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# Role of miRNA in the tumorigenesis of triple negative breast cancer integrated bioinformatics analysis

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**Abstract**
It is well recognized that microRNAs are essential for the growth and development of various cancer stages. Oncomirs and tumor suppressor miRNAs are two different types of miRNAs that can either target oncogenes or tumor suppressor genes. Depending on the tissue type and developmental phases, certain miRNAs solely function as tumor suppressors or oncomirs, whereas a few others may function as both. In the clinic, many methods of overexpressing the tumor suppressor miRNAs have been explored for therapeutic intervention. Another potential method for cancer therapy utilizing RNAi is the down-regulation of oncomirs employing inhibitors of miRNAs such as locked nucleic acid (LNA) miRNA inhibitors. It has been demonstrated that miR-491 inhibits MMP-9 expression in glioblastomas. Its precise mode of action in numerous cancer types is still unknown. MMP-9 is important for the development of breast cancer. According to reports, breast cancer's aggressiveness and greater metastatic potential are both correlated with MMP-9 levels. These patients also have a worse survival percentage due to their elevated MMP-9 expression. The role of miR-491 in breast cancer was the main topic of this investigation. Studies *in vitro* were conducted to determine how miR-491 affected MMP-9 and EGFR, two well-known oncogenes linked to the aggressiveness of breast cancer. Additionally, based on a systems biology approach, computational and bioinformatics methodologies were used to determine the targets of miR-491 in breast cancer as well as the potential anti-cancer mechanism of this miRNA.

**Keywords:** miRNA, tumorigenesis, triple negative, breast cancer

## Introduction
Cancer develops when cells lose the ability to control cell division, resulting in unchecked cell replication. These aberrant cells can create secondary tumors, which increase the tumor burden, and they can spread to surrounding areas as well as distant organs. The primary causes of loss of control over cell division are genetic alterations, which can be inherited or brought on by environmental influences, as well as flaws in the replication machinery that result in mistakes in DNA replication. As people age, their risk of developing cancer rises. A tumor is a mass of tissue formed when cells actively divide due to an aberration in the control of cell division. There are two sorts of tumors: benign tumors, which do not spread to other body parts, and malignant tumors, which can spread to other body parts and are cancerous. The four major types of cancer (growing from immune cells) are leukemia (growing from blood cells), sarcoma (growing from connective tissues), carcinoma (growing from epithelial cells), and lymphoma.

Typically, tumor suppressor miRNA levels decline as cancer develops. As a result, researchers are working hard to figure out how to slow tumor growth by over expressing or restoring down regulated miRNAs. MiRNA sponge therapy is applied when cancer cells overexpress incomers. The development of miRNA-based treatments frequently faces difficulties due to the low uptake of miRNAs inside the cell, a lack of an effective delivery route to target miRNAs to tumor tissues, and the negative effects of miRNA overexpression. MiRNAs and miRNA sponges are delivered to tumor cells using liposomes, viruses, and nanoparticles.

Oncomirs are down regulated in cancer cells using microRNA sponge treatment. Antisense oligonucleotides, which act as sponges, can bind to the target miRNA and render it inactive. Numerous non-coding RNAs found in living things have been shown to bind to miRNAs and render them inactive. HCV infection is a well-known example of naturally occurring RNAs acting as the sponge. HCV genomic RNA binds to the liver-specific microRNA miR-122 like a sponge. The HCV genome is shielded by this binding. Additionally, miR-122's binding to the 5' UTR sections of viral RNA triggers the replication of the virus.

Clinical trials for the treatment of HCV infection are being conducted with the miR-122 inhibitor Miravirsen. A second possible application for miRNA sponge was seen in hepatocellular cancer (HCC). In HCC patients, MicroRNA-221 was upregulated, and greater levels of this miRNA are linked to accelerated carcinogenesis. MiR-221 activity was downregulated when adeno-associated viral vectors with several miR-221 binding sites were used. This prevented the progression of tumors.

**MicroRNAs as anti-cancer and anti-metastatic agents**

Non-coding regulatory RNAs with a length of 17–25 nucleotides, known as microRNAs (miRNAs), can post-transcriptionally control the expression of genes by partnering with the complementary sequences in the mRNA. Numerous studies have demonstrated the crucial roles miRNAs play in a wide range of illnesses, including cancer, viral infections, immune-related diseases, and neurological diseases. Ambros and colleagues found the first miRNA (lin-4) in *Caenorhabditis elegans* in 1993. Seven years later, let-7, the second miRNA, was identified. There are now about 2578 mature miRNA sequences known. Many of these miRNA sequences' biological roles, however, are yet unknown.

Numerous biological processes, including cell division, proliferation, differentiation, development, metabolism, cell death, and immunological responses, are known to be significantly influenced by microRNAs. Over the past few decades, there has been discussion about miRNAs' potential participation in carcinogenesis because of their role in the regulation of numerous biological processes. Based on their function in targeting oncogenes and tumor suppressor genes, respectively, microRNAs are known to behave as both tumor suppressors and oncogenes. Restoring the homeostasis of the miRNA profile in tumor cells can be used to stop carcinogenesis, demonstrating the possibility of employing miRNAs as a therapeutic approach. Deregulation of miRNAs is seen in many malignancies. The potential use of miRNA profiles for the detection and categorization of cancer is yet another significant application of miRNAs. Some miRNAs may be used as biomarkers to identify diseases and as tools to categorize diseases into different stages. As a result, detailed research into the function of miRNAs in carcinogenesis has the potential to be applied in clinics as a supplemental intervention method and a diagnostic biomarker for the early detection of cancer progression.

**Research Methodology**

**Role of miRNA dysregulation in tumorigenesis and its progression**

Hanahan and Weinberg list a number of characteristics of cancer, such as being self-sufficient in growth signals, insensitive to anti-growth signals, evasion of apoptosis, prolonged angiogenesis, tissue invasion and metastasis, unlimited replicative potential, etc. It is thought that miRNAs regulate one or more of these hallmarks of cancer during the onset and spread of the disease. They are categorized as oncomirs or tumor suppressor miRNAs depending on whether they promote or suppress tumors. While tumor suppressor miRNAs target oncogenes to reduce both tumor start and development, oncomirs suppress tumor suppressor proteins in the cells that are promoting tumor advancement. Depending on a variety of variables, including tissue type, developmental phases, etc., several miRNAs can function as both tumor suppressors and oncomirs. However, it is well recognized that miRNAs play important roles in controlling certain cancer hallmarks.

**Exploring the advantages of miRNAs in cancer therapy**

Numerous studies have been done in the last ten years to investigate the translational uses of miRNAs in the fight against cancer. MiRNAs and their target proteins are in a state of balance in healthy tissues. Changes in miRNA expression occur during cancer, which causes abnormal expression of its target proteins. MicroRNAs can function as both tumor suppressors and oncomirs depending on whether they target oncogenes or tumor suppressor genes. Up-regulation of tumor suppressor miRNAs has been extensively researched since it can halt the growth of tumors. Oncomirs could similarly be targeted using various techniques to lower their levels in cancer cells. As a result, miRNA substitution or inhibition may be investigated as a possible therapeutic target for tumor growth. The use of miRNAs as a diagnostic biomarker for cancer is another way that they are used in cancer treatment. Additionally, miRNA expression levels can be exploited in cancer therapy as a predictive biomarker.

**Mir-491 downregulates the expression of MMP-9.**

Both stable cell lines and miR-491-transfected cells underwent qRT-PCR to ascertain the impact of miR-491 overexpression on MMP-9. MMP-9 expression was significantly reduced in both miR-491 overexpressed stable cells.

**MiR-491 directly targets MMP-9**

By binding to the 3'UTR of MMP-9 mRNA or by targeting genes that control MMP-9 production, microRNAs have the ability to inhibit the expression of MMP-9. A reporter construct made of CMV promoter-driven Renilla Luciferase upstream of cloned MMP-9 3'UTR was used to confirm the type of interaction between miR-491 and MMP-9 3'UTR. Co-expression of the reporter and miR-491 allowed for the establishment of both transient transfections and persistent cell lines. When compared to control cells, miR-491-overexpressed MDA-MB-231 cells showed a substantial decrease in the production of Luciferase (GFP). The expression of Luciferase did not significantly change when the MMP-9 3’ UTR deficient reporters was utilized. This proves that miR-491 and MMP-9's 3'UTR can interact.

**Results and Discussion**

**HSA-miR-491 is down regulated in breast cancer**

Depending on the types of tissues, microRNA expression patterns and their activities may change. A microRNA that is known to reduce tumors in one type of tissue might not behave similarly in other tissues. The miRNAs' targets in various tissues can also differ greatly from one another. A web-based bioinformatics application called OncomiR was utilized to determine the miR-491 expression pattern in cancer tissues. Table 1 demonstrates that the mean expression levels of mir-491 in breast cancer tissues are 1.4 times lower than those in healthy breast tissues. Another tool, star Base (http://starbase.sysu.edu.cn), a server for miRNA expression analysis from TCGA data sets, confirmed the outcome. MiR-491 is downregulated in breast cancer tissues, as seen in Fig. 1. These findings show that decreased miR-491 levels are linked to the development of breast cancer.

**Table 1:** OncomiR analysis of miR-491 in cancer cells

| miRNA Name | Cancer Abbreviation | T-Test P-value | T-Test FDR | Upregulated in: | Tumor Log2 Mean Expression | Normal Log2 Mean Expression |
|---|---|---|---|---|---|---|
| hsa-miR-491-5p | BRCA | 1.89e-08 | 7.39e-08 | Normal | 1.55 | 2.25 |
| hsa-miR-491-5p | ESCA | 3.24e-02 | 1.08e-01 | Normal | 0.26 | 0.69 |
| hsa-miR-491-5p | HNSC | 2.07e-02 | 4.25e-02 | Normal | 0.36 | 0.70 |
| hsa-miR-491-5p | KICH | 1.43e-02 | 3.07e-02 | Tumor | 0.44 | 0.19 |
| hsa-miR-491-5p | KIRP | 2.42e-02 | 4.66e-02 | Tumor | 0.45 | 0.12 |
| hsa-miR-491-5p | PRAD | 1.22e-06 | 6.62e-06 | Tumor | 1.05 | 0.38 |
| hsa-miR-491-5p | THCA | 6.66e-05 | 2.17e-04 | Normal | 2.75 | 3.26 |
| hsa-miR-491-5p | UCEC | 1.37e-04 | 4.69e-04 | Tumor | 2.03 | 0.76 |

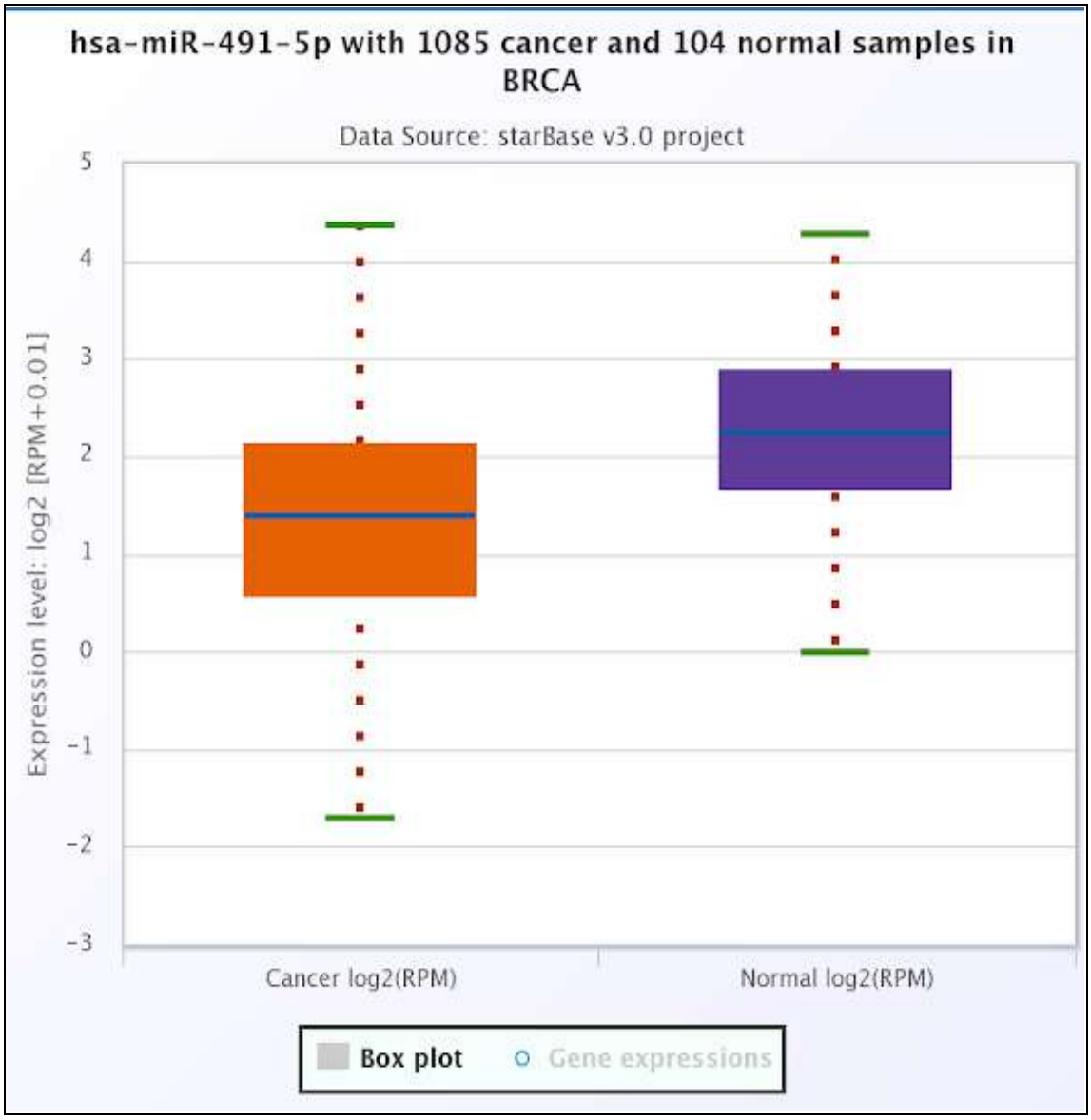


**Fig 1:** Expression levels of miR-491 in breast cancer tissues and corresponding normal tissues.

**HSA-miR-491 down-regulation is associated with reduced survival of triple- negative breast cancer patients**

Among the different types of breast cancer patients, KM plotter pan-cancer survival analysis showed a significant association between miR-491 expression and survival in triple-negative breast cancer patients. Patients with lower expression of miR-491 had reduced survival. Kaplan–Meier analysis of TCGA data sets showed a hazard ratio of 0.28 (Fig. -2).

**Over-expression of miR-491 in the MDA-MB-231 triple-negative breast cancer cell line**

Since miR-491 specifically exhibited a correlation with the survival of triple-negative breast cancer patients, the MDA-MB-231 triple-negative breast cancer cell line was used for further studies. Pre-miRNA Expression Lentivectors from System Biosciences, expressing precursor miRNA under the CMV promoter, were used to make lentiviruses for miRNA over-expression cell line development. The vector has copGFP under the EF1 promoter, which serves as a marker of successful overexpression of miRNA from the vector. Stable cell lines were made by lentiviral transduction, whereas transient transfection was performed using commercial transfection reagents.

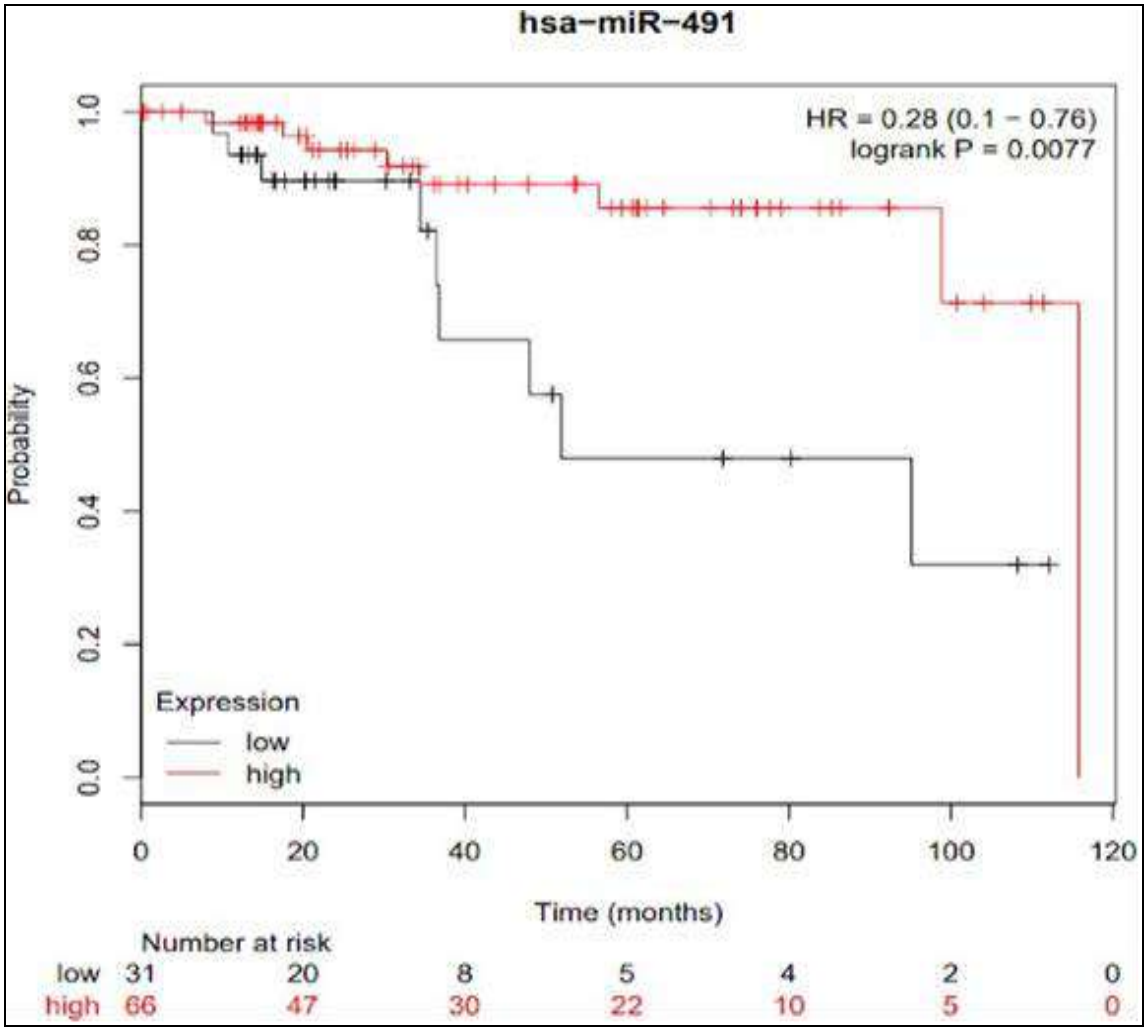


**Fig 2:** Reduced expression of hsa-miR-491 is associated with reduced survival of triple-negative breast cancer patients

**MMP-9 and EGFR are potential targets of miR-491**

The extracellular matrix is linked to a number of tumor growth mechanisms, including migration, invasion, and metastasis, angiogenesis, and cell death. MMP-9's activities in the growth of tumors are extensively researched because of its very important responsibilities in the regulation of ECM remodeling. MMP-9 expression is generally observed to encourage the development of tumors. According to reports, EGFR causes the expression of MMP-9 in cancer cells. MMP-9 and EGFR expression were found to be highly correlated in non-small cell lung cancer (NSCLC). The activation of the PI3K/Akt-mediated NF-κB pathway results from the induction of EGFR by IL-1, which also increases the production of MMP-9. Heparin-binding EGF-like factor (HB-EGF), which is bound to the plasma membrane, is then released after being broken down by the secreted MMP-9. The EGFR pathway is stimulated by HB-EGF, which ultimately induces the production of MMP-9. So, inhibiting EGFR could upregulate MMP-9 expression, and vice versa.

mRNA interactions are useful in reducing the list of potential genes and miRNAs for experimental validation, according to bioinformatics methods to predict miRNA. The sensitivity and specificity of these predictions are decreased by the possibility of false-positive and false-negative results. Programs for bioinformatics have strengths and limitations. Results from many prediction systems were thoroughly verified in order to improve specificity and sensitivity [56, 357]. For the purpose of identifying putative miR-491 targets, the target identification tools DIANAmT, miRanda, miRDB, miRWalk, RNAhybrid, PICTAR4, PICTAR5, PITA, RNA22, and Targetscan were obtained via the miRWalk website. Target prediction software identified the 3' UTR locations of MMP-9 and EGFR as potential miR-491 binding sites. While miRwalk, miRanda, PITA, RNAhybrid, and Targetscan suggested a potential contact between MMP-9 3'UTR and miR-491, DIANAmT, miRanda, miRDB, miRWalk, PICTAR5, and Targetscan predicted an interaction between EGFR 3'UTR and miR-491.

**MMP-9 is overexpressed in breast cancer and is negatively associated with patient survival.**

Through the GEPIA server, the MMP-9 expression profile in the Cancer Genome Atlas (TCGA) database was retrieved. With the exception of thymoma, MMP-9 is found to be elevated in all cancer types examined. MMP-9 expression was significantly upregulated in breast cancer tissues, according to an examination of 1085 tumor tissues and 112 normal tissues. MMP-9 expression and patient survival were significantly correlated, according to KMplotter's analysis of survival data. The calculated hazard ratio was 1.16. While the median survival of the high expression cohort was determined to be 45 months, that of the low expression cohort was 85 months.

**miR-491 downregulates EGFR expression in MDA-MB-231 cells.**

In stable MDA-MB-231 cells overexpressing miR-491, EGFR expression was examined. Results clearly demonstrated that miR-491-expressing MDA-MB-231 cells significantly reduced EGFR expression when compared to control cells transfected with the control GFP plasmid. The bioinformatics prediction reports that were earlier acquired are supported by this observation.

**EGFR is a direct target of miR-491**

Reporter constructs were created by cloning the EGFR 3'UTR downstream of the CMV promoter-driven Renilla Luciferase gene in order to demonstrate the direct interaction between the EGFR 3'UTR and miR-491. In MDA-MB-231 cells and 293T cells, the reporter plasmid and miR-491 were co-transfected. Transfection in 293T cells was accomplished using two different types of transfection reagents (Lipofectamine 2000 and Polyethyiemine). A notable decrease in Luciferase expression was seen in every experiment that was run. This demonstrates unequivocally that miR-491 may bind to the EGFR 3'UTR directly and suppress its expression.

**miR-491 does not affect the cell viability or cell cycle profiles of MDA-MB-231 cells**

Cell survival and cell cycle patterns of miR-491-transduced MDA-MB-231 cells were examined. The drop in MMP-9 and EGFR seen in miR-491 overexpressing cells is not the consequence of cell death, as shown by our findings, which showed that miR-491 overexpressing MDA-MB-231 cells did not exhibit any significant change in cell proliferation or cell cycle patterns. These findings do not, however, rule out the possibility that miR-491 can influence cell viability and cell cycle profiles. In both nasopharyngeal carcinoma and prostate cancer cells, microRNA-491 has been shown to control cell viability. The stable cell lines employed in this investigation were developed using single-cell cloning using the GFP tag after lentivirus transduction. In order to employ them for mRNA expression research, the cells that can avoid miR-491's negative effects on cell cycle patterns and proliferation were grown into separate colonies.

**miR-491 suppresses MDA-MB-231 cell migration and adhesion**

The most important phase in the process of cancer spread is cell migration. Higher MMP-9 and EGFR expression are linked to cancer cells' increased ability to migrate and metastasize. MiR-491 was examined for its impact on cellular migration because it was able to target both MMP-9 and EGFR. Our findings demonstrated that miR-491 overexpression in MDA-MB-231 cells effectively inhibited migration by a factor of ten. The migratory cells must cling to the substrate for the development of secondary tumors in distant areas to be successful. It was interesting to find that the MDA-MB-231 cells' ability to adhere to the substrate was severely reduced (37 times) by the overexpression of miR-491. These findings unequivocally show that miR-491 could target many stages of the metastatic cascade when it is overexpressed.

**EGFR expression positively correlates with MMP-9 expression in breast cancer**

The TCGA and GTEx datasets containing information on MMP-9 and EGFR expression were obtained via the GEPIA server in order to perform the Spearman technique to determine the connection between the two genes. When compared to the equivalent normal tissues in the TCGA database, the analysis revealed a positive correlation between EGFR and MMP-9 (R = 0.15, Value = 0.0074) in tumor tissues, whereas the association was not statistically significant. Interestingly, GTEx database examination of MMP-9 and EGFR expression in breast tissues demonstrated a strong negative connection. These findings demonstrate a positive correlation between EGFR and MMP-9 in tumor tissues but a negative correlation in normal tissues. The loss of some shared regulators of these genes may be one of the causes of the positive connection between MMP-9 and EGFR during tumor growth. One of these may be miR-491, which has been found to be downregulated in tumor tissues. EGFR must be expressed at a basal level in order for normal tissues to survive, however MMP-9 expression is tightly regulated, which could explain the negative association.

**Overview of MMP-9 and EGFR regulation by miR-491**

Compared to healthy tissues, microRNA-491 expression is downregulated in breast cancer. MiR-491 can directly bind to the 3'UTR of both MMP-9 and EGFR, therefore when its expression is downregulated, the levels of these two genes are increased in the cells. There is evidence that the EGFR and MMP-9 can both induce each other's expression, though. By stimulating NF-B, a crucial MMP-9 transcription factor, EGFR activation can induce the production of MMP-9. Through PI3K/Akt signaling, EGFR was able to promote MMP-9 expression in non-small cell lung cancer (NSCLC) cells. Similar regulatory mechanisms were found in Glioblastoma Multiforme (GBM), where EGFR stimulated MMP-9 expression via the PI3K/Akt and ERK1/ERK2 pathways. MMP-9 expression was also found to be increased as a result of NF-B activation mediated by STAT3 and STAT5 [206]. The heparin-bound epidermal growth factor (HB-EGF) can be broken down by MMP-9, which causes THP1 cells to express the EGFR receptor and activate downstream signaling cascades. In A549, HepG2, and MDA-MB-231 cells, HB-EGF has been shown to activate EGFR via metalloproteinase. These observations and the findings of the current investigation suggest that miR-491 can suppress the expression of MMP-9 and EGFR through both direct and indirect methods. MiR-491 reduces MMP-9 expression via down-regulating EGFR, which in turn causes MMP-9 to express less. The ability of breast cancer cells to spread through the body is decreased when MMP-9 and EGFR levels are decreased. According to a paper, miR-491 can lower TPX2 expression in breast cancer

cells, which lowers cell migration and invasion. As a result, additional processes might possibly be involved in the reduction of cell migration caused by miR-491.

**Identification of miR-491 targets in TNBC**

Bioinformatics studies were followed by network analysis in order to identify probable candidate targets of miR-491 in TNBC and examine this miRNA's function in the development of cancer. DisGeNET, a database of genes linked to various human diseases, was used to find the genes connected to TNBC. The analysis revealed 632 genes in total. Different bioinformatics tools, including miRWalk, miRanda, miRMap, RNA22, RNAhybrid, and Targetscan, were used to determine the targets of miR-491. Only the genes predicted by all six tools were taken into consideration as miR-491 target genes. Following the methods employed in related research that had already been published, 52 genes that were present in the list of genes linked with TNBC and the list of miR-491 targets were determined to be the miR-491 targets in TNBC. Gene ontology, functional annotation, KEGG pathway enrichment, and protein-protein interaction network analysis were all performed on all of these genes.

**Gene ontology (GO) analysis of target genes**

DAVID was used to carry out GO analysis to better understand the functional role of the miR-491 target genes. We only considered phrases that have a false discovery rate (FDR) of less than 0.05. Within the FDR cutoff, eight GO keywords were enriched within the biological process (BP) category.

**KEGG pathway analysis of target genes**

Gene set enrichment analysis in the KEGG pathway database was carried out to find relevant pathways related to miR-491 target genes. Significant data was defined as an FDR value less than 0.05. The target genes were enriched in 7 pathways, such as "pancreatic "non-small cell lung " "ERBB signaling "bladder "neurotrophin signaling pathway," and "prostate cancer." According to KEGG enrichment, the targets of miR-491 are enriched in pathways linked to the development of cancer. According to the current investigation, the targets of miR-491 may have a significant impact on the emergence of cancers such as pancreatic, lung, brain, prostate, and bladder cancers. According to the importance level of KEGG pathway enrichment, "ERBB signaling pathway" is rated fourth and is crucial to the advancement of breast cancer.

**Protein-protein interaction network of deregulated miRNA target genes**

The STRING database was used to build the PPI network, which was then used to detect interactions between the target proteins. Protein interactions were found to be very strong and significant in the generated network (p-value 1.0e-16). The presence of substantial interactions in a PPI network indicates that the proteins are connected, at least in part, which raises the likelihood that they will be controlled by comparable processes. The PPI network had 52 nodes and 106 edges, with an average node degree of 4.08 and a local clustering coefficient of 0.536. Software called Cytoscape was used to export and display the PPI network. The topology of the network was examined using the Network Analyzer plugin for the Cytoscape platform. The network was discovered to have a radius of 3 and a diameter of 5. Network characteristics included 0.345 network centralization, 2.208 characteristic path length, 0.080 network density, and 1.278 network heterogeneity. The shortest path length, aggregation coefficient, and node degree distribution were determined using Network Analyzer. The investigation revealed that one of the key characteristics of the biological network, the small-world network, is followed by the PPI network of miR-491 target genes. The network followed a power-law distribution and was scale-free.

**Identification of hub genes in the network**

Cytoscape add-on The hub genes in the network, which are the nodes in the network with a high degree of connection, were found using cytoHubba. As the top interacting nodes in the PPI network, MAPK1, MAPK3, TP53, MAPK8, SRC, PGR, EGFR, BCL2L1, ERBB4, and SMAD3 are formed. The top two hub genes, MAPK1 and MAPK3, have the highest connection levels among the top 10. The hub genes all have important functions in various malignancies.

**Hub genes play a critical role in breast cancer**

Breast cancer patients' overall survival was significantly correlated with MAPK1, PGR, TP53, and SRC, according to a survival analysis. Patient survival was adversely linked to MAPK1, TP53, and SRC. Patients with low expression of MAPK1 had a median survival of 215.2 months, whereas those with high expression had a median survival of 113.93 months. Patients with lower expression levels of TP53 and SRC had median survival times of 219,77 months and 148,53 months, respectively, whereas those with higher expression levels of TP53 and SRC had median survival times of 115.4 months and 115 months, respectively. MAPK1, TP53, and SRC had hazard ratios of 1.59, 1.43, and 1.33, respectively. The downregulation of its regulators may possibly be the cause of the higher levels of p53. The decreased levels of miR-491 may be potentially responsible for the elevated p53 levels in breast cancer patients. To determine the function of miR-491 down-regulation in the elevation of p53 levels in breast cancer, it is important to further examine the specificity of miR-491-mediated regulation of p53.

**A single functional module is obtained from the MCODE analysis of the PPI network**

In cells, biological processes are arranged and carried out in a modular fashion. Modules are nodes that are physically or functionally coupled and work together to carry out particular tasks in biological systems. As a result, modules are made up of a network's most connected and active nodes. The PPI network's MCODE analysis produced a single functional module with six nodes. The genes PEA15, PIN1, MAPK3, MAPK1, TLN1, and PTPN11 were all contained in the module. With an MCODE score of 3.73, further investigation revealed that PTPN11 serves as the seed gene of this functional module. These genes will co-express in breast cancer tissues because it is anticipated that they will interact to perform certain tasks. The module's genes (PEA15, MAPK3, MAPK1, TLN1, and PIN1) all have positive correlations with PTPN11, according to a Spearman correlation study of the module's genes and the seed gene. This suggests that the bulk of the genes in the modules are present in the tissues at the same time that the tumor develops.

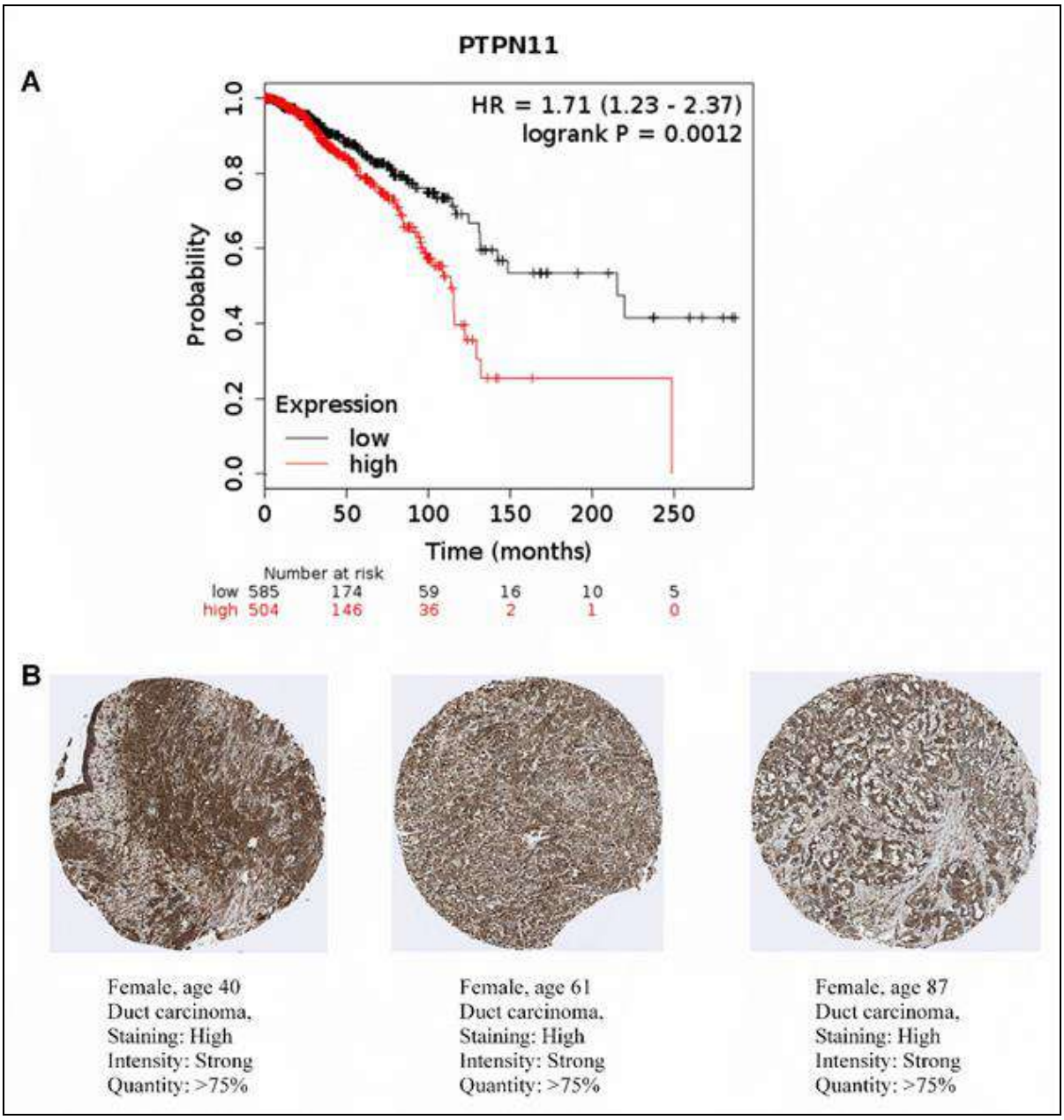


**Fig 3:** Survival and protein expression analysis of PTPN11 in breast cancer patients

**The seed gene PTPN11 is associated with patient survival**

With a hazard ratio of 1.71, the survival analysis of PTPN11 in BRCA patients revealed a significant impact of this gene on patient survival. The median survival for the PTPN11 low expression cohort was 215.2 months, whereas the median survival for the PTPN11 high expression cohort was 113.63 months. As a powerful oncogene and potential target for anti-cancer therapy, PTPN11 is linked to a number of different malignancies. Breast cancer, myelogenous leukemia, gastric cancer, glioblastoma multiforme (GBM), and large cell lymphoma have all been linked to the mutation that activates PTN11.

PTPN11 expression was discovered in 72% of infiltrating ductal carcinomas of the breast, which exhibited a significant link with higher tumor grade, lymph node metastases, and the accumulation of hormone receptors in the nucleus. Receptor tyrosine kinase activation of the RAS-MEK-ERK pathway was reduced as a result of PTPN11 expression inhibition. The elimination of acquired resistance to numerous targeted cancer medicines is another benefit of PTPN11 downregulation. Inhibiting PTPN11 may be a useful tactic for controlling cancer-activated pathways that promote tumor growth. This suggests that miR-491's ability to control PTPN11 has to be further investigated, and its use as a therapeutic agent needs to be established. MiR-491 may be employed as a viable method for the clinical treatment of cancer progression in conjunction with targeted medicines because PTPN11 is linked to acquired resistance to targeted therapy.

**Hub genes are associated with various cancer hallmarks.**

Cancer GeneNet's analysis of the hub gene associations in the PPI interaction network revealed that these miR-491 targets are associated with a number of cancer-related characteristics, including "Glycolysis," "Differentiation," "Proliferation," "Inflammation," "DNA Repair," "Angiogenesis," "Immortality," "Metastasis," and "Cell Death." The functional module genes showed a similar pattern of relationship. These findings suggest that miR-491 has the potential to target a variety of pathways involved in the growth of cancer.

**Conclusion**

According to this study, miR-491 may act as a tumor suppressor in breast cancer. In order to slow the spread of breast cancer, miR-491 microRNA restoration therapy may be employed. The ability of miR-491 to suppress two oncogenes, MMP-9 and EGFR, linked to carcinogenesis and tumor metastasis, was experimentally verified in this study. The migratory and adhesion abilities of the cancer cells, which are crucial for successful metastasis and the development of new tumors, were eliminated by the overexpression of miR-491 in MDA-MB-231 cells. A comprehensive overview of the tumor-suppressive pathways of miR-491 in breast cancer was provided by bioinformatics analysis. The majority of this miRNA's targets were linked to the development of tumors. This research lays the groundwork for further investigation into miR-491's potential as a therapeutic intervention in the vertical targeting of oncogene networks by partially blocking a number of genes linked to pathways that promote cancer.

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