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Plasma miR-210 levels correlate with sensitivity to trastuzumab and tumor presence in breast cancer patients

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Abstract

BACKGROUND—Trastuzumab is part of the standard treatment for HER-2 positive breast cancer patients, but not all patients respond to trastuzumab. Altered expression levels for microRNAs in cancer cells have been correlated with prognosis and response to chemotherapy. We hypothesized that altered expression levels for miRNAs in plasma are associated with sensitivity to trastuzumab in patients with HER-2 positive breast cancer.

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CONFLICT OF INTEREST DISCLOUSRES

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METHODS—We performed quantitative RT-PCR in plasma samples including breast cancer patients enrolled in a clinical trial of neoadjuvant trastuzumab-based chemotherapy. We analyzed expression levels for miR-210, -21, -29a, and -126 according to the type of response (pCR (n = 18) vs. residual disease (n = 11)). We also compared expression levels of miRNAs in trastuzumab-sensitive and –resistant breast cancer cells derived from BT474 cells and in an independent set of preoperative (n=39) and postoperative plasma (n=30) from 43 breast cancer patients not given any treatment.

RESULTS—At baseline before neoadjuvant chemotherapy combined with trastuzumab, circulating miR-210 levels were significantly higher in patients who had residual disease than in those who had pathologic CR ($P = 0.0359$). Mean expression ratio for miR-210 was significantly higher in trastuzumab-resistant BT474 cells and miR-210 expression was significantly higher before surgery than after surgery ($P = 0.0297$) and in patients whose cancer metastasized to the lymph nodes ($P = 0.0030$).

CONCLUSIONS—Circulating miR-210 levels were associated with trastuzumab sensitivity, tumor presence, and lymph node metastases. This suggests that plasma miR-210 may be used to predict and perhaps monitor response to therapies containing trastuzumab.

Keywords

microRNA; plasma; breast cancer; trastuzumab- resistance

INTRODUCTION

The human epidermal growth factor receptor 2 (HER2) is amplified in 20 – 30% of invasive breast cancer cases, and its amplification is associated with poor patient prognosis.[1] Since the advent of treatment with the anti-HER2 monoclonal antibody trastuzumab, survival of HER2-positive breast cancer patients has significantly improved, and trastuzumab is now part of the standard treatment for HER2-positive breast cancer. Monotherapy or combination treatment with trastuzumab results in a 30 – 50% response rate; however, most patients develop resistance to trastuzumab within 1 year. [2–5] Although one study found that combining trastuzumab with chemotherapy in a neoadjuvant setting resulted in a pathologic complete response (pCR) rate of more than 65%, almost 35% of patients treated with trastuzumab and chemotherapy still had residual disease.[6]

Although breast cancer can respond to chemotherapy, its sensitivity to a given drug regimen varies with each patient. Identification of patients who would benefit from specific chemotherapeutic agents prior to treatment could increase the proportion of cancers that respond to treatment and potentially help patients avoid the toxicity of ineffective chemotherapy. Amplification and consequent overexpression of HER2 in breast cancer is necessary for the cancer to respond to trastuzumab, but it is apparently not the only factor involved: about 70% of cancers with HER2 amplification fail to respond to trastuzumab.[7] One interesting study reported that HER2 blockade (trastuzumab) improves tumor oxygenation in Her2/neu+ tumors[7], and this study suggested that response to trastuzumab treatment correlates with tumor hypoxia. Several studies revealed that tumor hypoxia has been associated with poor prognosis and resistance to chemotherapy and radiation therapy.[8,9] In particular, the robust induction of miRNA-210 in hypoxic MCF-7 cell lines in one study proved to be a marker for hypoxia levels in tumors.[10]

MicroRNAs (miRNAs), endogenous RNAs that are about 20–23 nucleotides long, are known to play important regulatory roles in animals and plants by targeting messenger RNA transcripts for cleavage or translational repression.[11] The emergence of miRNAs as regulators of gene expression suggests that they might be able to serve as novel diagnostic

and prognostic biomarkers for disease and as predictive biomarkers for treatment. Although studies of miRNA expression in breast cancer have suggested that some miRNAs are promising candidates for these roles, most of the studies used samples from tumor tissues,[10,12–14] which are frequently not available in amounts necessary for detailed molecular investigation. Blood can be sampled much less invasively than tissue, and studies have found levels of miRNAs in blood from cancer patients to be detectable and remarkably stable.[15] Many studies have sought to identify miRNA markers in plasma or serum from cancer patients,[16–18] but none, to our knowledge, has examined miRNA levels in breast cancer as they relate to drug resistance.

We hypothesized that expression levels of miRNAs in plasma would be related to trastuzumab resistance in patients with HER-2 positive invasive breast cancer. To test this hypothesis, we measured the expression levels for miRNAs in plasma from patients with HER2-positive breast cancer before and after neoadjuvant chemotherapy that included trastuzumab, and we analyzed the relationship between mean relative miRNA expression levels and response to treatment. In a separate analysis, we also examined the relationship between mean relative miRNA expression levels and the presence of breast cancer.

PATIENTS AND METHODS

Patients in Analysis of Treatment Response

After obtaining Institutional Review Board approval from The University of Texas MD Anderson Cancer Center and written informed consent from the participants, we collected whole blood samples from 29 consecutive breast cancer patients who were treated with neoadjuvant chemotherapy and trastuzumab at MD Anderson and from 28 healthy, age-matched female volunteers recruited from MD Anderson who served as controls. We collected the blood samples at baseline (before chemotherapy) and at 24 weeks after the start of chemotherapy from patients in the treatment group and we collected blood samples at the time of study enrollment from healthy women in the control group. All 29 breast cancer patients in this study had histological confirmed breast cancer and tested HER2-positivity by either fluorescence in situ hybridization (FISH) or immunohistochemical analysis. Relevant demographic and clinicopathologic data for the patients were obtained from a prospectively maintained breast cancer database (Table 1). The pCR rate for this group (defined as no evidence of residual cancer in either breast or in the axilla) was 65%, as reported previously. [6] The median age of the 28 healthy volunteer was 53.5 years (range, 22–71 years), which was not significantly different from that of the treatment group (median age 52.0 years, range 21–70).

Each patient in the treatment group received 4 cycles of paclitaxel followed by 4 cycles of fluorouracil, epirubicin, and cyclophosphamide (FEC). Paclitaxel was administered at a dose of 225 mg/m^2 as a 24-hour continuous intravenous (IV) infusion; cycles were repeated every 3 weeks for 4 cycles. FEC consisted of 500 mg/m^2 of IV fluorouracil on days 1 and 4, 500 mg/m^2 of IV cyclophosphamide on day 1 only, and 75 mg/m^2 of IV epirubicin on day 1 only. Patients received trastuzumab at a dose of 4 mg/kg as a 90-minute IV infusion on day 1 of the first FEC cycle. Subsequent weekly treatments with trastuzumab were administered at a dose of 2 mg/kg as 30-minute IV infusions. Patients received weekly doses of trastuzumab for a total of 24 weeks.

Patients in Analysis of Tumor Presence

To analyze any potential relationship between plasma miRNA expression levels and tumor presence, we collected prospectively 39 preoperative and 30 postoperative plasma samples from a separate cohort of 43 Korean breast cancer patients who did not receive neoadjuvant

or adjuvant chemotherapy. This study was approved by the Institutional Review Board of Gyeongsang National University Hospital and informed consent was obtained from all patients. All patients had histological confirmed breast cancer. Plasma samples were collected preoperatively and in the second week postoperatively. Among the 43 patients, 13 tested positive for HER2 by immunohistochemical analysis (minimum score of 3+); FISH analyses were not performed in patients whose tumors were negative for HER2 on immunostaining (Table 2).

Plasma Collection

Up to 8 mL of whole blood was collected from each participant in an EDTA tube. Blood samples were centrifuged at 1,200 *g* for 10 minutes at 4°C to separate the blood cells, and the supernatant was transferred into microcentrifuge tubes and then centrifuged a second time at 12,000 *g* for 10 minutes at 4°C to completely remove the cellular components. Plasma was aliquoted and stored at −80°C until use. Blood samples were processed and plasma was frozen within 4 hours of collection.

Establishment of a Trastuzumab - Resistant BT474 Breast Cancer cell clone

Wild-type BT474 cells were obtained from the American Type Culture Collection (Manassas, VA) and were seeded in 6-well cell-culture plates and continuously treated with trastuzumab (Genentech) at a concentration of 10 μg/mL for 6 months. Cultures were replenished with fresh medium containing trastuzumab every week. After 6 months, cells were tested for sensitivity to trastuzumab based on their levels of upregulation of $p27^{Kip1}$ protein and cell-cycle arrest. Individual colonies resistant to trastuzumab (ie, those without $p27^{Kip1}$ induction and cell-cycle arrest at the G1 phase) were chosen microscopically, expanded, and rechecked for resistance to trastuzumab. Clone 65 (which we called BTR65) was the clone that exhibited the maximal resistance when compared to wild-type BT474 cells. Both wild-type BT474 and BTR65 cells seeded on 100-mm cell-culture dishes were treated once with trastuzumab 10μg/mL for 48 h. More details were published previously.[19]

RNA Extraction

Total RNAs were isolated from plasma samples using Norgen's RNA Purification Kit (Norgen Biotek Corp, Ontario, Canada) according to the manufacturer's protocol. Briefly, lysis solution was added to 100 μL of plasma, and then ethanol was added. The lysates were then loaded onto the provided column, and most of the contaminating cellular proteins were removed as they flowed through it. The column was then washed 3 times with 400 μL of wash solution. The purified total RNA was eluted into as much as 50 μL or as little as 20 μL of elution buffer. Eluted RNA samples were quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE). The mean amount of total eluted RNA in each sample was 182.39 ng (range, 111–378 ng).

Total RNA was extracted from BT474 cells and trastuzumab-resistant BTR65 cells using the TRIzol Reagent (Invitrogen, Carlsbad, CA). The concentrations of all RNA samples were quantified using the NanoDrop ND-1000 spectrophotometer.

Quantitative RT-PCR for Evaluation of MiRNA Expression

We selected a group of 4 miRNAs (miR-210, -21, -29a, and -126) that were abnormally expressed in the initial study and in other studies reporting that some miRNAs have altered expression profiles in breast cancer.[12,20–22] The miRNAs we chose have also been reported to be influenced by hypoxia in breast cancer cells and high expression level for miR-21 was correlated with trasuzumab resistance in breast cancer.[23,24]

Expression levels for miRNA-210, -21, -29a, and -126 were detected by qRT-PCR using the TaqMan MicroRNA Assays kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Twenty nanograms of total RNA from each sample was reverse transcribed using the TaqMan MicroRNA Reverse Transcription kit (Applied Biosystems). PCR amplifications were carried out in final volumes of 10 μL using the CFX384 real-time PCR detection system (Biorad). Amplifications were initiated with 10-minute incubation at 95°C followed by 40 cycles at 95°C for 15 seconds and 60°C for 60 seconds. Each amplification reactions were performed in duplicate wells and measured independently in two different days. To normalize the expression levels of miRNAs, we used U6 RNA as an internal control. The relative expression of each miRNA was calculated from the following equation: relative expression = $2^{-\Delta Ct}$, where Ct is the threshold cycle for a sample and ΔCt = mean Ct_{miRNA} − mean $Ct_{control\ U6}$. The mean relative expression levels for each miRNA were compared using 2-sided Student *t* tests ($P < 0.05$).

Statistical Analysis

All values are expressed as means ± standard deviations. Independent sample t- test was used to compare miRNA levels between breast cancer patients at baseline and healthy controls, between pCR and residual disease groups according to trastuzumab treatment response, wild-type BT474 cell line and clone 65. Paired Korean samples were analyzed by paired t-test analysis. We dichotomized miR-210 levels (low, and high) by median level for analysis of correlation between miR-210 and tumor size or lymph node status. Corresponding distribution plots were generated using GraphPad Prism 5.0 (GraphPad Software). Statistical analyses were performed using the SPSS 16.0 (SPSS Inc). All tests were two sided, and the difference of miRNAs expression was considered to be statistically significant at $P < 0.05$.

RESULTS

Expression of MiRNAs in Plasma of Breast Cancer Patients

First, we determined whether miR-210, -21, -29a, and -126 were expressed in the plasma of breast cancer patients in the treatment group (n=29) and healthy women in the control group (n=28) in the MD Anderson cohort. In 2 plasma samples from the baseline none of the tested miRNAs was expressed and therefore we analyzed miRNAs expressions in 27 samples at baseline and in 29 samples at 24 weeks timeline. The mean Ct values for miR-210, -21, -29a, and -126 in the treatment group were 34.97 (range, 31.94–38.01), 26.26 (range, 23.77–28.95), 31.36 (range, 27.59–34.38), and 27.45 (range, 25.09–30.23), respectively. In the control group, mean Ct values were 35.96, 27.89, 32.89, and 28.26 for miR-210, -21, -29a, and -126, respectively. These values indicate that the miRNAs were expressed in both groups. Mean Ct values for U6 (internal control) in the treatment and control groups ranged from 32.75 to 34.67, and differences in U6 Ct values between the treatment and control groups were not statistically significant (Figure 1).

We next determined whether mean relative miRNA expression levels differed between the treatment group and the control group. The mean relative expression levels for miR-210, -21, -29a, and -126 were significantly higher at baseline plasma samples (n=27) in the treatment group than in the control group ($P = 0.0119$, $P = 0.0430$, $P < 0.0001$, and $P < 0.0001$, respectively; Figure 1), and the ratios of mean relative miRNA expression levels in the treatment group to mean relative miRNA expression levels in the control group (tumor:control expression ratios) were 2.42, 3.09, 4.28, and 2.82, respectively (Table 3). Thus, we detected measurably higher expression levels for the 4 miRNAs in the plasma of breast cancer patients than in the plasma of healthy women of the same age.

Expression Levels of MiRNAs in Plasma from Breast Cancer Patients Before and After Neoadjuvant Trastuzumab-based Chemotherapy (HTFEC)

To assess the relationship between miRNA expression levels and response to trastuzumab treatment, we measured the expression levels for the 4 miRNAs before and 24 weeks after initiation of neoadjuvant chemotherapy that included trastuzumab. Among the treated patients, 18 achieved pCR and 11 still had residual disease after chemotherapy (Table 1). Baseline miRNAs expression level were technically not available for two samples and therefore we analyzed 16 baseline samples that achieved pCR. Among 29 patients, 10 patients had residual disease in breast and 3 patients had residual in lymph node. Two patients had residual disease in both places. For miR-29a, baseline mean relative expression level was not related to patients' ability to achieve pCR. For miR-210, miR-21 and miR-126, mean relative baseline expression levels were higher in samples from the residual disease group than in samples from the pCR group, but only the difference in mean relative expression levels for miR-210 reached statistical significance ($P = 0.0359$; Figure 2). Therefore, high baseline mean relative miR-210 expression levels were associated with resistance to treatment with trastuzumab in this set of patients.

Expression of MiRNAs in Trastuzumab-Sensitive and -Resistant Cell lines

To determine whether miR-210 variations were directly related to trastuzumab resistance, we measured the mean relative expression levels in BTR65 cells (clone 65 derived from the HER2-overexpressing BT474 breast cancer cell line), which exhibited resistance to trastuzumab.[19] As shown in Figure 3A, wild-type BT474 cells were sensitive to trastuzumab, as evidenced by up-regulation of the $p27^{Kip1}$ protein. In contrast, trastuzumab did not induce $p27^{Kip1}$ protein expression in BTR65 clone cells (Figure 3A).

We then measured the ratio of mean relative expression levels after 48 hours of treatment with trastuzumab to mean relative expression levels with no treatment (trastuzumab:control expression ratio) for each of the 4 miRNAs in wild-type BT474 cells and BTR65cells (Figure 3B) For miR-21 and -29a, trastuzumab:control expression ratios were slightly higher (but not significantly so) in BTR65 cells than in wild-type BT474 cells. For miR-126, the expression ratios did not differ between BT474 cells and BTR65 cells. For miR-210, the mean trastuzumab:control expression ratio was significantly higher in BTR65 cells than in BT474 cells (0.93 and 0.71, respectively; $P = 0.0075$), suggesting that high levels of miR-210, but not miRNA-21, -29a, or -126, indicate trastuzumab resistance.

Circulating MiRNA-210 Level and Tumor Presence

Finally, to understand the relationship between plasma miR-210 and tumor burden in breast cancer patients, we measured mean relative expression levels for miRNAs in preoperative (n = 39) and postoperative (n = 30) samples from 43 Korean patients who did not receive either neoadjuvant or adjuvant chemotherapy. Although we hypothesized that only miRNA-210 was related to tumor burden, we analyzed mean relative expression levels for all 4 miRNAs in the preoperative and postoperative plasma samples, as well as in the 25 paired samples that were available

We first analyzed mean relative expression levels for the 4 miRNAs in all samples and found that the miR-21, -29a, and -126 these were slightly, but not significantly, higher in postoperative samples than in preoperative samples (Figure 4). Mean relative expression levels for miR-210 alone were significantly lower in the postoperative samples than in the preoperative samples ($P = 0.0297$).

Similar findings were evident in the 25 paired samples. The mean relative expression levels for miR-210 were higher in the preoperative sample than in the postoperative sample in each

pair (mean relative expression levels were 0.92 and 0.58 for preoperative and postoperative samples, respectively; $P = 0.0382$). Mean relative expression levels for the other 3 microRNAs did not differ between the preoperative and the postoperative samples in each pair.

When we analyzed the relationship between expression levels for miR-210 and clinical characteristics, the mean relative expression levels from the 39 preoperative samples were significantly higher in patients whose disease had spread to the lymph nodes than in patients whose disease had not spread to the lymph nodes ($P = 0.0030$), but there was no relationship between mean relative expression levels for miR-210 and tumor size (Figure 5). Similar analysis of the MD Anderson cohort did not reveal any relationship between mean relative expression levels of miRNAs and tumor size, but identified the same positive trend, although not significant, for the association with LN status.

DISCUSSION

Our results suggest that high relative expression levels for miRNA-210 in the plasma of breast cancer patients are associated with trastuzumab resistance and the presence of the tumor; furthermore miR-210 expression level was significantly associated with lymph node involvement in the preoperative group from Korean patients. We also found evidence of this in a non-clinical setting, in which mean relative expression levels for miR-210 were higher in cells from a trastuzumab-resistant cell line than in trastuzumab-sensitive cells. To our knowledge, this is the first time a link has been found between relative expression levels for miRNA-210 in patient plasma and trastuzumab sensitivity.

The identification of patients who would benefit from chemotherapeutic agents is of great importance because individualized selection of treatment could maximize treatment benefit and minimize patient exposure to the adverse effects of ineffective therapy. Therefore, large-scale studies of the relationship between relative miRNA expression levels and chemotherapy-response are needed. In addition, as mentioned, most studies of chemotherapy response-specific miRNAs have measured miRNA levels in tumor tissues or cell lines rather than in plasma.[10, 12–14] However, tumor tissues are frequently not available in amounts necessary for detailed molecular investigation, and blood can be sampled much less invasively than tissue. Thus, investigations focusing on relative miRNA expression levels in the plasma are particularly warranted.

Several studies have focused on the identification of miRNAs linked to the acquisition of a resistant phenotype in cancer cell lines. An investigation in cancer cell lines by Blower et al. established the influence of 3 cancer-related miRNAs (let-7i, miR-16, and miR-21) on anticancer drug sensitivity. By testing 14 different anticancer compounds, the authors found that increased levels of miR-21 reduced the efficacy of nearly half the tested anticancer compounds.[25] Meng et al. demonstrated that miR-21, miR-141, and miR-200b were highly overexpressed in malignant cholangiocytes, and that inhibition of miR-21 and miR-200b increased the sensitivity of cholangiocarcinoma cells to gemcitabine.[26]

A recent article reported that high expression levels for miR-21 were associated with trastuzumab resistance in breast cancer.[23] In our current study, we found instead that plasma mean relative expression levels for miR-210 only, not miR-21, were related to trastuzumab sensitivity. However, we were able to confirm our findings by comparing the mean relative expression levels of each miRNA in a trastuzumab-resistant cell line with the expression levels in a trastuzumab-sensitive cell line.

Our finding that miR-210 was associated with tumor presence and drug resistance is not surprising given the findings of previous studies on miR-210 expression. Recent clinical studies have reported that higher expression levels for miR-210 were found in breast, head and neck, and pancreatic cancer patients than in healthy controls and that high expression levels were associated with poor prognosis.[10,27–29] A study of miRNAs as prognostic markers in pancreatic cancer revealed that overexpression of miR-210 and 3 other miRNAs (miR-155, -201, and -222) was associated with shorter survival.[27] In fact, many reports on different types of cancer tissues and blood have revealed that overexpression of miR-210 is associated with poor prognosis.,[10, 28–30] In a study of plasma from cancer patients, Ho and colleagues reported that circulating miR-210 levels might serve as a diagnostic marker in pancreatic cancer.[28]

However, miR-210 is not always overexpressed in cancer. Mean expression levels for miR-210 are lower in esophageal squamous cell carcinoma tissue and cell lines than in healthy tissue, and this downregulation is associated with poor differentiation.[31] This result suggests that expression patterns of miR-210 are specific to tumor type. In our current study, we found that mean expression levels for all miRNAs tested—miR-210, -21, -29a, and -126—were significantly higher in plasma from breast cancer patients than in plasma from healthy women. However, when we compared plasma mean expression levels of these miRNAs in preoperative breast cancer patients to those in postoperative breast cancer patients, miR-210 expression alone was significantly lower after tumor resection, suggesting that expression levels for miR-210 may be directly related to the presence of breast tumor.

We also discovered that, in addition to predicting tumor presence, high expression levels for miR-210 were associated with positive lymph nodes in preoperative Korean patients and had the same trend although not significant in MDACC baseline group of patients. This can be explained by the smaller size of the MDACC group of samples and although these results were obtained from a limited number of patients with different tumor characteristics, the results were consistent and substantial. However, studies with large-scale cohorts are needed to determine how well miR-210 could act as a predictive marker. Our finding in this small cohort that mean expression levels for miR-210 were associated with tumor presence but not with the size of the tumor also suggests that the expression levels could potentially indicate early presence of a tumor, but this also needs further study.

The function of miR-210, which is directly regulated by hypoxia-inducible factor 1-alpha, may also depend on cancer type. MiR-210 inhibits apoptosis, bypasses cell-cycle arrest, and promotes cancer cell survival when overexpressed, but when underexpressed, as it is in esophageal squamous cell carcinoma, it represses the initiation of tumor growth by inducing cell death and cell-cycle arrest.[31–33] To date, known targets of miR-210 in cancer include the receptor tyrosine kinase ligand ephrin-A3, the transcription factor E2F3, DNA repair enzyme RAD52, and fibroblast growth factor receptor-like 1.[34] MiR-210 may also interact with genes involved in the trastuzumab-resistance pathway. We searched miRNA-210 targets via RNA22, miRanda, TargetScan, and PITA software and found more than 5,300 targets; among these targets, we found well-known molecular targets for trastuzumab resistance, including MET, insulin-like growth factor 1 receptor, and membrane-associated mucin 4.[35–37] Functional studies beyond the scope of this paper should be performed to clarify the biological role of miR-210 in the trastuzumab-resistance pathway and identify if this miRNA could act also after potential release from cancer cells[38].

In summary, our results show that high plasma mean expression levels for miR-210 are associated with tumor presence in breast cancer patients and trastuzumab resistance in HER2-positive breast cancer patients. Although these results were obtained from small cohorts, they provide an important basis for larger prospective, multi-institutional studies to

investigate the potential role of plasma miRNAs as prognostic, diagnostic, and therapeutic markers for invasive breast cancer.

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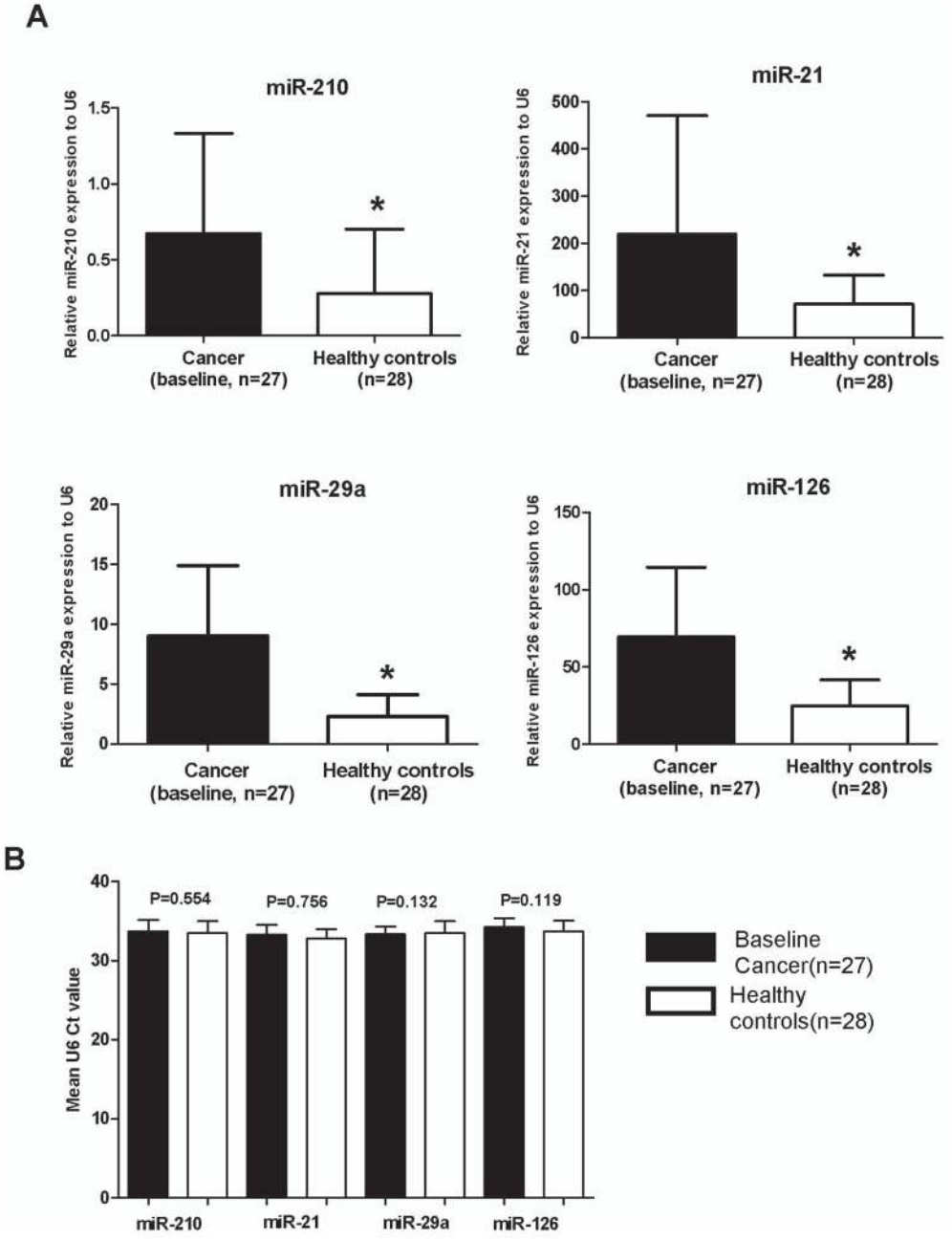

Figure 1. Plasma miRNAs levels in breast cancer patients

(A) Mean relative expression levels for microRNAs (miRNAs) in plasma from breast cancer patients at baseline before neoadjuvant chemotherapy in the treatment group and from healthy women in the control group. We analyzed miRNAs expressions in 27 samples at baseline and 29 samples at 24 weeks (two plasma samples from baseline were excluded because did not expressed any of the miRNAs). Mean relative expression levels for miR-210, -21, -29a, and -126 were significantly higher in the treatment group than in the control group ($P = 0.0119$, $P = 0.0430$, $P < 0.0001$, and $P < 0.0001$, respectively). *$P <$ 0.05. (B) Mean threshold cycle (Ct) values for U6 RNA (internal control) in plasma from breast cancer patients at baseline before neoadjuvant chemotherapy in the treatment group

and healthy women in the control group. Mean Ct values of U6 used for normalization are presented. No significant differences in mean U6 Ct values were found between the treatment group at baseline and the control group.

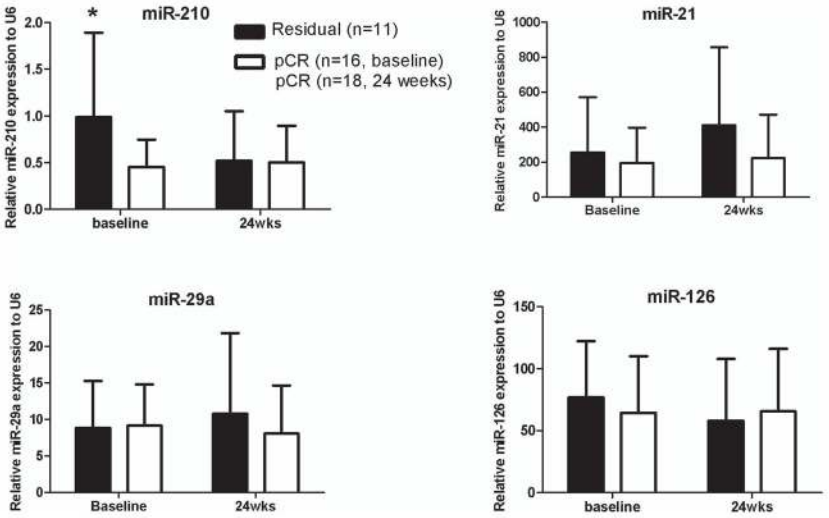

Figure 2. Expression of miR-210, -21, -29a, and -126 in plasma from breast cancer patients with residual disease or pathologic complete remission (pCR) to trastuzumab combined with neoadjuvant chemotherapy

At baseline before neoadjuvant chemotherapy combined with trastuzumab, expression of miR-210 was significantly higher in the residual disease group than in the pCR group (P = 0.0359). Baseline miRNAs expression level were technically not available for two samples (baseline pCR number, n=16).

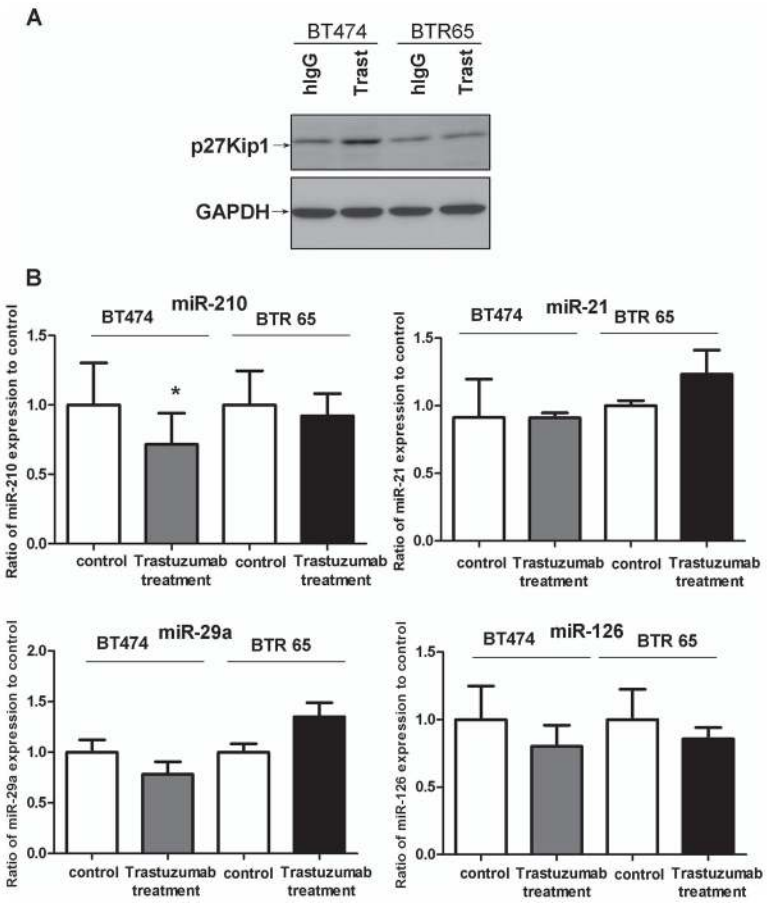

Figure 3. MiRNAs expression in Trastuzumab sensitive and resistant cells

A. The clone #65 (named BTR65) used in this study exhibited resistance (no p27 upregulation) to trastuzumab treatment compared to wild-type BT474 cells. B. MiR-210, -21, -29a and -126 expression ratios in BT474 cell and trastuzumab resistance BTR 65 cell. The ratio of miR-210 expression (trastuzumab:control miRNAs expression) was significantly lower in BT474 cell than trastuzumab resistance BTR 65 cell (P=0.0075). Trast = Trastuzumab. The graph legend is the same for all four panels.

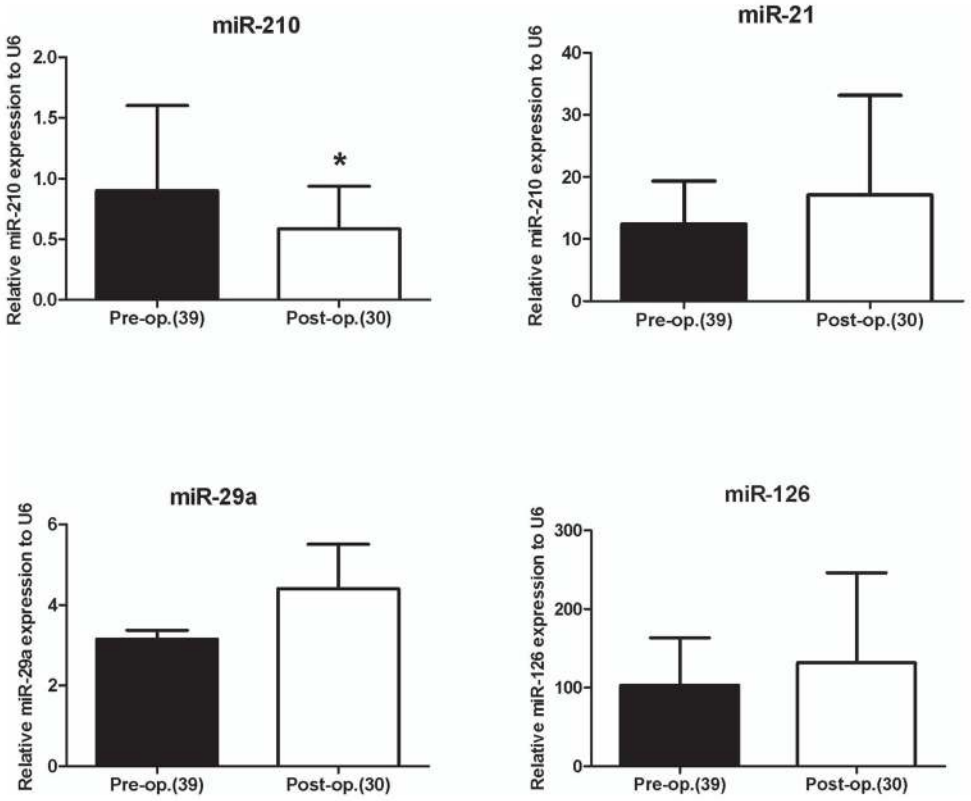

Figure 4. The plasma expression levels of miR-210, -21, -29a and -126 of breast cancer patients before (n = 39) and after surgery (n = 30)

None of the patients received any type of treatment for breast cancer. Only miR-210 was directly linked to presence of the tumor ($P = 0.0297$).

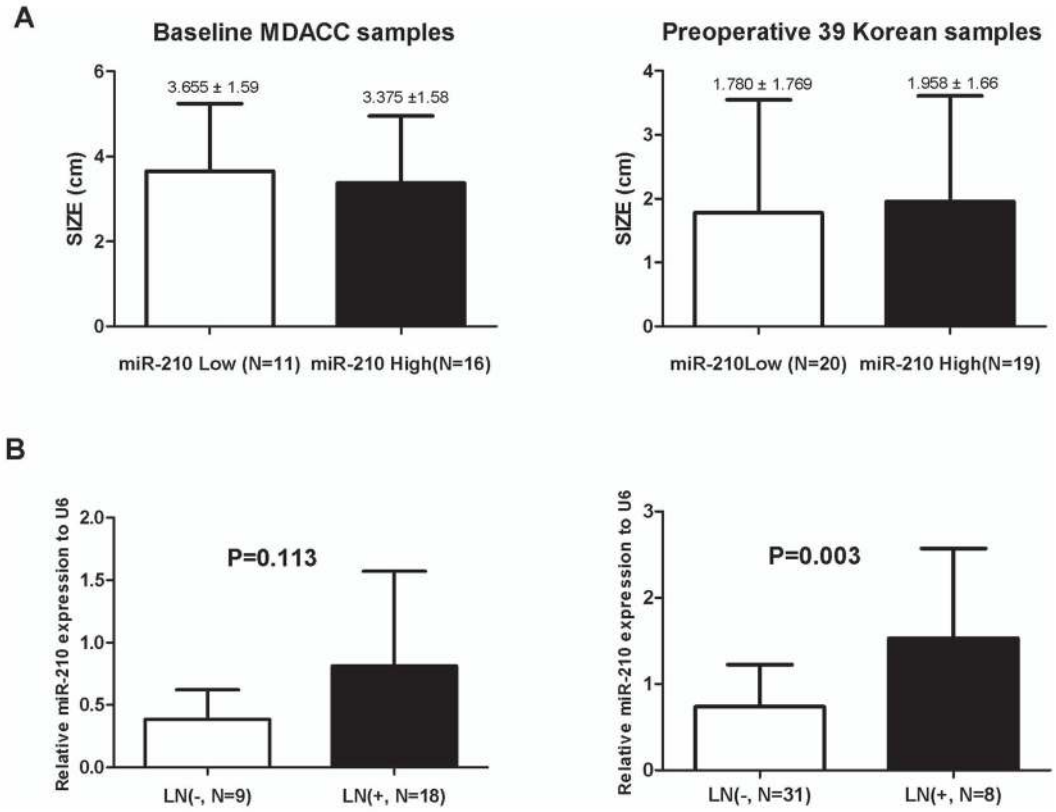

Figure 5. Correlation between miR-210 expression levels and tumor size (A), and lymph node involvement (B)

Preoperative Korean samples showed significant higher level of miR-210 in node positive group (p=0.0030), but not significant variations in relation with the tumor size. The miR-210 expression from baseline MDACC samples had the same type of variation in relation with the node involvement but not significant.

Table 1

Clinical characteristics of patients at MD Anderson who received chemotherapy (paclitaxel followed by fluorouracil, cyclophosphamide, and epirubicin) plus trastuzumab [*].

Characteristic	No. of patients (N = 29)
Median age (range)	52 years (21–70 years)
Tumor status	
T1	3
T2	20
T3	6
T4	0
Nodal status	
N0	10
N1	19
N2	0
N3	0
Hormonal receptor status	
Estrogen receptor (+)	13
Progesterone receptor (+)	8
HER2 status	
FISH (+)	28
IHC (+)[a]	1
Response	
Pathologic complete response	18
Residual disease	11
Residual disease in breast	
<1 cm	7
1–3 cm	1
>3 cm	2
No. of positive nodes	
1–3	3
4–10	0
>10	0

[*] HER2 indicates human epidermal growth factor receptor 2; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry.

[a] FISH results not available.

Table 2

Clinical characteristics of perioperative patients in the Korean cohort who did not receive adjuvant or neoadjuvant treatment *.

Characteristic	No. of patients (N = 43)
Sample group	
Preoperative	39
Postoperative	30
Median age (range)	53 years (35–78 years)
Tumor status	
T1	30
T2	11
T3	2
T4	0
Nodal status	
N0	35
N1	1
N2	5
N3	2
Hormonal receptor status	
Estrogen receptor (+)	29
Progesterone receptor (+)	35
HER2 status	
FISH (+)	ND
IHC (+)	13

* HER2 indicates human epidermal growth factor receptor 2; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; ND, not done.

Table 3

Ratios of mean relative microRNA (miRNA) expression levels in the plasma of breast cancer patients [*].

MiRNA	Ratios			
	Tumor:control	Residual:pCR, baseline	Residual:pCR, 24 weeks	Preoperative:postoperative
miRNA-210	2.42*	2.18*	0.97	1.53*
miRNA-21	3.09*	1.30	0.96	0.73
miRNA-29a	4.28*	1.17	1.09	0.72
miRNA-126	2.82*	1.19	0.88	0.78

[*] Tumor:control indicates the ratio of mean relative expression levels in breast cancer patients in the treatment group at baseline to mean relative expression levels in healthy women in the control group in the MD Anderson cohort; residual:pCR, mean relative expression levels in those who had residual disease to mean relative expression levels in those who achieved pathologic complete response in the MD Anderson cohort, at baseline before neoadjuvant chemotherapy and at 24 weeks after treatment; preoperative:postoperative, mean relative expression levels in breast cancer patients before surgery to mean relative expression levels in breast cancer patients after surgery in the Korean cohort.

[*] $P < 0.05$