miRNome and Functional Network Analysis of PGRMC1 Regulated miRNA Target Genes Identify Pathways and Biological Functions Associated With Triple Negative Breast Cancer

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Background: Increased expression of the progesterone receptor membrane component 1, a heme and progesterone binding protein, is frequently found in triple negative breast cancer tissue. The basis for the expression of PGRMC1 and its regulation on cellular signaling mechanisms remain largely unknown. Therefore, we aim to study microRNAs that target selective genes and mechanisms that are regulated by PGRMC1 in TNBCs.

Methods: To identify altered miRNAs, whole human miRNome profiling was performed following AG-205 treatment and PGRMC1 silencing. Network analysis identified miRNA target genes while KEGG, REACTOME and Gene ontology were used to explore altered signaling pathways, biological processes, and molecular functions.

Results: KEGG term pathway analysis revealed that upregulated miRNAs target specific genes that are involved in signaling pathways that play a major role in carcinogenesis. While multiple downregulated miRNAs are known oncogenes and have been previously demonstrated to be overexpressed in a variety of cancers. Overlapping miRNA target genes associated with KEGG term pathways were identified and overexpression/amplification of these genes was observed in invasive breast carcinoma tissue from TCGA. Further, the top two genes (CCND1 and YWHAZ) which are highly genetically altered are also associated with poorer overall survival.

Conclusions: Thus, our data demonstrates that therapeutic targeting of PGRMC1 in aggressive breast cancers leads to the activation of miRNAs that target overexpressed genes and deactivation of miRNAs that have oncogenic potential.

Keywords: PGRMC1, miRNA, miRNome, TNBC, KEGG, REACTOME, Gene Ontology
INTRODUCTION

Breast cancer is the most commonly diagnosed cancer in women in the U.S (1). Treatment for breast cancers are guided by the identification of hormone receptors, Estrogen receptor (ER), Progesterone receptor (PR), and Human Epidermal Growth Factor Receptor 2 (HER2) (2, 3). Based on receptor status, breast cancers are categorized into four major molecular subtypes: Luminal A, Luminal B, HER2-enriched, and triple negative/basal-like (3). Among these triple negative breast cancers (TNBCs) are the most aggressive breast cancers with an overall poorer prognosis compared to other subtypes (4, 5). Because TNBC lack ER, PR and HER2, endocrine and antibody-based therapy are ineffective (6–8). Therefore, it is important to identify novel molecular drivers that enable TNBC growth and metastasis and target or reprogram these markers to better treat patients with aggressive metastatic cancers.

Recent evidence in multiple cancers (9–13) including breast cancer (14–16) identify microRNAs (miRNAs) as novel gene expression regulators and potential biomarkers (17–19). miRNAs are small non-coding RNAs approximately 19 to 25 nucleotides in length; they control gene expression by targeting selective-sequences of mRNAs, inducing translational repression or complete mRNA degradation (20). miRNA expression profiles have the ability to identify molecular breast cancer subtypes (21, 22) and can differentiate between basal and luminal subtypes (23). Their effect on hormone receptor expression, regulation, and activity remains in its infant stage. Ongoing studies however, have a major focus for miRNAs that target genes that are altered in aggressive breast cancers while dysregulation of miRNAs has been directly linked to aggressive basal-like breast cancers (24–28). Although one miRNA can target hundreds of genes, treatments that can switch-on specific miRNAs could lead to direct targeted gene suppression of multiple genes that are overexpressed or have oncogenic potential.

PGRMC1 a member of the membrane-associated progesterone receptor (MAPR) family with the ability to initiate non-classical signaling has been described in breast cancers (29–33). PGRMC1 overexpression is observed in more aggressive phenotypes and is associated with poor prognosis in patients diagnosed with ER-negative breast cancers (34). In addition, in vitro and in vivo studies demonstrate that PGRMC1 possess the ability to promote the growth and survival of human breast cancer cells and xenografted breast tumors (35, 36). Although PGRMC1 expression has been observed in multiple cancers (36–40), it’s signaling mechanism remains unknown. Sequencing and microarray technology has opened new insights into the genetic and genomic landscape of all breast cancers including TNBC (41, 42). For example, amplification of MYC and loss-of-function mutation of BRCA1 are often described in TNBCs (43, 44). Further, the most frequently mutated or amplified genes in TNBCs include PI3KCA (55%), AKTI (13%) and CDH1 (13%) (45). These genes can activate downstream cell-cycle regulators that can either activate (cyclin D1) or repress (p53), leading to sustained proliferation and inhibition of apoptosis of breast cancers (46). Our recent work demonstrated that PGRMC1 activates EGFR and PI3K/AKT signaling pathways, leading to increased cell proliferation of TNBC cells (33). While, other studies have demonstrated cell-specific effects between PGRMC1 and AKT signaling (47–49). Historically, the PI3K/AKT pathway is one of the most altered signaling mechanisms in human cancers (50–53). It plays a key role in controlling cellular processes such as cell proliferation and tumor growth (54, 55). Although directly targeting amplified genes such as PI3KCA and AKT1 has proven to be difficult but promising (56, 57), novel genes that behave in a similar fashion should be identified.

To uncover genes and pathways associated with PGRMC1 in TNBCs we performed human miRNome profiling. We impaired PGRMC1 signaling using a chemical inhibitor and RNA interference. Whole human miRNome profiling identified miRNAs that were both up and down regulated following PGRMC1 impairment. Using an array of online databases and datasets we identified direct miRNA target genes. We proceeded to study these genes by identifying their involvement in the different signaling pathways that were altered following PGRMC1 suppression. More importantly, these genes were differentially expressed in human metastatic tumor samples. From all of the miRNA target genes observed, CyclinD1 (CCND1) and 14-3-3 protein zeta/delta (YWHAZ) had the highest gene expression in human tumors and were involved in various signaling pathways. Patient samples with high expression of either gene were associated with overall poorer survival probability. Increased relative gene expression and copy number variation of CCND1 and YWHAZ was observed in MDA-MB-468 breast cancer cells and silencing PGRMC1 reduced the expression of these genes. Interestingly, multiple miRNAs (miR-224, miR-550a, miR-181a, miR-664a, miR-30b, miR-345, miR-93) that were downregulated upon PGRMC1 impairment are known to be overexpressed in multiple cancers and are described as possible oncogenes. Our results demonstrate that targeting PGRMC1 regulates miRNAs that directly target amplified genes and downregulates oncogenic miRNAs in TNBCs.

MATERIALS AND METHODS

Cell Culture

MDA-MB-468 cells were obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured in RPMI-1640 media supplemented with 100 units/mL of penicillin, 100 μg/mL of streptomycin (Life Technologies, Grand Island, NY, USA), and 10% fetal bovine serum (FBS). Cells were incubated at 37°C in 5% CO2 and maintained at an atmosphere of 95% air.

Treatment With Small Molecule Inhibitor and Gene Silencing

MDA-MB-468 cells were plated in six-well plates at a density of 5x105 cells/well and allowed to attach overnight. Cells were then
either treated with 50 μM AG-205 for 24 h or transfected with PGRMC1 siRNA for 48 h. Using Mirus bio TransIT siQUEST transfection reagent (Mirus Bio) with either a control scrambled-sequence or siRNAs targeting PGRMC1-sequence (Origene). Three different siRNA sequences (A, B and C) and multiple concentrations ranging from 20 to 60 nM were used to effectively silence PGRMC1. To minimize toxicity, the ratio of siRNA to transfection reagent was maintained at 1:1, in accordance with the manufacturer’s protocol. siRNA sequences used were as follows:

SR323253A-rGrArUrCrArArCrUrUrUrArGrUrCrArUrGrArUrGrUrUUCT
SR323253B-rCrArUrUrGrArCrUrArGrUrGrArUrGrUrUUCT
SR323253C-rUrCrArArCrUrUrUrUrGrUrCrArUrGrArUrGrUrCrUGT

**Quantitative RT-PCR**

Total RNA was isolated from MDA-MB-468 breast cancer cells using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA was then reverse transcribed using the RT2 first strand kit (Qiagen; Cat. No. 330401). qRT-PCR was performed using the StepOnePlus real time PCR system (Applied Biosystems, Foster City, CA, USA). The comparative Ct (2-ΔΔCT) method was used to analyze the results. The primers used for PGRMC1, CCND1, YWHAZ and 18S are as follows:

PGRMC1
Forward: 5′-CGACGGGCGTCCAGGACCC-3′
Reverse: 5′-TCTTCTCTATCTGGATACACAG-3′
CCND1
Forward: 5′-ATGGAACATACGCTGCTGT-3′
Reverse: 5′-TCAGATGTCACATCCTCGGC-3′
YWHAZ
Forward: 5′-ATGGAACCAACACATCCTCTATC-3′
Reverse: 5′-GCATTATTAGCGTGCTGTCTT-3′
18S
Forward: 5′-GCATTATTAGCGTGCTGTCTT-3′

**miRNome Profiling**

Global microRNA profiling was generated using the SABiosciences PCR miScript PCR Array Human miRNome (Cat No. MIHS-216Z). Briefly, total RNA was extracted using TRIzol reagent (Life Technologies) from MDA-MB-468 cells treated with 50 μM AG-205 for 24 h or 48 h post siRNA transfection. Human miRNome array was performed following the synthesis of cDNA using miScript II RT kit (SABiosciences). miScript miRNA PCR array was performed using miScript SYBR Green PCR Kit (SABiosciences). All of the differentially expressed miRNAs were well-characterized in the human genome as annotated by miRNet (http://www.mirnet.ca/).

**Identifying Pathways Altered by PGRMC1 Using KEGG, Gene Ontology and Reactome**

Using KEGG and gene ontology terms we analyzed the signaling pathways that were significantly altered following PGRMC1 disruption. The Reactome Analysis Tool (http://reactome.org) (58, 59) was used to visualize the genome-wide hierarchy of enriched pathways in response to PGRMC1. The most significantly enriched pathways are represented as yellow and are maintained in the middle of the circular representation and the less or non-significantly enriched pathways are labeled in grey. A list of all the miRNA target genes was uploaded into the Reactome database and significantly enriched pathway analysis was defined by FDR < 0.05.

**Determining PGRMC1-Induced Genetic Alterations Using In Silico Analysis**

To study possible genetic alterations such as inframe, missense, truncating mutations as well as gene amplification and deep deletion of the miRNA target genes observed following PGRMC1 disruption. We uploaded the DEG dataset onto the cbioportal (http://www.cbioportal.org/) database and analyzed it in reference to the cancer genome atlas (TCGA). Oncoprint diagrams were used to visualize genetic alterations from invasive breast carcinoma samples (60). Because we impaired PGRMC1 in TNBC cells, using the xena platform (https://xenabrowser.net) database, we studied the altered gene expression in response to PGRMC1 disruption. More specifically we obtained data from the breast cancer cell line Heiser 2012 (54 breast and breast cancer cell lines), breast cancer cell line encyclopedia (68 breast and breast cancer cell lines) as well as TCGA Breast Cancer (BRCA) dataset (n = 1,247 samples).

**Assessing PGRMC1 Signaling and Overall Survival in Breast Cancer Patients Using KM Plotter and Interaction of miRNA Target Genes Using Genemania**

The cBioportal (http://www.cbioportal.org/) database was used to study overall cumulative survival of patients with high and low expression of the miRNA target genes observed following PGRMC1 impairment. Kaplan-Meier plots were generated from TCGA breast invasive carcinoma samples (n=817). To study the impact of individual genes on overall survival probability, we used the KM plotter (http://kmplot.com/) database and generated Kaplan-Meier plots from ER-negative/HER2-negative breast cancer samples (n=869). Finally, using genemania 3 (http://genemania.org) we explored the interconnection between miRNA target genes involved in the pathways that were significantly altered following PGRMC1 impairment.

**Statistical Analysis**

All data are expressed as the mean ± SD. The differences between control and experimental groups were compared using Student’s t-test. P < 0.05 was considered to be statistically significant. Statistical analysis was conducted using GraphPad Prism 7 software, version 7.0 (GraphPad Prism Software, San Diego, CA, USA).
RESULTS

Disrupting PGRMC1 Signaling the Human miRNome

To identify miRNAs regulated by PGRMC1, whole human miRNome profiling was performed using a miScript miRNA PCR array (miRNome V16) where a total of 1,084 mature miRNAs including their respective controls were measured. MDA-MB-468 breast cancer cells were treated with 50 µM AG-205. AG-205 is known to disrupt the downstream signaling of PGRMC1 possibly causing it to accumulate in the membrane. Therefore, it was not surprising to observe an increase in PGRMC1 mRNA expression (Figure 1A) as earlier studies have shown increased protein expression of PGRMC1 following AG-205 treatment (33, 38). Human miRNome profiling following AG-205 treatment identified alterations in the expression of various miRNAs (Figure 1B). The 20 most upregulated and downregulated miRNAs were observed (Figures 1C, D). Because AG-205 increased PGRMC1 mRNA expression, we proceeded to silence PGRMC1 to further study its impact on miRNA expression (Figure 1E). Following successful PGRMC1 silencing, human miRNome profiling identified alterations to 776 miRNAs (Figure 1F). Here again, the 20 most upregulated and downregulated miRNAs were identified (Figures 1G, H). We then identified the target genes for the 20 most altered miRNAs using the miRNet database. Following AG-205 treatment the 20 most upregulated miRNAs targeted 2,898 genes while the 20 most downregulated miRNAs targeted 2,501 genes (Figure 1I and Supplementary Tables 1, 2). Similarly, the top 20 most upregulated miRNAs accounted for 1,788 target genes. While, the 20 most downregulated miRNAs targeted 3,029 genes after PGRMC1 was silenced (Figure 1J and Supplementary Tables 3, 4).

PGRMC1 Signal Disruption Alters miRNAs Involved in Pathways Associated With Cancers

Since our earlier analysis with the top 20 miRNAs altered by PGRMC1 resulted in a large number of target genes, we proceeded to study the network analysis of the top 10 most upregulated and downregulated miRNAs following AG-205 treatment. Network analysis of the top 10 most upregulated miRNAs (hsa-miR-523-3p, hsa-miR-3167, hsa-miR-3176, hsa-miR-570-3p, hsa-miR-410-3p, hsa-miR-646, hsa-miR-1256, hsa-miR-576-3p, hsa-miR-378a-5p and hsa-miR-1224-5p) identified 1,479 target genes (Figure 2A and Supplementary Table 5) while the top 10 most downregulated miRNAs (hsa-miR-3681-5p, hsa-miR-3617-5p, hsa-miR-34a-5p, hsa-miR-101-5p, hsa-miR-224-5p, hsa-miR-550a-3p, hsa-miR-181a-3p, hsa-miR-1914-3p, hsa-miR-664a-3p and hsa-miR-3605-3p) targeted 1,402 genes (Figure 2B and Supplementary Table 6).
the top miRNAs made our study more focused on miRNAs that may be more effectively regulated by PGRMC1. To identify miRNA target genes that could have a significant impact, we narrowed down our search by performing KEGG and gene ontology analysis. KEGG terms of the computed 1,479 target genes allowed us to pin-point and identify target genes of PGRMC1 altered miRNAs that are uniquely involved within the top signaling pathways, which interestingly included, p53 signaling pathway, cell cycle and pathways in cancers (Figure 2C; Supplementary Figure 1 and Supplementary Table 7). Interestingly, the downregulated miRNAs also significantly altered pathways in cancer, cell cycle and p53 signaling pathways (Figure 2D; Supplementary Figure 2 and Supplementary Table 8). Further, gene functions including kinase binding, single-stranded DNA binding, gene silencing, intrinsic apoptotic signaling pathway, regulated program cell death, enzyme binding, and nucleotide binding were classified using gene ontology based molecular functions and biological processes of both up and downregulated miRNAs (Figures 2E, F). The candidate 10 most up and downregulated miRNAs following AG-205 treatment and their respective target genes were listed (Tables 1, 2).

miRNAs Regulated Signaling Pathways Identified Following PGRMC1 Silencing

Network analysis following PGRMC1 silencing identified 1,015 genes as targets of the 10 most upregulated miRNAs (hsa-miR-617, hsa-miR-3138, hsa-miR-3150b-3p, hsa-miR-101-5p, hsa-miR-483-5p, hsa-miR-1267, hsa-miR-221-5p, hsa-miR-3201, hsa-miR-1273d and hsa-miR-642b-3p) (Figure 3A and Supplementary Table 9). While, 2,010 genes were identified to be direct targets of the top 10 most downregulated miRNAs (hsa-miR-135a-5p, hsa-miR-3200-5p, hsa-miR-139-5p, hsa-miR-224-5p, hsa-miR-30b-3p, hsa-miR-181a-3p, hsa-miR-345-5p, hsa-miR-93-3p, hsa-miR-4291 and hsa-miR-128-3p) (Figure 3B and Supplementary Table 10). KEGG analysis of the upregulated (Figure 3C; Supplementary Figure 4 and Supplementary Table 11) and downregulated (Figure 3D; Supplementary Figure 5 and Supplementary Table 12) miRNAs following PGRMC1 silencing identified enrichment to similar KEGG terms observed in the AG-205 treatment group, such as p53 signaling pathway, cell cycle and pathways in cancers. Gene ontology terms, identified important molecular functions and biological processes including protein kinase binding, transcription factor binding, MAPK kinase activity, inactivation of MAPK activity, intrinsic apoptotic signaling pathway, purine nucleotide binding, adenyl nucleotide binding, protein phosphorylation, and regulation of phosphorylation (Figures 3E, F). The candidate 10 most up and downregulated miRNAs following PGRMC1 silencing and their respective target genes were listed (Tables 3, 4).

PGRMC1 Signal Disruption and Silencing Alters miRNAs That Target Genes Involved in Breast Cancers

Once we identified the altered pathways following PGRMC1 signal disruption by AG-205 treatment we wanted to identify if the genes that are directly involved within these pathways are observed in breast cancer patient samples. Therefore, the identified genes were taken and computed into the xenabrowser database. TCGA data
| miRNA ID | Target Gene | Gene ID | Accession | Target ID | Experiment | PubMed ID |
|----------|-------------|---------|-----------|-----------|------------|-----------|
| hsa-mir-3167 | CALM2 | 805 | MIMAT0015042 | PAR-CLIP | 23592263 |
| hsa-mir-3167 | AURKA | 6790 | MIMAT0015042 | PAR-CLIP | 26701625 |
| hsa-mir-3167 | VPS4A | 27183 | MIMAT0015042 | PAR-CLIP | 22012620 |
| hsa-mir-3167 | WASF2 | 10163 | MIMAT0015042 | HITS-CLIP | 23592263 |
| hsa-mir-3167 | ZNF274 | 10782 | MIMAT0015042 | HITS-CLIP | 23824327 |
| hsa-mir-3167 | CYCS | 54205 | MIMAT0015042 | HITS-CLIP | 22012620 |
| hsa-mir-3167 | TTC37 | 27183 | MIMAT0015042 | PAR-CLIP | 23592263 |
| hsa-mir-3167 | ANAPC7 | 51434 | MIMAT0015042 | HITS-CLIP | 23824327 |
| hsa-mir-3167 | LSM3 | 27258 | MIMAT0015042 | PAR-CLIP | 23824327 |
| hsa-mir-3167 | RAB11FIP4 | 84440 | MIMAT0015042 | PAR-CLIP | 23824327 |
| hsa-mir-570-3p | HHIP | 64399 | MIMAT0003235 | PAR-CLIP | 23592263 |
| hsa-mir-570-3p | CALM3 | 808 | MIMAT0003235 | PAR-CLIP | 23592263 |
| hsa-mir-570-3p | PMAIP1 | 5366 | MIMAT0003235 | PAR-CLIP | 23592263 |
| hsa-mir-570-3p | TTC37 | 9652 | MIMAT0003235 | PAR-CLIP | 23592263 |
| hsa-mir-570-3p | ANAPC7 | 10163 | MIMAT0003235 | PAR-CLIP | 23592263 |
| hsa-mir-570-3p | LSM3 | 27258 | MIMAT0003235 | PAR-CLIP | 23592263 |
| hsa-mir-570-3p | RAB11FIP4 | 84440 | MIMAT0003235 | PAR-CLIP | 23592263 |
| hsa-mir-570-3p | ACTB | 60 | MIMAT0003235 | PAR-CLIP | 23592263 |
| hsa-mir-410-3p | VEGFA | 7422 | MIMAT0002171 | PAR-CLIP | 23824327 |
| hsa-mir-410-3p | CRK | 1398 | MIMAT0002171 | PAR-CLIP | 23824327 |
| hsa-mir-410-3p | PPP2R5E | 5929 | MIMAT0002171 | PAR-CLIP | 23824327 |
| hsa-mir-410-3p | CDKN1A | 1026 | MIMAT0002171 | PAR-CLIP | 23824327 |
| hsa-mir-410-3p | TPM3 | 3480 | MIMAT0002171 | PAR-CLIP | 23824327 |
| hsa-mir-410-3p | MDM2 | 4193 | MIMAT0002171 | PAR-CLIP | 23824327 |
| hsa-mir-410-3p | CDK1 | 983 | MIMAT0002171 | PAR-CLIP | 23824327 |
| hsa-mir-410-3p | LDLR | 3949 | MIMAT0002171 | PAR-CLIP | 23824327 |
| hsa-mir-410-3p | TFDP1 | 7027 | MIMAT0002171 | PAR-CLIP | 23824327 |
| hsa-mir-646 | ZMAT3 | 64399 | MIMAT0003316 | PAR-CLIP | 23824327 |
| hsa-mir-646 | CCND1 | 808 | MIMAT0003316 | PAR-CLIP | 23824327 |
| hsa-mir-646 | CRK | 1398 | MIMAT0003316 | PAR-CLIP | 23824327 |

(Continued)
| miRNA ID | Accession | Target Gene | Target ID | Experiment | Literature PubMed ID |
|----------|-----------|-------------|-----------|------------|---------------------|
| hsa-mir-646 | MIMAT0003316 | VEGFA | 7422 | HITS-CLIP/ PAR-CLIP | 23592263 | 24398324 | 21572407 | 20371350 | 26701625 |
| hsa-mir-646 | MIMAT0003316 | BTG2 | 7832 | PAR-CLIP | 21572407 | 20371350 | 22012620 | 23131552 | 24398324 | 23446348 | 21572407 | 20371350 | 27292025 |
| hsa-mir-646 | MIMAT0003316 | PPP2R5C | 5527 | PAR-CLIP | 21572407 | 20371350 | 22012620 | 23131552 | 24398324 | 23446348 | 21572407 | 20371350 | 27292025 |
| hsa-mir-646 | MIMAT0003316 | CSNK2A1 | 1457 | PAR-CLIP | 21572407 | 20371350 | 22012620 | 23131552 | 24398324 | 23446348 | 21572407 | 20371350 | 27292025 |
| hsa-mir-646 | MIMAT0003316 | ORC4 | 5000 | PAR-CLIP | 21572407 | 20371350 | 22012620 | 23131552 | 24398324 | 23446348 | 21572407 | 20371350 | 27292025 |
| hsa-mir-646 | MIMAT0003316 | PRKAR2A | 5576 | PAR-CLIP | 21572407 | 20371350 | 22012620 | 23131552 | 24398324 | 23446348 | 21572407 | 20371350 | 27292025 |
| hsa-mir-646 | MIMAT0003316 | RBL1 | 5933 | PAR-CLIP | 21572407 | 20371350 | 22012620 | 23131552 | 24398324 | 23446348 | 21572407 | 20371350 | 27292025 |
| hsa-mir-646 | MIMAT0003316 | BIRC5 | 332 | PAR-CLIP | 21572407 | 20371350 | 22012620 | 23131552 | 24398324 | 23446348 | 21572407 | 20371350 | 27292025 |
| hsa-mir-646 | MIMAT0003316 | WEE1 | 7465 | PAR-CLIP | 21572407 | 20371350 | 22012620 | 23131552 | 24398324 | 23446348 | 21572407 | 20371350 | 27292025 |
| hsa-mir-646 | MIMAT0003316 | CDK6 | 1021 | PAR-CLIP | 21572407 | 20371350 | 22012620 | 23131552 | 24398324 | 23446348 | 21572407 | 20371350 | 27292025 |
| hsa-mir-646 | MIMAT0003316 | CSNK2A1 | 1457 | PAR-CLIP | 21572407 | 20371350 | 22012620 | 23131552 | 24398324 | 23446348 | 21572407 | 20371350 | 27292025 |
| hsa-mir-646 | MIMAT0003316 | ORC4 | 5000 | PAR-CLIP | 21572407 | 20371350 | 22012620 | 23131552 | 24398324 | 23446348 | 21572407 | 20371350 | 27292025 |
| hsa-mir-646 | MIMAT0003316 | PRKAR2A | 5576 | PAR-CLIP | 21572407 | 20371350 | 22012620 | 23131552 | 24398324 | 23446348 | 21572407 | 20371350 | 27292025 |
| hsa-mir-646 | MIMAT0003316 | RBL1 | 5933 | PAR-CLIP | 21572407 | 20371350 | 22012620 | 23131552 | 24398324 | 23446348 | 21572407 | 20371350 | 27292025 |
| hsa-mir-646 | MIMAT0003316 | BIRC5 | 332 | PAR-CLIP | 21572407 | 20371350 | 22012620 | 23131552 | 24398324 | 23446348 | 21572407 | 20371350 | 27292025 |
| hsa-mir-646 | MIMAT0003316 | WEE1 | 7465 | PAR-CLIP | 21572407 | 20371350 | 22012620 | 23131552 | 24398324 | 23446348 | 21572407 | 20371350 | 27292025 |
| hsa-mir-646 | MIMAT0003316 | CDK6 | 1021 | PAR-CLIP | 21572407 | 20371350 | 22012620 | 23131552 | 24398324 | 23446348 | 21572407 | 20371350 | 27292025 |
| hsa-mir-646 | MIMAT0003316 | STK11 | 6794 | PAR-CLIP | 21572407 | 20371350 | 22012620 | 23131552 | 24398324 | 23446348 | 21572407 | 20371350 | 27292025 |
| hsa-mir-646 | MIMAT0003316 | PPP2R5C | 5527 | PAR-CLIP | 21572407 | 20371350 | 22012620 | 23131552 | 24398324 | 23446348 | 21572407 | 20371350 | 27292025 |
| hsa-mir-646 | MIMAT0003316 | PPP2R5C | 5527 | PAR-CLIP | 21572407 | 20371350 | 22012620 | 23131552 | 24398324 | 23446348 | 21572407 | 20371350 | 27292025 |
| hsa-mir-646 | MIMAT0003316 | PPP2R5C | 5527 | PAR-CLIP | 21572407 | 20371350 | 22012620 | 23131552 | 24398324 | 23446348 | 21572407 | 20371350 | 27292025 |
from primary and metastatic tumor samples was downloaded and plotted. Genes from p53 signaling pathway, cell cycle neutrophin signaling pathways, pathways in cancer, adherens junction, insulin signaling pathway, oocyte meiosis, mTOR signaling pathway, RNA degradation, and endocytosis were differentially expressed in both metastatic and primary tumor tissue samples (Figure 4). Target genes of downregulated miRNAs were also differentially expressed in similar pathways including pathways in cancer, cell cycle, and p53 signaling pathway (Supplementary Figure 5). Identified genes involved within each pathway following PGRMC1 silencing were similarly computed into the xenabrowser database. TCGA data analyzed from metastatic tumor samples identified upregulated miRNA target genes to be involved in pathways in cancer, T cell receptor signaling pathway, cell pathway, p53 signaling pathway, B cell receptor signaling pathway, MAPK signaling pathway, JAK-STAT signaling pathway, ErbB signaling pathway, NOD-like receptor signaling pathway, and mRNA surveillance pathway (Figure 5). Intriguingly, downregulated miRNAs had similarly altered miRNA target genes in pathways in cancer, p53 signaling pathway, T cell receptor signaling pathway and ErbB signaling pathway (Supplementary Figure 6). However, some miRNA target genes were also observed in adherens junctions, focal adhesion, neutrophin signaling pathway, regulation of actin cytoskeleton, aldosterone-regulated sodium reabsorption and chemokine signaling pathway (Supplementary Figure 6).

### PGRMC1 Regulates miRNAs Involved in Cell Cycle, Disease Signal and Transduction Processes

Gene network analysis allowed us to identify novel target genes and we were able to classify them using KEGG term enrichment following AG-205 treatment of PGRMC1 silencing. We employed the Reactome database to study pathway-topology analysis using the miRNA target genes from KEGG and GO analysis. Using the Reactome pathway identifier we were able to observe genes that are mapped to pathways and over-represented within those pathways (58, 61). Following AG-205 treatment, we identified over-representation of miRNA target genes in pathways involved in cell cycle, gene expression (Transcription), disease, and signal transduction (Figure 6A). Similarly, following PGRMC1 silencing we observed over-representation of miRNA target genes in pathways involved in immune system, signal transduction, gene expression (transcription), and cell cycle (Figure 6B).

### Functional Annotation Analysis of PGRMC1 Altered miRNA Target Genes in Invasive Breast Carcinomas Samples Using TCGA Dataset

TCGA data was used to study possible genetic alterations of the miRNA target genes due to miRNA alterations in response to PGRMC1 disruption. From the miRNA target genes observed, the top 22 that displayed increased mRNA expression within the spectrum of signaling pathways identified by KEGG were further analyzed. Using the cBioportal database we were able to observe and differentiate between the miRNA target genes based on genetic alteration. Using oncoprint we visualized the genetic alterations in the 22 miRNA target genes (CCND1, YWHAZ, TPM3, BTG2, PAPPC1, IGF1R, RAB11FIP1, PRKDC, MAPKAPK2, MAPK3, THBS1, CALM2, PIK3R1, RPS6, ACTB, PTPRF, ITGB1, RH0A, MAPK1, BCL2L1, RAC1 and PPP2R1A) (Figure 7A and Supplementary Figure 7). However, the percentage of genetic alteration varied within each gene and most miRNA target genes that displayed an alteration in > 5 percent were mainly amplified (Figure 7A). Patients that displayed high expression of these genes had a cumulative lower survival rate (Figure 7B). Network analysis by the Genemania database demonstrated that these amplified genes have tight interactions within signaling pathways. The light-red lines connect genes that are known to directly interact with one another within signaling pathways that are well studied (Figure 7C). Although, cumulatively these genes displayed a lower survival rate, only high expression of CCND1 and YWHAZ in ER-negative breast cancer patients displayed significant overall lower survival probability (Figure 7D and Supplementary Figure 8). Finally, gene expression data analysis from the breast cancer cell line dataset and copy number variation from the cancer cell line encyclopedia dataset similarly demonstrated increased expression/CN variation of CCND1 and YWHAZ in TNBC cell lines (Figure 7E). Further, we also confirmed the decreased expression of CCND1 and YWHAZ in PGRMC1 silenced MDA-MB-468 cells (Figure 7F). Overall, our in vitro and in silico analysis demonstrates that PGRMC1 plays a major role in influencing the miRNome in such a way that these alterations favor breast tumor growth and progression.

### DISCUSSION

TNBCs account for approximately 12-14% of breast cancers diagnosed in the United States, with most exhibiting BRCA1/2
| miRNA ID | Accession | Target Gene | Target ID | Experiment | Literature PubMed ID |
|----------|-----------|-------------|-----------|------------|---------------------|
| hsa-mir-181a-3p | MIMAT0000270 | ARHGDIA | 396 | PAR-CLIP | 26701625 |
| mir-3605-3p | None | | | | |
| hsa-mir-664a-3p | MIMAT0005949 | WNT7A | 7476 | PAR-CLIP | 22012620 |
| hsa-mir-664a-3p | MIMAT0005949 | WEE2 | 494551 | HITS-CLIP | 23824327 |
| hsa-mir-664a-3p | MIMAT0005949 | CALM1 | 801 | PAR-CLIP | 21572407 |
| hsa-mir-664a-3p | MIMAT0005949 | RPS6KA5 | 9252 | PAR-CLIP | 21572407 |
| hsa-mir-664a-3p | MIMAT0005949 | YWHAE | 7531 | PAR-CLIP | 23824327 |
| hsa-mir-664a-3p | MIMAT0005949 | PLCG1 | 5335 | CLASH | 23622248 |
| hsa-mir-664a-3p | MIMAT0005949 | E2F3 | 1871 | PAR-CLIP | 22929263 |
| hsa-mir-664a-3p | MIMAT0005949 | NRG4 | 145957 | PAR-CLIP | 23446348 |
| hsa-mir-664a-3p | MIMAT0005949 | CALM3 | 808 | PAR-CLIP | 26701625 |
| hsa-mir-1914-3p | MIMAT0007890 | YWHAE | 7531 | PAR-CLIP | 23592263 |
| hsa-mir-1914-3p | MIMAT0007890 | PLCG1 | 5335 | CLASH | 23622248 |
| hsa-mir-1914-3p | MIMAT0007890 | E2F3 | 1871 | PAR-CLIP | 22929263 |
| hsa-mir-1914-3p | MIMAT0007890 | STAT5B | 6776 | PAR-CLIP | 22291592 |
| hsa-mir-1914-3p | MIMAT0007890 | TAB2 | 23118 | PAR-CLIP | 23592263 |
| hsa-mir-1914-3p | MIMAT0007890 | NRG4 | 145957 | PAR-CLIP | 23446348 |
| hsa-mir-1914-3p | MIMAT0007890 | CALM3 | 808 | PAR-CLIP | 26701625 |
| hsa-mir-3617-5p | MIMAT0017997 | CDKN1A | 1026 | PAR-CLIP | 26701625 |
| hsa-mir-3617-5p | MIMAT0017997 | CDKN2B | 1030 | HITS-CLIP | 23313552 |
| hsa-mir-3617-5p | MIMAT0017997 | MAPK10 | 5602 | HITS-CLIP | 23824327 |
| hsa-mir-3617-5p | MIMAT0017997 | MDM2 | 4193 | PAR-CLIP | 21572407 |
| hsa-mir-3617-5p | MIMAT0017997 | CDK1 | 983 | PAR-CLIP | 21572407 |
| hsa-mir-3617-5p | MIMAT0017997 | PMAIP1 | 5366 | PAR-CLIP | 27292025 |
| hsa-mir-224-5p | MIMAT0000281 | CCND1 | 595 | PAR-CLIP | 26701625 |
| hsa-mir-224-5p | MIMAT0000281 | BCL2 | 596 | Microarray//qRT-PCR//Western blot | 22989374 |
| hsa-mir-224-5p | MIMAT0000281 | CASP3 | 836 | Luciferase reporter assay//Western blot | 26307684 |
| hsa-mir-224-5p | MIMAT0000281 | IGF1R | 3480 | PAR-CLIP | 20371350 |
| hsa-mir-224-5p | MIMAT0000281 | SMAD4 | 4089 | Luciferase reporter assay//qRT-PCR//Western blot | 23446348 |
| hsa-mir-224-5p | MIMAT0000281 | CCND1 | 595 | PAR-CLIP | 26701625 |
| hsa-mir-224-5p | MIMAT0000281 | BCL2 | 596 | Microarray//qRT-PCR//Western blot | 22989374 |
| hsa-mir-224-5p | MIMAT0000281 | CASP3 | 836 | Luciferase reporter assay//Western blot | 26307684 |
| hsa-mir-224-5p | MIMAT0000281 | IGF1R | 3480 | PAR-CLIP | 20371350 |
| hsa-mir-224-5p | MIMAT0000281 | MAPK3 | 5595 | /Luciferase reporter assay//qRT-PCR//Western blot | 27292025 |
| hsa-mir-224-5p | MIMAT0000281 | HSP90AA1 | 3320 | PAR-CLIP | 23446348 |
| hsa-mir-224-5p | MIMAT0000281 | MAPK2K2 | 5605 | HITS-CLIP | 23824327 |
| hsa-mir-224-5p | MIMAT0000281 | RAC1 | 5879 | Luciferase reporter assay | 27229231 |
| hsa-mir-224-5p | MIMAT0000281 | TPR | 7175 | PAR-CLIP | 22229281 |
| hsa-mir-224-5p | MIMAT0000281 | GSK3B | 2932 | Luciferase reporter assay | 25588771 |
| hsa-mir-550a-3p | MIMAT0003257 | MAPK3 | 5595 | /Luciferase reporter assay//qRT-PCR//Western blot | 22989374 |
| hsa-mir-101-5p | MIMAT0004513 | FOS | 5386 | Luciferase reporter assay | 27292025 |
| hsa-mir-101-5p | MIMAT0004513 | VEGFA | 7422 | Luciferase reporter assay//qRT-PCR//Western blot | 22989374 |
| hsa-mir-101-5p | MIMAT0004513 | STK4 | 472 | Luciferase reporter assay//qRT-PCR | 20617180 |
| hsa-mir-101-5p | MIMAT0004513 | PRKDC | 5591 | Luciferase reporter assay//qRT-PCR | 20617180 |

(Continued)
| miRNA ID   | Accession  | Target Gene | Target ID | Experiment                          | Literature                  | PubMed ID                  |
|------------|------------|-------------|-----------|-------------------------------------|----------------------------|---------------------------|
| hsa-miR-101-5p | MIMAT0004513 | PMAIP1      | 5366      | PAR-CLIP                            | 23446348|2012620|2157240|23446348|2012620 |
| hsa-miR-3681-5p | MIMAT0018108 | FZD6        | 8323      | HITS-CLIP//PAR-CLIP                  | 15936157                    | 25692263                  |
| hsa-miR-3681-5p | MIMAT0018108 | MALT1       | 10892     | PAR-CLIP                            | 25692263                  |
| hsa-miR-3681-5p | MIMAT0000255 | AKT1        | 207       | Flow//qRT-PCR//Western blot          | 27073535                  |
| hsa-miR-3681-5p | MIMAT0000255 | BIRC2       | 329       | PCR array                           | 28097098                  |
| hsa-miR-3681-5p | MIMAT0000255 | BIRC3       | 330       | Microarray//Northern blot           | 17540599                  |
| hsa-miR-3681-5p | MIMAT0000255 | XIAP        | 331       | PCR array                           | 28097098                  |
| hsa-miR-34a-5p  | MIMAT0000255 | FASLG       | 356       | PCR array                           | 28097098                  |
| hsa-miR-34a-5p  | MIMAT0000255 | AR          | 367       | qRT-PCR//Western blot               | 23415211                  |
| hsa-miR-34a-5p  | MIMAT0000255 | BAX         | 581       | Luciferase reporter assay//Western blot | 27610823                  |
| hsa-miR-34a-5p  | MIMAT0000255 | CCND1       | 595       | Reporter assay//Sequencing//Western blot | 18406335[19461653]        |
| hsa-miR-34a-5p  | MIMAT0000255 | CDK2        | 596       | //qRT-PCR//qRT-PCR//Reporter assay//Western blot | 21240262[21128241]       |
| hsa-miR-34a-5p  | MIMAT0000255 | CDKn1B      | 1027      | PAR-CLIP                            | 19773441[21240262]       |
| hsa-miR-34a-5p  | MIMAT0000255 | CDK2       | 1029      | Western blot                        | 23035210[23592263]       |
| hsa-miR-34a-5p  | MIMAT0000255 | CSF1R       | 1436      | Luciferase reporter assay//qRT-PCR   | 24198819                  |
| hsa-miR-34a-5p  | MIMAT0000255 | CTNNB1      | 1499      | Proteomics                          | 2566225                  |
| hsa-miR-34a-5p  | MIMAT0000255 | DAPK1       | 1612      | PCR array                           | 28097098                  |
| hsa-miR-34a-5p  | MIMAT0000255 | E2F1        | 1869      | Luciferase reporter assay//qRT-PCR//Western blot | 1785987[21128241]       |
| hsa-miR-34a-5p  | MIMAT0000255 | E2F3        | 1871      | //Microarray//PAR-CLIP//qRT-PCR//Western blot | 2395432[212986779]      |
| hsa-miR-34a-5p  | MIMAT0000255 | ERBB2       | 2064      | Luciferase reporter assay//Western blot | 28097290[23839657]       |
| hsa-miR-34a-5p  | MIMAT0000255 | FOS         | 2353      | ChIP//mRNA decay//qRT-PCR//Western blot | 27513856                  |
| hsa-miR-34a-5p  | MIMAT0000255 | GRB2        | 2885      | Sequencing                          | 20371350                  |
| hsa-miR-34a-5p  | MIMAT0000255 | HDAC1       | 3065      | //qRT-PCR//Reporter assay//Western blot | 21566225[28836167]      |
| hsa-miR-34a-5p  | MIMAT0000255 | IGF1R       | 3480      | CLASH                                | 26362248                  |
| hsa-miR-34a-5p  | MIMAT0000255 | ITGA6       | 3655      | Proteomics                          | 2566225                  |
| hsa-miR-34a-5p  | MIMAT0000255 | KIT         | 3815      | Luciferase reporter assay//Western blot | 24009008[27056900]       |
| hsa-miR-34a-5p  | MIMAT0000255 | SMAD4       | 4089      | //PAR-CLIP//qRT-PCR//Western blot   | 20371350[28348487]       |
| hsa-miR-34a-5p  | MIMAT0000255 | MET         | 4233      | //Northern blot//qRT-PCR//Western blot | 24983493[26313360]      |
| hsa-miR-34a-5p  | MIMAT0000255 | MYC         | 4609      | //Reporter assay//Sequencing//TRAP//Western blot | 26238271[27513895]      |
| hsa-miR-34a-5p  | MIMAT0000255 | NFkB1       | 4790      | PCR array                           | 21297663[22159222]      |
| hsa-miR-34a-5p  | MIMAT0000255 | PDGFRA      | 5156      | //Microarray//qRT-PCR//Western blot  | 20371350[24510096]      |
| hsa-miR-34a-5p  | MIMAT0000255 | PDGFRB      | 5159      | Luciferase reporter assay//qRT-PCR//Western blot | 23805317[24837198]      |
| hsa-miR-34a-5p  | MIMAT0000255 | PKC3G       | 5294      | Flow//qRT-PCR//Western blot          | 2566225                  |
| hsa-miR-34a-5p  | MIMAT0000255 | PLCG1       | 5335      | Proteomics                          | 21128241[21128241]      |
| miRNA ID  | Accession | Target Gene | Target ID | Experiment | Literature PubMed ID |
|----------|-----------|-------------|-----------|------------|---------------------|
| hsa-mir-34a-5p | MIMAT0000255 | MAPK3 | 5595 | CLASH | 23622248 |
| hsa-mir-34a-5p | MIMAT0000255 | MAP2K1 | 5604 | Luciferase reporter assay/Northern blot/qRT-PCR/Western blot | 20299489 |
| hsa-mir-34a-5p | MIMAT0000255 | RALB | 5899 | Proteomics | 21566225 |
| hsa-mir-34a-5p | MIMAT0000255 | SP11 | 6688 | Luciferase reporter assay/Reporter assay | 20598588 |
| hsa-mir-34a-5p | MIMAT0000255 | STAT1 | 6772 | Proteomics | 21566225 |
| hsa-mir-34a-5p | MIMAT0000255 | TGF7 | 6932 | Luciferase reporter assay/qRT-PCR/Western blot | 25498980 |
| hsa-mir-34a-5p | MIMAT0000255 | TGFB2R2 | 7048 | PAR-CLIP | 22012820 |
| hsa-mir-34a-5p | MIMAT0000255 | TP53 | 7157 | Northern blot/qRT-PCR/qRT-PCR/Western blot | 26403328/26177460 |
| hsa-mir-34a-5p | MIMAT0000255 | TRAF2 | 7186 | PCR array | 28097098 |
| hsa-mir-34a-5p | MIMAT0000255 | TRAF3 | 7187 | PCR array | 28097098 |
| hsa-mir-34a-5p | MIMAT0000255 | VEGFA | 7422 | ELISA/Luciferase reporter assay | 18320040 |
| hsa-mir-34a-5p | MIMAT0000255 | WNT1 | 7471 | Luciferase reporter assay/Microarray/qRT-PCR/Western blot | 19336450/19398721 |
| hsa-mir-34a-5p | MIMAT0000255 | CCNE2 | 9134 | Luciferase reporter assay/Microarray/PAR-CLIP/Western blot | 19416565/19714404 |
| hsa-mir-34a-5p | MIMAT0000255 | CYCS | 54205 | PCR array | 28097098 |
| hsa-mir-34a-5p | MIMAT0000255 | KRAS | 3845 | qRT-PCR/Western blot | 23667495 |
| hsa-mir-34a-5p | MIMAT0000255 | CCND3 | 896 | Western blot | 18406353 |
| hsa-mir-34a-5p | MIMAT0000255 | CDC20 | 991 | CLASH/Proteomics | 21566225/23622248 |
| hsa-mir-34a-5p | MIMAT0000255 | CDC25A | 993 | Western blot | 18406353 |
| hsa-mir-34a-5p | MIMAT0000255 | CDC25C | 995 | Microarray | 19461653 |
| hsa-mir-34a-5p | MIMAT0000255 | CDKN1B | 1027 | PCR array | 23446348 |
| hsa-mir-34a-5p | MIMAT0000255 | CDKN2A | 1029 | Western blot | 21128241 |
| hsa-mir-34a-5p | MIMAT0000255 | CDKN2C | 1031 | qRT-PCR/Reporter assay | 21128241 |
| hsa-mir-34a-5p | MIMAT0000255 | GADD45A | 1647 | PCR array | 21566225/23622248 |
| hsa-mir-34a-5p | MIMAT0000255 | E2F1 | 1869 | Luciferase reporter assay/qRT-PCR/Western blot | 20371350/28348487 |
| hsa-mir-34a-5p | MIMAT0000255 | E2F5 | 1875 | Microarray/PAR-CLIP/qRT-PCR/Reporter assay/Western blot | 19461653/19714404 |
| hsa-mir-34a-5p | MIMAT0000255 | E2F3 | 1871 | Luciferase reporter assay/Microarray/PAR-CLIP/qRT-PCR/Western blot | 21240262/21128241 |
| hsa-mir-34a-5p | MIMAT0000255 | CDK6 | 1021 | Microarray/PAR-CLIP/qRT-PCR/Reporter assay/Western blot | 19461653/19714404 |
| hsa-mir-34a-5p | MIMAT0000255 | CDK11B | 1027 | PAR-CLIP | 23592263 |
| hsa-mir-34a-5p | MIMAT0000255 | CDK2A | 1029 | Western blot | 23448348 |
| hsa-mir-34a-5p | MIMAT0000255 | CDK2C | 1031 | qRT-PCR/Reporter assay | 23448348 |
| hsa-mir-34a-5p | MIMAT0000255 | GADD45A | 1647 | PCR array | 21566225 |
| hsa-mir-34a-5p | MIMAT0000255 | E2F3 | 1871 | Luciferase reporter assay/qRT-PCR/Western blot | 21566225/23622248 |
| hsa-mir-34a-5p | MIMAT0000255 | SFN | 2810 | Proteomics | 26035691/28123637 |
| hsa-mir-34a-5p | MIMAT0000255 | HDAC1 | 3065 | /Proteomics/qRT-PCR/Reporter assay/Western blot | 26035691/28123637 |
| hsa-mir-34a-5p | MIMAT0000255 | SMAD4 | 4089 | Luciferase reporter assay/PAR-CLIP/qRT-PCR/Western blot | 23448348 |
| hsa-mir-34a-5p | MIMAT0000255 | MOM2 | 4171 | Proteomics | 26077733 |
| hsa-mir-34a-5p | MIMAT0000255 | MOM3 | 4172 | Proteomics | 21566225 |
| hsa-mir-34a-5p | MIMAT0000255 | MOM4 | 4173 | Proteomics | 21566225 |
| hsa-mir-34a-5p | MIMAT0000255 | MOM5 | 4174 | Proteomics | 21566225 |
| hsa-mir-34a-5p | MIMAT0000255 | MOM6 | 4175 | Proteomics | 21566225 |
| hsa-mir-34a-5p | MIMAT0000255 | MOM7 | 4176 | Proteomics | 21566225 |
| hsa-mir-34a-5p | MIMAT0000255 | CDC23 | 8697 | Proteomics | 21566225 |
| hsa-mir-34a-5p | MIMAT0000255 | CCNE2 | 9134 | Luciferase reporter assay/Microarray/PAR-CLIP/Western blot | 19461653/19714404 |
| hsa-mir-34a-5p | MIMAT0000255 | STAG2 | 10735 | Proteomics | 23448348 |
| hsa-mir-34a-5p | MIMAT0000255 | FZR1 | 51343 | PAR-CLIP | 21566225 |
| hsa-mir-34a-5p | MIMAT0000255 | ANAPC5 | 51433 | CLASH | 26076125 |
| hsa-mir-34a-5p | MIMAT0000255 | CASP8 | 841 | PCR array | 23622248 |
| hsa-mir-34a-5p | MIMAT0000255 | CASP9 | 842 | PCR array | 23622248 |
| hsa-mir-34a-5p | MIMAT0000255 | TNFRSF10B | 8795 | PCR array | 23622248 |
| hsa-mir-34a-5p | MIMAT0000255 | CYCS | 54205 | PCR array | 23622248 |
| hsa-mir-34a-5p | MIMAT0000255 | AKT1 | 207 | Flow/qRT-PCR/Western blot | 27073535 |

(Continued)
and p53 germline mutations (62, 63). TNBCs are the most aggressive type of breast cancer and most patients do not respond well to conventional chemotherapy (64, 65). The concept of gene therapy has been brought up as an alternative to chemotherapy to treat these aggressive cancers (66, 67). In this case RNAi could be used to target mutated proteins which are a product of missense mutations, leading to high constitutive expression of mutated proteins such as TP53 (68). However, suppressing genes with RNAi requires effective delivery methods, which have proven to be effective in some cases but difficult in both in vivo and in vitro systems (69–71). Therefore, other means of gene targeting therapies could be valued options.

miRNAs have emerged as important biological regulators of normal development (72) and evidence suggest that they play a major role in human cancers (73). miRNAs are abundantly found in multiple human cells and have the ability to regulate gene expression of approximately 60% of all mammalian genes (74, 75) hence they promote themselves as an attractive therapeutic option. Several miRNAs have been shown to be altered in TNBCs (24–28). Two examples of this are through the activation of STAT3, a transcription factor that is well documented in cancers (76). Activation of STAT3 is observed in TNBC tumors where epigenetic suppression of miR-146b leads to constitutive STAT3 activation and tumor growth (77, 78). Secondly, through the activation of the miRNA-200 family, these miRNAs are known to negatively regulate the epithelial to mesenchymal transition (EMT) and can specifically target ZEB1/2 (79, 80). Therefore, other means of gene targeting therapies could be valued options.

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PGRMC1 has been deemed a novel tumor biomarker due to its elevated levels in human cancers (49, 81–84). Because PGRMC1 plays a role in chemoresistance, tumor progression and growth it has become an attractive therapeutic target (36). Intriguingly, PGRMC1 is commonly observed in aggressive TNBC tissue (35). This is particularly interesting because TNBCs lack the classical signaling hormone receptors, ER and PR yet TNBCs that overexpress PGRMC1 could respond to steroid hormones via PGRMC1. Our previous studies showed that PGRMC1 is clearly overexpressed in the TNBC cell line MDA-MB-468 and using a known inhibitor (AG-205) and

**FIGURE 3** | PGRMC1 silencing alters pathways that are have miRNA target genes involved. Silencing PGRMC1 upregulates different miRNAs (from AG-205 treatment) that target similar miRNA target genes which are upregulated in metastatic breast cancer samples. (A) Target genes highlighted in pink of the top ten most upregulated miRNAs highlighted in green. (B) The top ten most downregulated miRNAs highlighted in green and their direct targets highlighted in grey. (C) and (D) The top 10 most significantly enriched pathways (non-disease related) were identified by KEGG analysis, adjusted p < 0.05. (E, F) miRNA target genes show involvement in GO: terms Molecular functions and Biological process.

| miRNA ID | Accession | Target Gene | Target ID | Experiment | Literature PubMed ID |
|----------|------------|-------------|-----------|------------|---------------------|
| hsa-mir-34a-5p | MIMAT0000255 | BIRC2 | 329 | PCR array | 28097098 |
| hsa-mir-34a-5p | MIMAT0000255 | BIRC3 | 330 | Microarray/Northern blot | 17540599 |
| hsa-mir-34a-5p | MIMAT0000255 | XIAP | 331 | PCR array | 28097098 |
| hsa-mir-34a-5p | MIMAT0000255 | FASLG | 356 | PCR array | 28097098 |
| miRNA ID | Accession | Target Gene | Target ID | Experiment | Literature PubMed ID |
|----------|-----------|-------------|-----------|------------|----------------------|
| hsa-mir-617 | MIMAT003286 | PABPC1 | 26886 | HITS-CLIP | 19536157 |
| hsa-mir-3138 | MIMAT0015006 | PPP2RSE | 5529 | PAR-CLIP | 23592263 |
| hsa-mir-3138 | MIMAT0015006 | PPP2R1A | 5518 | PAR-CLIP | 26701625 |
| hsa-mir-3138 | MIMAT0015006 | CDC25A | 993 | PAR-CLIP | 23592263 |
| hsa-mir-3138 | MIMAT0015006 | CDK6 | 1021 | PAR-CLIP | 26701625 |
| hsa-mir-3138 | MIMAT0015006 | FZD6 | 8323 | HITS-CLIP//PAR-CLIP | 24398324|21572407|23313552 |
| hsa-mir-3138 | MIMAT0015006 | PIAS4 | 5518 | PAR-CLIP | 26701625 |
| hsa-mir-3150b-3p | MIMAT0018194 | CBL | 867 | PAR-CLIP | 26701625 |
| hsa-mir-3150b-3p | MIMAT0018194 | BBC3 | 27113 | PAR-CLIP | 23592263 |
| hsa-mir-3150b-3p | MIMAT0018194 | WNT7B | 7477 | PAR-CLIP | 23592263|26701625 |
| hsa-mir-3150b-3p | MIMAT0018194 | RBM8A | 9939 | PAR-CLIP | 23592263|23446348|22012620|20371350|26701625|27292025 |
| hsa-mir-3150b-3p | MIMAT0018194 | YWHAZ | 7534 | PAR-CLIP | 26701625 |
| hsa-mir-3150b-3p | MIMAT0018194 | SUGT1 | 10910 | PAR-CLIP | 23592263|20371350 |
| hsa-mir-3150b-3p | MIMAT0018194 | RALBP1 | 10928 | PAR-CLIP | 26701625 |
| hsa-mir-3150b-3p | MIMAT0018194 | PABPC1L2B | 645974 | PAR-CLIP | 23592263 |
| hsa-mir-3150b-3p | MIMAT0018194 | FZD7 | 8324 | PAR-CLIP | 26701625 |
| hsa-mir-3150b-3p | MIMAT0018194 | IKBKG | 8517 | PAR-CLIP | 24398324 |
| hsa-mir-3150b-3p | MIMAT0018194 | PLK1 | 5347 | PAR-CLIP | 26701625 |
| hsa-mir-3150b-3p | MIMAT0018194 | PABPN1 | 8106 | PAR-CLIP | 26701625 |
| hsa-mir-3150b-3p | MIMAT0018194 | BCL2L1 | 598 | PAR-CLIP | 23592263|26701625 |
| hsa-mir-3150b-3p | MIMAT0018194 | MAPK14 | 1432 | PAR-CLIP | 26697839 |
| hsa-mir-3150b-3p | MIMAT0018194 | CRK | 1398 | PAR-CLIP | 26697839 |
| hsa-mir-3150b-3p | MIMAT0018194 | CDK4 | 1019 | PAR-CLIP | 26697839 |
| hsa-mir-3150b-3p | MIMAT0018194 | SMAD2 | 4087 | PAR-CLIP | 27292025 |
| hsa-mir-3150b-3p | MIMAT0018194 | RPS6KA5 | 9252 | PAR-CLIP | 23824327 |
| hsa-mir-3150b-3p | MIMAT0018194 | CUL2 | 8453 | PAR-CLIP | 21572407 |
| hsa-mir-3150b-3p | MIMAT0018194 | WEE1 | 7465 | PAR-CLIP | 27418678 |
| hsa-mir-3150b-3p | MIMAT0018194 | NFkBIB | 4793 | PAR-CLIP | 27418678 |
| hsa-mir-3150b-3p | MIMAT0018194 | CDKN1B | 1027 | PAR-CLIP | 23446348 |

(Continued)
TABLE 3 | Continued

| miRNA ID | Accession | Target Gene | Target ID | Experiment | Literature PubMed ID |
|----------|-----------|-------------|-----------|------------|---------------------|
| hsa-mir-221-5p | MIMAT0004568 | CDKN1B | 1027 | Chromatin immunoprecipitation//Co-immunoprecipitation//qRT-PCR//Western blot | 26153983 |
| hsa-mir-221-5p | MIMAT0004568 | ABL1 | 25 | PAR-CLIP | 26701625 |
| hsa-mir-221-5p | MIMAT0004568 | CDKN1C | 1028 | Chromatin immunoprecipitation//Co-immunoprecipitation//qRT-PCR//Western blot | 26153983 |
| hsa-mir-221-5p | MIMAT0004568 | ITGB1 | 3688 | PAR-CLIP | 20371350 |
| hsa-mir-221-5p | MIMAT0004568 | GRB2 | 2885 | PAR-CLIP | 26701625 |
| hsa-mir-221-5p | MIMAT0004568 | CARD8 | 22900 | HTS-CLIP | 23313552 |
| hsa-mir-221-5p | MIMAT0004568 | STAT2 | 6773 | PAR-CLIP | 20371350 |
| hsa-mir-221-5p | MIMAT0004568 | FZD2 | 2535 | HTS-CLIP | 23824327 |
| hsa-mir-221-5p | MIMAT0004568 | IL6R | 3570 | Luciferase reporter assay//qRT-PCR//Western blot | 26645045 |
| hsa-mir-3201 | MIMAT0015086 | LAMC1 | 3915 | PAR-CLIP | 23446348|2021026020371350|26701625|27292025 |
| hsa-mir-3201 | MIMAT0015086 | SPRED1 | 161742 | PAR-CLIP | 23592263 |
| hsa-mir-3201 | MIMAT0015086 | TNFRSF10B | 8795 | HTS-CLIP | 23313552 |
| hsa-mir-3201 | MIMAT0015086 | PTEN | 5728 | PAR-CLIP | 23592263 |
| hsa-mir-3201 | MIMAT0015086 | EGLN1 | 54583 | PAR-CLIP | 21572407 |
| hsa-mir-3201 | MIMAT0015086 | DUSP10 | 11221 | HITS-CLIP | 23824327 |
| hsa-mir-3201 | MIMAT0015086 | CD4 | 920 | PAR-CLIP | 23592263 |
| hsa-mir-3201 | MIMAT0015086 | VAV2 | 7410 | PAR-CLIP | 26701625 |
| hsa-mir-3201 | MIMAT0015086 | CDC25B | 994 | PAR-CLIP | 23824327 |
| hsa-mir-3201 | MIMAT0015086 | CBL | 867 | HTS-CLIP | 23824327 |
| hsa-mir-3201 | MIMAT0015086 | VAV2 | 7410 | PAR-CLIP | 26701625 |
| hsa-mir-3201 | MIMAT0015086 | CD4 | 920 | PAR-CLIP | 23592263 |
| hsa-mir-3201 | MIMAT0015086 | SERPINE1 | 5054 | PAR-CLIP | 22012620 |
| hsa-mir-642b-3p | MIMAT0018444 | CAON1 | 774 | HTS-CLIP | 23824327 |
| hsa-mir-642b-3p | MIMAT0018444 | CDC25B | 994 | PAR-CLIP | 23592263 |
| hsa-mir-642b-3p | MIMAT0018444 | SYK | 6850 | HTS-CLIP | 240643019282157 |
| hsa-mir-642b-3p | MIMAT0018444 | MAP3K5 | 4217 | PAR-CLIP | 21572407|27292025 |
| hsa-mir-642b-3p | MIMAT0018444 | NRAS | 4893 | PAR-CLIP | 21572407 |
| hsa-mir-642b-3p | MIMAT0018444 | CDKN1A | 1026 | PAR-CLIP | 26701625 |

PGRMC1 silencing we demonstrated that it promotes TNBC cell proliferation through the EGFR/P13K/AKT pathway (33). However, our study also focused on signaling pathways associated with ER-positive breast cancers (33). Here, we mainly focused on TNBCs as alternative mechanisms regulated by PGRMC1 in TNBCs should be further explored. To study and uncover novel mechanisms behind PGRMC1 we performed miRNome profiling following AG-205 treatment and PGRMC1 silencing. Studying the human miRNome enabled us to identify miRNAs that were significantly altered following PGRMC1 signal disruption and silencing. This presents itself as an important way to identify signaling pathways and genes involved within these pathways that could be associated with PGRMC1.

Human miRNome profiling identified alteration of 1,008 miRNAs following AG-205 treatment and 776 miRNAs after PGRMC1 siRNA transfection. Using a variety of gene mining platforms (miRNet, xenobrowser, cbioportal, Reactome, Kaplan-Meier plotter and GeneMANIA) we identified miRNA-mRNA network hubs that are altered when PGRMC1 is impaired. Network analysis by miRNet, an all in one, high-performance, analytics tool was used to predict PGRMC1 altered miRNAs targets (85). miRNet, incorporates data from TarBase, miRTarBase, starBase, Epimir, Pharmacomir, SM2mir, Phenomir, HMD2, miR2Disease, miRanda and miRecords making it a reliable data mining source (86). The top 10 most upregulated and downregulated miRNAs following AG-205 treatment and PGRMC1 silencing were identified. KEGG pathway analysis identified matching enriched pathways between the two treatment groups which included, pathways in cancer, cell cycle and p53 signaling pathway. In addition, TCGA derived gene expression data analysis taken from metastatic tissue identified the 22 most overexpressed genes in response to PGRMC1 signaling inhibition and silencing. Based on the above data, miRNAs that were upregulated following PGRMC1 impairment directly target and have the capability to suppress genes that are overexpressed in TNBC patient samples. However, because of their function we proceeded to study the downregulated miRNAs but considered them to be possible biomarkers. Interestingly, miR-30b, miR-664a-3p and miR-93-3p, miR-224-5p all which were downregulated following PGRMC1 impairment are commonly observed in multiple cancers including ovarian (87), prostate (88), gastric (89) and metastatic breast cancer (90–92). Furthermore, miR-181a-3p, miR-224-5p, miR-345-5p and miR-93-3p act like oncogenes and all have been associated with chemoresistance, migration, metastasis and stemness (87, 88, 91, 93). Based on the available literature disrupting PGRMC1 downregulates miRNAs that display oncogenic potential.

To get a better understanding of the signaling mechanism involved within the upregulated miRNA target genes we employed the Reactome pathway analyzer. This enabled us to study different signaling pathways that are not associated with the KEGG analysis from the miRNet database. We observed the upregulated genes to be involved in cell cycle and signal transduction mechanisms. This agrees with our previous findings of cell cycle involvement;
| miRNA ID     | Accession | Target Gene | Target ID | Experiment                  | Literature PubMed ID |
|-------------|-----------|-------------|-----------|------------------------------|----------------------|
| hsa-miR-139-5p | MIMAT0000250 | BCL2       | 596       | Luciferase reporter assay/qRT-PCR/Western blot | 27244080             |
| hsa-miR-139-5p | MIMAT0000250 | FOS        | 2353      | qRT-PCR/Western blot         | 23001723|27668889 |
| hsa-miR-139-5p | MIMAT0000250 | HRAS       | 3265      | Luciferase reporter assay     | 24158791             |
| hsa-miR-139-5p | MIMAT0000250 | HSP90AA1   | 3320      | PAR-CLIP                     | 21572407             |
| hsa-miR-139-5p | MIMAT0000250 | IGF1R      | 3480      | Luciferase reporter assay/qRT-PCR/Western blot | 2258005|124942287|26097570 |
| hsa-miR-139-5p | MIMAT0000250 | JUN        | 3725      | Luciferase reporter assay/qRT-PCR/Western blot | 25499265             |
| hsa-miR-139-5p | MIMAT0000250 | MET        | 4233      | Luciferase reporter assay/qRT-PCR/Western blot | 26497851             |
| hsa-miR-139-5p | MIMAT0000250 | NFKB1      | 4790      | Luciferase reporter assay     | 24158791             |
| hsa-miR-139-5p | MIMAT0000250 | PIK3CA     | 5290      | Luciferase reporter assay     | 24158791             |
| hsa-miR-139-5p | MIMAT0000250 | WNT1       | 7471      | Luciferase reporter assay/Western blot | 25529604             |
| hsa-miR-139-5p | MIMAT0000250 | IGF1R      | 3480      | Luciferase reporter assay/qRT-PCR/Western blot | 2258005|124942287|26097570 |
| hsa-miR-139-5p | MIMAT0000250 | MET        | 4233      | Luciferase reporter assay     | 26497851             |
| hsa-miR-139-5p | MIMAT0000250 | BCL2       | 596       | Luciferase reporter assay/qRT-PCR/Western blot | 27244080             |
| hsa-miR-139-5p | MIMAT0000250 | HRAS       | 3265      | Luciferase reporter assay     | 24158791             |
| hsa-miR-139-5p | MIMAT0000250 | IGF1R      | 3480      | Luciferase reporter assay/qRT-PCR/Western blot | 2258005|124942287|26097570 |
| hsa-miR-139-5p | MIMAT0000250 | JUN        | 3725      | Luciferase reporter assay/qRT-PCR/Western blot | 25499265             |
| hsa-miR-139-5p | MIMAT0000250 | MET        | 4233      | Luciferase reporter assay/qRT-PCR/Western blot | 26497851             |
| hsa-miR-139-5p | MIMAT0000250 | PIK3CA     | 5290      | Luciferase reporter assay     | 24158791             |
| hsa-miR-139-5p | MIMAT0000250 | RAP1B      | 5908      | PAR-CLIP//qRT-PCR/Western blot | 24942287|23592263 |
| hsa-miR-139-5p | MIMAT0000250 | ROCK2      | 9475      | Luciferase reporter assay/qRT-PCR/Western blot | 24942287             |
| hsa-miR-224-5p | MIMAT0000281 | BCL2       | 596       | Microarray/qRT-PCR/Western blot | 22989374             |
| hsa-miR-224-5p | MIMAT0000281 | ROCK2      | 9475      | Luciferase reporter assay/qRT-PCR/Western blot | 24942287             |
| hsa-miR-224-5p | MIMAT0000281 | HRAS       | 3265      | Luciferase reporter assay     | 26037864             |
| hsa-miR-224-5p | MIMAT0000281 | CASP3      | 836       | Luciferase reporter assay/Western blot | 20023766|17777217 |22989374 |
| hsa-miR-224-5p | MIMAT0000281 | CDC42      | 998       | Microarray/qRT-PCR/Western blot | 27315344             |
| hsa-miR-224-5p | MIMAT0000281 | MTOR       | 2475      | Luciferase reporter assay/qRT-PCR/Western blot | 27315344             |
| hsa-miR-224-5p | MIMAT0000281 | GSK3B      | 2932      | Luciferase reporter assay     | 25588771             |
| hsa-miR-224-5p | MIMAT0000281 | KRAS       | 3845      | qRT-PCR/Western blot          | 23667495             |
| hsa-miR-224-5p | MIMAT0000281 | PIK3CA     | 5290      | Luciferase reporter assay     | 24158791             |
| hsa-miR-224-5p | MIMAT0000281 | ROCK2      | 9475      | Luciferase reporter assay/qRT-PCR/Western blot | 24942287             |
| hsa-miR-224-5p | MIMAT0000281 | ROCK2      | 9475      | Luciferase reporter assay/qRT-PCR/Western blot | 24942287             |
| hsa-miR-224-5p | MIMAT0000281 | ROCK2      | 9475      | Luciferase reporter assay/qRT-PCR/Western blot | 24942287             |
| hsa-miR-224-5p | MIMAT0000281 | ROCK2      | 9475      | Luciferase reporter assay/qRT-PCR/Western blot | 24942287             |
| hsa-miR-224-5p | MIMAT0000281 | ROCK2      | 9475      | Luciferase reporter assay/qRT-PCR/Western blot | 24942287             |
| hsa-miR-224-5p | MIMAT0000281 | ROCK2      | 9475      | Luciferase reporter assay/qRT-PCR/Western blot | 24942287             |
| hsa-miR-224-5p | MIMAT0000281 | ROCK2      | 9475      | Luciferase reporter assay/qRT-PCR/Western blot | 24942287             |
| hsa-miR-224-5p | MIMAT0000281 | ROCK2      | 9475      | Luciferase reporter assay/qRT-PCR/Western blot | 24942287             |
| hsa-miR-224-5p | MIMAT0000281 | ROCK2      | 9475      | Luciferase reporter assay/qRT-PCR/Western blot | 24942287             |
| hsa-miR-224-5p | MIMAT0000281 | ROCK2      | 9475      | Luciferase reporter assay/qRT-PCR/Western blot | 24942287             |
| hsa-miR-224-5p | MIMAT0000281 | ROCK2      | 9475      | Luciferase reporter assay/qRT-PCR/Western blot | 24942287             |
| hsa-miR-224-5p | MIMAT0000281 | ROCK2      | 9475      | Luciferase reporter assay/qRT-PCR/Western blot | 24942287             |
| miRNA ID | Accession | Target Gene | Target ID | Experiment | Literature PubMed ID |
|----------|-----------|-------------|-----------|------------|---------------------|
| hsa-mir-135a-5p | MMAT0000428 | NRF2 | 4306 | Luciferase reporter assay/qRT-PCR | 19944075 |
| hsa-mir-128-3p | MMAT0000424 | CASP3 | 1871 | Luciferase reporter assay | 18810376 | 19013014 |
| hsa-mir-128-3p | MMAT0000424 | MTOR | 5979 | Luciferase reporter assay | 22853714 |
| hsa-mir-128-3p | MMAT0000424 | BAX | 581 | Luciferase reporter assay | 23526655 |
| hsa-mir-128-3p | MMAT0000424 | RUNX1 | 861 | Luciferase reporter assay | 23313552 |
| hsa-mir-128-3p | MMAT0000424 | PTEN | 5728 | Luciferase reporter assay | 24132591 | 25250865 |
| hsa-mir-128-3p | MMAT0000424 | PTGS2 | 5743 | Luciferase reporter assay | 17612493 |
| hsa-mir-128-3p | MMAT0000424 | MAP2K1 | 5604 | Luciferase reporter assay | 20371350 |
| hsa-mir-128-3p | MMAT0000424 | PTEN | 5728 | Luciferase reporter assay | 24132591 | 25250865 |
| hsa-mir-128-3p | MMAT0000424 | PTGS2 | 5743 | Luciferase reporter assay | 17612493 |
| hsa-mir-128-3p | MMAT0000424 | MAP2K1 | 5604 | Luciferase reporter assay | 20371350 |
| hsa-mir-128-3p | MMAT0000424 | PTEN | 5728 | Luciferase reporter assay | 24132591 | 25250865 |
| hsa-mir-128-3p | MMAT0000424 | PTGS2 | 5743 | Luciferase reporter assay | 17612493 |
| hsa-mir-128-3p | MMAT0000424 | MAP2K1 | 5604 | Luciferase reporter assay | 20371350 |

(Continued)
| miRNA ID | Accession | Target Gene | Target ID | Experiment | Literature | PubMed ID |
|----------|-----------|-------------|-----------|------------|-----------|-----------|
| hsa-mir-128-3p | MMAT0000424 | EGFR | 1956 | Western blot | 22853714 |
| hsa-mir-128-3p | MMAT0000424 | PIK3R1 | 5295 | Luciferase reporter assay/Microarray/qRT-PCR | 27903811 |
| hsa-mir-128-3p | MMAT0000424 | MAP2K1 | 5604 | Sequencing | 23313552 |
| hsa-mir-128-3p | MMAT0000424 | SOS1 | 6654 | HITS-CLIP | 23313552 |
| hsa-mir-128-3p | MMAT0000424 | MAP2K1 | 5604 | Sequencing | 20371350 |
| hsa-mir-128-3p | MMAT0000424 | WASL | 8976 | PAR-CLIP | 23622248 |
| hsa-mir-128-3p | MMAT0000424 | GNG12 | 55970 | PAR-CLIP | 24398324 | 21572407 |
| hsa-mir-93-3p | MMAT0004509 | CDC42 | 998 | CLASH | 23622248 |
| hsa-mir-93-3p | MMAT0004509 | MAP2K1 | 5604 | CLASH | 23622248 |
| hsa-mir-93-3p | MMAT0004509 | HSP90AB1 | 3326 | CLASH | 23622248 |
| hsa-mir-93-3p | MMAT0004509 | LAMA4 | 3910 | CLASH | 23622248 |
| hsa-mir-93-3p | MMAT0004509 | STAT5B | 6777 | CLASH | 23622248 |
| hsa-mir-93-3p | MMAT0004509 | NCOA4 | 8031 | CLASH | 23622248 |
| hsa-mir-93-3p | MMAT0004509 | CUL2 | 8453 | CLASH | 23622248 |
| hsa-mir-93-3p | MMAT0004509 | SUFU | 51684 | CLASH | 23622248 |
| hsa-mir-93-3p | MMAT0004509 | CYGS | 54205 | CLASH | 23622248 |
| hsa-mir-93-3p | MMAT0004509 | FYN | 3326 | CLASH | 23622248 |
| hsa-mir-93-3p | MMAT0004509 | ACTB | 60 | CLASH | 23622248 |
| hsa-mir-93-3p | MMAT0004509 | FER | 87 | CLASH | 23622248 |
| hsa-mir-93-3p | MMAT0004509 | PARD3 | 56288 | HITS-CLIP | 23824327 |
| hsa-mir-93-3p | MMAT0004509 | PPP1R12A | 4659 | HITS-CLIP | 23622248 |
| hsa-mir-93-3p | MMAT0004509 | IRAK1 | 3654 | HITS-CLIP | 23622248 |
| hsa-mir-93-3p | MMAT0004509 | EIF4EBP1 | 1978 | HITS-CLIP | 23622248 |
| hsa-mir-93-3p | MMAT0004509 | CDKN1A | 1026 | HITS-CLIP | 23622248 |
| hsa-mir-93-3p | MMAT0004509 | XIAP | 331 | HITS-CLIP/PAR-CLIP | 23446384 | 23824327 |
| hsa-mir-30b-3p | MMAT0004589 | IGF1 | 3479 | HITS-CLIP | 23313552 |
| hsa-mir-30b-3p | MMAT0004589 | CDKN1A | 1026 | HITS-CLIP | 23622248 |
| hsa-mir-30b-3p | MMAT0004589 | XIAP | 331 | HITS-CLIP/PAR-CLIP | 23313552 |
| hsa-mir-30b-3p | MMAT0004589 | BCL2L1 | 598 | PAR-CLIP | 26701625 |
| hsa-mir-30b-3p | MMAT0004589 | CRKL | 1399 | HITS-CLIP | 23824327 |
| hsa-mir-30b-3p | MMAT0004589 | ITGA3 | 3675 | HITS-CLIP | 23701625 | 23313552 |
| hsa-mir-30b-3p | MMAT0004589 | MDM2 | 4193 | PAR-CLIP | 27920225 |
| hsa-mir-30b-3p | MMAT0004589 | PDGFRA | 5156 | HITS-CLIP/PAR-CLIP | 23463848 | 23313552 |
| hsa-mir-30b-3p | MMAT0004589 | RARA | 5914 | PAR-CLIP | 23592263 |
| hsa-mir-30b-3p | MMAT0004589 | CTNND1 | 1500 | PAR-CLIP | 23592263 | 26701625 |
| hsa-mir-30b-3p | MMAT0004589 | COL5A1 | 1289 | PAR-CLIP | 23592263 |
| hsa-mir-30b-3p | MMAT0004589 | ITGB3 | 3690 | HITS-CLIP | 23824327 |
| hsa-mir-30b-3p | MMAT0004589 | TNN1 | 7094 | HITS-CLIP | 23824327 |
| hsa-mir-30b-3p | MMAT0004589 | YWAH4 | 7554 | PAR-CLIP | 23824327 |
| hsa-mir-30b-3p | MMAT0004589 | YWAH4 | 7554 | PAR-CLIP | 27920225 |
| hsa-mir-30b-3p | MMAT0004589 | IRAK3 | 11213 | HITS-CLIP/PAR-CLIP | 21572407 | 20371350 | 23824327 |

(Continued)
TABLE 4 | Continued

| miRNA ID     | Accession | Target Gene | Target ID | Experiment       | Literature               | PubMed ID       |
|--------------|-----------|-------------|-----------|------------------|--------------------------|-----------------|
| hsa-mir-345-5p | MIMAT0000772 | NTRK3      | 4916      | Luciferase reporter assay |                          | 19370765        |
| hsa-mir-4291  | MIMAT0016922 | CDKN1A     | 1026      | PAR-CLIP         |                          | 26701625        |
| hsa-mir-4291  | MIMAT0016922 | LAMA4      | 3910      | PAR-CLIP         |                          | 23592263        |
| hsa-mir-4291  | MIMAT0016922 | CDK6       | 1021      | PAR-CLIP         |                          | 23446348[21572407| 20371350        |
| hsa-mir-4291  | MIMAT0016922 | FGF2       | 2247      | PAR-CLIP         |                          | 23592263[23446348| 20371350        |
| hsa-mir-4291  | MIMAT0016922 | LAMA4      | 3910      | PAR-CLIP         |                          | 23592263        |
| hsa-mir-4291  | MIMAT0016922 | RAF1       | 5894      | PAR-CLIP         |                          | 21572407        |
| hsa-mir-4291  | MIMAT0016922 | VASP       | 7408      | PAR-CLIP         |                          | 26701625        |
| hsa-mir-4291  | MIMAT0016922 | RAF1       | 5894      | PAR-CLIP         |                          | 21572407        |
| hsa-mir-4291  | MIMAT0016922 | RAF1       | 5894      | PAR-CLIP         |                          | 21572407        |
| hsa-mir-4291  | MIMAT0016922 | RAF1       | 5894      | PAR-CLIP         |                          | 21572407        |
| hsa-mir-4291  | MIMAT0016922 | RAF1       | 5894      | PAR-CLIP         |                          | 21572407        |
| hsa-mir-4291  | MIMAT0016922 | RAF1       | 5894      | PAR-CLIP         |                          | 21572407        |
| hsa-mir-4291  | MIMAT0016922 | RAF1       | 5894      | PAR-CLIP         |                          | 21572407        |
| hsa-mir-4291  | MIMAT0016922 | RAF1       | 5894      | PAR-CLIP         |                          | 21572407        |
| hsa-mir-4291  | MIMAT0016922 | RAF1       | 5894      | PAR-CLIP         |                          | 21572407        |
| hsa-mir-181a-3p | MIMAT0000772 | ARHGDIA   | 396       | PAR-CLIP         |                          | 26701625        |

FIGURE 4 | Network analysis identified miRNA target genes to be upregulated in breast cancers following AG-205 treatment. miRNAs target differentially expressed genes miRNA target genes that are upregulated in metastatic breast tumor samples. A Log2 (normalized_counts) expression of upregulated miRNA target genes in metastatic breast tumor samples downloaded from TCGA database. miRNA target genes are involved in term pathways identified by KEGG analysis and are direct targets of the top miRNAs.
Interestingly upregulated genes involved in signal transduction mechanisms could be directly regulated by PGRMC1, as signal transduction mechanisms are known to be directly involved in cellular membranes where PGRMC1 is primarily located (94). To further study the clinical impact of these genes, we studied genetic alterations using OncoPrint. It was particularly interesting to see that only 10 genes displayed significant genetic alteration among the 22 genes that were overexpressed. However, of the ten genes the top two most genetically altered, \textit{CCND1} and \textit{YWHAZ} seemed to be overexpressed due to amplification and had overall lower survival probability. \textit{CCND1} has long been considered an oncogene and has been demonstrated to be amplified in 10-20\% in one study while in another study \textit{CCND1} amplification was seen in 78.6\% of breast cancer cases (95–97). \textit{CCND1} is thought to play a major role in ER-positive but not in ER-negative breast cancers (98). One of the reasons could be because it is a known downstream target of PR that can promote breast cancer cell proliferation (99, 100). One interesting thought could be that in TNBCs that overexpress PGRMC1, it could be enhancing the transcription of \textit{CCND1} even in tumors that lack ER and PR making it a potential target in TNBCs. The \textit{YWHAZ} gene has been described in multiple cancers including non-small lung cancer (101), hepatocellular carcinoma (102), gastric cancer (103), bladder cancer (104), and in breast cancers (105). Overexpression of \textit{YWHAZ} in breast cancers has been associated with chemoresistance to anthracyclines particularly associated with metastatic recurrence (105). This is also extremely interesting as PGRMC1 has been linked to chemoresistance (106) and it would be strongly warranted to further explore the possibility of a PGRMC1/YWHAZ axis in metastatic breast cancers that do not respond to chemotherapy.

**CONCLUSION**

In summary, our study identified that impairing PGRMC1 can alter miRNAs, specifically hsa-mir-646 that directly targets \textit{CCND1} (107) as well as hsa-mir-410-3p and hsa-mir-3150b-3p.
which target YWHAZ (108–113). Interestingly, both genes were amplified in patients with aggressive TNBCs and patients that express high levels of either gene have lower overall survival probability. Lastly, PGRMC1 impairment downregulates oncogenic miRNAs (miR-30b, miR-664a-3p and miR-93-3p, miR-224-5p, miR-181a-3p and miR-345-5p) in TNBC cells. Therefore, targeting PGRMC1 with AG-205 or a novel compound that can downregulate PGRMC1 expression could

FIGURE 6 | Reactome pathway analysis of the genes identified by KEGG term analysis. (A) Reactome pathways analysis of the miRNA target genes (n = 112) identified following AG-205 treatment illustrates increased pathway involvement. (B) Top pathways involved within the miRNA target genes (n = 84) observed following PGRMC1 silencing were also mapped. Over-represented pathways are highlighted in yellow. All overexpressed pathways are from gene lists of formerly annotated and published signatures.
be potential therapeutic options for TNBC patients that overexpress PGRMC1.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

**AUTHOR CONTRIBUTIONS**

Conception and design: RL and DP. Methodology was developed by DP and VR. Data acquisition: DP, MR, and VR. Data was interpreted by RL, DP, MR, VR, RS, and AE. The manuscript was written and/or revised by DP, MR, RS, VM, TG, and RL. This study was supervised by RL. All authors contributed to the article and approved the submitted version.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.710337/full#supplementary-material

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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