RESEARCH ARTICLE

Association of miR-1266 with Recurrence/Metastasis Potential in Estrogen Receptor Positive Breast Cancer Patients

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Abstract

The *Homeobox B13 (HOXB13):Interleukin 17 Receptor B (IL17BR)* index of estrogen receptor (ER)-positive breast cancer (ER (+) BC) patients may be a potential biomarker of recurrence/ metastasis. However, effects of microRNA (miRNA) binding to the 3' untranslated region (3´UTR) of *HOXB13* and *IL17BR* and its function on recurrence/metastasis in ER (+) BC remains elusive. The aims of this study were to determine the expression of miRNAs that bind to 3´UTR of *HOXB13* and *IL17BR* in ER (+) BC patients and asess the effects of these miRNAs on recurrence/metastasis. The expression profiles of *HOXB13* and *IL17BR* were evaluated using RT-PCR in tumors and normal tissue samples from 40 ER (+) BC patients. The expression level of 4 miRNAs, which were predicted to bind the 3´UTR of *HOXB13* and *IL17BR* using TargetScan, microRNA.org and miRDB online databases, were further evaluated with RT-PCR. Our findings demonstrated that high miR-1266 levels might be significant prognostic factor for recurrence/metastasis occurrence (3.05 fold p=0.004) and tamoxifen response (3.90 fold; p=0.2514) in ER (+) BC cases. Although we suggest that modulation of miR-1266 expression may be an important mechanism underlying the chemoresistance of ER (+) BC, advanced studies and validation are required.

Keywords: miR-1266 - recurrence/metastasis - estrogen receptor - breast cancer

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Introduction

Breast cancer (BC) remains the most common malignancy and is one of the major causes of cancer-related mortality among women worldwide (Benson et al., 2009; Pedraza et al., 2010). It is a major health problem for females in industrialized countries with more than 1 million new cases and approximately 500,000 deaths per year worldwide (Porter, 2008). Despite advances in early detection and the understanding of the molecular bases of BC biology, the heterogeneity of BC makes it a challenging solid tumor to diagnose and treat (Hutchinson, 2010). Almost 75% of all BC patients are estrogen receptor (ER) (+) invasive cases (Perou et al., 2000). Morphologically, this subtype of BC is well-differentiated and has a relatively good prognosis. Additionally, ER (+) BC patients have longer relapse-free survival and overall survival compared to the ER (-) breast carcinomas (Hoch et al., 1999; Zhang et al., 2014). As part of BC treatment, the presence of ER has become an important determinant for predicting the response to adjuvant endocrine therapy with tamoxifen (Dowsett et al., 2008; Ng et al., 2014). However, 30-50% of these cases do not respond to or develop resistance to tamoxifen (Girault et al., 2006). There are various studies to investigate the molecular mechanism of tamoxifen resistance in ER (+) BC. Recent studies show evidence that the *Homeobox B13 (HOXB13): Interleukin 17 Receptor B (IL17BR)* index of these patients may be a potential biomarker of tamoxifen response (Ma et al., 2004; Jansen et al., 2007; Jerevall et al., 2008). However, this molecular network has not been completely elucidated. Recently, increasing evidence has shown that microRNAs (miRNAs) have multiple functions related to drug resistance in BC (Kayani et al., 2011; Tekiner and Basaga, 2013; Chen et al., 2014; Chen et al., 2014). Currently, more than two thousands microRNAs have been identified (www.mirbase.org). To date, although a number of miRNA have been link to drug resistance in BC (Chen et al., 2013; Wang et al., 2013; Gan et al., 2014; Jiang et al., 2014; Ward et al., 2014), miRNAs that are involved in ER(+) BC and their effect on tamoxifen response have not been sufficiently investigated. Moreover, whether miRNAs that bind to 3' untranslated region (3´UTR) of *HOXB13* and *IL17BR* have functions related to tamoxifen resistance in ER(+) BC remains elusive.

The aims of this study were to determine the expression of miRNAs that bind to the 3´UTR region of *HOXB13* and *IL17BR* in ER(+) BC patients and to investigate the effect of these miRNAs on the response to tamoxifen, which is widely used as a selective ER modulator in

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ER(+) BC cases.

Materials and Methods

Patient selection and tumor samples

Forty patients in this study underwent surgical resection of the primary tumor at the Department of General Surgery of the Medical Faculty of Uludag University, Bursa, Turkey between 1994 and 2011. All of the patients were diagnosed with ER(+) primary invasive ductal BC and treated with standard breast surgery, with or without postoperative radiation or chemotherapy and with or without five years adjuvant tamoxifen monotherapy. Clinical and follow-up data were derived from the patient archive at the Department of General Surgery.

All tumor specimens were archived in the form of formalin-fixed, paraffin-embedded (FFPE) tissue and obtained from repositories at the Department of Pathology of the Medical Faculty of Uludag University. Histopathological characteristics of the tumor samples were evaluated by an expert pathologist. Normal breast tissue samples of patients were used as negative controls. The study was approved by the local Ethics Committee (2011-2/23) and conformed to the ethical standards of the Helsinki Declaration.

RNA extraction and mRNA expression of HOXB13 and IL17BR

Before RNA extraction, each tissue sample was subjected to paraffin removal using the BiOstic Paraffin Removal Reagent (MO BIO Laboratories, Carlsbad, CA) and washed with a 100% alcohol series. Total RNA was isolated from 0.2-0.5 cm FFPE tissue sections for each sample using the RNeasy FFPE Kit (Qiagen, Germantown, MD) according to the manufacturer's instructions. The amount and purity of total RNA for all of the samples were determined using a Nanodrop 2000 spectrophotometer (Thermo Scientific, Wilmington, Delaware, USA).

To evaluate the expression of the target genes of the significantly altered miRNAs, RNAs were reverse transcribed using a cDNA Synthesis Kit (New England Biolabs, UK). The samples were then analyzed using RT-qPCR to profile the expression levels of *HOXB13* (NM_006361) and *IL17BR* (NM_018725) using Real Time Ready Catalog Assay (Roche Diagnostics, USA) according to manufacturer's instruction; we also evaluated the expression level of the human Beta Actin (ACTB) housekeeping gene. The absence of genomic DNA contamination was confirmed by performing a no reverse-transcription control with RNA samples using an ACTB RT-qPCR primer assay. The initial copy number of the samples and the threshold cycle (Ct) for mRNA expression was determined using Light Cycler 480II software (Roche Diagnostics, Indianapolis, USA). The 2^-ΔCt method was used to calculate the fold change in mRNA expression between the tested samples (Livak and Schmittgen, 2001). The *HOXB13:IL17BR* ratio of the cases was analyzed as described previously (Ma et al., 2004; Ma et al., 2006).

Determining miRNAs that bind to 3´UTR region of HOXB13 and IL17BR

miRNAs that bind to the 3´UTR region of *HOXB13* and *IL17BR* were identified using the TargetScan (http://www.targetscan.org/), microRNA.org (http://www.microrna.org/microrna/home.do) and miRDB (http://mirdb.org/miRDB/) online databases and a literature search.

miRNA expression analysis

cDNA synthesis was performed with 5 ng RNA using a pool of miRNA-specific primers using the Taqman microRNA Reverse Transcription Kit (Applied Biosystems, USA) according to the manufacturer's instructions. The samples were analyzed for the presence and differential expression of miR-661 (MIMAT0003324), miR-1260 (MIMAT0005911), miR-1266 (MIMAT0005920) and miR-1278 (MIMAT0005936) using Taqman® MicroRNA Assays (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Thermal cycling conditions for all assays were as follows: 2 min at 50˚C for UNG activity,10 min at 95˚C for 1 cycle initial denaturation, followed by 40 cycles of 15s at 95˚C denaturation, 1 min at 60˚C annealing, and cooling at 40˚C for 30s in the LightCycler 480II (Roche Diagnostics, Indianapolis, USA). RNA input was normalized to endogenous control RNU19 for miRNAs and the TATA-binding protein for protein encoding genes. The initial copy number in the samples and the threshold cycle (Ct) for miRNA expression were determined using the Light Cycler 480II software (Roche Diagnostics, Indianapolis, USA). The miRNA Reverse Transcription Control Assay was used to test the efficiency of the Taqman microRNA Reverse Transcription Kit reaction using a primer set to detect a template synthesized from the kit's built-in miRNA External RNA Control. Positive PCR control assays were used to test the efficiency of the polymerase chain reaction chemistry and of the instrument using a predispensed artificial DNA sequence and a primer set designed to detect the sequence. The 2(-Delta Delta C(T)) (2^-ΔCt) method was used to calculate the fold change in miRNA expression between the tested samples (Livak and Schmittgen, 2001).

Statistical analysis

An independent sample t-test was used to evaluate the relationship of the *HOXB13:IL17BR* ratio and miRNA expression levels and tamoxifen resistance and recurrence and/or metastasis status. Pearson correlation analysis was performed to determine the association of miRNAs and target genes. A Kaplan-Meier analyses was performed for survival analyses. All statistical analyses were performed using the MedCalc 12.4.0 statistical software programme. A p value<0.05 was considered statistically significant and all results were represented with a 95% Confidence Interval (CI).

Results

Clinical data

Forty patients diagnosed with ER (+) BC entered this study. The 1 man and 39 women were aged 33-83 years, and the median age at the time of diagnosis was

55.00±0.85 years. Primary tumors were localized in right breast in 18 cases and the left breast in 22 cases. Thirty three of the cases had tamoxifen therapy and 13 of the patients had recurrence or distant metastasis.

HOXB13 and IL17BR expression status of tumors

The expression levels of *HOXB13* was 1.31-fold up-regulated and the expression level of *IL17BR* was 1.25-fold down-regulated in ER(+) BC tumors (n=40) in compare to non-tumor tissues (n=40).

HOXB13:IL17BR index

Numerous studies have shown that the *HOXB13* and *IL17BR* expression ratio (*HOXB13:IL17BR*) may have a potential to be a biomarker for predicting the treatment outcome in tamoxifen monotherapy in ER(+) breast cancer (Ma et al., 2004; Ma et al., 2006; Jansen et al., 2007; Jerevall et al., 2008; Zhao et al., 2014). In the current study, the *HOXB13:IL17BR* ratio of the cases was analyzed. According to our findings, the *HOXB13:IL17BR* index was significantly increased in recurrence/metastasis (+) cases (n=13) compared to recurrence/metastasis (-) cases (n=27; p<0.0001). However, when this ratio was evaluated in the cases of treated with tamoxifen (n=33), there wasn't a significant difference between recurrence/metastasis (+) (n=9) and recurrence/metastasis (-) patients (n=24).

Identification of differentially expressed miRNAs that target HOXB13 and IL17BR using bioinformatic analysis

The miRNAs that bind to the 3´UTR regions of *HOXB13* and *IL17BR* were identified using the TargetScan (http://www.targetscan.org/), microRNA.org (http://www.microrna.org/microrna/home.do) and miRDB (http://mirdb.org/miRDB/) online databases and a literature search. Although there is no published data regarding the miRNAs that target these genes, according to TargetScan, microRNA.org and miRDB, the common miRNAs targeting *HOXB13* are miR-661, miR-1260 and 1278, and according on microRNA.org, the miR-1266 miRNA targets IL17BR.

Correlation between HOXB13, IL17BR and target miRNAs

We evaluated the correlation between the expression level of miR-661, miR-1260 and miR-1278 with *HOXB13* and miR-1266 with IL17BR. According to Pearson correlation analyses, although they were not at significant levels, miR-661, miR-1260 and miR-1278 expressions revealed negative correlations with *HOXB13* mRNA expression. In addition, miR-1266 expression was negatively correlated with *IL17BR* mRNA expression (Table 1).

The relationship of miRNA expression and clinical parameters

Depending on the miRNA expression status of tumors, basic clinical and tumor characteristics such as tumor localization, grade, *in situ* components, extracapsular, perineural and lymphatic invasions and Karnofsky performance status (KPS) of patients were analyzed using

Table 1. Correlations between miRNAs and Target Gene Expression Levels

miRNA	Correlation (r value) with *HOXB13* expression	pvalue*
miR-661	-0.0524	0.748
miR-1260	-0.09234	0.57
miR-1278	-0.0447	0.784
Correlation (r value) with *IL17BR* expression		
miR-1266	-0.05167	0.751

*Derived using Pearson correlation analyses

Table 2. The Association of the Biopathological Features of Cases and miRNA Expression Levels

Characteristics	miR-661			miR-1260			miR-1266			miR-1278		
	up (%)	down (%)	p value	up (%)	down (%)	p value	up (%)	down (%)	p value	up (%)	down (%)	p value
Tumor localization			0.149**			0.412*			1.000**			1.000**
Left	7 (17.5)	15 (37.5)		7 (17.5)	15 (37.5)		2 (5)	20 (50)		1 (2.5)	21 (52.5)	
Right	2 (5)	16 (40)		8 (20)	10 (25)		1 (2.5)	17 (42.5)		1 (2.5)	17 (42.5)	
Grade			0.441**			0.505*			0.553**			1.000**
1 and 2	4 (10)	20 (50)		10 (25)	14 (35)		1 (2.5)	23 (57.5)		1 (2.5)	23 (57.5)	
3 and 4	5 (12.5)	11 (27.5)		5 (12.5)	11 (27.5)		2 (5)	14 (35)		1 (2.5)	15 (37.5)	
In- situ component			1.000**			1.000**			1.000**			1.000**
>25 %	1 (2.5)	4 (10)		2 (5)	3 (7.5)		0 (0)	5 (12.5)		0 (0)	5 (12.5)	
<25 %	8 (20)	27 (67.5)		13 (32.5)	22 (55)		3 (7.5)	32 (80)		2 (5)	33 (82.5)	
Extracapsular invasion			1.000**			0.935*			0.098**			0.219**
+	5 (12.5)	16 (40)		8 (20)	13 (32.5)		0 (0)	21 (52.5)		0 (0)	21 (52.5)	
-	4 (10)	15 (37.5)		7 (17.5)	12 (30)		3 (7.5)	16 (40)		2 (5)	17 (42.5)	
Lymphatic invasion			1.000**			0.285*			1.000**			1.000**
+	3 (7.5)	9 (22.5)		6 (15)	6 (15)		1 (2.5)	11 (27.5)		0 (0)	12 (30)	
-	6 (15)	22 (55)		9 (22.5)	19 (47.5)		2 (5)	26 (65)		2 (5)	26 (65)	
Perineural invasion			1.000**			1.000**			1.000**			1.000**
+	2 (5)	6 (15)		3 (7.5)	5 (12.5)		0 (0)	8 (20)		0 (0)	8 (20)	
-	7 (17.5)	25 (62.5)		12 (30)	20 (50)		3 (7.5)	29 (72.5)		2 (5)	30 (75)	
KPS			0.348**			1.000**			1.000**			1.000**
>90 %	6 (15)	26 (65)		12 (30)	20 (50)		3 (7.5)	29 (72.5)		2 (5)	30 (75)	
<90 %	3 (7.5)	5 (12.5)		3 (7.5)	5 (12.5)		0 (0)	8 (20)		0 (0)	8 (20)	

*Derived using the χ^2 test; **Derived using Fisher's exact test

Table 3. miR-661, miR-1260, miR-1266 and miR-1278 Expression Levels depend on Recurrence/metastasis Status

miRNAs	Recurrence/Metastasis (-) Tumors	Recurrence/Metastasis (+) Tumors					
	2^(-Avg.(Delta(Ct))	2^(-Avg.(Delta(Ct))	%95 (CI)	t statistic	Fold change	Fold regulation	p value*
miR-661	0.00617	0.00576	-3.7728 to 4.0337	0.995	0.93	No difference	0.32
miR-1260	0.07887	0.0984	-2.3147 to 1.5833	-0.464	1.24	No difference	0.64
miR-1266	0.00233	0.00711	-5.4063 to 1.9961	2.905	3.05	Up	0.004
miR-1278	0.00007	0.00005	-0.6197 to 0.8218	3.324	0.69	No difference	0.001

*Derived using Independent samples t-test

Table 4. Differential Expression Status of miR-661, miR-1260, miR-1266 and miR-1278 depend on Recurrence/ Metastasis Status in Tamoxifen-treated Cases

miRNAs	Recurrence/Metastasis (-) at the end of Tamoxifen Theraphy	Recurrence/Metastasis (+) at the end of Tamoxifen Theraphy					
	2^(-Avg.(Delta(Ct))	2^(-Avg.(Delta(Ct))	%95 (CI)	t statistic	Fold change	Fold regulation	p value*
miR-661	0.00642	0.00744	-0.01662 to 0.01458	-0.133	1.15	No difference	0.895
miR-1260	0.06843	0.05256	-0.06310 to 0.09484	0.41	0.76	No difference	0.684
miR-1266	0.0026	0.01016	-0.02075 to 0.00563	-1.169	3.9	Up	0.251
miR-1278	0.00008	0.00006	-0.00019 to 0.00021	0.117	0.85	No difference	0.907

*Derived using Independent samples t-test

the χ^2 and Fisher's exact tests. There was no significant relationship between the clinical profiles and pathological features of patients and the miRNA expression status of tumors (Table 2).

The effect of miRNA expressions on metastasis and/or recurrence

To evaluate the alterations in miRNA expressions in cases with recurrence and/or metastasis, we compared the expression status of miR-661, miR-1260, miR-1266 and miR-1278 between these cases (n=13) and the recurrence and/or metastasis (-) cases (n=27). The independent samples t-test demonstrated that the expression level of miR-1266 was 3.05-fold higher in recurrence/metastasis (+) cases compared to (-) cases (p=0.004; Table 3).

The role of miRNAs in tamoxifen response

Recurrence and/or metastasis have occurred in 9 of 33 (27.3%) tamoxifen-treated cases. According to independent samples t-tests, although the differences were not significant, the expression of miR-661 was slightly increased and miR-1260 and miR-1278 were slightly reduced. However, the expression of miR-1266 was 3.90 fold increased (p = 0.251; Table 4).

The effect of miRNA expression on survival

The median follow-up time of patients was 51.30±5.36 months (range 12-204 months). Both overall and disease-free survival were shorter in the cases with up-regulated miR-1266, but the difference was not significant (log-rank p=0.7514; 0.7201, respectively). Kaplan-Meier plots comparing the overall and disease-free survival rates of patients with different regulation of miR-1266 are presented in Figure 1.

When the effect of this miRNA on survival was analyzed in tamoxifen-treated cases, induced expression levels of miR-1266 caused shorter overall and disease-free survival in patients treated with tamoxifen (log-rank P = 0.7707; 0.7514, respectively). Kaplan-Meier plots

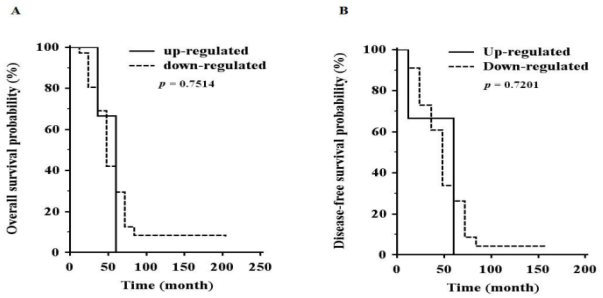

Figure 1. Kaplan-Meier Plots of the (A) Overall and (B) Disease-free Survival Probability of ER(+) BC Patients with Upregulated versus Downregulated Expression of miR-1266

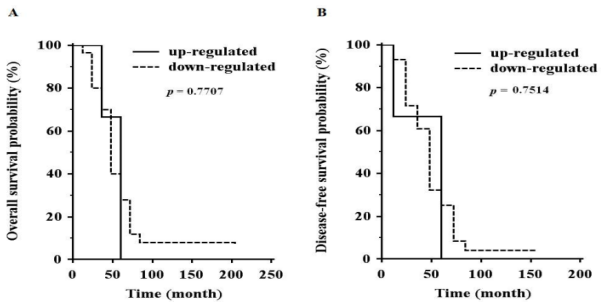

Figure 2. Kaplan-Meier Plots of the (A) Overall and (B) Disease-free Survival Probability of Tamoxifen treated ER(+) BC Patients with Upregulated versus Downregulated Expression of miR-1266

comparing the overall and disease-free survival rates of patients with different regulation of miR-1266 in tamoxifen-treated cases are presented in Figure 2.

Discussion

The majority of ER (+) BC cases sufficiently benefit from adjuvant tamoxifen therapy. However, approximately

40% of these patients develop recurrence or metastasis (Vendrell et al., 2007; Gao et al., 2014). Numerous studies showed that the altered *HOXB13:IL17BR* expression ratio may be a sign of metastasis risk and tamoxifen response in these cases (Ma et al., 2004; Jansen et al., 2007; Jerevall et al., 2008; Ma et al., 2008; Sgroi, 2009; Zhao et al., 2014). According to Jansen et al., a high *HOXB13:IL17BR* ratio expression level was associated with both tumor aggressiveness and tamoxifen therapy failure (Jansen et al., 2007). In addition, Ma et al. demonstrated the shorter disease-free survival of patients with high ratios compared to patients with low ratios (Ma et al., 2004). Similarly, Zhao et al. revealed the significantly worse outcomes of patients with higher *HOXB13:IL17BR* expression ratios that were treated with tamoxifen (Zhao et al., 2014). In the present study, the *HOXB13:IL17BR* expression index was evaluated depending on the recurrence/metastasis status of 40 ER(+) BC patients. However we indicated that, although the *HOXB13:IL17BR* index was significantly increased in recurrence/metastasis (+) cases compared to recurrence/metastasis (-) cases ($p<0.0001$), when this ratio was evaluated in the cases of treated with tamoxifen, there wasn't a significant difference between recurrence/metastasis (+) and recurrence/metastasis (-) patients. Therefore, our findings support the previously reported data regarding the association of the *HOXB13:IL17BR* index with the recurrence/metastasis potential but the utility of tamoxifen monotherapy in ER (+) BC cases may be affected by other factors such as hereditary and epigenetic mechanisms.

Recent studies on BC focused on the effect of epigenetic factors such as miRNAs in treatment response and the metastasis development process (Li et al., 2012; Avcı et al., 2013; Ell et al., 2014; Perez-Rivas et al., 2014, Corcoran et al., 2014). Nevertheless, the functions of miRNAs that bind to the 3'UTR of *HOXB13* and *IL17BR* in recurrence/metastasis development remain unknown. Based on the TargetScan, microRNA.org and miRDB databases, we predicted that miR-661, miR-1260 and miR-1278 bind to the 3'UTR of *HOXB13* and miR-1266 binds to the 3'UTR of IL17BR. Although there were not significant p values, according to Pearson correlation analyses, miR-661, miR-1260 and miR-1278 expression levels were negatively correlated with HOXB13, and miR-1266 expression was negatively correlated with *IL17BR* mRNA expression (r=-0.05240; -0.09234; -0.04470; -0.05167, respectively). This finding implies that these miRNAs might play roles in the regulation of *HOXB13* and IL17BR. Although the function of miR-661 in BC is examined in a several studies, the regulation of this miRNA in these cases is controversial. While Reddy et al linked the reduced expression of miR-661 to invasiveness and tumorigenicity; Vetter et al emphasized the relationship between induced expression of this miRNA and invasion in BC cells (Reddy et al., 2009; Vetter et al., 2010). In the current study, although miR-661 has a potential to be a one of the regulators of HOXB13, the expression status of this miRNA is controversial. In the current study, fold regulation of miR-661 tended to decrease in recurrence/metastasis (+) tumors in comparison to recurrence/metastasis (-) tumors (1.07 fold; p=0.32). However, in the cases treated with tamoxifen, the expression level of miR-661 tended to induce in recurrence/ metastasis occurrence (1.15-fold; p=0.895). According to Hoffman et al., the low miR-661 expression correlates with the poor outcome in BC in a p53-dependent manner (Hoffman et al., 2014). Thus, miR-661 may either suppress or promote cancer aggressiveness, depending on the p53 status (Hoffman et al., 2014). Therefore, we suggest that the p53 status of the cases may affect the miR-661 expression status of our cases; thus, to clarify the function of this miRNA in recurrence/metastasis and tamoxifen response, advanced studies are required. According to web-based analyses, another miRNA that has potential to play a role in the regulation of *HOXB13* was miR-1260. Previous studies focused on the regulation of miR-1260 in hepatocellular carcinoma cells and primary cutaneous malignant melanoma (Sand et al., 2013; Yan et al., 2013). In the current study, the expression status of miR-1260 is uniquely evaluated in BC. However, no relation was found between miR-1260 recurrence/metastasis and tamoxifen response.

The third miRNA that binds the *HOXB13* 3´UTR was miR-1278. Despite miR-1278 being a potential regulator of HOXB13, as indicated by the TargetScan, microRNA.org and miRDB databases, there has not been any study to evaluate the function of this miRNA. Therefore, the current study addresses the role of miR-1278 in cancer progression for the first time. We found that the expression of miR-1278 was 1.45-fold lower in recurrence/metastasis (+) tissues (p=0.001). Thus, although fold difference of miR-1278 was seemed to be negligible, because the difference was at significant level, the altered regulation of miR-1278 may have a possibility to be a candidate to be a prognostic factor for the occurrence of recurrence/metastasis in ER (+) BC cases. Therefore, we suggest that, confirmative studies with increased number of cases are required to reveal the importance of this miRNA for recurrence/metastasis occurrence. On the other hand, no relation was found between miR-1278 and tamoxifen response (p=0.907). In addition, although the median survival time of the cases with up regulated miR-1278 was shorter than cases with down regulated miR-1278, these patients are still alive and their Kps is >90%. For this reason, a sufficient Kaplan-Meier test to evaluate the effect of miR-1278 on survival could not be performed. Therefore, we suggest that, a longer follow up time is required to obtain confident data about the effect of miR-1278 on the survival of patients.

According to microRNA.org, the only miRNA that was complementary to the 3´UTR of IL17BR, was miR-1266. A previous study in BGC823, MKN28, SGC-7901, KATO-III, U2OS and HF cell lines displayed the tumor suppressor effect of miR-1266 in gastric cancer (Chen et al., 2014). According to this study, miR-1266 was determined to be a hTERT suppressor. However, in the current study we determined the oncogenic effect of miR-1266 in ER (+) BC. The low expression of *IL17BR* and its negative correlation with miR-1266 are also validated by the regulation of this miRNA in our study group (r=-0.05167). When the regulation of mir-1266 was evaluated between the cases with and without recurrence/

metastasis, it was determined that the expression of miR-1266 was significantly induced (3.05-fold, p=0.004). Moreover, depending on the Kaplan-Meier analyses, the altered expression of miR-1266 caused lower overall and disease-free survival (log-rank p=0.7514, 0.7201, respectively). Therefore, we emphasized the potential of high miR-1266 expression in the presence of recurrence/ metastasis. Furthermore, the expression of this miRNA was 3.90-fold higher in cases of recurrence/ metastasis although tamoxifen monotherapy (p=0.251). According to the Kaplan-Meier analyses, induced expression of miR-1266 also caused lower overall and disease-free survival (log-rank p=0.7707, 0.7514, respectively) in tamoxifen-treated cases. Thus, we suggest that miR-1266 may have a potential to be a biomarker for recurrence/ metastasis and tamoxifen-response as a negative regulator of IL17BR.

In summary, although miR-661, miR-1260 and miR-1278 are potential miRNAs that bind the 3´UTR of HOXB13, the function of these miRNAs is unclear. In contrast, according to the present findings, miR-1266 may regulate the expression of IL17BR. Although we suggest that the function of miR-1266 may affect the *HOXB13:IL17BR* ratio via epigenetic mechanisms, the validation of present data will require a larger study group. Thus, advanced studies may clarify the role of miR-1266 in determining recurrence/metastasis possibility and in the prediction of tamoxifen response in ER (+) BC cases.

In conclusion, we demonstrated that high miR-1266 level might be significant prognostic factors for recurrence/metastasis occurrence in ER (+) BC cases (p = 0.004). The expression status of this miRNA may also assist with prediction of tamoxifen response. Thus, these observations suggest that modulation of miRNA expression may be another important mechanism underlying the chemoresistance of ER (+) BC. To the best of our knowledge, this is the first time that the role miR-1266 has been examined in cancer progression, and altered expression of this miRNA has been linked to recurrence/metastasis occurrence and tamoxifen resistance in ER (+) BC patients. Therefore, advanced studies of the silencing of miR-1266 in ER (+) BC may help to clarify the relationships of this miRNA with the *HOXB13:IL17BR* ratio and recurrence/metastasis occurrence.

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