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RESEARCH

Complexity in Regulation of microRNA Machinery Components in Invasive Breast Carcinoma

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Abstract Altered expression of microRNA (miRNA) machinery components may play an important role in breast cancer progression. The objective of the current study was to evaluate Drosha, the DiGeorge syndrome critical region gene 8 (DGCR8), Dicer, and Argonaute 2 (AGO2) mRNA expression in invasive breast carcinoma (IBC) and to assess the value of clinical parameters on their expression. By using quantitative real-time PCR, we examined the expression of the four miRNA machinery components in 52 breast tumor tissues which are diagnosed as invasive ductal carcinoma and adjacent non-neoplastic tissues. In the present study, decreased mRNA expression levels of major miRNA machinery components were observed in IBC. The altered mRNA expression levels of DGCR8 and AGO2 are positively correlated with to each other. This study revealed for the first time that expression alterations of DGCR8 are significantly associated with estrogen receptor and Ki-67 status in IBC. Moreover, AGO2 mRNA expression level was significantly correlated with N stage. These results provided evidences that down-regulated the four miRNA machinery components may play an important role in breast pathobiology and that DGCR8 and AGO2 might be associated with important clinical factors.

Keywords microRNA biogenesis · DGCR8 · AGO2 · Ki-67 · Invasive breast carcinoma

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Introduction

Invasive breast carcinoma (IBC) represents a heterogeneous group of lesions in terms of clinical, histopathological, and their molecular complexity and biological diversity. Molecular subtypes of IBC are classified by the immunohistochemical results for estrogen receptor (ER), progesterone receptor (PR) and epidermal growth factor receptor type 2 (HER2) protein expression: luminal A (ER+ or PR+, and HER2-), luminal B (ER+ or PR+, and HER2+), HER2-enriched (ER-, PR- and HER2+), and triple-negative (TN) (ER-, PR- and HER2-) [1–4]. According to these subtypes, IBC has different incidences, prognosis, and responses to chemotherapy [1, 2, 4–6]. Immunohistochemical assessment of the proportion of cells staining for the nuclear antigen Ki-67 is a well-established method for evaluating proliferative and prognostic potential in cancers, including breast carcinoma [7, 8].

MicroRNAs (miRNAs) are a new class of highly conserved, small noncoding RNAs that regulate gene expression on the post-transcriptional level by translational repression or cleavage of the target mRNA [9]. The biogenesis of miRNA occurs in a well-organized process, referred to as the "miRNA machinery" [10] (Fig. 1). A number of interesting reports have provided proof 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 Drosha, the DiGeorge syndrome critical region gene 8 (DGCR8), Dicer, and

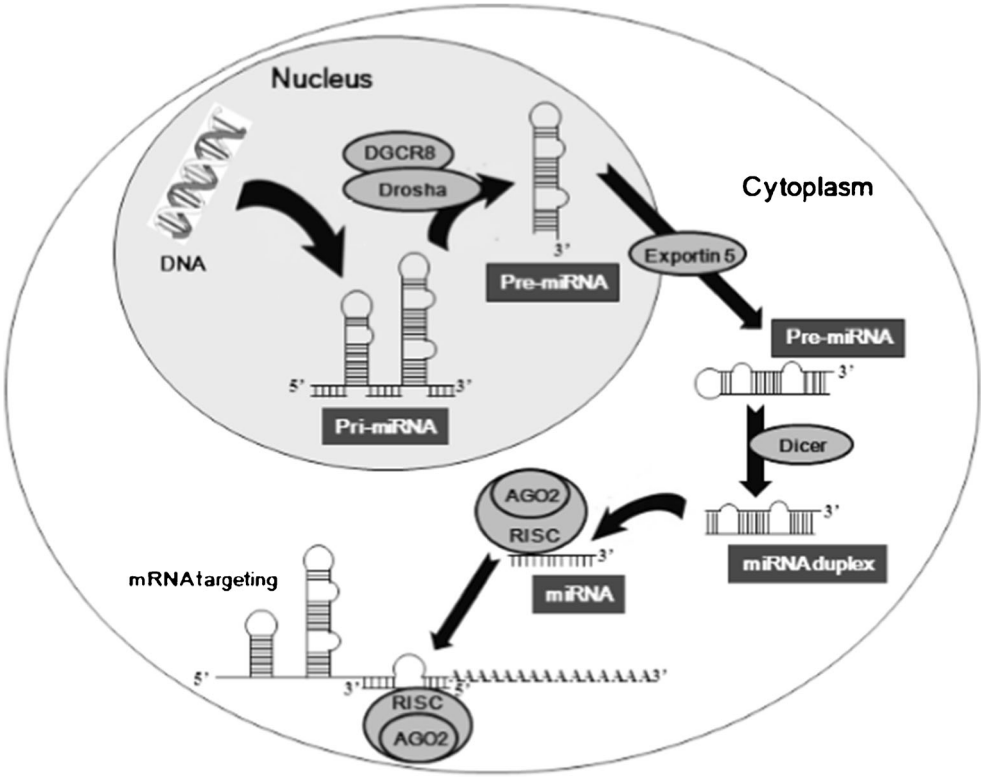

Fig. 1 microRNA machinery. microRNA (*miRNA*) is a non-coding RNA. miRNA genes are transcribed by RNA polymerase II and the primary miRNA (*pri-miRNA*) is cleaved into precursor miRNA (*pre-miRNA*) by RNases in the nucleus, such as Drosha, complexed with another protein DGCR8. Pre-miRNA is exported from nucleus to the cytoplasm by Exprotin 5. In the cytoplasm, Dicer processes the exported pre-miRNA into miRNA duplex. A short double stranded RNA (*miRNA duplex*) is processed into each strand and one of the strands is incorporated into the RNA induced silenced complex (*RISC*), which is consist of several different proteins, including AGO2. The incorporated single-stranded RNA is mature miRNA. The mature miRNAs interact with complementary target mRNA that leads to protein translation repression or mRNA destabilization

Argonaute (AGO) [11]. Drosha, an RNAse III endonuclease, is a part of a multiprotein complex, the microprocessor, which cleaves primary miRNAs (pri-miRNAs; consisting of a hair-pin 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 [12]. DGCR8 is also a part of the microprocessor complex and has been shown to be essential for miRNAs maturation [13]. Within the 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 [14]. 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 [9]. 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 [15].

The expression of miRNA machinery components could directly influence expression patterns of various genes. If any miRNA machinery component is dysregulated, miRNA may be incompletely matured. The dysregulated expression of important individual miRNA machinery component has recently been shown in various human diseases, including malignancies [15–17]. Furthermore, especially, rapidly accumulating evidence has revealed that dysregulation of Dicer or Drosha is closely associated with clinical characteristics of breast cancers [18–22]. These studies showed that down-regulation of miRNA machinery components in tumors may be associated with aggressive clinical behavior, resulting poor prognosis. However, it was still controversial because opposite results were found in some tumors [23–25], to which more and more oncologists have paid their attention.

In this study, we compared the expression levels of miRNA machinery components between breast cancer tissues and corresponding adjacent non-neoplastic tissues from patients with same cancer, and investigated whether the expression levels of miRNA machinery components are associated with clinicopathological characteristics.

Materials and Methods

Patients and Tissues

Altogether, 52 patients diagnosed with IBC were included in the study. Breast carcinomas and adjacent non-neoplastic tissues were obtained from the patients undergoing surgery in Keimyung University Dongsan Medical Center (Daegu, Korea) between April 2008 and January 2010. Tissue samples were immediately frozen in liquid nitrogen and stored at −196 °C until RNA isolation. Tissue samples were provided from Keimyung Human Bio-resource Bank, Korea. All patients were explained the study purpose 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, #2013-07-026).

Immunohistochemistry

ER, PR, Her2 status and Ki-67 labeling index were established by immunohistochemistry (IHC) using the Ventana BenchMark® XT automated slide stainer (Ventana Medical Systems, Tucson, AZ, USA) according to the manufacturer's recommendations. Four-micrometer-thick sections were deparaffinized, and hydrated through graded series of alcohols to distilled water. The primary antibodies used were ER (predilution, SP1 Clone; Ventana, Tucson, AZ, USA), PR (predilution, 1E2 Clone; Ventana, Tucson, AZ, USA), Her2 (predilution, 4B5 Clone; Ventana, Tucson, AZ, USA), and Ki-67 (1:1000, MIB-1 Clone; Dako, Glostrup, Denmark).

HER2 Flurorescent In Situ Hybridization (FISH)/Sliver In Situ Hybridization (SISH)

Unstained sections with two-micrometer-thickness were prepared and the status of *HER2* gene amplification of 52 IBC was determined by FISH using PathVysion™ LSI HER2/CEP probe (PathVysion, Abott/Vysis, Downers Grove, USA) or SISH using the INFORM HER2 DNA and CHR 17 probes (Ventana, Tucson, AZ, USA) according to the manufacturer's protocols. All of SISH cases were used with the Ventana BenchMark® XT automated slide stainer (Ventana Medical Systems, Tucson, AZ, USA).

Immunohistochemical Staining Analysis

Immunohistochemical staining of ER and PR were assessed by Allred score which is semi-quantitative measuring system [26]. A score for intensity, 0 through 3, was assigned for none, weak, moderate, and strong nuclear staining and a score for the proportion of nuclear staining was categorized as 0 (no staining), 1 (less than 1 %), 2 (1 % to 10 %), 3 (11 % to 33 %), 4 (34 % to 66 %) and 5 (67 % to 100 %). The intensity score was added to the proportion score to make total scores 0 through 8. A score of 0 to 2 was regarded as negative. Contrarily, a score of 3 to 8 was regarded as positive. The status of immunohistochemical staining of Her2 was scored in four categories according to the ASCO and CAP guidelines [27]. Score 0 and 1+ were regarded as negative and score 2+ was determined to be equivocal. Score 3+ was considered as positive. In cases of score 2+, we confirmed HER2 gene amplification by FISH/SISH. To analysis the correlation between mRNA expression levels and HER2 status, we classified HER2 status into 2 groups: one group is HER2 negative (IHC status of HER2 0, 1+); the other group is HER2 positive (IHC status of HER2 3+ or HER2 gene amplification by FISH/SISH).

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 form 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 a no template sample was used as a negative control.

Statistical Analysis

Prior to statistical analysis, raw qPCR data of Dicer, Drosha, DGCR8, and AGO2 mRNA expression were normalized to reference gene, β-actin. Then, the qPCR data were analyzed by the $2^{-\Delta\Delta ct}$ method [28]. Statistical analysis was performed with SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Comparisons for statistical significance of paired samples were performed by using Wilcoxon signed-rank test. The mean value was used as cut-off value in the present study. Clinical characteristics of the mRNA expression levels of miRNA biogenesis-related component were analyzed using Chi-square test for categorical variables. 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 of miRNA Machinery Components in Breast Cancer Tissues and Adjacent Non-neoplastic Breast Tissues of IBC Patients

To investigate the mRNA expression levels of miRNA machinery components, we examined the mRNA expression levels of important four miRNA machinery components, including Drosha, DGCR8, Dicer, and AGO, in 52 pairs of human breast cancer tissues and their respective non-neoplastic breast tissues by using qPCR. In approximately

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

Components	Position	Sequence
Dicer	Forward	5′-TTAACCTTTTGGTGTTTGAT GAGTGT-3′
	Reverse	5′-AGGACATGATGGACAATT-3′
Drosha	Forward	5′-CTGTCGATGCACCAGATT-3′
	Reverse	5′-TGCATAACTCAACTGTGCAGG-3′
AGO2	Forward	5′-TCATGGTCAAAGATGAGATG ACAGA-3′
	Reverse	5′-TTTATTCCTGCCCCCGTAGA-3′
DGCR8	Forward	5′-CAAGCAGGAGACATCGGACAAG-3′
	Reverse	5′-CACAATGGACATCTTGGGCTTC-3′
β-actin	Forward	5′-CAGCCATGTACGTTGCTATCCAGG-3′
	Reverse	5′-AGGTCCAGACGCAGGATGGCATG-3′

80 % of tumors, all of the miRNA machinery components except DGCR8 (63.5 %) appears down-regulated (Dicer: 79.2 %; Drosha: 78 %; AGO2: 76.9 %), respectively (Table 2). However, it has been identified that only two components, such Dicer and AGO2 had statistical significances between IBC tissues and adjacent non-neoplastic breast tissues (Figs. 2 and 3).

Relationship Between mRNA Expression Levels of Inter-individual miRNA Machinery Components in Patients with IBC

To investigate the significant correlation between mRNA levels of inter-individual miRNA machinery components in IBC, we evaluated the correlations of the four selected miRNA machinery components. As shown in Fig. 4, there was a significant association between DGCR8 and AGO2 with Pearson correlation coefficient value of 0.945 in IBC ($P<0.001$). However, there was no significant association between the other components (data not shown).

Relationship Between mRNA Expression Levels of Individual miRNA Machinery Components and the Clinical Parameters in Patients with IBC

To evaluate the influence of the clinical parameters on mRNA expression of each miRNA machinery component, patients were classified according to each clinical characteristic. The clinicopathological characteristics of all 52 patients (mean age; 52.4±10.3 years) with IBC were presented in Table 3. mRNA expression levels of miRNA machinery components were not statistically associated with age, T stage, M stage, histological differentiation, PR status, HER2 status, and Luminal classification in our breast cancer specimens (Table 3). On the other hand, AGO2 mRNA expression level was significantly correlated with N stage ($P=0.033$, Table 3). Furthermore, DGCR8 mRNA expression level was significantly associated with ER and Ki-67 status ($P=0.021$ and $P=0.04$, respectively) (Table 3).

Discussion

MiRNAs are involved in the regulation of several physiological cellular processes, including cellular development, differentiation, proliferation, cell death, and metabolism and are closely related with the development of cancer [29–31]. The approximately ~21 nucleotides mature miRNA is processed from longer precursor molecules following series of well-coordinated process, which is accomplished by a set of molecules, including Drosha, DGCR8, Dicer, and AGO [9, 13]. Deregulation of miRNAs in various cancers may be related with altered expression and function of the genes involved in the miRNA machinery components [32–34].

The aim of this study was to elucidate the expression patterns of four selected miRNA machinery components by RT-qPCR method in pair-matched breast specimens and analyze their correlation with different clinical characteristics. We therefore identified the mRNA expression levels of the miRNA machinery components in IBC tissue compared with adjacent non-neoplastic breast tissue using RT-qPCR in a total number of 52 breast cancer patients. Compared with adjacent non-neo-plastic tissue, a wide variation of miRNA machinery components mRNA levels was detected in IBCs. We found that the mRNA expression patterns of each component were similar. For more than 75 % of specimens, the down-regulated mRNA expression levels of each component except DGCR8 were observed (Table 3). The results indicated that mRNA expression levels of four selected miRNA machinery components were down-regulated in a large fraction of IBCs and suggested down-regulations of the components could play an important role in tumorigenesis of IBC.

Recent studies have shown that inter-individual miRNA machinery components are significantly correlated with each other in strictly pair-matched samples of breast cancerous and adjacent non-neoplastic tissues: Dicer and Drosha in TN breast cancer specimens [20]; DGCR8 and AGO2 in colorectal cancer specimens [17]. So, in the present study, the correlation between expression levels of inter-individual miRNA machinery components in breast cancer was evaluated by using the Pearson's correlation coefficient analysis. mRNA expression levels of DGCR8 and AGO2, in common with our reported study [17], are positively correlated in IBC (Fig. 4). However, our statistical analyses revealed that the mRNA levels of

Table 2 Dicer, Drosha, AGO2, and DGCR8 mRNA expression levels in relation to clinicopathological parameters

	Dicer (number, %)			Drosha (number, %)			AGO2 (number, %)			DGCR8 (number, %)		
	Low	High	*p*	Low	High	*p*	Low	High	*p*	Low	High	*p*
Total	38 (79.1)	10 (20.9)		39 (78.0)	11 (22.0)		40 (76.9)	12 (23.1)		33 (63.5)	19 (36.5)	
Age			0.65			0.73			1.00			0.58
≤50	16 (42.1)	5 (50.0)		18 (46.2)	4 (36.4)		17 (42.5)	5 (41.7)		15 (45.5)	7 (36.8)	
>50	22 (57.9)	5 (50.0)		21 (53.8)	7 (63.6)		23 (57.5)	7 (58.3)		18 (54.5)	12(63.2)	
T stage			0.67			0.63			0.97			0.36
T1	13 (34.2)	2 (20.0)		12 (30.8)	4 (36.4)		12 (30.0)	4 (33.3)		8 (24.4)	8 (42.1)	
T2	21 (55.3)	7 (70.0)		24 (61.5)	7 (63.6)		24 (60.0)	7 (58.3)		22 (66.7)	9 (47.4)	
T3	4 (10.5)	1 (10.0)		3 (7.7)	0 (0)		4 (10.0)	1 (8.3)		3 (9.1)	2 (10.5)	
N stage			0.25			0.41			0.033			0.17
N0	18 (47.4)	6 (60.0)		21 (53.8)	5 (45.5)		20 (50.0)	6 (50.0)		14 (42.4)	12 (63.2)	
N1	14 (36.8)	1 (10.0)		9 (23.1)	5 (45.5)		14 (35.0)	1 (8.3)		13 (39.4)	2 (10.5)	
N2	2 (5.3)	2 (20.0)		5 (12.8)	1 (9.1)		2 (5.0)	4 (33.3)		3 (9.1)	3 (15.8)	
N3	4 (10.5)	1 (10.1)		4 (10.3)	0 (0)		4 (10.0)	1 (8.3)		9 (9.1)	2 (10.5)	
M stage			1.00			0.22			1.00			0.53
M0	36 (94.7)	10 (100)		39 (100)	10 (90.9)		38 (95.0)	12 (100)		31 (93.9)	19 (100)	
M1	2 (5.3)	0 (0)		0 (0)	1 (9.1)		2 (5.0)	0 (0)		2 (6.1)	0 (0)	
Histological grade			0.68			1.00			1.00			1.00
1/2	8 (21.1)	3 (30.0)		9 (23.1)	2 (18.2)		9 (22.5)	3 (25.0)		8 (24.2)	4 (21.1)	
3	30 (78.9)	7 (70.0)		30 (76.9)	9 (81.8)		31 (77.5)	9 (75.5)		25 (75.8)	15 (78.9)	
Ki-67			0.66			0.28			0.32			0.021
<5 %	6 (15.8)	2 (20.0)		8 (20.5)	0 (0)		7 (17.5)	2 (16.7)		8 (24.2)	1 (5.3)	
5–10 %	9 (23.7)	1 (10.0)		7 (17.9)	3 (27.3)		9 (22.5)	1 (8.3)		7 (21.2)	3 (15.8)	
11–25 %	4 (10.5)	0 (0)		4 (10.3)	0 (0)		3 (7.5)	1 (8.3)		3 (9.1)	1 (5.3)	
26–50 %	8 (21.1)	3 (30.0)		7 (17.9)	4 (36.4)		10 (25.0)	1 (8.3)		9 (27.3)	2 (10.5)	
>50 %	11 (28.9)	4 (40.0)		13 (33.3)	4 (36.4)		11 (27.5)	7 (58.3)		6 (18.2)	12 (63.2)	
ER status			1.00			0.16			0.74			0.04
Negative	15 (39.5)	4 (40.0)		18 (46.2)	2 (18.2)		16 (40.0)	6 (50.0)		10 (30.3)	12 (63.2)	
Positive	23 (60.5)	6 (60.0)		21 (53.8)	9 (81.8)		24 (60.0)	6 (50.0)		23 (69.7)	7 (36.8)	
PR status			1.00			0.29			1.00			0.77
Negative	23 (60.5)	6 (60.0)		26 (66.7)	5 (45.5)		25 (62.5)	8 (66.7)		20 (60.6)	13 (68.4)	
Positive	15 (39.5)	4 (40.0)		13 (33.3)	6 (54.5)		15 (37.5)	4 (33.3)		13 (39.4)	6 (31.6)	
HER2			0.16			0.17			0.51			0.56
Negative	13 (34.2)	6 (60.0)		14 (35.9)	7 (63.6)		15 (37.5)	6 (50.0)		12 (36.4)	9 (47.4)	
Positive	25 (65.8)	4 (40.0)		25 (64.1)	4 (36.4)		25 (62.5)	6 (50.0)		21 (63.6)	10 (52.6)	
Subtype according to IHC			0.48			0.14			0.61			0.07
Luminal A	10 (26.3)	4 (40.0)		8 (20.5)	6 (54.5)		11 (27.5)	3 (25.0)		10 (30.3)	4 (21.1)	
Luminal B	13 (34.2)	2 (20.0)		13 (33.3)	3 (27.3)		13 (32.5)	3 (25.0)		13 (39.4)	3 (15.8)	
HER2-enriched	12 (31.6)	2 (20.0)		12 (30.8)	1 (9.1)		12 (30.0)	3 (25.0)		8 (24.2)	5 (36.8)	
TN	3 (7.9)	2 (20.0)		6 (15.4)	1 (9.1)		4 (10.0)	3 (25.0)		2 (6.1)	5 (26.3)	

ER estrogen receptor; *PR* progesterone receptor; *HER2* epidermal growth factor receptor type2; *IHC* immunohistochemistry; *TN* triple-negative; *Luminal A* ER+ or PR+, and HER2-; *Luminal B* ER+ or PR+, and HER2+; *HER2-enriched* ER-,PR-, and HER2+; *TN* ER-,PR-, and HER-

inter-individual miRNA machinery components except DGCR8 and AGO2 were not correlated to each other (data not shown). These observations suggested that DGCR8 and AGO2 may share partially common mechanisms which are involved in breast carcinogenesis.

Recently, rapidly accumulating evidences have revealed that miRNA dysregulation is closely associated with development and progression of many cancers, including breast cancer [35, 36]. Because Drosha and Dicer are two critical enzymes required for post-transcriptional miRNA processing, we

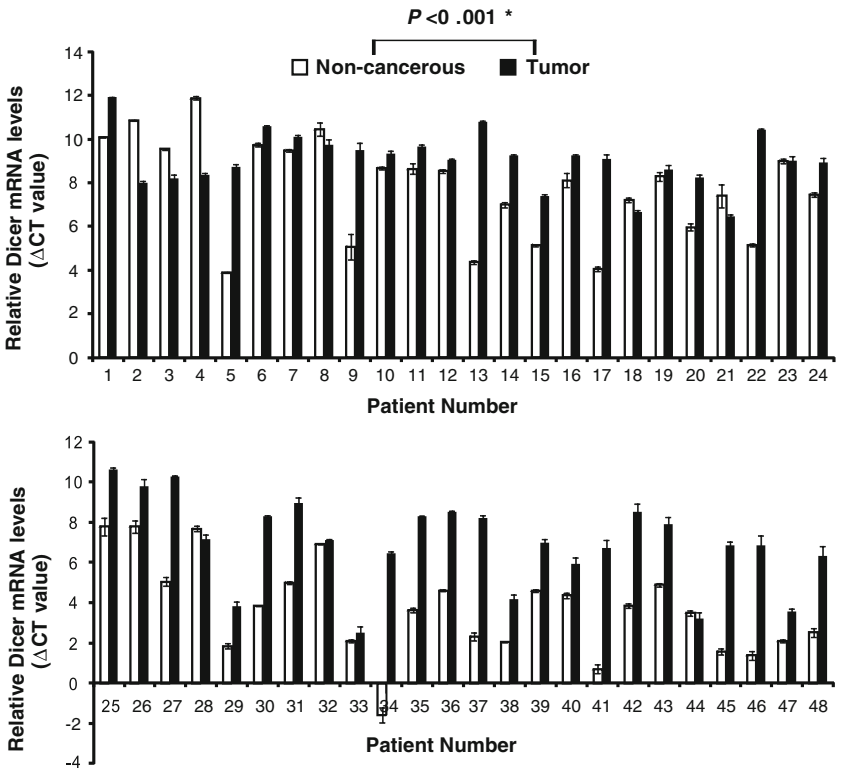

Fig. 2 The relative Dicer mRNA level (normalized to the corresponding β-actin mRNAs) in tumor tissues compared to adjacent non-cancerous breast tissues. *Asterisk* indicates Wilcoxon signed-rank test

therefore investigated whether the altered mRNA expressions of Dicer and Drosha are associated with the development of breast cancer. Unfortunately, there was no association between altered expressions of the two enzyme and clinical parameters, including Age, TNM stage, histological differentiation, Ki-67 status, ER status, PR status, HER2 status, and Lumina

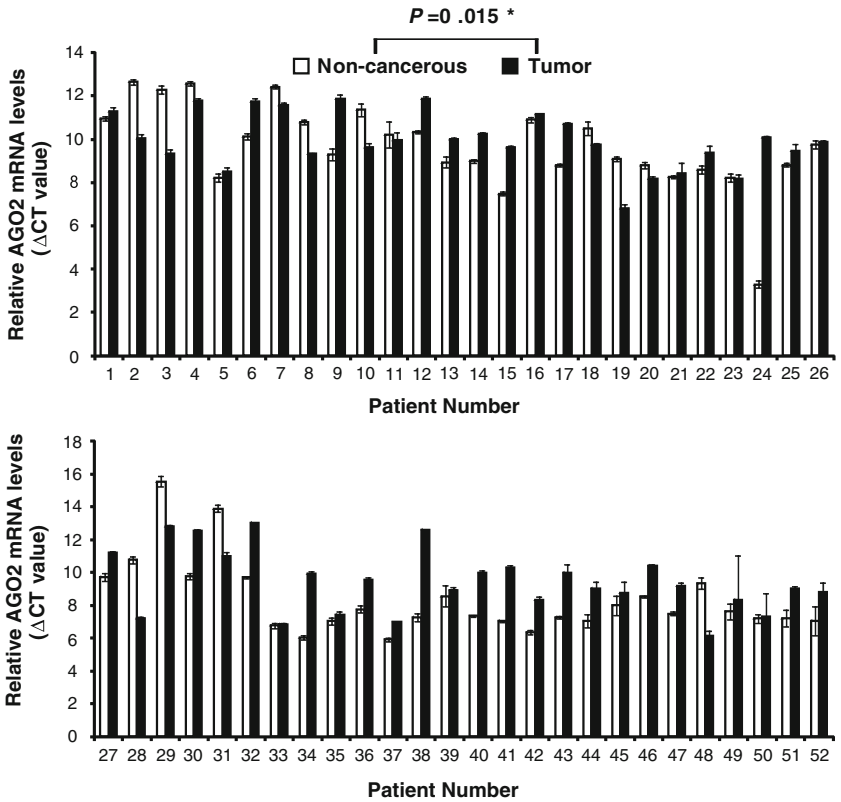

Fig. 3 The relative AGO2 mRNA level (normalized to the corresponding β-actin mRNAs) in tumor tissues compared to adjacent non-cancerous breast tissues. *Asterisk* indicates Wilcoxon signed-rank test

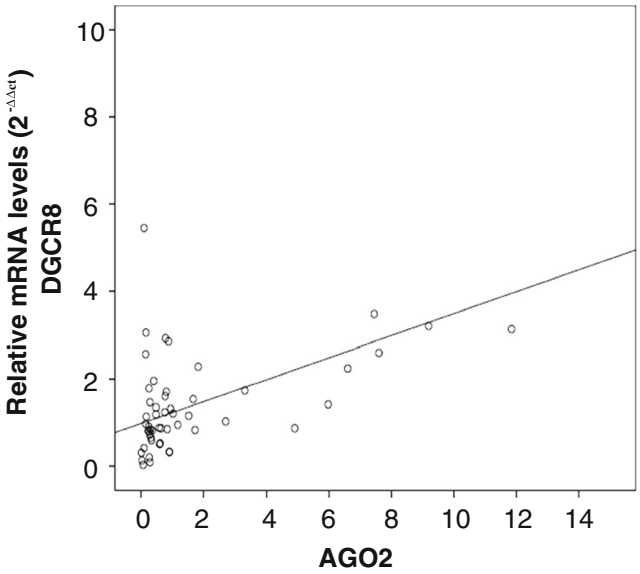

Fig. 4 Correlation between mRNA expression levels of inter-individual components in breast cancer. DGCR8 and AGO2. * $P<0.001$

classification (Table 3). However, as shown in Table 3, a component of the RISC factors AGO2 was associated with the clinical parameters except N stage. Especially, DGCR8 up-regulation was significantly associated with highly expressed Ki-67 and ER-positive status. Moreover, DGCR8 down-regulation was significantly observed in ER-negative breast cancers (69.7 %). It has been reported that lymph node metastasis, ER status, and Ki-67 status in breast cancer are very important prognostic factors in breast cancer [37–39]. Therefore, these results suggested that DGCR8 regulation in IBC is associated with prognosis and hormonal therapeutic responsiveness. However, due to a short follow-up period, we could not analyze the prognostic value of each miRNA machinery component in our study group. Therefore, further investigation with longer follow-up period will resume and prognostic impact of the components will be analyzed as soon as possible.

TN breast cancers are defined in accordance with their phenotype and consisted with tumors that insufficiently express ERs, PRs and HER2 [40]. It has been reported that Drosha mRNA expression level in TN breast cancers was significantly higher than those in normal breast tissues [20]. Unfortunately, in the present study, specimens of TN breast cancer were too small to evaluate the association between mRNA expression levels of miRNA machinery components and clinical parameters. So, more research is needed on the relationship between altered mRNA expression levels of the components and clinical parameters.

In this study, we investigated the expression patterns of four selected miRNA machinery components and their clinical association in breast cancer. Here, we revealed for the first time that dysregulated DGCR8 and AGO2 mRNA expression levels are significantly associated with clinical characteristics, including ER status, Ki-67, and N stage in breast cancers. Finally, our observations would helpful to explain the

Table 3 Patient's clinicopathological parameters

Variables	Number	%
Total	52	100
Age		
≤50	22	42.3
>50	30	57.7
T stage		
T1	16	30.8
T2	31	59.6
T3	5	9.6
N stage		
N0	26	50.0
N1	15	28.8
N2	6	11.5
N3	5	9.6
M stage		
M0	50	96.2
M1	2	3.8
Histological grade		
1/2	12	23.1
3	40	76.9
Ki-67		
<5 %	9	17.3
5–10 %	10	19.2
11–25 %	4	7.7
26–50 %	11	21.2
>50 %	18	34.6
ER status		
Negative	22	42.3
Positive	30	57.7
PR status		
Negative	33	63.5
Positive	19	36.5
HER2		
Negative	21	40.4
Positive	31	59.6
Subtype according to IHC		
Luminal A	14	26.9
Luminal B	16	30.8
HER2-enriched	15	28.8
TN	7	13.5

ER estrogen receptor; *PR* progesterone receptor; *HER2* epidermal growth factor receptor type2; *IHC* immunohistochemistry; *TN* triple-negative; *Luminal A* ER+ or PR+, and HER2-; *Luminal B* ER+ or PR+, and HER2+ ; *HER2-enriched* ER-,PR-, and HER2+; *TN* ER-, PR-, and HER-

correlation between altered expression of miRNA machinery components and breast carcinogenesis.

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