Effects of miR-592 on the metastatic behavior of MDA-MB-231 triple negative breast cancer cells

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Abstract

Triple-negative breast cancer (TNBC) is a disease characterized by its aggressive metastasis and poor prognosis. Developing targeted therapies that increase the tissue selectivity of drugs would be beneficial to the improvement of the overall efficacy of anti-cancer treatment and the suppression of the mortality rate of TNBC patients. The type 1 insulin-like growth factor receptor (IGF-1R) and focal adhesion kinase (FAK) pathways are involved in cell migration and cell invasion of the metastatic cascade in TNBC. The upregulation of miR-592 is has been shown to reduce cell proliferation and invasion in cancer cells, however, its role in TNBC remains unknown. It is demonstrated in this study that the overexpression of miR-592 resulted in decreased FAK expression, cell migration, and colony formation in the MDA-MB-231 cell line. Comprehensively, miR-592 is proposed as an important repressor of the development and progression of TNBC linked to epithelial-mesenchymal transition and tumorigenesis. Understanding the exact mechanism of action of miR-592 can reveal new therapeutic targets for the treatment of mesenchymal TNBCs.

Introduction

Triple-negative breast cancer (TNBC) involves the lack of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth receptor 2 (HER2) (1–3). This subtype exhibits aggressive metastasis and accounts for 10-20% of invasive breast cancers as of 2009 (3). The prevalence of TNBC and similar basal-like breast cancer subtypes is significantly higher in pre-menopausal African American and Latino women (4,5). The TNBC subtype is characterized by high risk of recurrence, distant metastasis and high mortality rates within 5 years (6). Current gene-targeted therapies, such as tamoxifen and Herceptin, target transmembrane receptors that the TNBC subtype does not possess (1). The use of chemotherapeutic drugs and radiation also has not displayed promising prevention of the poor prognosis of TNBC patients (7). Due to the lack of ER, PR, and HER2 receptors in TNBC, there still remains a great need for targeted gene therapy through the understanding of cellular mechanisms involves in the aggressive behavior of TNBC.

The tyrosine kinase type 1 insulin-like growth factor receptor (IGF-1R) has been recognized for its role in tumor cell proliferation and increased cancer risk (8–10). Its ligand, the IGF-1 hormone, is abundant in human serum and released primarily from the liver. The upregulation of intracellular signaling of IGF-1R leads to the activation of the PI3K/AKT and MAPK pathways promoting cancer development and progression through maintenance of cell proliferation and survival (9,11,12). Significant crosstalks between IGF-1R and a variety of other pathways, including those involving mesenchymal-epithelial transition factor (MET) and vascular endothelial growth factor (VEGFR), been shown to promote the development and progression of various cancers (10). A particular crosstalk between the IGF-1R and focal adhesion kinase (FAK) signaling pathways have been shown to be involved in the regulation of migratory and invasive behaviors associated with metastasis in mesenchymal TNBCs (13). Due to its interaction in complex networks, IGF-1R has been noted as an interesting therapeutic target.

microRNAs (miRNAs) are short single-stranded non-coding RNA involved in the post-transcriptional regulation (14). miRNAs negatively regulate gene expression through mechanisms involving translation repression by forming the RISC complex and binding to the 3' untranslated region (UTR), preventing interaction of polyadenylate-binding protein (PABP) on the 3' UTR with the 5' UTR that is required for ribosomal assembly or mRNA degradation by perfect complementary binding to mRNA (15,16). Studies have recently displayed that tumor suppressor or oncogenic miRNAs interact in pathways involving metastasis, apoptosis, and cell proliferation (14,17,18). As a result of its attractive potential therapeutic function, recent studies have analyzed the effect of expression of miRNA in a variety of cancers as they are located in cancer-linked genomic regions and involved in cancer-related pathways (18–20).

IGF-1R has been shown to be a direct target gene for microRNA-592 in hepatocellular carcinoma cells and the upregulation of the miR-592 resulted in decreased cell proliferation and cell invasion (20). The upregulation of microRNA-592 yielded significantly reduced levels of cell proliferation and invasion in lung cancer, hepatocellular carcinoma, and in gliomas (10,20–22). However, miRNA-592 regulation has not been extensively studied in breast cancer cells, especially in triple negative breast cancer cells. A recent study investigated the overexpression of miRNA-592 in breast cancer cell lines which resulted in downregulation of TGFβ-2, a direct target oncogene of miRNA-592 (23). miRNA profiling of TNBC cells in Latin American patients also revealed that miRNA-592 is expressed in TNBC cell lines and could be involved in cancer-related pathways (24).

It was hypothesized that the overexpression of miRNA 592 will inhibit the migration and cell growth of TNBC cancer cells by affecting the signaling of IGF-1R and FAK. This current study displays the connection with the expression of miRNA-592 and the regulation of the FAK pathway in TNBC cells through immunoblotting, wound healing assay, and clonogenicity assay. The results of this study demonstrate that miRNA-592 could be a worthy candidate for investigation in TNBC cell lines as a possible tumor suppressor.

Materials and Methods

Reagents

Radioimmunoprecipitation assay (RIPA) cell lysis buffer was from Cell Signaling Technology, Inc. (Beverly, MA, USA), Nonidet P-40 (NP40) cell lysis buffer was from Boston BioProducts (Ashland, MA, USA), phenylmethylsulfonyl fluoride (PMSF) was from NOVUS (Littleton, CO, USA), and EZ Block protease and phosphatase inhibitor cocktail was purchased from Bio Vision (San Francisco, CA, USA). Phosphate buffer saline (PBS) was from Mediatech (Manassas, VA, USA).

Cell Culture

MDA-MB-231 cell line was obtained from American Type Culture Collection (Manassas, VA, USA). This cell line was authenticated. Cells were routinely maintained in complete media, including Dulbecco's Modification of Eagle's Medium (DMEM) (CORNING) supplemented with 10% Fetal Bovine Serum (FBS) (CORNING) and 2?M L-glutamine (Invitrogen, Carlsbad, CA, USA). Cells were cultured in humidified incubators at 37°C with 5% CO2.

miR-592 Transfection

miR-592 Mimic (HMI0001-HMI2785) was a small, double-stranded RNA molecule utilized to imitate endogenous mature miR-592 molecules. miR-592 Mimic was commercially available from Sigma-Aldrich (St. Louis, MO, USA). A scrambled sequence miRNA (miR-NC) (HMC0002) was also used as a negative control (Sigma-Aldrich). To rehydrate the miR-592 and miR-NC, each pellet was mixed with 250?L of Molecular Grade Water from G-Biosciences (St. Louis, MO, USA) to produce 20?M stocks. INTERFERin transfection reagent was obtained from Polyplus (New York, NY, USA). FuGENE transfection reagent was obtained from Promega (Madison, WI, USA). Both reagents were utilized to optimize miRNA transfection according to the manufacturer's instruction. miR-592 and miR-NC were gently mixed with INTERFERin or FuGENE in serum-free DMEM and incubated at

room temperature for 10 minutes for complex formation. MDA-MB-231 cells were plated and grown to 70-80% confluency. After incubation, the complex was added to the plated cells. The untreated cells contained complete media only. Forty-eight hours post-transfection, cells were subsequently used in Western blotting, wound-healing assay, and clonogenicity assay as detailed below.

Western Blotting

Following 48-72 hours treatments, MDA-MB-231 cells were washed with PBS and harvested using RIPA or NP40 lysis buffer containing PMSF and protease/phosphatase inhibitors according to the manufacturer's protocol. The total protein concentration of each treatment following transfection was measured utilizing the Bradford Assay (Bio Rad; Hercules, CA, USA). Equal amounts of protein, 20 µg, for each sample was separated via SDS-PAGE at 80V for 20 minutes and 125 V for 45 minutes. The polyvinylidene difluoride (PVDF) membranes (Bio Rad) were activated with methanol for 5 minutes. The separated proteins were transferred to either methanol-activated PVDF or nitrocellulose membranes (Bio Rad) overnight at 4?C at 23V. The antibody against ?-actin, an internal loading control, was purchased from NOVUS (Littleton, CO, USA) were used for Western Blots according to standard protocol. Primary antibodies against IGF1R, anti-pIGF-1R were from Cell Signaling Technologies, Inc. (Danvers, MA, USA). Primary antibodies anti-FAK, anti-p-FAK were from BD Biosciences (San Jose, CA, USA). The goat anti-rabbit and goat anti-mouse secondary antibodies were from SouthernBiotech (Birmingham, AL, USA). Following a blocking incubation with 5% non-fat milk at room temperature for 2 hours, the membranes were incubated with rocking at 4@C overnight with primary anti IGF 1R, anti-pIGF-1R (dilution, 1:1,000) and anti-FAK, anti-p-FAK (dilution, 1:1,000), followed by incubation at room temperature for 1 hour with the goat anti-rabbit or goat anti-mouse horseradish peroxidase-conjugated secondary antibody (1:5,000-10,000 dilution). The proteins were developed with Luminata Classico Western horseradish peroxidase substrate from Millipore (Billerica, MA, USA) and visualized using the c300 Azure imaging system (Dublin, CA, USA).

Wound-Healing Assay

Cells were seeded in a 24-well and transfected at 80-90% confluency. At 100% confluency, the monolayer cells were wounded by scratching with a sterilized 200uL pipette tip, ensuring the width of each wound was the same. The wells were rinsed with PBS three times to remove floating cells and debris. Complete media was added, and wounds were immediately imaged (0hr time point). Cells were then incubated for 24 hours at 37°C. Pictures were acquired using a Motic AE31 microscope (Carlsbad, CA, USA), moticam 2500 digital camera from Motic software (Carlsbad, CA, USA) at indicated time (0hr, 24hr, and 48hr).

Clonogenicity Assay

Single cell suspensions were seeded in a 6-well plate at a density of 250 cells per well and allowed to grow. The following day, cells will be transfected with miR-592 or miR-NC as described above. After a 6-day treatment period, the media was removed. Cells were fixed in 10% ice-cold methanol, stained with crystal violet (0.1% in 20% methanol), and allowed to dry overnight. Colony numbers will be assessed visually and colonies containing >25 normal appearing cells were counted. Pictures were acquired using a Motic AE31 microscope (Carlsbad, CA, USA), moticam 2500 digital camera from Motic software (Carlsbad, CA, USA).

Statistical Analysis

For the clonogenicity assay results, the average colonies observed for each treatment and standard error bars produced were determined utilizing Microsoft Excel.

Results

miR-592 overexpression inhibits FAK but not IGF-1R expression in TNBC cells

The crosstalk between the IGF-1R and FAK pathways have been shown to be associated with the metastasis of TNBC tumor cell (13). To obtain evidence of the effects of overexpression of miR-592 on FAK expression in cultured TNBC cells, FAK protein levels were determined by Western blotting in the MDA-MB-231 cell line (Figure 1). Without treatment, MDA-MB-231 cells expressed high levels of p-FAK and FAK protein. The

introduction of miR-NC into TNBC with FuGENE for 48 hours resulted in less p-FAK protein expression than in the untreated cells. This negative control also caused much lower expression of FAK protein (Figure 1). As expected, following the transfection of miR-592 with the FuGENE reagent for 48 hours, the TNBC cells low levels of p-FAK and FAK, specifically undetectable levels for FAK protein.
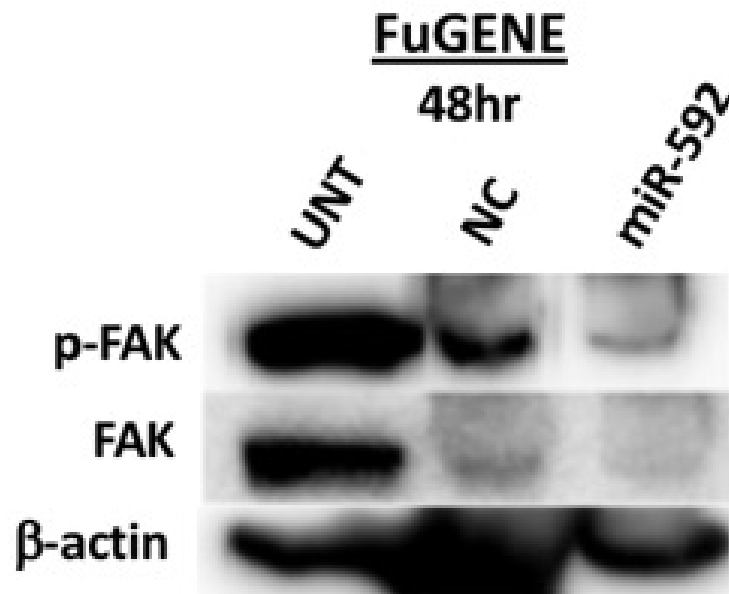

Figure 1. **Overexpression of miR-592 inhibits FAK expression in TNBC cells.** *Lysates of MDA-MB-231 cells transiently expressing miR-592 and miR-NC 48h post transfection with INTERFERin were immunoblotted with specific antibodies for pFAK, FAK, and beta actin loading control.*

To obtain evidence of the effects of overexpression of miR-592 on IGF-1R expression in cultured TNBC cells, IGF-1R protein levels were determined by Western blotting in the MDA-MB-231 cell line (Figure 2). Without treatment, the TNBC cells displayed moderate levels of p-IGF1R and low levels of IGF-1R. The transfection of the negative control with INTERFERin for 48 hours led to a significant reduction in p-IGF-1R levels and increased IGF-1R levels in comparison to the untreated cells (Figure 2). The introduction of miR-592 in TNBC cells with INTERFERin for 48 hours caused similar levels of p-IGF1R to the untreated cells. This transfection of miR-592 also led to higher levels of IGF-1R protein compared to both the untreated cells and miR-NC treatment (Figure 2).

To determine the optimal transfection time with INTERFERin in the inhibition of FAK protein, FAK protein levels were determined again by Western Blotting in the MDA-MB-231 cell line. It was noted that the first 48hr transfection displayed similarly high levels of p-FAK for the untreated cells, miR-NC transfected cells as shown in Figure 3. The second 48hr transfection displayed a gradual increase in p-FAK protein levels for the untreated, negative control, and miR-592 treatments groups. The untreated cells displayed undetectable levels of p-FAK protein. The miR-NC- and miR-592-transfected TNBC cells displayed similar levels of p-FAK protein. A similar gradual increase was noted in FAK protein expression in which the untreated cells expressed extremely low FAK protein and the TNBC cells transfected with miR-592 expressed the highest level of FAK. The cells transfected with miR-NC displayed low levels of FAK protein that was higher than that of the untreated cells. It was noted that the 72 hours was the optimal transfection duration for INTERFERin as the untreated and miR-
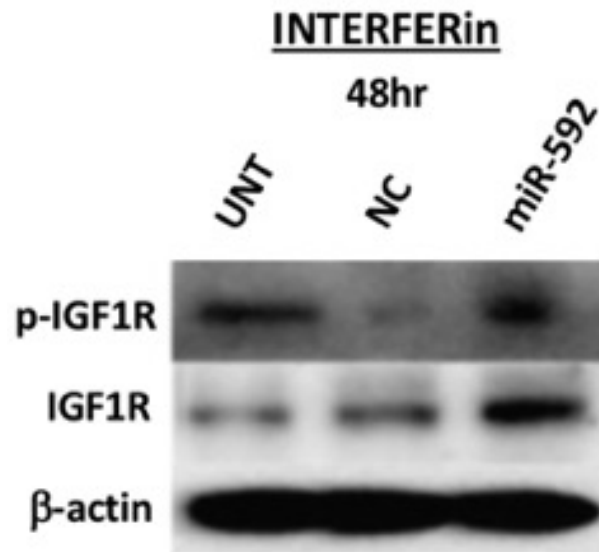
INTERFERin

48hr

UNT   NC   miR-592

p-IGF1R

IGF1R

β-actin

Figure 2. ***Overexpression of miR-592 inhibits FAK expression in TNBC cells.*** *Lysates of MDA-MB-231 cells transiently expressing miR-592 and miR-NC 48h post transfection with INTERFERin were immunoblotted with specific antibodies for pFAK, FAK, and beta actin loading control.*

NC-transfected cells displayed similarly high levels of both p-FAK and FAK protein. The cells transfected with miR-592 displayed the lowest levels of both p-FAK and FAK proteins with the 72hr transfection.
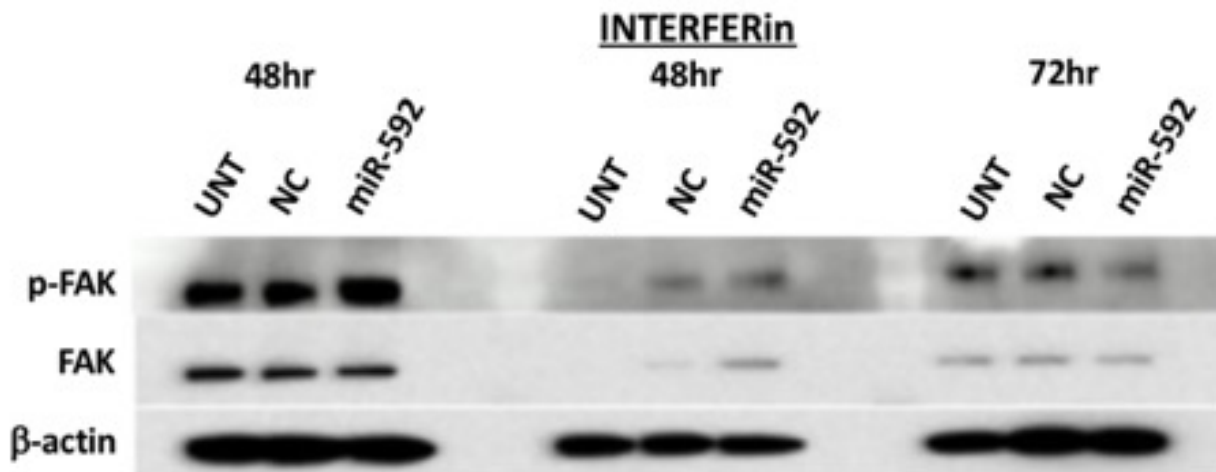

INTERFERin

48hr   **48hr**   **72hr**

UNT NC miR-592 UNT NC miR-592 UNT NC miR-592

p-FAK

FAK

β-actin

Figure 3. ***Optimization of miR-592 overexpression in TNBC cells using INTERFERin.*** *Lysates of MDA-MB-231 cells transiently transfected with miR-592 or miR-NC for 48-72 hours were immunoblotted with antibodies specific for pFAK, total FAK, and beta actin.*

miR-592 overexpression inhibits cell migration in TNBC cells

Cell migration is essential in the metastatic properties of malignant cells. In the present study, the effects of miR-592 on TNBC cell migration was assessed using wound-healing assay. As expected, the results displayed an involvement of miR-592 in the migratory abilities of TNBC cells. The overexpression of miR-592 resulted in a significant decrease in cell migration in MDA-MB-231 cells compared with that of the untreated cells and miR-NC-transfected cells. The introduction of miR-NC also displayed a low degree of cell migration inhibition in comparison to the untreated TNBC cells.
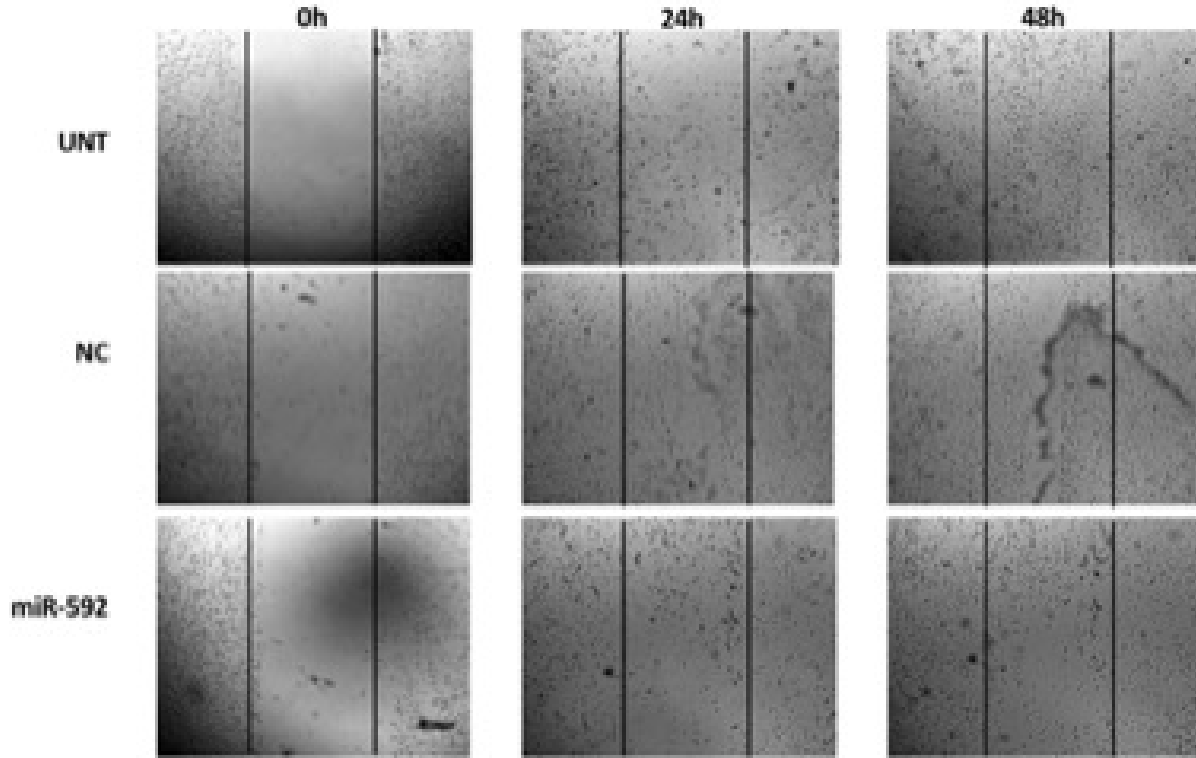

Figure 4. *Overexpression of miR-592 suppresses cell migration in TNBC. Following transfection with FuGene at 90% confluency, cell monolayers were carefully wounded and incubated in complete mediat. Representative images of different treatments of wound-healing assay in which miR-592 inhibited the migration of MDA-MB-231 cells over a 48 hour time period (t=0h, 24h, and 48h).*

miR-592 overexpression suppresses TNBC cell colony formation

Colony formation is also essential to the metastatic properties and tumorigenesis capabilities of metastatic cells. Interestingly, the overexpression of miR-592 resulted in a substantial decrease in colony formation of MDA-MB-231 (26 fold) cells in comparison to the untreated cells. The results of this study show that the introduction of miR-NC in MDA-MB-231 cells also resulted in a substantial decrease in colony formation (7.5 fold). The standard error of the average number colonies for UNT, miR-NC, and miR-592 were 15.4, 3.5, 1.76, respectively. These results indicate that miR-592 effectively inhibits colony formation in TNBC cells.

Discussion

Although it has been previously shown to be involved in cancer-related pathways, including Hippo, TGF-?, and Ras signaling pathways, miR-592 overexpression has never been specifically associated with the IGF-1R-FAK
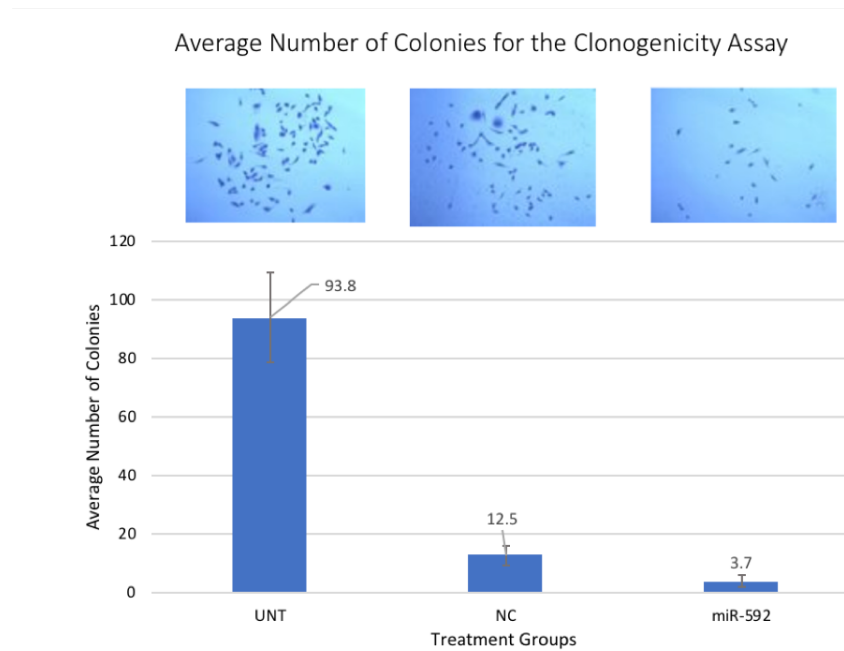
Average Number of Colonies for the Clonogenicity Assay

Figure 5. *Overexpression of miR-592 inhibits colony formation in TNBC cells.* *Clonogenicity assays were performed as described above using MDA-MB-231 transiently transfected with miR-NC or miR-592. 7-10 days post transfection, colonies containing >20 normal-appearing cells were counted. Representative colonies of each treatment.*
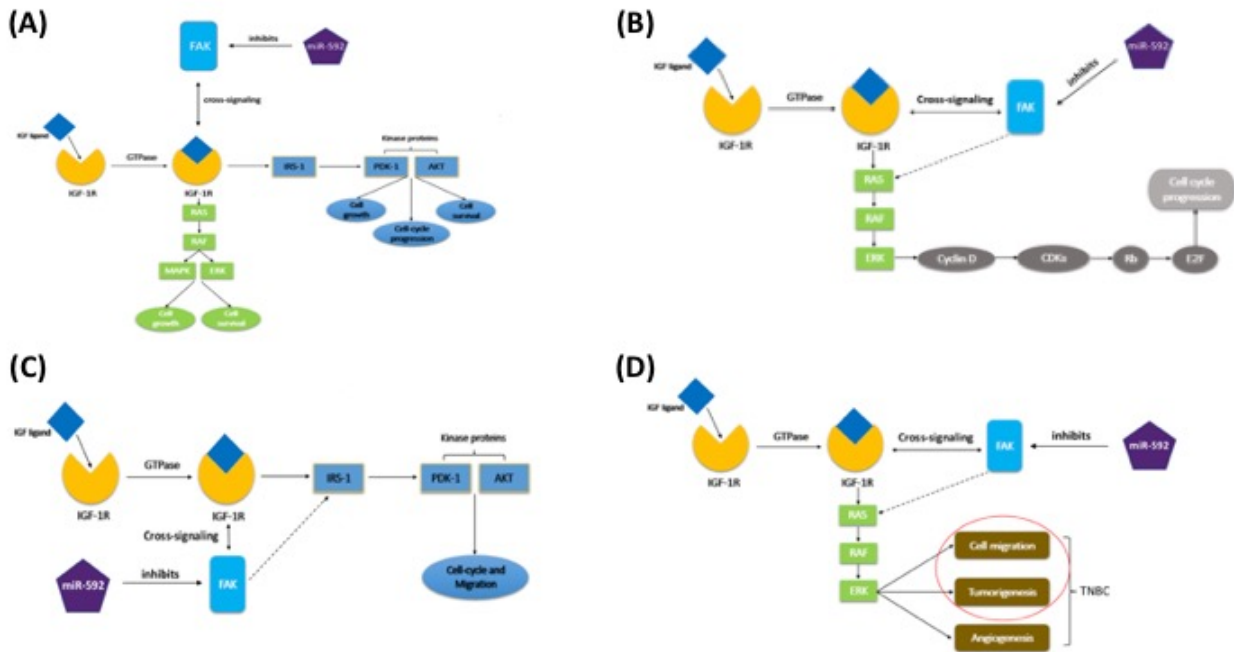

Figure 6. *Proposed models of miR-592's inhibitory effects on the IGF1R/FAK signaling pathway in TNBC cells.*

signaling pathway in TNBC (24). The present study demonstrates a novel role of the overexpression of miR-

592 in FAK expression, the regulation of cell migration and colony formation, and also provides a new insight in downstream processes regulated by IGF-1R and FAK in TNBC cells. Because TNBC is greatly in need of new therapies, the role of miR-592 in the particular subtype was analyzed. It was hypothesized that the overexpression of miRNA 592 will inhibit the migration and cell growth of TNBC cancer cells by affecting the signaling of IGF-1R and FAK.

This is the first study to demonstrates that FAK expression is reduced by the overexpression of miR-592 in TNBC cell line MDA-MB-231. It was found that the optimal transfection elongation of miR-592 for significant suppression of FAK expression with INTERFERin was 72 hours. Utilizing FuGENE to conduct transfections at different durations would further exhibit the effect of transfection optimization in FAK expression levels. It was also demonstrated that miR-592 overexpression is critical for inhibiting the potentiation of cell migration and colony formation of TNBC cells.

Another major finding from this study is that miR-592 does not decrease the expression of IGF-1R with 48hr transfection. This was unexpected as the crosstalk between IGF-1R and FAK in TNBC has been previously shown (13). The unexpected results may be explained by the inadequacy of the primary antibodies for p-IGF-1R and IGF-1R that constantly demonstrated streaking upon development in imaging. Another experiment could be run primary antibodies from a different manufacturer and also utilizing the FuGENE transfection reagent to truly evaluate the role of miR-592 on IGF-1R expression in TNBC.

For transfection, the miR-NC did not display expected results. The immunoblotting aimed to determine the effect of the overexpression of miR-592 on FAK expression in TNBC. Interestingly, the miR-NC-transfected cells did not display the high levels of the FAK exhibited in the untreated cells. Instead, the FAK levels with the negative control were intermediate between those of the untreated cells and the miR-592-transfected cells. Similarly, it was found that cell migration was decreased slightly by the transfection of miR-NC in TNBC cells. The rate of cell migration was similar to that of the untreated cells, but it was clear that there was a slight inhibitory effect. Surprisingly, there was an unexpected and significant decrease in colony formation resulting from the introduction of the miR-NC Mimic in TNBC cells. These unexpected results in FAK expression levels, cell migration, and colony formation by the ineffectiveness of the scramble sequence of miR-NC that was intended to exhibit no effect on known miRNA function in the cells. There are possibly common sequences between miR-NC and miR-592 that contribute to the intermediate effects displayed. Utilizing a miR-NC Mimic that has been tested to display no effect on miRNA expression in the MDA-MB-231 cells would be beneficial in the future.

IGF signaling is a crucial factor in the limited survival associated with drug resistance in breast cancer (25). Interestingly, activation of IGF-1R by the IGF ligand can result in the signaling of both the RAS/MAPK and PDK-1/AKT pathways. Decreasing the expression of IGF-1R reduces cellular proliferation in the RAS/MAPK kinase cascade and reduces cell cycle progression and cell growth/survival via the PDK-1/AKT pathway (11). Considering the cross-signaling between IGF-1R and FAK protein, a model was proposed of the regulation of cell growth, cell survival, and cell-cycle progression through the reduction of FAK expression by miR-592 overexpression in TNBC cells (Figure 6A).

Cyclin D1 is a known regulator of the progression from the G1 to S phase. FAK signaling pathways can increase the transcription of cyclin D1 which is rate-limiting in cellular proliferation (26). A model was proposed of the suppression of cyclin D through the overexpression miR-592 (Figure 6B). FAK activates RAS is a kinase cascade resulting in the activation of ERK which leads to increase expression of the cyclin D1 (26). As shown in this study, the overexpression of miR-592 has depressive effects on FAK levels in TNBC. This decrease of FAK expression leads to the decrease of cyclin D1 available to progress cells from G1 to S phase is cellular proliferation, resulting in reduced cell growth in TNBC.

Epithelial-mesenchymal transition (EMT) is a major source of mesenchymal cells involved in metastasis and tumor invasiveness (27). The metastasis of epithelial cancer cells involves the cell migration of differentiated cells. As it was observed in this study, cell migration was decreased by miR-592 overexpression in TNBC. A pathway was proposed in which the reduction FAK expression by miR-592 overexpression prevents the activation of IRS-1 in a kinase cascade resulting in cell migration (Figure 6C). This is a possible explanation of the means by which cell migration was reduced by the overexpression of miR-592 in this study. The overexpression miR-7 has been shown inhibit EMT and metastasis of breast cancer cells by reducing FAK expression (19). miR-592 may exhibit similar effects involving EMT and tumorigenesis in TNBC.

In esophageal squamous cells, Rab25 phosphorylates FAK via a deregulated MAPK/ERK signaling pathway to drive tumorigenesis, angiogenesis, and migration invasion (28). It was also shown the miR-2324 inhibits EMT and cell invasion by directly regulating Rab25 (16). Colony formation is an indication of tumorigenesis potential of cancer cells. It was demonstrated that colony formation was reduced in TNBC with the overexpression of miR-592. Noting the Rab25 signaling pathway, a model was proposed in which the overexpression of miR-592 leads to decreased activation of RAF by FAK in the signaling pathway leading to tumorigenesis and cell formation (Figure 6D). It remains to be determined if miR-592 overexpression affects Rab25 directly in its decrease of FAK expression. Identifying the other signaling pathways in TNBC miR-592 effects would give more information about potential sites of therapeutic intervention.

p53 is a tumor repressor gene known for inhibiting the progression of the cell cycle through affecting levels of proteins involved in cell cycle progression, such as GADD45, p21, 14-3-3σ, and cyclin B (29). miR-30a is involved in the restraint of ZEB2 expression by p53. This restraint prevents EMT, tumor cell migration, and drug resistance in TNBC (29). Demonstrating the relationship that miR-592 has with p53 levels or ZEB2 expression would be beneficial in determining biological markers to target for treatment development.

This study may be improved by including a vehicle control in which the transfection reagent, INTERFERin or FuGENE, is administered to the MDA-MB-231 cells in the same manner as it was in the miR-NC- and miR-592-transfected cells. This would ensure that the decreased levels of FAK expression, cell migration, and colony formation displayed resulted solely from the involvement of miR-592 and not the transfection reagent utilized. A comparison to a vehicle control would also reveal the direct effects of the scramble sequence incorporated in the miR-NC Mimic utilized in transfection.

The development of targeted gene therapies aimed at the prevention of tumorigenesis and epithelial-mesenchymal transition is essential to attacking the aggressively metastatic behavior of TNBC. Through this study, the role of the overexpression of miR-592 on the suppression of FAK expression, cell migration, and colony formation in TNBC cells is displayed. The data obtained in this study suggests that through its inhibition of FAK expression, miR-592 may inhibit cell cycle progression, cell growth, survival and migration relevant to EMT in cancer cells through various signaling pathways. Based on what this study demonstrates, miR-592 is a potentially important biomarker in TNBC. These findings also warrant future investigation of the mechanism of action of miR-592 in the IGF-1R/FAK signaling pathway in targeting drug resistance in TNBC.

References

1.   Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, et al. Triple-Negative Breast Cancer: Clinical Features and Patterns of Recurrence. Clin. Cancer Res. 2007; 13:4429-34.

2.   Papa A, Caruso D, Tomao S, Rossi L, Zaccarelli E, Tomao F. Triple-negative breast cancer: investigating potential molecular therapeutic target. Expert Opin Ther Targets. 2015;19:55–75.

3.   Tomao F, Papa A, Zaccarelli E, Rossi L, Caruso D, Minozzi M, et al. Triple-negative breast cancer: new perspectives for targeted therapies. OncoTargets Ther. 2015;8:177–93.

4.   Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, et al. Race, Breast Cancer Subtypes, and Survival in the Carolina Breast Cancer Study. JAMA. 2006;295:2492–502.

5.   Lund MJ, Trivers KF, Porter PL, Coates RJ, Leyland-Jones B, Brawley OW, et al. Race and triple negative threats to breast cancer survival: a population-based study in Atlanta, GA. Breast Cancer Res Treat. 2009;113:357–70.

6.   Choi YJ, Seong MH, Choi SH, Kook SH, Kwag HJ, Park YL, et al. Ultrasound and Clinicopathological Characteristics of Triple Receptor-Negative Breast Cancers. J Breast Cancer. 2011;14:119–23.

7.   Lin Y, Liu R, Zhao P, Ye J, Zheng Z, Huang J, et al. Discovery of novel mifepristone derivatives via suppressing KLF5 expression for the treatment of triple-negative breast cancer. Eur J Med Chem. 2018;146:354–67.

8.   Pollak M. The insulin and insulin-like growth factor receptor family in neoplasia: an update. Nat Rev Cancer. 2012;12:159–69.

9.   Chen HX, Sharon E. IGF-1R as an anti-cancer target—trials and tribulations. Chin J Cancer. 2013;32:242–52.

10.   Liu Z, Wu R, Li G, Sun P, Xu Q, Liu Z. MiR-592 inhibited cell proliferation of human colorectal cancer cells by suppressing of CCND3 expression. Int J Clin Exp Med. 2015;8:3490–7

11.   Denduluri SK, Idowu O, Wang Z, Liao Z, Yan Z, Mohammed MK, et al. Insulin-like growth factor (IGF) signaling in tumorigenesis and the development of cancer drug resistance. Genes Dis. 2015;2:13–25.

12.   Tao Y, Pinzi V, Bourhis J, Deutsch E. Mechanisms of Disease: signaling of the insulin-like growth factor 1 receptor pathway—therapeutic perspectives in cancer. Nat Rev Clin Oncol. 2007;4:591–602.

13.   Taliaferro-Smith L, Oberlick E, Liu T, McGlothen T, Alcaide T, Tobin R, et al. FAK activation is required for IGF1R-mediated regulation of EMT, migration, and invasion in mesenchymal triple negative breast cancer cells. Oncotarget. 2015;6:4757–72.

14.   Iorio MV, Croce CM. microRNA involvement in human cancer. Carcinogenesis. 2012;33:1126–33.

15.   Fabian MR, Sonenberg N. The mechanics of miRNA-mediated gene silencing: a look under the hood of miRISC. Nat Struct Mol Biol. 2012;19:586–93.

16.   Yin C, Mou Q, Pan X, Zhang G, Li H, Sun Y. MiR-577 suppresses epithelial-mesenchymal transition and metastasis of breast cancer by targeting Rab25. Thorac Cancer. 2018;9:472–9.

17.   Tessitore A, Cicciarelli G, Mastroiaco V, Vecchio FD, Capece D, Verzella D, et al. Therapeutic Use of MicroRNAs in Cancer [Internet]. Anticancer Agents Med. Chem. 2015 [cited 2018 May 2]. Available from: http://www.eurekaselect.com/134277/articl

18.   Lu M, Wang Y, Zhou S, Xu J, Li J, Tao R, et al. MicroRNA-370 suppresses the progression and proliferation of human astrocytoma and glioblastoma by negatively regulating β-catenin and causing activation of FOXO3a. Exp Ther Med. 2018;15:1093–8.

19.   Kong X, Li G, Yuan Y, He Y, Wu X, Zhang W, et al. MicroRNA-7 Inhibits Epithelial-to-Mesenchymal Transition and Metastasis of Breast Cancer Cells via Targeting FAK Expression. PLoS ONE [Internet]. 2012 [cited 2018 May 2];7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3410899/

20.   Wang W, Zhang H, Tang M, Liu L, Zhou Z, Zhang S, et al. MicroRNA-592 targets IGF-1R to suppress cellular proliferation, migration and invasion in hepatocellular carcinoma. Oncol Lett. 2017;13:3522–8.

21.   Li Z, Li B, Niu L, Ge L. miR-592 functions as a tumor suppressor in human non-small cell lung cancer by targeting SOX9. Oncol Rep. 2017;37:297–304.

22.   Peng T, Zhou L, Qi H, Wang G, Luan Y, Zuo L. MiR-592 functions as a tumor suppressor in glioma by targeting IGFBP2. Tumor Biol. 2017;39:1010428317719273.

23.   Hou W, Zhang H, Bai X, Liu X, Yu Y, Song L, et al. Suppressive role of miR-592 in breast cancer by repressing TGF-β2. Oncol Rep. 2017;38:3447–54.

24.   Sugita B, Pereira SR, Almeida R, Lima RS, Urban CA, Boca S, et al. Abstract A28: Copy number and miRNA profiling in triple-negative breast cancer patients from Latin America. Clin Cancer Res. 2018;24:A28–A28.

25.   Florea A-M, Büsselberg D. Breast cancer and possible mechanisms of therapy resistance. J Local Glob Health Sci. 2013;2.

26.   Zhao J, Pestell R, Guan J-L. Transcriptional Activation of Cyclin D1 Promoter by FAK Contributes to Cell Cycle Progression. Mol Biol Cell. 2001;12:4066–77.

27.   Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest. 2009;119:1420–8.

28.   Tong M, Chan KW, Bao JYJ, Wong KY, Chen J-N, Kwan PS, et al. Rab25 Is a Tumor Suppressor Gene with Antiangiogenic and Anti-Invasive Activities in Esophageal Squamous Cell Carcinoma. Cancer Res. 2012;72:6024–35.

29. Gennaro A di, Damiano V, Brisotto G, Armellin M, Perin T, Zucchetto A, et al. A p53/miR-30a/ZEB2 axis controls triple negative breast cancer aggressiveness. Cell Death Differ. 2018;1.