-F	5′-CAAGATCATCAGCAATGCCTCC-3′	122	63.2
GAPDH-R	5′-GCCATCACGCCACAGTTTCC-3′		62.1

(fold change = 3.7, $P = 0.0014$) were significantly upregulated in tumoral tissues in relation to marginal tissues (Fig. 1).

The correlation analysis between the mRNA expression level of Dicer, Drosha, and DGCR8 and clinicopathological characters was conducted. It was observed that mRNA expression levels of Dicer, Drosha, and DGCR8 were not significantly correlated with clinicopathological and demographic parameters with regard to age, sex, smoking, alcohol consumption, nodal status, distant metastasis, and tumor grade in the patients (Table 3).

Discussion

Epidemiological evidence reports that gastric cancer is the third leading cause of cancer-related death in all over the world. In spite of a decreased incidence in some geographical regions, there is a challenge in clinical aspects of gastric cancer due to the disease diagnosis in an advanced stage, poor prognosis, and inefficient treatment choices. Helicobacter pylori infection has been considered as the most common cause of the gastric cancer, and then Epstein-Barr virus infection as well as familial genetic background is important. Among the

most critical predisposing factors for gastric cancer are smoking, high salt intake and other dietary contributing factors [21]. The prevalence of the gastric cancer in East-Azerbaijan, Iran, where the patients in this study were taken from, is 8.3% (the most prevalent after skin, esophageal, and breast cancers) in female and 15.6% in males [22]. Therefore, sophisticated hygiene programing appears to be crucial in reducing the prevalence as well as treating the patients. This could be possible through identification of novel diagnostic and prognostic approaches, such as miRNAs, which seems to be promising within this context [16, 23].

Previous studies have documented the crucial role of miRNAs machinery system components in some types of cancers [24]. According to these studies, Drosha, Dicer, and DGCR8 demonstrated different expression pattern in each tumor type. Furthermore, aberrant expression profile of these genes has been associated with dysregulation of miRNAs, which in turn was related to the onset and development of malignancies [19, 25]. However, there is no obvious evidence indicating how expression pattern of miRNAs is controlled directly by these genes [26, 27].

In the ongoing study, we evaluated the mRNA expression level of Dicer, Drosha, and DGCR8 in tumoral tissues of

Fig. 1 Illustration of mRNA expression level of Drosha, Dicer, and DGCR8 in tumor tissues of gastric cancer patients in comparison to marginal tissues (FC fold change)
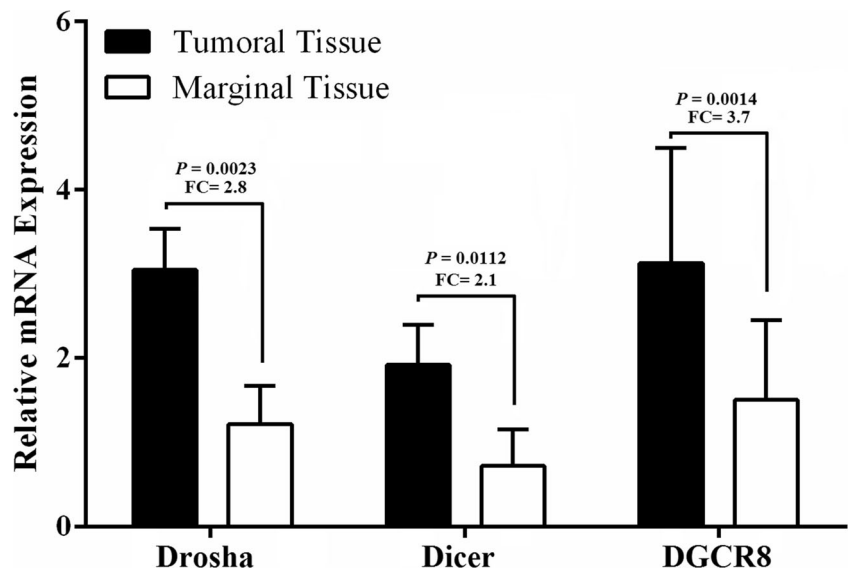
Table 3 Association of mRNA expression level of Drosha, Dicer, and DGCR8 genes and clinicopathological data of gastric cancer patients (*P* values are represented)

Characteristic	Drosha	Dicer	DGCR8
Sex	0.861	0.742	0.796
Smoking	0.814	0.543	0.731
Age	0.731	0.878	0.748
Alcohol consumption	0.561	0.739	0.813
Nodal status	0.341	0.542	0.248
Distant metastasis	0.251	0.142	0.248
Tumor grade	0.186	0.127	0.211

gastric cancer patients in comparison to marginal non tumor tissues. It was found that there were significant upmodulations in expression levels of these three genes in tumoral samples in comparison to marginal tissues. Alteration in Dicer, Drosha, and DGCR8 expression levels has previously been demonstrated in gastric and hepatocellular cancer cell lines [28]. Moreover, the altered expression of Dicer and Drosha has been associated with deregulated expression pattern of some miRNAs in gastric cancer [16, 27].

Our experiments also demonstrated that there was no any relation between expression levels of these genes and clinical symptoms of the gastric cancer patients, which was also previously shown [5].

Studies have shown that knockdown of miRNA machinery system components can culminate in total decreased expression level of miRNAs in various cell types [29, 30]. Furthermore, it has been observed that functional mutations may occur in genes encoding miRNA machinery components and may be contributing to cancer development [31]. It seems logical that increased levels of Drosha, Dicer, and DGCR8 result in increased production of miRNAs in general. However, even though the miRNA biogenesis enzymes are upregulated, there might be decreased levels of several miRNAs. This is because of regulatory mechanisms, including that occur after the biogenesis of miRNAs, such as untemplated nucleotidyl addition to the 3′ end of miRNA, miRNA editing through the conversion of adenosine to inosine, miRNA methylation, regulation through miRNA stability that contains are cleavage at the terminal loop, etc. [32].

In consideration of all, Dicer, Drosha, and DGCR8 expression patterns, as the most important enzymes in miRNA processing system, were upregulated in tumoral tissues compared with normal marginal tissues from patients with gastric cancer. But there were no significant communications between expression profile of these genes and clinical and pathological symptoms of the patients. However, this conclusion is premature and could not be determined based on our data only and further investigations are still mandatory.

Compliance with Ethical Standards

The Human Research Ethics Committees from the Tabriz University of Medical Sciences approved the protocol of this study and written informed consent was taken by all patients.

Conflict of Interest The authors declare that they have no conflict of interest.

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