MicroRNA-192-5p inhibits migration of triple negative breast cancer cells and directly regulates Rho GTPase activating protein 19

Beate Vajen | Luisa Greiwe | Vera Schäffer | Marlies Eilers | Nicole Huge | Amelie Stalke | Brigitte Schlegelberger | Thomas Illig | Britta Skawran

Department of Human Genetics, Hannover Medical School, Hannover, Germany

Abstract

Among the different breast cancer subtypes, triple-negative breast cancer (TNBC) is associated with a poor prognosis, low survival rates, and high expression of histone deacytases. Treatment with histone deacytase inhibitor trichostatin A (TSA) leads to an increased expression of potential tumor-suppressive miRNAs. Characterization of these miRNAs can help to find new molecular targets for treatment of TNBC. We identified differentially expressed miRNAs by microarray analyses after treatment with TSA in the TNBC cell lines HCC38, HCC1395, and HCC1935. The gene locus of hsa-miRNA-192-5p (miR-192) and hsa-miR-194-2 (miR-194-2) with its host gene, long noncoding RNA miR-194-2HG, has been linked to inhibition of migration in different tumor types. Therefore, we examined tumor-relevant functional effects using WST-1-based proliferation, capsase-3/7-based apoptosis, and trans-well migration assays after transfection with miRNA mimics or specific siRNAs. We demonstrated the tumor-suppressive capacity of miR-192 in TNBC cells, which was exerted through inhibition of proliferation, induction of apoptosis, and reduction of migration.

Gene expression and bioinformatics analyses of TNBC cell lines transfected with miR-192 mimics, identified a number of genes involved in migration including the Rho GTPase Activating Protein ARHGAP19. Through RNA immunoprecipitation we demonstrated the direct binding of miR-192 and ARHGAP19. Downregulation of ARHGAP19 expression by either miR-192 or siRNA inhibited migration of TNBC cells significantly. Our findings demonstrate that overexpression of epigenetically deregulated miR-192 decreases proliferation, promotes apoptosis, and inhibits migration of TNBC cell lines.

Abbreviations: 3'UTR, 3' untranslated region; Ago2, Argonaute RISC catalytic component 2; ATCC, American Type Culture Collection; ARHGAP19, Rho GTPase activating protein 19; BSA, BRCA1/2; ChIP, chromatin immunoprecipitation; DNA, deoxyribonucleic acid; EGTA, Ethylene glycol-bis-[β-aminoethyl ether]-N,N,N',N'-tetraacetic acid; HDAC, histone deacytase; HER2, human epidermal growth factor receptor 2; HRP, horseradish peroxidase; IF, immunofluorescence; IP, immunoprecipitation; miR-192, microRNA-192; miR-194, microRNA-194; IncRNA miR194-2HG, long noncoding RNA miR194-2HG; PBS, phosphate buffered saline; PIPES, piperazine-N,N'-bis(2-ethanesulfonic acid); qRT-PCR, quantitative real time PCR; RIPA, radioimmunoprecipitation; RNA, ribonucleic acid; siRNA, small interfering ribonucleic acid; TNBC, triple negative breast cancer; TSA, trichostatin A; WST, water soluble tetrazolium; wt, wildtype.

Beate Vajen and Luisa Greiwe contributed equally to this study.

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1 | INTRODUCTION

TNBC is defined by the lack of expression of estrogen and progesterone receptors and no overexpression or amplification of HER2, an epidermal growth factor receptor.1 TNBC is an aggressive tumor type associated with a poor prognosis. So far, there are only limited therapeutic options.2 Hence, it is absolutely necessary to discover new treatment options for triple negative breast cancers.

HDACs are overexpressed in many tumor entities, resulting in hypoacetylation in different areas of the genome that leads to reduced transcription and is associated with poor prognosis.3,4 In addition to altered gene expression, miRNA expression may also be reduced. These short, noncoding RNAs regulate gene expression post-transcriptionally by binding to complementary sites at 3' UTR of specific target mRNAs.5 Global downregulation of miRNAs results in disordered proliferation, apoptosis, and differentiation and, therefore, makes them interesting therapeutic targets.6 We have previously reported that increased acetylation leads to induced expression of different miRNAs such as the miR-449 family or miR-129-5p in liver cancer.7 Since HDAC inhibition opposes a reduction of acetylation, the HDAC-inhibitor TSA was here used to identify epigenetically regulated tumor-suppressive miRNAs.

2 | MATERIALS AND METHODS

2.1 | Cell culture and transfection

TNBC cell lines HCC38, HCC1395, and HCC1937, and immortalized normal breast cell line MCF12A (all obtained from the American Type Culture Collection [ATCC], Manassas in 2015) were cultured as recommended by the ATCC. All cell lines were recently authenticated by STR analyses by Eurofins, (Ebersberg, Germany) and tested for mycoplasma contamination by PCR Mycoplasma Test Kit (Promo Cell, Heidelberg, Germany). For treatment with TSA (100 ng/mL) or ethanol vehicle containing 1% FCS for serum starvation. After 24 h serum starvation, 50 000 cells were seeded in 250 μl RPMI-1640 medium containing 1% FCS in the upper part of a transwell (8-μm pore size, Corning, Corning, New York) with the lower part filled with 750 μl RPMI-1640 containing 10% FCS. After 24 h, nonmigrated cells on the upper surface of the transwell were removed twice with a cotton swab. Migrated cells on the lower surface of the transwell were fixed with methanol, stained with Giemsa, and inspected by light microscopy. Cells were photographed and counted in six randomly selected fields.

2.2 | Chromatin immunoprecipitation

ChIP was performed as described previously.7 In brief, after TSA treatment of HCC38 cells for 1 h, DNA and proteins were crosslinked with 1% formaldehyde for 10 min and the reaction was terminated by the addition of glycine to a final concentration of 0.125 M. Cells were washed twice with PBS, lysed and sonicated with a Covaris S2/E210 (Covaris, Woburn, Massachusetts). Per reaction, 75 μg DNA was precleared overnight with protein G magnetic beads (Merck, Darmstadt, Germany). After removing the beads, samples were incubated with 3 μl antibodies for 6 h and then with 40 μl beads overnight. Complexes of DNA/protein were eluted and crosslinking was reversed at 65°C overnight. DNA was purified with a PCR purification kit (Qiagen), and quantitative PCR was performed using primers specific for the IncRNA miR194-2HG promoter. Details are provided in the Supporting information (Appendix S1).

2.3 | Proliferation, apoptosis, and migration assay

Cell viability and apoptosis were measured in triplicate using the WST-1 Proliferation Reagent (Roche, Basel, Switzerland) and the Caspase3/7 Glo Assay (Promega, Madison, Wisconsin, USA), respectively. For migration assays, cells were transfected in six-well plates. After 24 h, the medium was changed to RPMI-1640 medium containing 1% FCS for serum starvation. After 24 h serum starvation, 50 000 cells were seeded in 250 μl RPMI-1640 medium containing 1% FCS in the upper part of a transwell (8-μm pore size, Corning, Corning, New York) with the lower part filled with 750 μl RPMI-1640 containing 10% FCS. After 24 h, nonmigrated cells on the upper surface of the transwell were removed twice with a cotton swab. Migrated cells on the lower surface of the transwell were fixed with methanol, stained with Giemsa, and inspected by light microscopy. Cells were photographed and counted in six randomly selected fields.

2.4 | Analysis of mRNA, miRNA, and protein expression

Total RNA, including miRNAs, was isolated using the Qiazol Lysis Reagent and the miRNeasy Mini Kit (both Qiagen). Expression of mRNA and miRNA was measured in triplicate by quantitative real-time PCR (qRT-PCR) using Taqman Gene Expression Assays and Taqman MicroRNA Assays (both Life Technologies, details provided in the Supporting Information, Appendix S1).

For protein analysis, whole cell lysates were prepared with RIPA buffer employing equal numbers of cells. Lysates were sonicated three times for 10 s with 1 min breaks on ice. Cell debris was removed by centrifugation at 4°C and 16 200 x g for 20 min. Proteins were separated on 12% SDS polyacrylamide gels and transferred to nitrocellulose membranes. Membranes were blocked with 5% milk in PBS.
including 0.05% Tween-20 (PBS-T) for 1 h. Membranes were incubated with primary antibodies overnight at 4°C. After washing with PBS-T 3× for 5 min, membranes were incubated with HRP-conjugated secondary antibodies for 1 h. All antibodies used are listed in the Supporting Information (Appendix S1). After washing, the membranes with PBS-T 3× for 5 min, protein bands were detected with

**FIGURE 1**  Histone deacetylase inhibitor TSA induces expression of miR-192/miR-194-2 cluster in TNBC cell lines. (A) Intersection of upregulated miRNAs (≥log2 fold change) after TSA treatment in HCC38, HCC1395, and HCC1937 compared to ethanol treated cells (unpaired Students t-test, cutoff p < 0.05). (B) miR-192 and miR-194-2 are encoded in a cluster in the second exon of IncRNA miR-194-2HG. Sequences of miR-192 and miR-194-2 are given with seed sequences highlighted in gray. (C) Relative expression of miR-192, miR-194-2, and IncRNA miR194-2HG in TSA treated TNBC cell lines and in non-tumorigenic cell line MCF12A compared to ethanol treated cells measured by qPCR. (D) ChIP of histone acetylation levels of H3K9, H4K5, H4K8, H4K12, and H4K16 in the miR194-2HG promoter in HCC38 cells after TSA treatment in relation to the total amount of H3 or H4.
2.5 | RNA immunoprecipitation

In brief, miRNA-192 vs control transfected HCC38 cells were lysed in 100 μl polysomal lysis buffer. Then, dynabeads (Invitrogen) were prepared and incubated with control IgG or Ago2 antibody. To control and monitor successful immunoprecipitation (IP), an aliquot of beads was taken and Ago2 pulldown was analyzed by Western Blot. After successful IP, RNA was isolated by the use of TRIZOL (Sigma-Aldrich). Isolated RNA from Ago2-IP was subjected to qPCR for validation. Details are provided in the Supporting Information (Appendix S1).

2.6 | Statistics

Data are represented as mean ± SD of at least three independent experiments unless stated otherwise. In figure legends, n represents the number of independent experiments. Statistical significance was determined by two-tailed Student’s t-test, by one-way ANOVA followed by Dunnett’s multiple comparison test or by two-way ANOVA with the Geisser-Greenhouse correction. All statistical analyses were completed with the GraphPad Prism software 8 (GraphPad Software, La Jolla, California).

3 | RESULTS

3.1 | Histone deacetylase inhibitor TSA induces expression of miR-192/miR-194-2 in TNBC cell lines

To identify epigenetically regulated microRNAs, TNBC cell lines HCC38, HCC1937 and HCC1937, as well as the immortalized breast cell line MCF12A, were treated with TSA. After treatment with TSA we measured a significant induction of acetylation in all cell lines (Figure S1). Expression analyses of at least twofold deregulated miRNAs after TSA treatment identified 82 downregulated and 140 upregulated miRNAs in breast cancer cell lines (Tables S1 and S2). By comparing miRNA expressions that were at least two-fold increased after TSA treatment in all analyzed cell lines we identified five miRNAs: miR-129-2-3p, miR-449a, miR-4730, miR-192, and miR-194-2 to be upregulated (Figure 1A). By viewing literature focusing on the prognostic role of the different miRNAs in human tumors, we decided to analyze miR-192 and miR-194-2. Analyses by the public cBioPortal site of the Center for Molecular Oncology of the Memorial Sloan Kettering Cancer Center showed that the highest copy number alteration frequency of miR-192 and miR-194-2 with 3.2% was found in the BRCA (INSERM 2016) cohort within all analyzed breast cancer cohorts (Figure S2).10 Although the percentage of breast cancer patients with altered miR-192/miR-194-2 copy numbers is low, breast cancer patients with altered miR-192 copy number show a significantly poorer survival compared to patients with unaltered miR-192 copy number (Figure S2). To analyze the expression of miR-192 and miR-194-2 in different breast cancer subtypes and normal breast-like tissue, we performed meta-analyses on miRNA expression using bc-GenExMiner 4.5,11 a statistical mining tool of published annotated breast cancer transcriptomic data from DNA microarrays and RNA-sequencing. For the expression analysis of miR-192 the following datasets GSE55348,12 GSE36295,13 GSE37751,14 and GSE8616615 were used. The different breast cancer subtypes defined by different expression patterns by Sorlie et al.16 showed no significantly different expression of miR-192 compared to normal breast-like tissue (Figure S3A). Furthermore, there was no significant difference in expression comparing non-TNBC and TNBC samples (Figure S3B). The expression data of miR-194-2 were available in dataset GSE96058.17 Here, we did not observe significant differences in miR-194-2 expression of the different breast cancer subtypes and normal breast-like tissue (Figure S3C) or when comparing non-TNBC and TNBC samples (Figure S3D).

Both miRNAs (miR-192 and miR-194-2) are encoded as a cluster within the long noncoding RNA IncRNA miR194-2HG (Figure 1B). The gene for IncRNA miR194-2HG is located within the cytogenetic band 11q13.1 on position 64,889,560-64,893,449.18 The UCSC Genome Browser19 shows an increase in H3K27 acetylation within the transcription start site of miR-194-2HG (Figure S4). Based on this, we measured the expression of miR-192, miR-194-2 (hereafter referred to miR-194), and of IncRNA miR194-2HG in all cell lines after TSA treatment by quantitative PCR. Expression of miR-192, miR-194, and IncRNA miR194-2HG was strongly induced by TSA in the normal breast cell line MCF12A and in the TNBC cell lines HCC1395, HCC1937, and HCC38a (Figure 1C). The strongest induction was observed in HCC38 cells with a 5- to 14-fold induction of expression. In order to further investigate the HDAC-dependent induction of the miR-192/miR-194 cluster, we performed ChIP experiments. Following TSA treatment of the TNBC cell line HCC38 we detected an increased acetylation at histones H3 (H3K9) and H4 (H4K5, H4K8, H4K12, and H4K16) in the promoter region of IncRNA miR194-2HG (Figure 1D).

3.2 | High expression of miR-192 leads to reduced cell viability, induced apoptosis and decreased migratory capacity

In order to investigate tumor-suppressive effects of miR-192 and miR-194, we performed transient transfections of TNBC cells with miR-192 or miR-194 mimics and, in addition, a miR-192/miR-194 combination. High expression of miR-192, miR-194, and the combination miR-192/miR-194 resulted in decreased proliferation in the TNBC cell lines HCC38, HCC1395, and HCC1937 (Figure 2A). The strongest reduction in cell viability was observed by transfection of miR-192 mimics. Cell viability was reduced by miR-192 mimics by 75% in HCC38 cells and by 50% in HCC1937 cells. Furthermore, we detected a strong induction of apoptosis after transfection of miR-192, miR-194, and the combination miR-192/
miR-194 in all TNBC cell lines (Figure 2B). Here, the most prominent effect was obvious by transfection of miR-192 mimics. The relative apoptosis was seven-fold induced in HCC38 cells and three-fold in HCC1937 cells after 96 h. In MCF12A cells, we detected no significant effect on proliferation or apoptosis by transfection of these miR mimics (Figure S5). Notably, by performing migration assays, we observed a significant reduction in the migration capacity of HCC38 cells after transfection with miR-192 mimics and the combination miR-192/miR194 (Figure 2C,D). The reduction in migration capacity by miR-192 mimics was also obvious, when migration
was normalized by proliferation (Figure S6). Furthermore, transfection of miR-192 mimics reduced the migration capacity of the other TNBC cell lines, HCC1395 and HCC1937, although not significant and to a lesser extent (Figures S7 and S8). We, therefore, decided to focus on the identification of target genes that promote the tumor-suppressive effect of miR-192 in TNBC.

**FIGURE 3** ARHGAP19 is a direct target of miR-192. (A) Deregulated mRNAs after transfection with miR-192 mimics identified by intersection of downregulated mRNAs (≥log1.5-fold change) in the TNBC cell lines HCC38 and HCC1937 compared to miR-control transfected cells (unpaired Student’s t-test, cutoff p < 0.05). Only putative target genes of miR-192, identified by targetscan are shown. (B) Expression of ARHGAP19 on mRNA and protein level after transfection of HCC38 cells with miR-192 mimics compared to miR-control transfected cells. The full-length blot is presented in Figure S9. (C) RIP was performed after transfection with miR-192 mimics. ARHGAP19 expression was measured by RT PCR before RIP. (D) Enrichment of ARHGAP19 measured by RT PCR after RIP. *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001, ****p ≤ 0.0001, Student’s t-test.
3.3 | ARHGAP19 is a new direct target of miRNA-192

To identify new target genes regulated by miR-192, we performed mRNA expression analyses in the TNBC cell lines HCC38 and HCC1937. We identified 70 differentially expressed genes after miR-192 mimic transfection, that were downregulated (FC < 1.5) in both cell lines and predicted targets of miR-192 by online target identification tools (ie, miRmap, Targetscan, and miRanda) (Table S2 and Figure 3A). Based on literature data, focusing on migration, we decided to analyze the putative target gene ARHGAP19 (Rho GTPase-activating protein 19). Indeed, ARHGAP19 expression on thr mRNA as

FIGURE 4 Functional effects of reduced ARHGAP19 expression in HCC38 cells. (A) ARHGAP19 expression on mRNA and protein level 72 h after transfection of HCC38 cells with siARHGAP19 #6 and siARHGAP19 #7 compared to control transfected cells. The full-length blot is presented in Figure S11. (B) Cell viability analyzed by WST-1 assay, normalized to si-control (dotted line). (C) Apoptosis analyzed by caspase3/7 activity, normalized to cell viability and si-control (dotted line). (D) Transwell assays 48 h after transfection. Representative photographs are shown. (E) Analyses of migrated cells per well. *p < 0.05, **p < 0.01, one-way ANOVA/Dunnett’s multiple comparison test
well as on the protein level was significantly reduced by 50–52% in all three TNBC cell lines after transfection with miR-192 mimics (Figure 3B, corresponding original Western Blot; Figure S9). To analyze if ARHGAP19 is a direct target of miR-192, we performed RNA immunoprecipitation. After confirming decreased expression of ARHGAP19 in HCC38 cells transfected with miR-192 mimics (Figure 3C), we detected a significant enrichment (seven-fold vs enrichment of input) of ARHGAP19 following transfection with miR-192 in comparison to control transfected cells (Figure 3D). This shows the direct binding of miR-192 and its target gene ARHGAP19.

3.4 | MiRNA-192 reduces migration of TNBC cells by downregulation of ARHGAP19

Due to the fact that ARHGAP19, a Rho GTPase activating protein, is known to play a role in developmental processes and is expressed more in fetal than in adult tissue, we performed metaanalyses on expression data of different breast cancer samples. Expression data of ARHGAP19 were available in 54 different data sets. The expression of ARHGAP19 was significantly lower in breast cancer subtypes compared to normal breast-like tissue (Figure S10A). There was no significant difference between non-TNBC and TNBC samples (Figure S10B).

To assess the effect of ARHGAP19 on cell viability, apoptosis, and migration, we silenced the mRNA expression of ARHGAP19 by siRNAs. Transfection of HCC38 cells with two different siRNAs (siARHGAP19 #6 and siARHGAP19 #7) against ARHGAP19 resulted in decreased mRNA expression of ARHGAP19 to 23% compared to the mRNA expression in control transfected cells (Figure 4A). Reduction of protein levels after transfection of siARHGAP19 #6 and siARHGAP19 #7 was also observed (Figure 4A, corresponding original Western Blot Figure S11). The viability (Figure 4B) and apoptosis (Figure 4C) of HCC38 cells as well as HCC1395, HCC1937 and MCF12A (Figure S12) were not influenced by the reduction of ARHGAP19 expression. Notably, the migration capacity of HCC38 cells was significantly reduced by both siRNAs against ARHGAP19 (Figure 4D,E). The reduction of migration capacity was also obvious, when migration was normalized to viable cells (Figure S13). Furthermore, we observed a slight, not significant reduction in migration capacity by ARHGAP19 silencing in HCC1395 (Figure S14) and in HCC1937 (Figure S15). Summarized, we identified a role of ARHGAP19 in the migration capacity of TNBC cells.

4 | DISCUSSION

Here, we demonstrate the tumor-suppressive role of miR-192 in different TNBC cell lines. Beside induction of apoptosis and reduction of proliferation, we detected a significant influence of miR-192 on migration of TNBC cells. Furthermore, we identified ARHGAP19 as a new target of miR-192 and detected a strong influence of reduced expression of ARHGAP19 on migration capacity of the cells.

Epigenetic mechanisms such as histone acetylation have a great impact on cell proliferation, apoptosis, cell differentiation and migration by regulating gene expression. In cancer cells, the expression of tumor-suppressive miRNAs is often decreased. Treatment with HDAC inhibitors results in an increase in expression of these miRNAs. Here, we identified epigenetically regulated miRNAs by treating three different TNBC cell lines with the HDAC inhibitor TSA. Although TNBC is not a homogeneous group of breast cancers and the expression of genes is very different among this subtype of breast cancer, we detected induced expression of miR-192 and miR-194 in all three TNBC cell lines after TSA treatment. The transcription of miR-192 and miR-194 is coregulated, because they are encoded as a cluster within the coding region of the long non-coding RNA miR194-2HG. Our further analysis, therefore, focused on these two miRNAs.

Rhodes et al. have described an induction of expression of miR-194 by TSA in the breast cancer cell line MCF-7. In line with this, we observed an induction in expression of both miR-192 and miR-194 as well as their host gene lncRNA miR194-2HG. Furthermore, it has been reported that expression of miR-192 and miR-194 is induced by HNF1α and HNF4α. Using chromatin immunoprecipitation, we showed that increased expression of miR-192 and miR-194 was likely caused by an increased acetylation in the promoter region of miR-194-2HG. Next, we demonstrated the antitumor capacity of miR-192 and miR-194 in the TNBC cell lines HCC38, HCC1395 and HCC1937. We detected increased apoptosis and decreased cell viability in cells transfected with miR-192 mimics, miR-194 mimics, or a combination of both mimics, compared to control transfected cells. MiR-192 showed the strongest effect on apoptosis and cell viability in the cell line HCC38. In line with our results, there are studies that show an inhibition of proliferation by miR-192 in the TNBC cell line MDA-MB-231. This effect was explained by the direct regulation of RB1. Furthermore, it has been shown that miR-194 inhibits proliferation of Her2-positive breast cancer cells. Beside apoptosis and proliferation, the migration capacity plays a fundamental role in cancer progression. Metastases are more frequent and develop earlier in TNBC compared to other breast cancer subtypes. Metastasis is observed in about 34% of patients with TNBC, in contrast to 20% of patients with other breast cancer types.

Therefore, it is very important for women with TNBC to characterize molecules involved in migration since these molecules are interesting putative therapeutic targets. Here, we observed a strong influence of miR-192 on migration. The migration capacity was significantly reduced by miR-192 mimic transfection of HCC38 cells. For miR-194 we detected a reduction in the migration capacity too, even though the effect was not significant compared to controls. The inhibition of the migration capacity has been described for miR-192 in the breast cell line MCF7 by regulation of deltaEF1. Another explanation has been given by Morimoto et al, who have shown that miR-192 decreases the expression of MSN and, thereby, inhibits migration. In HER2-overexpressing breast cancer cells, it has been reported that miR-194 reduces the migratory capacity significantly.
Low miR-192 expression in human tumors is predictive of poor clinical outcome in several cancer types as multiple myeloma, colorectal cancer, renal cell carcinoma, and hepatocellular carcinoma. In ovarian and kidney carcinomas, miR-192 has shown antiangiogenic effects. The decreased tumoral miR-192 levels were associated with increased angiogenesis and poor overall survival in patients with ovarian or renal clear cell carcinomas. Using 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC) nanoliposomes, they developed a miR-192 therapy and showed that miR-192 delivery leads to inhibition of tumor angiogenesis in multiple ovarian and renal tumor models.

By regulation of MAP4K4, miR-194 is able to diminish proliferation of hepatocellular carcinoma cells. However, contrasting results, including our findings reported here, have shown an oncogenic capacity of miR-192 in stomach carcinoma and miR-194 in prostate cancer and pancreas carcinoma.

By analyzing publically available datasets, we observed similar expression level of miR-192 and miR-194 in breast cancer samples compared to normal breast tissue. This is in contrast to observations of Hu et al., who have detected decreased expression of miR-192 in breast cancer samples compared to the corresponding normal tissue. The deviating results could be explained by the different breast cancer cohorts that were analyzed. Furthermore, TNBC is a very heterogeneous group of breast cancer and there are patients even in the analyzed data sets that show a very low level of miR-192 expression.

By mRNA expression arrays after transfection with miR-192 mimics followed by analyzing different miRNA-mRNA interaction prediction tools, we identified the gene ARHGAP19 as a potential target of miR-192. ARHGAP19 is a Rho GTPase activating protein and is usually located in the nucleus. The expression of ARHGAP19 is high in fetal tissue compared to adult tissues. Lv et al. have hypothesized that ARHGAP19 has an important function in developmental processes. By analyzing metaanalyses of expression data, we observed significant differences between the breast cancer subtypes and normal like breast tissue, but none between TNBC and non-TNBC. It has been demonstrated that ARHGAP19 regulates cell elongation and cytokinesis during lymphocyte mitosis acting through the RhoA/ROCK pathway. In this study, ARHGAP19 was validated as a new direct target of miR-192 by RNA immunoprecipitation. An involvement of ARHGAP19 in epithelial mesenchymal transition has also been suggested by Howe et al. in breast cancer cells. They have identified ARHGAP19 as a direct target of miR-200c that inhibits migration and invasion. Furthermore, high expression of ARHGAP19 is an unfavorable prognostic marker in patients with diffuse large B-cell lymphoma.

In conclusion, our findings demonstrate that miRNA-192 acts as a tumor-suppressive miRNA in triple negative breast cancer by increasing apoptosis, reducing proliferation and migration. In addition, we identified the GAP activating protein ARHGAP19 as a new target gene of miR-192. Further experiments are necessary to evaluate the ability of ARHGAP19 to reduce the migration capacity of TNBC cells.

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AUTHOR CONTRIBUTIONS
The general experimental scheme was conceived by Beate Vajen, Luisa Greiwe, Thomas Illig, Brigitte Schlegelberger and Britta Skawran. Beate Vajen, Luisa Greiwe, Vera Schäffer, Marlies Eilers, Nicole Hube, and Amelie Stalke designed and performed experiments and analyzed data. Beate Vajen wrote the manuscript, which was critically reviewed by Thomas Illig, Brigitte Schlegelberger and Britta Skawran. All authors have read and approved the manuscript.

CONFLICT OF INTERESTS
The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT
Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on request.

ORCID
Beate Vajen https://orcid.org/0000-0002-6973-3339

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.