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Complexity in Regulation of MicroRNA Machinery Components in Cervical Cancer

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Abstract

Altered expression of microRNA (miRNA) machinery components may play an important role in cervical cancer progression. The objective of current study was to evaluate Drosha, the DiGeorge syndrome critical region gene 8 (DGCR8), Dicer, and Argonaute 2 (AGO2) mRNA expression in cervical cancer. By using quantitative real-time PCR, mRNA expression levels of the four miRNA machinery components were examined in 31 cervical cancer tissues and adjacent histologically non-neoplastic tissues. In the present study, mRNA expression levels of major miRNA machinery components were variable among cervical cancer tissues. The altered mRNA expression levels of AGO2 and DGCR8 were positively correlated with each other. For the first time, this study revealed that the positive correlation between altered mRNA expression levels of each miRNA machinery components in cervical cancer tissues.

Key Words : AGO2, Biogenesis, Cervical cancer, DGCR8, Dicer, Drosha, MicroRNA

Introduction

Cervical cancer is the third most common malignancy among women worldwide according to the most recent estimates of the global cancer incidence [1]. It has been reported that seven leading primary cancer sites in female during 2009 in Korea were lung, stomach, colon and rectum, liver, breast, and cervix uteri [2]. In Korea, changes of annual percentage in age-standardized incidence rates for cervical cancer were-4.4% in women between 1999 and 2009 based on world

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standard population as a standard population [2]. Although the incidence of cervical cancer has been shown to decrease, the definite pathogenesis of cervical cancer is still unclear. So it would be necessary to assess the mechanisms of pathogenesis by approaching strategy through diverse methods.

MicroRNA (MiRNA) is a recently described class of highly conserved, small non-protein coding RNA molecules which regulates gene expression on the post-transcriptional level by translational repression or cleavage of the target mRNA [3]. miRNAs have sequences of approximately 17~21 nucleotides and have been shown to involve in various important biological processes including cellular development, differentiation, proliferation, cell death, metabolism, and carcinogenesis [4-6]. The biogenesis of miRNA is accomplished by a set of enzymes, collectively referred to as the "miRNA machinery" [7]. MiRNA biogenesis is regulated by several miRNA machinery components including Drosha, Dicer, the DiGeorge syndrome critical region gene 8 (DGCR8), and Argonaute (AGO). Drosha, a nuclear ribonuclease, is a part of a multiprotein complex, the microprocessor, which cleaves primary miRNAs (pri-miRNAs; consisting of a hairpin stem, a terminal loop, and 5' and 3' single-stranded RNA extensions) into precursor miRNAs (pre-miRNAs; approximately 60-70 nucleotide stem-loop structure) in nucleus [8]. In addition to Drosha, DGCR8 is also a part of the microprocessor complex and has been shown to be essential for miRNAs maturation [9]. Within cytoplasm, the pre-miRNAs are further processed by a multidomain Dicer, which also belongs to the class of RNAse III endonucleases, into short double-stranded molecules, mature miRNAs [10]. The gene expression regulating effects of miRNAs are accomplished by the RNA-induced silencing complex (RISC), multiprotein effector complex with endonuclease activity, which integrates mature miRNA strands [3]. The RISC is the main element of the RNA silencing process and consist of several different proteins that comprise a multiprotein complex, including AGO1, AGO2, and the dsRNA-binding protein PACT [11]. A number of interesting reports have provided that human disorders, including malignant tumors, are frequently associated with global alterations in the miRNA machinery components, comprising irregular expression and function of the key factors such as Drosha, DGCR8, Dicer, and AGO [12].

Therefore, the mRNA expression levels of miRNA machinery components were compared and analyzed in cervical cancer tissues and corresponding adjacent non-neoplastic tissues from same patients. Moreover, the mRNA expression levels of AGO2 and DGCR8 were correlated with each other.

Materials and Methods

Patients and tissues

Altogether, thirty-one patients diagnosed with cervical cancer (24 squamous cell carcinoma, 5 adenocarcinoma, 1 glassy cell carcinoma, 1 adenosquamous carcinoma) were included in the study. Cervical carcinomas and adjacent non-neoplastic tissues were obtained from the patients who received surgery in Dongsan Medical Center (Daegu, Korea) from May 2008 to June 2010. Tissue samples were immediately frozen in liquid nitrogen and stored at-80℃ until RNA isolation. Tissue samples were provided from Keimyung Human Bio-resource Bank, Korea. The purpose of this study was explained to all patients and informed consent was obtained from each study participant. The protocols were approved by the

Institutional Review Board of Keimyung University Dongsan Medical Center (approval #11-199-1).

Isolation of RNA and quantitative real-time PCR

Total cellular RNA was extracted from tissues using the TRIzol reagent (Molecular Research Center, Inc., Cincinnati, OH, USA). RNA was quantified using Nanodrop 1000 (Thermo Scientific, Wilmington, Denmark). Each cDNA was synthesized from 2 μg of total RNA using M-MLV reverse transcriptase (Promega, Madison, WI, USA) according to the manufacturer's protocol. By using the specific primer pairs described in Table 1 and SYBR GREEN Premix (TOYOBO, Japan), quantitative real-time PCR (qPCR) was performed on the LightCycler® 480 real-time PCR system (Roche Diagnostics, Mannheim, Germany). β-actin was used as a housekeeping gene for normalization, and no template sample was used as a negative control.

Statistical analysis

Statistical analysis was performed with SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Statistical comparisons for significance were made with Wilcoxon signed-rank test for paired samples. Correlations between relative mRNA expressions of inter-individual miRNA machinery components were analyzed by the Pearson's correlation coefficient analysis. Generally, p value of less than 0.05 was established to denote significance in all statistical analyses performed in the study.

Results

Expression levels of miRNAs machinery components in cervical cancer

The mRNA expression levels of miRNAs machinery components were quantified by qPCR in paired samples of cancer tissues and adjacent non-cancer tissues from 31 patients with cervical

Table 1. Primer sequences of miRNA machinery components used in quantitative PCR

Components	Components	Cases (s)
Dicer	Forward	5'-TTAACCTTTTGGTGTTTGATGAGTGT-3'
	Reverse	5'-AGGACATGATGGACAATT-3'
Drosha	Forward	5'-CTGTCGATGCACCAGATT-3'
	Reverse	5'-TGCATAACTCAACTGTGCAGG-3'
AGO2	Forward	5'-TCATGGTCAAAGATGAGATGACAGA-3'
	Reverse	5'-TTTATTCCTGCCCCCGTAGA-3'
DGCR8	Forward	5'-CAAGCAGGAGACATCGGACAAG-3'
	Reverse	5'-CACAATGGACATCTTGGGCTTC-3'
β-actin	Forward	5'-CAGCCATGTACGTTGCTATCCAGG-3'
	Reverse	5'-AGGTCCAGACGCAGGATGGCATG-3'

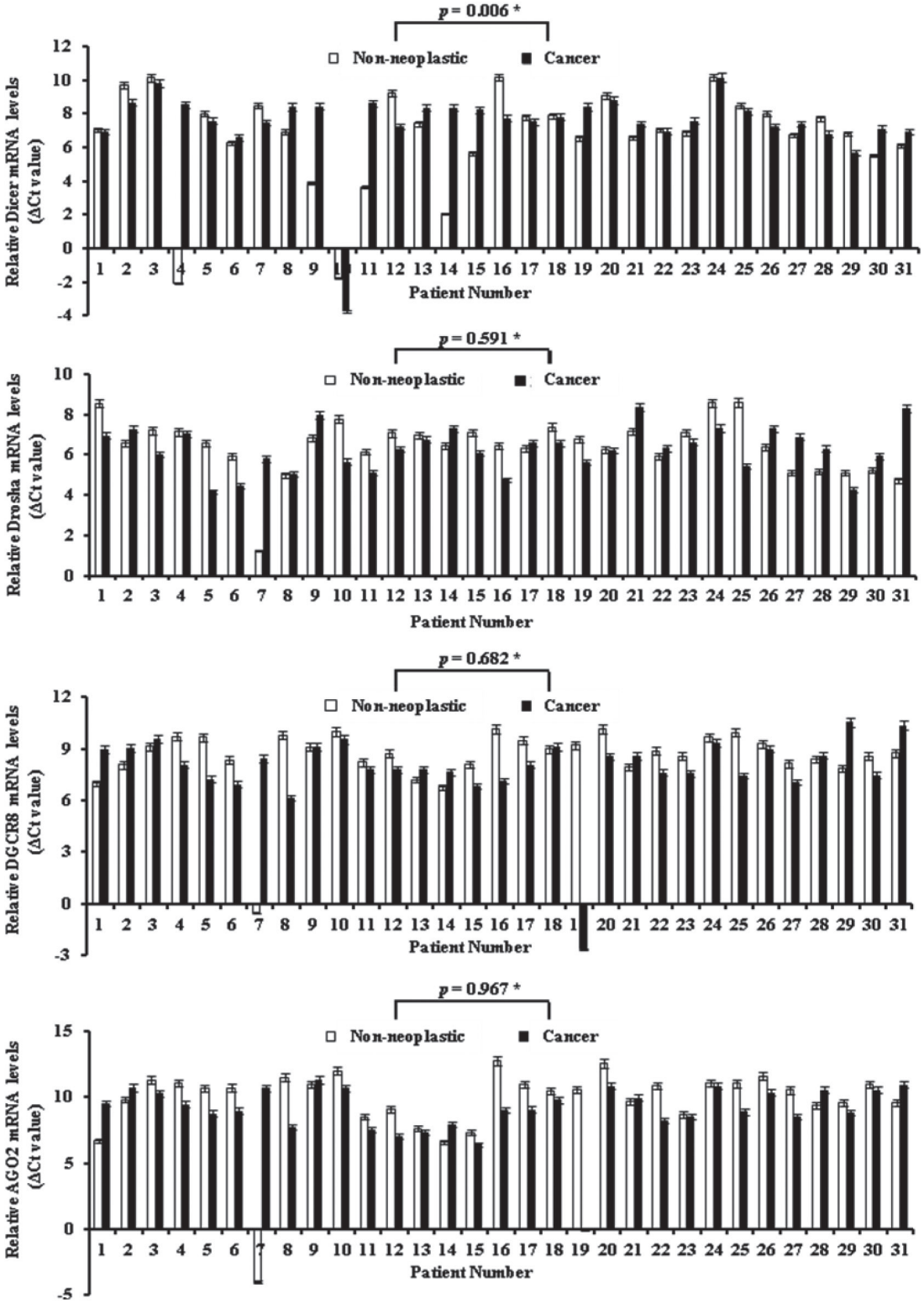

Fig. 1. The relative Dicer, Drosha, DGCR8, and AGO2 mRNA levels (normalized to the corresponding β-actin mRNAs) in cervical cancer tissues compared to adjacent non-neoplastic tissues. *Wilcoxon signed rank Test.

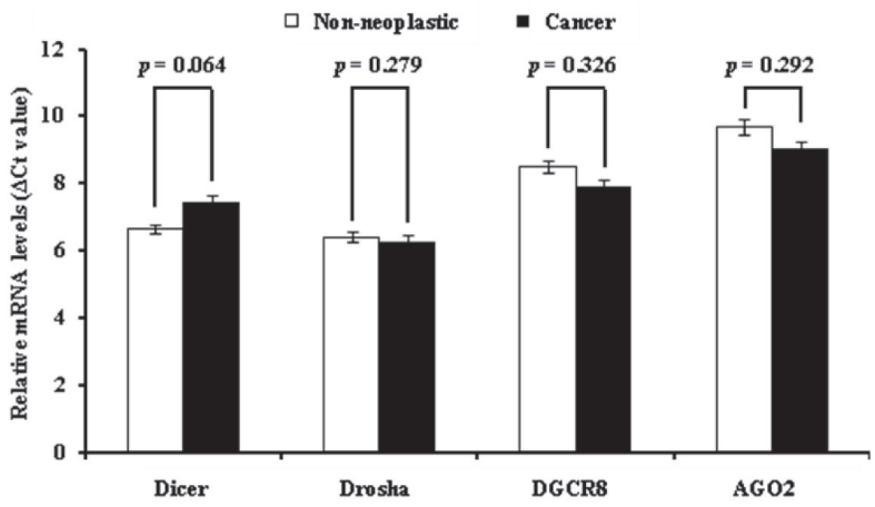

Fig. 2. The relative Dicer, Drosha, DGCR8, and AGO2 mRNA expression in cervical cancer group and in the non-neoplastic group.

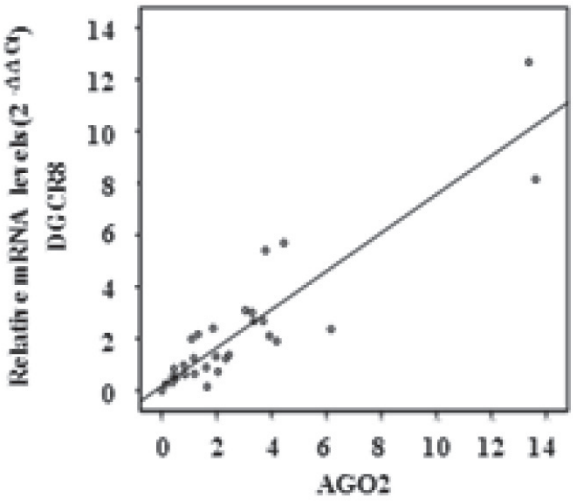

Fig. 3. Correlation between mRNA expressions of AGO2 and DGCR8 in cervical cancer. * $p < 0.001$.

cancer. Each mRNA level of components was normalized to the level of β-actin mRNA. Among four components only Dicer mRNA expression was significantly different between cervical cancer tissues and the corresponding non-neoplastic tissues (p=0.006, Fig. 1). However, the mean value of Dicer mRNA expression level in cervical tissues was not different between cancerous and non-neoplastic cervical tissues (Fig. 2).

Relationship between mRNA expression levels of inter-individual miRNA machinery components in patients with cervical cancer

Prior to statistical analysis, raw qPCR data of Dicer, Drosha, DGCR8, and AGO2 mRNA expression were normalized to reference gene, β-actin. Then, qPCR data were analyzed by the $2^{-\Delta\Delta ct}$ method [13]. To investigate significant correlation between mRNA levels of inter-individual miRNA machinery components in cervical cancer, correlations of the four selected miRNA machinery components were evaluated. As shown in Fig. 3, there were significant associations between AGO2 and DGCR8 with Pearson correlation coefficient value of 0.903 in cervical cancer ($p < 0.001$).

Discussion

Dysregulations of the miRNA machinery components have previously been linked to a variety of different cancers [14]. Although various papers have revealed alterations of microRNAs (miRNAs) expression profiles and their functional roles in cervical cancer [15-17], there have been few studies on the miRNA machinery itself.

In this study, the expression of the miRNA machinery components, which consists of extra- and intranuclear miRNA maturation components (Dicer, Drosha and DGCR8) and the extracellular miRNA effector RISC (AGO2) have been systematically investigated in cervical cancer. It has been reported that Drosha is up-regulated but DGCR8 is unchanged in cervical cancer [18]. In the present study, Dicer, Drosha, DGCR8, and AGO2 mRNA expressions are variable among cervical cancer specimens. Although there was a significance in statistical comparisons with Wilcoxon signed-rank test for paired samples of Dicer mRNA expression level, up-regulated Dicer mRNA level was shown more in malignant tissues than compared to the corresponding non-neoplastic tissues in 17 of the 31 patients with PTC (54.8%). Furthermore, only 4 cases were more than doubled in Dicer mRNA expression levels. Drosha, DGCR8, and AGO2 mRNA expression levels were not significantly different between cervical cancer tissues and the corresponding non-neoplastic tissues (Fig. 1). Although it would be necessary to evaluate the regulations of miRNA machinery components in more cases of cervical cancer, these results suggested that the mRNA expression levels of Dicer, Drosha, DGCR8, and AGO2 are not remarkably changed in cervical cancer tissues compared to corresponding non-neoplastic tissues.

Although mRNA expression levels of Dicer, Drosha, and AGO2 were strongly and positively correlated to each other in colorectal carcinoma [7], there were few studies to compare inter-individual miRNA machinery components in strictly pair-matched samples of cervical cancerous tissues and adjacent non-neoplastic tissues. Thus, in present study, the correlation between expression levels of inter-individual miRNA machinery components in cervical cancer was evaluated by using the Pearson's correlation coefficient analysis. It appeared that mRNA expression levels of AGO2 and DGCR8 are positively correlated with one another (Fig. 3).

For the first time, I investigated the expression patterns of four selected miRNA machinery components and their inter-relation in cervical cancer. The mRNA expression levels of major miRNA machinery components were variable in cervical cancer tissues. However, the mRNA expression levels of AGO2 and DGCR8 were positively correlated with one another. These results suggested that altered mRNA expression levels of miRNA machinery components may not be related with cervical carcinogenesis and AGO2 and DGCR8 may share partially common regulating mechanisms in cervical cancer.

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