MicroRNA-496 inhibits triple negative breast cancer cell proliferation by targeting Del-1

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Abstract
Del-1 has been linked to the pathogenesis of various cancers, including breast cancer. However, the regulation of Del-1 expression remains unclear. We previously reported the interaction between microRNA-137 (miR-137) and the Del-1 gene. In this study, we investigated miR-496 and miR-137 as regulators of Del-1 expression in triple negative breast cancer (TNBC). Del-1 mRNA and miR-496 were measured by quantitative PCR in breast cancer cells (MDA-MB-231, MCF7, SK-BR3, and T-47D) and tissues from 30 patients with TNBC. The effects of miR-496 on cell proliferation, migration, and invasion were determined with MTT, wound healing, and Matrigel transwell assays, respectively. In MDA-MB-231 cells, miR-496 levels were remarkably low and Del-1 mRNA levels were higher than in other breast cancer cell lines. Luciferase reporter assays revealed that miR-496 binds the 3′-UTR of Del-1 and Del-1 expression is downregulated by miR-496 mimics. Furthermore, miR-496 inhibited the proliferation, migration, and invasion of MDA-MB-231 cells. The effects of miR-496 on cell proliferation were additive with those of miR-137, another miRNA that regulates Del-1 expression. Moreover, in the 30 TNBC specimens, miR-496 was downregulated (P < .005) and the levels of Del-1 in the plasma were significantly elevated as compared with in normal controls (P = .0142). The Cancer Genome Atlas (TCGA) data showed the correlation of miR-496 expression with better overall survival in patients with early TNBC. In in silico and in vitro analyses, we showed that Del-1 is a target of miR-496 in TNBC and thereby affects cancer progression. Our findings suggest that miR-496 and miR-137 additively target Del-1 and act as modulating factors in TNBC. They are potentially new biomarkers for patients with TNBC.

Abbreviations: DEL-1 = developmental endothelial locus-1, miRNAs = MicroRNAs, TCGA = The Cancer Genome Atlas, TNBC = triple negative breast cancer, WT = wild type.

Keywords: breast cancer, cancer biomarker, microRNA

1. Introduction
Triple negative breast cancer (TNBC) accounts for only 15% to 20% of all breast cancer cases, but it accounts for the majority of breast cancer-related deaths.[1] Therefore, it is important to identify novel biomarkers for TNBC for the purpose of accurately classifying and hopefully treating TNBC.

Developmental endothelial locus-1 (Del-1; also called EGF Like Repeats and Discoidin Domains 3 or Edil-3) is widely expressed and Del-1 levels are high in circulating extracellular vesicles in the plasma of patients with breast cancer. After curative surgery, plasma Del-1 levels are decreased, suggesting it is a new diagnostic marker for early breast cancer.[2,3] Notably, Del-1 expression is significantly associated with unfavorable histology, high Ki67 expression, and the TNBC subtype, indicating its potential as a prognostic marker for TNBC.[4] However, it is not known how Del-1 expression is regulated in normal or tumor cells.

MicroRNAs (miRNAs) suppress gene expression through sequence-specific base paring with the 3′ untranslated region (3′UTR) of their target mRNAs, resulting in their translational repression or degradation. Evidence has suggested that miRNAs regulate gene expression by controlling diverse cellular and metabolic pathways in cancer cells as either tumor suppressors or oncogenes and, therefore, could emerge as promising biomarkers for a variety of cancers.[5–8] We hypothesized that specific miRNA(s) may affect Del-1 expression in TNBC and, if so, such miRNA(s) could be novel therapeutic target(s) together with Del-1. Recently, we identified the functional role of miR-137 and
the interaction between miR-137 and the Del-1 gene. Since miR-496 has also been predicted to bind to the Del-1 gene, we investigated its interaction with miR-137 and role in Del-1 expression in TNBC.

2. Material and methods

2.1. Selection of miR-496 as a candidate
Since miRNAs negatively regulate gene expression, any miRNA upregulated in cancer cells can be a candidate to downregulate mRNAs of target genes. The miRNA candidates possibly affecting Del-1 expression were selected from a list created using 3 web-based algorithms: miRanda (http://www.microrna.org/microrna/home.do), Target Scan (http://www.targetscan.org/vert_71/), and miRDB (http://mirdb.org/miRDB/).

2.2. Clinical specimens to measure miR-496 and Del-1 expression
All clinical breast cancers and paired adjacent normal breast tissues were acquired from 30 patients with early TNBC (Stage I through IIIA). Total RNA was extracted using an RNaseasy Lipid Tissue Mini Kit (Qagen, Hilden, Germany) in accordance with the manufacturer’s instructions. Del-1 mRNA and miRNA expressions were measured and analyzed considering both clinical and pathological characteristics, such as age, tumor size, lymph node involvement, histological grade, lymphovascular invasion, and BRCA 1/2 mutation status. All procedures were performed under a protocol approved by the institutional review board at Kyungpook National University Chilgok Hospital (#2013-09-009-001). At the time of recruitment, patients were given an information leaflet and a consent form for storage and collection of biological materials, including blood and tissue samples, as well as future use of their samples for research purposes.

2.3. Breast cancer cell lines
A human breast epithelial cell line (MCF10A) and breast cancer cell lines (MDA-MB-231, MCF7, and SK-BR3) were purchased from the American Type Culture Collection (ATCC, Manassas, VA). MCF10A cells were maintained in Dulbecco Modified Eagle medium (DMEM)/F-12 (1:1) medium (Lonza, Walkersville, MD) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY), 10 ng/ml epidermal growth factor (Sigma-Aldrich Co., St. Louis, MO), 0.5 μg/ml hydrocortisone (Sigma-Aldrich), 100 ng/ml cholera toxin (Sigma-Aldrich), and 10 μg/ml insulin (Sigma–Aldrich). MDA-MB-231, MCF7, and SK-BR3 cells were maintained in DMEM (Gibco) supplemented with 10% FBS.

2.4. RNA extraction and quantitative PCR (qPCR)
Total RNA from cells was isolated using RNAiso Plus (TaKaRa, Otsu, Shiga, Japan) according to the manufacturer’s instructions. The Super Script III First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA) and TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) were used to reverse transcribe the mRNAs and miRNAs, respectively. qPCR was performed to assess the relative expressions of Del-1 using the Power SYBR Green PCR Master Mix (Applied Biosystems). Additionally, the abundance of miRNA was measured using TaqMan Universal Master Mix II (Applied Biosystems). The relative amount of each miRNA was normalized to U6 snRNA, whereas mRNA expression was normalized to β-actin. Relative expression levels were calculated using the \(2^{-\Delta\Delta C_t}\) method. The TaqMan probe sets for miR-496 and U6 were purchased from Applied Biosystems. The primers used for qPCR were as follows: Del-1 (5'-TCGAGACATTGCACTTGGC-3' and 5'-ACCCAGAGGCTCAGAAC-3') and β-actin (5'-TTGCCGACAGATGCAGAA-3' and 5'-GCGATCCACGAGTACT-3').

2.5. Enzyme-linked immunosorbent assay (ELISA)
Del-1 levels in culture medium were detected by ELISA since Del-1 is known to be secreted from the cells as previously described.

2.6. miRNA transfection
miR-496 mimics and the negative control were purchased from Sigma-Aldrich Co., LLC (St. Louis, MO, USA). miR-496 inhibitors were purchased from Ambion by Life Technologies Inc. (Carlsbad, CA). Cells were plated in 6-well plates (2 × 10^5 cells/well) for wound healing assays or 96-well plates (5 × 10^3 cells/well) for 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays. After culturing overnight, the cells were transfected using Lipofectamine RNAiMAX Transfection Reagent (Invitrogen) according to the manufacturer’s instructions.

2.7. Plasmid vector construction for luciferase reporter assays and mutagenesis
To construct a reporter plasmid containing the wild type (WT) Del-1 3’-UTR, a 533-bp fragment of the Del-1 3’-UTR was amplified by PCR using MDA-MB-231 cDNA as a template and cloned into the pTOP Blunt V2 vector (Enzymatics Co. Ltd, Daejeon, South Korea). Thereafter, the Del-1 3’-UTR was cloned in the pmirGLO Dual-Luciferase miRNA Target Expression Vector (Promega, Madison, WI). To construct the plasmids with a mutant (Mut) Del-1 3’-UTR, the putative miR-496 target sequence was mutated using a Muta-Direct Site-Directed Mutagenesis Kit (iNtRON Biotechnology, Gyeonggi-do, South Korea). All the products were confirmed by sequencing.

2.8. Dual luciferase reporter assays
MDA-MB-231 cells were plated into 24-well plates (4 × 10^4 cells/well). After 24 hours, the cells were co-transfected with 100 ng of the pmirGLO Dual-Luciferase expression construct containing the 3’-UTR of Del-1 (WT or Mut), and 10 pmol of miR-496 mimics or its negative control using Lipofectamine 3000 (Invitrogen) according to the manufacturer’s protocol. Forty-eight hours after transfection, the cell lysates were collected and set aside, and the luciferase activities were measured using a Dual-Luciferase Reporter Assay System (Promega) and normalized to Renilla luciferase activity.

2.9. MTT assays to assess cell proliferation
Cell proliferation was estimated using an MTT assay (Sigma-Aldrich). Briefly, cells were seeded in 96-well plates. After
transfection with 1 pmol of miRNA mimics, miRNA inhibitors, or the negative control, cells were incubated for 1, 2, or 3 days without changing the medium after transfection. At each time point, 50 μL of MTT (2 mg/mL) were added to the wells, and the cells were cultured at 37°C for 4 hours. After removing the media, 150 μL of dimethyl sulfoxide (DMSO) were added and the solution was mixed for 10 minutes. The optical density was determined at 570 nm using a microplate reader (BioTek Instruments, Inc., Winooski, VT).

2.10. Wound scratch assays for cell migration

To examine the effects of miR-496 on the migration of MDA-MB-231 cells, the cells were seeded in 6-well plates. After culturing overnight, a line was scratched into the cell monolayer using a sterile pipette tip and the medium was changed. Subsequently, the cells were transfected with 25 pmol of negative controls, miR-496 mimics or miR-496 inhibitors and further incubated for 48 hours. Images were then captured with the aid of a microscope and camera system (Olympus Co., Tokyo, Japan) after 0, 24, 48, or 72 hours of incubation.

2.11. Transwell assays for cell invasion

For the invasion assays, transwell chambers with 8 μm pores were coated with Matrigel (Corning Inc., Tewksbury, MA) and incubated at 37°C for 2 hours. After transfection for 24 hours with 25 pmol of the negative control, miR-496 mimics, or miR-496 inhibitors, the cells were resuspended in DMEM containing 1% FBS and 1 × 10^5 cells were plated in the upper chamber. The lower chamber contained the complete medium supplemented with 10% FBS. After incubation for 48 hours, the cells on the upper surface of the chamber were swabbed using a cotton swab, and those at the bottom were fixed with 0.5% paraformaldehyde, stained with 0.5% crystal violet, and then rinsed with phosphate-buffered saline. Finally, 4 random views were chosen for each culture well under a light microscope (200 × ; Olympus Co., Tokyo, Japan) and the number of invading cells in each view was counted.

2.12. Survival according to miR-496 levels

To validate whether the levels of miR-496 were related to the survival of patients, we used a The Cancer Genome Atlas (TCGA) dataset (n = 1,077) relative to patients with TNBC and divided it into subgroups based on the levels of miR-496 and Del-1. The prognostic value of miR-496 was estimated using the Kaplan–Meier Plotter (http://kmplot.com/analysis/).

2.13. Statistical analysis

All quantitative experiments were performed at least in triplicate. The data are expressed as the mean ± standard deviation and were analyzed using Student t test and Mann–Whitney U test with GraphPad Prism version 7 (GraphPad Software, San Diego, CA). P value of ≤ 0.05 was considered statistically significant.

3. Results

3.1. miR-496 expression in breast cancer cell lines and tumor tissues

Based on a bioinformatics search using 3 prediction algorithms, miR-496 was selected as a potential miRNA targeting Del-1. Next, we corroborated this interaction. We measured miR-496 levels in various breast cancer cell lines and found that miR-496 was significantly downregulated in a triple negative breast cell line (MDA-MB-231) compared with MCF10A, and it was similarly expressed in the other cancer lines (P < 0.005; Fig. 1A). Furthermore, the expression of miR-496 in breast tumor tissues of 30 patients with TNBC showed the level was significantly lower than that in the paired normal breast tissues, irrespective of clinical stage, pathologic characteristics, and BRCA mutation status (P = 0.0142; Fig. 1B, Supplementary Fig. 1, http://links.lww.com/MD2/A33).

![Figure 1](http://links.lww.com/MD2/A33)

**Figure 1.** Expression of miR-496 in breast cancer cell lines and tissues. (A) The expression of miR-496 was downregulated in all breast cancer cells including MDA-MB-231 cells compared with MCF10A breast epithelial cells. (B) Expression of miR-496 in patients with TNBC: the breast tumor tissues of patients with TNBC showed a significant downregulation of miR-496 expression irrespective of the patients’ clinical and pathological characteristics (n = 30 for each group). TNBC = triple negative breast cancer.
3.2. *miR-496* directly targets the 3′-UTR of Del-1

Bioinformatic analyses predicted the 380 to 386 regions in the 3′-UTR of Del-1 mRNA as a putative target of miR-496 (Fig. 2A). To determine whether the site was functional, a constructed mutant of the Del-1 plasmid was transfected into MDA-MB-231 cells and tested using luciferase assays with miR-496 mimics. As shown in Figure 2B, miR-496 mimics caused significant downregulation of the luciferase activity of the plasmid with WT Del-1 3′-UTR ($P < .01$). There was no effect on the plasmid with Mut Del-1 3′-UTR. These findings suggested that the 3′-UTR of Del-1 is a functional target of miR-496.

3.3. Del-1 expression is negatively regulated by miR-496

To investigate whether miR-496 negatively regulates Del-1 protein expression, we transfected miR-496 into MDA-MB-231 cells...
cells and measured Del-1 mRNA and protein levels using qPCR and ELISA, respectively. Del-1 expression was significantly downregulated (85%) in MDA-MB-231 transfected with miR-496 mimics, whereas miR-496 inhibitors reversed this effect \((P<.001\); Fig. 3A). Additionally, Del-1 protein levels were significantly decreased (72.5%) upon miR-496 mimics transfection and, again, miR-496 inhibitors reversed this effect \((P<.001\); Fig. 3B). These findings suggested that miR-496 may suppress the transcription of Del-1 or induce its degradation.

### 3.4. miR-496 inhibits the proliferation, migration, and invasion of MDA-MB-231 cells

Del-1 has been shown to promote cancer progression.\cite{3} Because miR-496 negatively regulates Del-1, it might reverse the oncogenic effect of Del-1 in TNBC. To investigate this hypothesis, we assessed the effect of miR-496 on the proliferation, migration, and invasion of MDA-MB-231. MTT assays showed that the proliferation of MDA-MB-231 cells were significantly decreased upon transfection of miR-496 mimics \((P<.0001\); Figure 4A), whereas miR-496 inhibitors had little effect on cell proliferation compared with the negative control miRNAs. The migration and invasion of MDA-MB-231 cells were significantly inhibited by miR-496 mimics, whereas the effect of miR-496 inhibitors was similar to that of the negative control miRNAs (Figure 4B and C). In addition, we evaluated the functional effects of miR-496 in another TNBC cell line, Hs578T, to exclude cell-line dependent effects. Transfection of miR-496 mimics significantly inhibited proliferation, migration, and invasion of Hs578T cells, which showed consistent results with MDA-MB-231 cells (Supplementary Fig. 2, http://links.lww.com/MD2/A33). Collectively, these results showed that miR-496 had a tumor suppressive role during the progression of TNBC via Del-1 downregulation.

### 3.5. Effects of miR-496 and miR-137 on MDA-MB-231 cells

Among several miRNAs predicted to target Del-1, our previous work revealed miR-137 also targets Del-1.\cite{19} We therefore tested whether miR-496 and miR-137 act additively or synergistically in MDA-MB-231 cells. As shown in Figure 5, co-transfection of the 2 miRNAs additively reduced Del-1 mRNA and protein expression \((P<.001\) and \(P<.01\), respectively). Additionally, we showed their additive effect on the proliferation of MDA-MB-231, but not on the invasion and migration of these cells.

### 3.6. High miR-496 expression predicts a better prognosis

We then used the Kaplan–Meier Plotter database to evaluate the relation between miR-496 levels and the prognosis of 97 patients with TNBC in whom miR-496 levels were available in the TCGA database. We set a cutoff value of miR-496 levels of 2 to separate the high and low expression groups. Although high miR-496 expression was slightly associated with better survival \((HR = 0.43; 95\% CI = 0.14–1.37; P=.14\); Fig. 6A), when node-negative TNBC cases \((n=53)\) were selected to minimize the impact of the clinical characteristics, a significant association was observed between miR-496 and survival \((HR and 95\% CI were not computed due to no death event in the high miR-496 group; \(P=.022\); Fig. 6B). Meanwhile, Del-1 expression was estimated as an unfavorable prognostic factor (Fig. 7). These results provide additional evidence that miR-496 or Del-1 may play a role in TNBC progression.

### 4. Discussion

Given the lack of validated molecular targets and the poor outcomes, a greater understanding of TNBC at all levels is critical.\cite{11} This study found that miR-496 as well as miR-137 is significantly downregulated in TNBC and Del-1 is a direct target of these miRNAs. Furthermore, we found that the overexpression of miR-496 inhibits TNBC cell proliferation, invasion, and migration by decreasing Del-1 expression, and these effects were reversed by miR-496 inhibitors. Since miR-496 can downregulate both Del-1 mRNA and protein, this miRNA might affect both Del-1 mRNA degradation and translation. In summary, this study identified novel mRNA/miRNA interactions that contribute to TNBC via Del-1 and therefore represent a potential target for the development of new therapeutic strategies for TNBC. Notably, it corroborated the findings of previous studies investigating Del-1 as a diagnostic and prognostic marker of breast cancer.\cite{2,4,9}

Del-1, initially identified as an extracellular matrix protein with epidermal growth factor-like domains, is over-expressed in several cancers and has been shown to induce tumorigenesis or cancer progression via direct mechanisms such as apoptosis and epithelial-mesenchymal transition\cite{11–13} or indirect mechanisms such as angiogenesis and immune tolerance against tumor cells.\cite{16–19} In breast cancer, Del-1 seems to enhance cancer progression via angiogenesis\cite{20} and a different study shows the direct impact of Del-1 on breast cancer invasion and metastasis through the integrin-FAK signaling pathway.\cite{3} The diagnostic and prognostic value of Del-1 has been first suggested in a proteomic study in 2 separate cohorts \((n=320\) and 242, respectively) showing that Del-1 was upregulated as in the plasma of patients with breast cancer as well as in the media of MDA-MB-231 cells compared with controls.\cite{2} Additionally, in a survival analysis of a Kyungpook National University Hospital cohort, Del-1 expression in tumor tissues has been found to be independently associated with worse survival in patients with TNBC.\cite{4} Similarly, in this study, a survival analysis of a TCGA dataset showed that high Del-1 expression was correlated with worse survival in TNBC early breast cancer. (Supplementary Table 1, http://links.lww.com/MD2/A36).

Despite evidence showing the role of Del-1 in tumor progression, how its expression is regulated in cancer cells is not clear. Since miRNAs act as post-transcriptional regulators of a variety of target genes, functioning as either tumor suppressors or oncogenes,\cite{21,22} the identification of one or more miRNAs regulating Del-1 expression might suggest their targeting ability to regulate Del-1. In this study, bioinformatic analyses pointed to miR-496 as a potential regulator of Del-1 expression. miR-496 was significantly downregulated in TNBC cell lines and was found to suppress Del-1 expression by directly binding to its mRNA. Additionally, functional studies demonstrated the inhibitory effect of miR-496 on the proliferation, migration, and invasion of TNBC cells.

Interestingly, the examination of a public dataset revealed that high levels of miR-496 correlate with the survival of patients in early-stages TNBC. Similarly, a survival analysis from a TCGA dataset relative to patients with pancreatic cancer has demonstrated the association of miR-496 with survival.\cite{23}
Figure 4. Proliferation, migration, and invasion of MDA-MB-231 cells following the overexpression and knockdown of miR-496: (A) MTT assays, (B) Wound scratch assays, and (C) Matrigel transwell assays. *: P < .05, **: P < .001.
Figure 5. Additive inhibitory effect of both miR-137 and -496 in Del-1 expression. (A) qRT-PCR analysis for Del-1 mRNA expression level: upregulation of both miR-137 and -496 in MDA-MB-231 cells by transfecting miR-137 and -496 mimics resulted in additive reduction in Del-1 mRNA transcription. (B) Western blot analysis for Del-1 protein levels in the cell lysate and soup from MDA-MB-231. (C) Proliferation of MDA-MB-231 cells was additively impaired by both miRNAs transfection in a MTT assay. (D) For cell migration using a wound scratch assay, both miRNAs respectively suppressed breast cancer cell migration compared with in normal controls, but there was an additive effect with both miRNAs. (E) Similarly, a transwell assay for cell invasion revealed that there was an additive effect on tumor cell migration and invasion although the significant decrease was observed with both miRNAs, separately under a light microscope (200×) (E upper) and confirmed by microplate reader at 590nm (E bottom). *: P < .05, **: P < .01, ***: P < .001. Del-1 = developmental endothelial locus-1.
To our knowledge, this is the first study describing the tumor suppressor role of miR-496, with the exception of a recent study showing that miR-496 reduces mTOR expression, a key molecule of the PI3K/Akt/mTOR signaling pathway. Thus, when considering the cross-talk between p53 and mTOR in cell growth, proliferation, and death, it is conceivable to hypothesize that miR-496 affects the Del-1/p53/mTOR axis.

Notably, the correlations of miR-496 with breast cancer have not been fully elucidated in a large cohort or prospective studies and, moreover, the underlying mechanisms are still unclear. In this study, we investigated the effect of miR-496 with miR-137 on Del-1 expression, and found they have an additive effect on cancer cell proliferation and invasion, suggesting their independent action on Del-1.

Breast cancer cell lines have been proven to be useful in laboratory and preclinical investigations to predict the outcome of endocrine or anti-HER2 therapeutics in clinical trials for hormone responsive or HER2-overexpressing breast cancer subtypes, respectively. TNBC, however, is a heterogeneous disease both pathologically and molecularly and comprises several subtypes based on gene expression. Therefore, there are several limitations in the investigation of TNBC with cell lines. Currently, there are ≥27 human breast cancer cell lines classified as TNBC but with different characteristics. MDA-MB-231 cells, for example, which were used in this study, are the most commonly studied cell line for TNBC, but they were established in the 1970s, before routine evaluation of the expression of hormone receptors and HER2. The development of molecular science has subsequently revealed that MDA-MB-231 is a basal cell line with features of high-grade histology, mutations in P53 or KRas, and wild type BRCA and genes in the PI3K pathway. As such, the preclinical success of targeted agents using TNBC/basal cell lines does not translate into the success of clinical trials. Given that TNBC is heterogeneous and the role of specific miRNAs are ultimately exclusive to different types of cells or tissues, further studies with different types of TNBC cell lines are necessary to validate the exact role of miRNAs in breast cancer. We have shown that miR-496 regulates Del-1 expression by interacting miR-137 and affects the proliferation, migration, and invasion of MDA-MB-231 cells.

In summary, miR-496 was found to be involved in Del-1 regulation with miR-137 via binding with the Del-1 gene, affecting cancer progression by altering Del-1 expression, which suggests that miR-137 and miR-496 exert a tumor suppressive role by targeting Del-1 in TNBC. Thus, we provide new insights into the potential mechanisms of Del-1-related oncogenesis and reveal the potential role of miR-496 as a new biomarker for TNBC. However, when considering that Del-1 is not the only target gene of miR-137 and miR-496, and it was not down-regulated in all the TNBC tissues, further studies are warranted to investigate the exact mechanisms of Del-1 regulation based on the heterogeneity of TNBC.
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References
[1] Anders CK, Carey LA. Biology, metastatic patterns, and treatment of patients with triple-negative breast cancer. Clin Breast Cancer 2009;9 (Suppl 2):S73–81.
[2] Moon PG, Lee JE, Cho YE, et al. Identification of developmental endothelial locus-1 on circulating extracellular vesicles as a Novel Biomarker for early breast cancer detection. Clin Cancer Res 2016;22:1757–66.
[3] Lee JE, Moon PG, Cho YE, et al. Identification of EDIL3 on extracellular vesicles involved in breast cancer cell invasion. J Proteomics 2016;131:17–28.
[4] Lee SJ, Lee J, Kim WW, et al. Del-1 expression as a potential biomarker in triple-negative early breast cancer. Oncology 2018;94:243–56.
[5] Lim LP, Lau NC, Garrett-Engele P, et al. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature 2005;433:769–73.
[6] Amb S, Prueitt RL, Yi M, et al. Genomic profiling of miRNA and messenger RNA reveals deregulated microRNA expression in prostate cancer. Cancer Res 2008;68:6162–70.
[7] Hobert O. Gene regulation by transcription factors and microRNAs. Science 2008;319:1783–6.
[8] Jorio MV, Groce CM. MicroRNAs in cancer: small molecules with a huge impact. J Clin Oncol 2009;27:5848–56.
[9] Lee SJ, Jeong JH, Kang SH, et al. MicroRNA-137 inhibits cancer progression by targeting Del-1 in triple-negative breast cancer cells. Int J Mol Sci 2019;20:
[10] Lanczyk A, Nagy A, Bottai G, et al. miRpower: a web-tool to validate some microRNAs downregulate large numbers of target mRNAs. Nature 2005;433:769–73.
[11] Goldhirsch A, Ingle JN, Gelber RD, et al. Thresholds for therapies: highlights of the St Gallen International Expert Consensus on the primary therapy of early breast cancer 2009. Ann Oncol 2009;20:1319–29.
[12] Beckham Cj, Olsen J, Yin PN, et al. Bladder cancer exosomes contain EDIL-3/Dell and facilitate cancer progression. J Urol 2014;192:583–92.
[13] Goldhirsch A. Personalized adjuvant therapies: lessons from the past: the opening address by the St. Gallen 2013 award recipient. Breast Cancer Res Treat 2013;22 (Suppl 2):S3–7.
[14] Kim H, Lee SH, Lee MN, et al. Choi EY. p53 regulates the transcription of the anti-inflammatory molecule developmental endothelial locus-1 (Del-1). Oncotarget 2013;4:1976–83.
[15] Lee SH, Kim DY, Jing F, et al. Del-1 overexpression potentiates lung cancer cell proliferation and invasion. Biochem Biophys Res Commun 2015;468:92–8.
[16] Esken MA, Jotwani R, Abe T, et al. The leukocyte integrin antagonist Del-1 inhibits IL-17-mediated inflammatory bone loss. Nat Immunol 2012;13:465–73.
[17] Choi EY, Chavakis E, Czabanka MA, et al. Del-1, an endogenous leukocyte-endothelial adhesion inhibitor, limits inflammatory cell recruitment. Science 2008;322:1101–4.
[18] Mitroulis I, Kang YY, Gahnberg CG, et al. Developmental endothelial locus-1 attenuates complement-dependent phagocytosis through inhibition of Mac-1-integrin. Thromb Haemost 2014;111:1004–6.
[19] Choi EY, Lim JH, Neuworth A, et al. Developmental endothelial locus-1 is a homeostatic factor in the central nervous system limiting neuroinflammation and demyelination. Mol Psychiatry 2015;20:880–8.
[20] Aoka Y, Johnson FL, Penta K, et al. The embryonic angiogenic factor Del1 accelerates tumor growth by enhancing vascular formation. Microvasc Res 2002;64:148–61.
[21] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004;116:281–97.
[22] Meister G, Tuschl T. Mechanisms of gene silencing by double-stranded RNA. Nature 2004;431:343–9.
[23] Doo D, Yang S, Lin Y, et al. An eight-miRNA signature expression-based risk scoring system for prediction of survival in pancreatic adenocarcinoma. Cancer Biomark 2018.
[24] Ruhle C, Kolsch K, Halajda B, et al. microRNA-496 - a new, potentially aging-relevant regulator of mTOR. Cell Cycle 2016;15:1108–16.
[25] Lehmann BD, Bauer JA, Chen X, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest 2011;121:2750–67.
[26] Chavez KJ, Garmella SV, Lipkowitz S. Triple negative breast cancer cell lines: one tool in the search for better treatment of triple negative breast cancer. Breast Dis 2010;32:35–48.
[27] Brinkley BR, Beall PT, Wible LJ, et al. Variations in cell form and cytoskeleton in human breast carcinoma cells in vitro. Cancer Res 1980;40:3118–29.
[28] Cailléau R, Young R, Olive M, et al. Breast tumor cell lines from pleural effusions. J Natl Cancer Inst 1974;53:661–74.
[29] O’Connor PM, Jackman J, Bar I, et al. Characterization of the p53 tumor suppressor pathway in cell lines of the National Cancer Institute anticancer drug screen and correlations with the growth-inhibitory potency of 123 anticancer agents. Cancer Res 1997;57:4783–800.
[30] Kao J, Salari K, Bocanegra M, et al. Molecular profiling of breast cancer cell lines: one tool in the search for better treatment of triple negative breast cancer cell lines. Breast Cancer Res Treat 2010;121:53–64.
[31] Brinkley BR, Beall PT, Wible LJ, et al. Variations in cell form and cytoskeleton in human breast carcinoma cells in vitro. Cancer Res 1980;40:3118–29.
[32] Cailléau R, Young R, Olive M, et al. Breast tumor cell lines from pleural effusions. J Natl Cancer Inst 1974;53:661–74.
[33] O’Connor PM, Jackman J, Bar I, et al. Characterization of the p53 tumor suppressor pathway in cell lines of the National Cancer Institute anticancer drug screen and correlations with the growth-inhibitory potency of 123 anticancer agents. Cancer Res 1997;57:4783–800.
[34] Kao J, Salari K, Bocanegra M, et al. Molecular profiling of breast cancer cell lines: one tool in the search for better treatment of triple negative breast cancer cell lines. Breast Cancer Res Treat 2010;121:53–64.
[35] Lee SH, Kim DY, Jing F, et al. Del-1 overexpression potentiates lung cancer cell proliferation and invasion. Biochem Biophys Res Commun 2015;468:92–8.