

THE ROLE OF HSA-MIR-451A IN THE GROWTH AND PROGRESSION OF LUMINAL BREAST CANCER

Andres Hernandez-Velandia^{1,*}, Angela García-Fonseca¹, Reggie Garcia-Robles² and Andres Aristizabal-Pachon¹

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Abstract

Numerous genetic and biological processes have been linked to the function of microRNAs which regulate gene expression by targeting messenger RNA mRNA. MicroRNAs play a role in the development of disease and the embryology of mammals. To further understand its function in the oncogenic process, the expression of the microRNA profile in cancer has been investigated.

Despite being referred to as a noteworthy microRNA in cancer, it is unknown whether hsa-miR-451a plays a part in the in vitro progression of the luminal A and luminal B subtypes of breast cancer. Activity of hsa-miR-451a was examined when it was transfected and expressed in breast cancer cell lines known as luminal A and luminal B, in terms of the normal stages of cancer progression in vitro, such as proliferation, survival, migration, and invasion. We also thoroughly investigated target score-based bioinformatics algorithms for predicting microRNA-mRNA interactions. The progression (proliferation, migration, invasion) of luminal A and B breast cancer cell lines in vitro was markedly slowed by the expression of exogenous hsa-miR-451a. Based on bioinformatics analysis, we found a powerful interaction between hsa-miR-451a and the genes for CAB39, AKT1, and BRAF. In silico and experimental results confirmed a detailed gene network that boosts the activation of signaling pathways required for cancer progression and under expression of hsa-miR-451a it was decreased.

Absence of hsa-miR-451a in breast cancer tissue and its correlation with prognosis and tumor behavior provides a significant contribution that supports current contexts such as liquid biopsies and precision oncology based on bioinformatics tools and data mining.

Keywords: Micrnas; Invasion; Migration; Cancer; Proliferation; hsa-miR-451a

Introduction

Worldwide breast cancer has an incidence of 2.261.419 cases (47.8%) and mortality rate of 684.996 deaths (13.6%) (Sung *et al.*, 2021) affecting female population and with a particularity related to different behavior according with phenotype and

molecular-anatomical classification, resulting in several conditions for determining patient survival (Feng *et al.*, 2018). The mechanisms associated to cancer incidence and progression involves biological, physical, chemical, and biochemical

¹ Departamento de Nutrición y Bioquímica, Facultad de Ciencias, Pontificia Universidad Javeriana, Bogotá D.C., Colombia. E-mail: y.hernandez@javeriana.edu.co

² Departamento de Ciencias Fisiológicas, Facultad de Medicina, Pontificia Universidad Javeriana, Bogotá D.C., Colombia.

* Corresponding author

interactions all of them regulated by molecules and proteins which try to maintain an equilibrium inside and outside tumor microenvironment. The extensive and fine network of actors related to cancer development, favor and support the genetic imprint which characterizes the recognized breast cancer phenotypes, highly relevant due to its impact on tumor heterogeneity that is one of the biggest challenges in cancer diagnosis, prognosis and effective treatment (Turajlic *et al.*, 2019). Breast cancer molecular subtypes involves a specific, unique and fine genetic profile expression that confers to cancer cells several abilities associated with its behavior like proliferative, survival, migratory and invasive abilities (Guo *et al.*, 2019) which has been used to enhance cancer diagnosis and effective antitumor specific treatment.

Most incident molecular subtypes have been reported with significant data for luminal breast cancer. The luminal breast cancer subtype has been classified with favorable clinical characteristics and an optimistic prognosis for patients, due to its intermediate aggressiveness and in situ permanence. In particular, the expression of the estrogen receptor (ER), progesterone receptor (PR), and fluctuating HER2 expression are shared by luminal breast cancer cells and are useful for potential therapeutic targets. Based on St. Gallen consensus (Goldhirsch *et al.*, 2013), luminal A cancer cells with the higher rate of presentation among patients diagnosed, near to 17%, exhibits ER positive, PR $\geq 20\%$, HER2 negative, Ki67 $< 14\%$ and their described particularities like lower migration, invasion and recurrence rates (Acheampong *et al.*, 2020; García-Cortés *et al.*, 2021). Luminal B cancer cells also represents an important epidemiological data for incidence near to 11% (Al-thoubaity, 2020), were defined as ER positive, PR negative or $< 20\%$, HER2 negative/positive and Ki67 $\geq 20\%$, this subtype have the characteristic of higher aggressive behavior than luminal A subtype (Bediaga *et al.*, 2016). Each breast cancer subtype's behavior is dependent on the up- or down-regulation of several molecules that have been extensively described in the development of molecular and genetic profiles, like miRNAs, membrane receptors, availability of ligands, among others.

One of the molecules widely reported in luminal breast cancer with specific profile expression are microRNAs (Pascut *et al.*, 2020). This broad family of small non-coding RNAs widely and specifically distributed across mammal tissues have been reported with pivotal roles in many biological processes, recognized them as strategic molecules with big responsibilities, participating as critical modulators in normal embryologic development, among others. In cancer

development, some miRNAs have been reported with antitumor participation and others promoting tumor progression, recognized as oncomiRs (Petri and Klinge, 2020). miRNAs are short molecules with approximately length of 25 nucleotides which are processed into the nucleus and after that, they are cleaved by enzymes like DICER to obtain in a canonical pathway a classical mature miRNA, following the formation of the RISC complex (cytoplasmic RNA-induced silencing complex), miRNAs can carry out their regulatory functions based on mRNA interaction (Kim and Kim, 2012).

Establishing a connection between placenta growth and tumor development supports the trophoblastic model which is based on molecular and biological mechanisms shared by placenta and tumor development, characterized by proliferation, invasion, and migration across uterus or tumor surrounding tissues. The highly controlled process occurring in placenta are mediated among others by miRNAs, some of them are extremely expressed and impact pivotal signaling pathways. Several miRNAs were reported with high expression levels in healthy placenta tissue, in that set of molecules, hsa-miR-451a has been recognized as highly expressed and influence gene signaling mechanisms targeting mRNAs (Vidal *et al.*, 2018). Unlike placental growth, these processes are dysregulated in tumor progression causing serious injuries in surrounding tissue (Ross, 2015). Based on the hypothesis that placental growth is controlled under mechanisms favoring self-regulation mediated by overexpressed microRNAs among other biomolecules, the aim of this research was to evaluate the hsa-microRNA-451a as a target molecule to explore its role in vitro in luminal A and B breast cancer progression.

Materials and methods

Placenta samples

RNA obtained from healthy placenta samples were provided by the Human Genetics Institute of the Pontificia Universidad Javeriana and San Ignacio Hospital. Informed consent and ethical considerations were approved by the ethics and research committee of the science faculty and San Ignacio Hospital.

Cell culture

The human MCF-7 (luminal A) and BT-474 (luminal B) cell lines were obtained from the American Type Culture Collection (ATCC®) and maintained in Dulbecco's modified Eagle's medium (DMEM, Lonza, Walkersville, USA), supplemented with 10% fetal bovine serum (FBS, Eurobio, Paris, France). Cell cultures were incubated in

a humidified atmosphere and 5% CO₂ at 37°C, defined as standard conditions.

Transient Transfection

MCF-7 and BT-474 cell lines were transfected in 6-well plates, hsa-miR-451a mirVana mimic® mature sequence AAACCGUUACCAUACUGAGUU (ThermoFisher Scientific, Waltham, USA) was mixed with lipofectamine 2000® (Invitrogen, Carlsbad, CA, USA) in 1500 µL of Opti-MEM® (ThermoFisher Scientific, Waltham, USA). Transfection mix was added to cells and seeded. Cell plates were incubated at standard conditions for 24h. Culture media was removed 24h after transfection and 1500 µL cell culture media DMEM was added. Transfected cells were used to subsequent assays. Cell line control was transfected with scramble sequence and used in all experiments.

RNA Extraction and gene expression

To validate the successful transfection, cell culture media was removed, and plates were washed with PBS 10X. TRIzol® (ThermoFisher Scientific, Waltham, USA) was added for cell lysis and RNA extraction, following manufacturer's instructions. RNA extracted was quantified in Nanodrop® (ThermoFisher Scientific, Waltham, USA). After that, RNA samples were treated with DNase I (New England Biolabs, Massachusetts, USA) and subsequent processing to obtain cDNA with TaqMan advanced miRNA cDNA synthesis kit® (ThermoFisher Scientific, Waltham, USA). qPCR was performed according to TaqMan miRNA advanced assays® (ThermoFisher Scientific, Waltham, USA).

For predicted miRNA-mRNA target score, qPCR was performed from the RNA samples to amplify the targets CAB39 forward 5-3' GAGCATGGCTGTTCTGGAAAAGC, reverse 5-3' GCTACTGCTTCTGTCTGAGGCT; AKT1 forward 5-3' TGGACTACCTGCACTCGGAGAA, reverse 5-3' GTGCCGCAAAAGGTCTTCATGG; BRAF forward 5-3' AACGAGACCGATCCTCATCAGC, reverse 5-3' GGTAGCAGACAAACCTGTGGTTG.

Colony formation assays

Following protocol for clonogenic in vitro assay (Brix *et al.*, 2021), MCF-7 and BT-474 cells were seeded into 6-well plates at 500 cells per well and cultured for 10 days under standard conditions. Subsequently, the cells were fixed and stained with Giemsa (Sigma Aldrich, USA). ImageJ software (National Institutes of Health, Bethesda, Maryland, USA) was used to determine the colony formation rate.

Proliferation cell assays

MCF-7 and BT-474 cell lines were seeded into 96-well plates at 2.5×10³ cells per well. Reading points were set at 4h, 24h, 48h, 72h and 96 hours after transfection by CytoPainter Cell Proliferation Staining Reagent-Green fluorescence (ABCAM, Cambridge, UK). Cell proliferation was monitored under fluorescence change at wavelength at Ex/Em=485/520 in FLUOstar Omega plate reader (BMG Labtech, Offenburg, Germany).

Migration and invasion assays

Migration assays were conducted using 24-well plates with Thincert® cell culture inserts (Greiner Bio-one, Monroe, USA). MCF-7 and BT-474 5.0×10⁴ cells, were seeded in the upper side of the matrix well and each culture well contained DMEM without serum and was located at the top of the well. Separated by a migration membrane, the bottom of the well contained DMEM supplemented with 10% FBS to stimulate migration. After incubation for 12 h, cells remaining in the top well were removed with a cotton swab and the migrating cells adhered to membrane insert were fixed and dyed with Giemsa. ImageJ software, (National Institutes of Health, Bethesda, Maryland, USA) was used to determine the migration rate.

The cell invasion assays were conducted based on Boyden chamber modified method (Chen, 2005) using 96-well plates coated with basement membrane extract and 8 µm pore diameter (ABCAM, Cambridge, UK). Experiments were performed in Cell Invasion Assay Basement Membrane (ABCAM, Cambridge, UK), according with manufacturer's instructions. MCF-7 and BT-474 5.0×10⁴ cells were seeded in the upper well of the invasion chamber in DMEM without serum. The lower well of the chamber contained DMEM supplemented with 10% FBS to stimulate cell invasion. After incubation for 24 h, invasion chamber was disassembled, and the MCF-7 and BT-474 cells were washed. Invading cells in the bottom well were dyed with cell dye ABCAM Invasion assay® (ABCAM, 235697), after that, each well were evaluated by fluorescence intensity with FLUOstar Omega plate reader (BMG Labtech, Offenburg, Germany) at Ex/Em=485/570nm. RFU (Relative Fluorescence Units) was obtained.

hsa-miR-451a target interaction prediction

Based on the findings regarding the hsa-miR-451a's functional role, potential gene targets were looked for to explain the results. The MirPath TarBase v7.0 modules of the bioinformatics programs DianaTools and mirTarBase were used to

conduct the search. 'hsa-miR-451a' was used as the search term across all platforms. From each platform, a list of genes with which hsa-miR-451a interacts was obtained. From this initial list, the level of interaction evidence was filtered, predetermined in the bioinformatics platform as 'strong evidence' or 'less strong evidence' and those targets with interaction values greater than 75% were considered, obtaining a second list of target genes. Subsequently, the microRNA-mRNA interaction was filtered considering the variables: a) level of evidence; b) impact on cellular processes related to tumor progression; c) available scientific literature. Next, the selected genes were evaluated on the KEGG (Kyoto Encyclopedia of Genes and Genomes) platform. The search methodology was carried out under the prefix 'map'. Subsequently, the signaling pathways were obtained in relation to each search criteria. Table 1

Statistical Analysis

All experiments were performed three times in triplicate. Depending on data normality, parametric and non-parametric tests were applied. All the results are presented as the mean standard error. P value <0.05 was considered as significant.

Results

hsa-miR-451a expression in MCF7 and BT474 cell lines

qPCR was performed to assess the hsa-miR-451a expression levels in breast cancer cell lines MCF7 and BT474. Results showed no expression of hsa-miR-451a (Ct >38) in both of two cell lines, compared to placenta tissue (Ct = 24 - 25).

Table 1. Interaction network for hsa-miR-451a based on bioinformatics analysis, filtering the most relevant results related to cell proliferation, survival and metabolism.

ID	Species(miRNA)	Species(Target)	miRNA	Target
MIRT000046	hsa	hsa	hsa-miR-451a	MIF
MIRT000500	hsa	hsa	hsa-miR-451a	CAB39
MIRT005656	hsa	hsa	hsa-miR-451a	ABCB1
MIRT005740	hsa	hsa	hsa-miR-451a	AKT1
MIRT005742	hsa	hsa	hsa-miR-451a	MMP2
MIRT005743	hsa	hsa	hsa-miR-451a	MMP9
MIRT005744	hsa	hsa	hsa-miR-451a	BCL2
MIRT006358	hsa	hsa	hsa-miR-451a	MYC
MIRT006533	hsa	hsa	hsa-miR-451a	RAB14
MIRT006597	hsa	hsa	hsa-miR-451a	TMED7
MIRT016628	hsa	hsa	hsa-miR-451a	ARPP19
MIRT016629	hsa	hsa	hsa-miR-451a	UBE2H
MIRT052920	hsa	hsa	hsa-miR-451a	CPNE3
MIRT052921	hsa	hsa	hsa-miR-451a	RAB5A
MIRT054788	hsa	hsa	hsa-miR-451a	DCBLD2
MIRT054843	hsa	hsa	hsa-miR-451a	IL6R
MIRT438803	hsa	hsa	hsa-miR-451a	IKBKB
MIRT438841	hsa	hsa	hsa-miR-451a	FRZB
MIRT438842	hsa	hsa	hsa-miR-451a	OSR1
MIRT438843	hsa	hsa	hsa-miR-451a	PKD1
MIRT438844	hsa	hsa	hsa-miR-451a	ROR2
MIRT538246	hsa	hsa	hsa-miR-451a	CUX2
MIRT665973	hsa	hsa	hsa-miR-451a	SZRD1
MIRT733208	hsa	hsa	hsa-miR-451a	mTOR
MIRT733466	hsa	hsa	hsa-miR-451a	ATF2
MIRT733467	hsa	hsa	hsa-miR-451a	ADAM10
MIRT733490	hsa	hsa	hsa-miR-451a	CDKN2D
MIRT733492	hsa	hsa	hsa-miR-451a	PSMB8
MIRT731810	hsa	hsa	hsa-miR-451a	BRAF
MIRT735338	hsa	hsa	hsa-miR-451a	ETS1
MIRT735369	hsa	hsa	hsa-miR-451a	DGCR8
MIRT735371	hsa	hsa	hsa-miR-451a	ERH
MIRT735550	hsa	hsa	hsa-miR-451a	HELLS
MIRT735631	hsa	hsa	hsa-miR-451a	CAV1
MIRT735632	hsa	hsa	hsa-miR-451a	TSC1
MIRT735715	hsa	hsa	hsa-miR-451a	CA1
MIRT736199	hsa	hsa	hsa-miR-451a	FNTA
MIRT736200	hsa	hsa	hsa-miR-451a	S1PR3
MIRT736606	hsa	hsa	hsa-miR-451a	YWHAZ

Subsequently, qPCR was performed to confirm the transfection efficacy of the complex hsa-miR-451a and lipofectamine in breast cancer cells. Results and comparative analysis between scramble and hsa-miR-451a transfected cells showed increased expression in the transfected group with media values in fold change obtained: MCF7 transfected (Ct=12) and BT474 transfected (Ct=<20).

hsa-miR-451a expression - enrichment analysis in cancer

Bioinformatic analysis was performed in curated databases to understand and approximate knowledge about the expression relationship of hsa-miR-451a in various types of cancer with solid tumor and its correlation with biological processes related to cell and tumor viability. We found a significant correlation rate in the expression of hsa-miR-451 with biological processes such as fat cell differentiation, cell response to stress and cell proliferation (Figure 1a and Figure 1b).

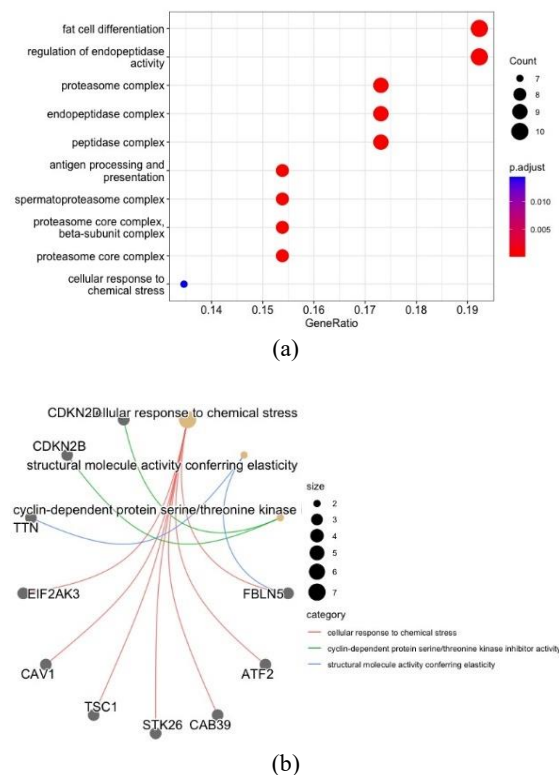


Figure 1. a) hsa-miR-451a and expression from bioinformatics database and its relationship with cancer cell activity. b). hsa-miR-451a gene targets.

hsa-miR-451a reduces proliferation and survival capacities in breast cancer cells

MCF7 cells transfected with hsa-miR-451 significantly reduced proliferative abilities on a timeline with 5 reading points (Figure 2a). Data analysis showed significant statistical differences between scramble and transfected cells (p value < 0,0001). For BT474 cells transfected with hsa-miR-451a and maintained under same culture conditions, proliferative abilities were slightly reduced compared to scramble (p value < 0,0001). Results showed coherent data related to population doubling time in BT474 cell line (Figure 2b).

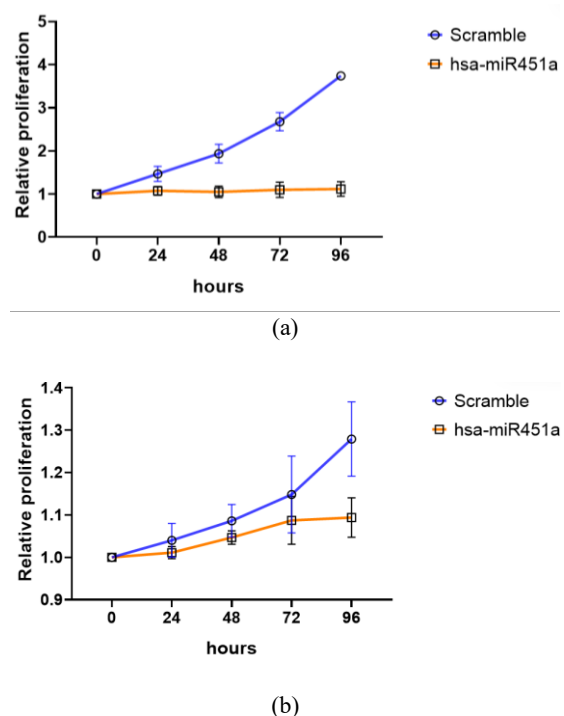
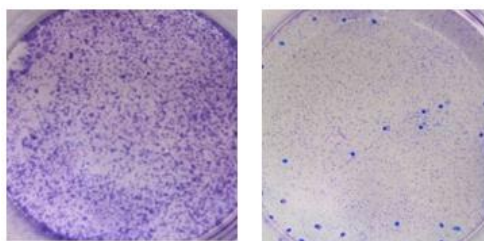
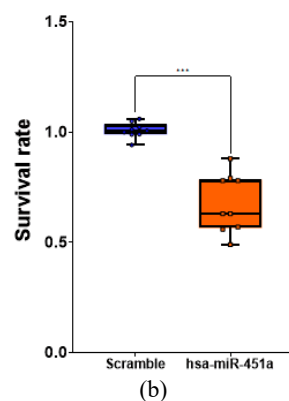
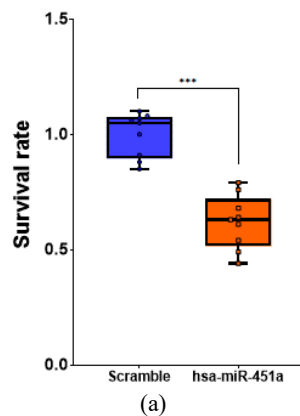


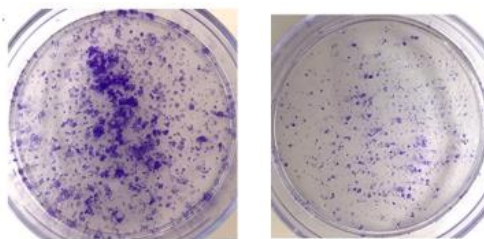
Figure 2. a) MCF7 in proliferation assay. Scramble compared to cells transfected with hsa-miR-451a. b). BT474 in proliferation assay. Scramble compared cells transfected with hsa-miR-451a.

Survival abilities in cancer cells transfected with hsa-miR-451a showed a significant decrease in the survival rate (SR), revealing that overexpression of hsa-miRNA-451a impacts cell survival and weakening the possibilities to form colonies in vitro, our results showed a significantly difference between scramble and transfected group (p <0,0001). MCF7 cancer cells (Figure 3a) and BT474

cancer cells reduced (Figure 3b) survival capacities in vitro compared to scramble. MCF7 and BT474 cell lines were dyed according to procedure to count survival and colony formation capacities in vitro, MCF7 (Figure 3c), BT474 (Figure 3d).



(c)



(d)

Figure 3. Survival rate with expression of hsa-miRNA-451a.

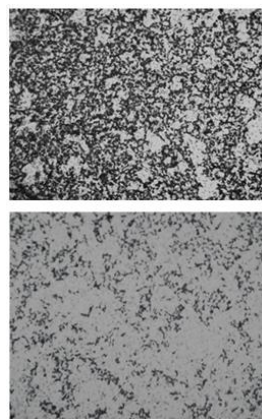
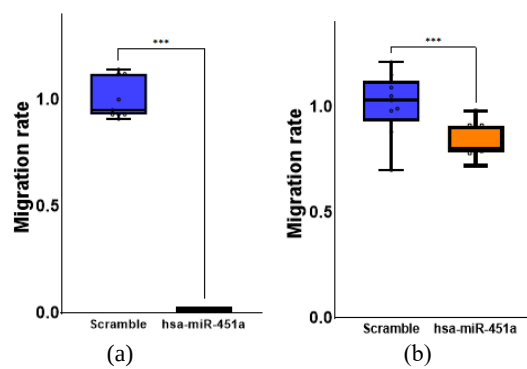
Figure 3c MCF7 cell line survival rate with expression of hsa-miRNA-451a compared to scramble. Figure 3d BT474 cell line survival rate with expression of hsa-miRNA-451a compared to scramble. Image 1 Survival clonogenic assay dyed with Giemsa. Figure 3c MCF7 survival clonogenic assay, left MCF7 cell line scramble. Right MCF7 cell line under hsa-miRNA-451a expression. Figure 3d BT474 survival clonogenic assay, left BT474 cell line scramble. Right BT474 cell line under hsa-miRNA-451a expression. Results of colony formation showed a substantially different value ($p = <0,0001$).

hsa-miR-451a impairs migration abilities of breast cancer cells

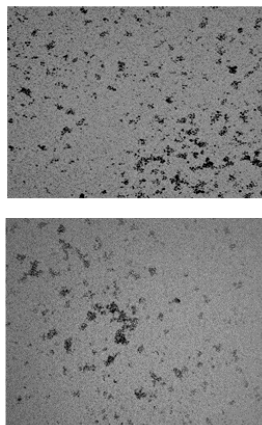
MCF7 cells were tested and evaluated in a Boyden chamber modified to assess the migration abilities under controlled conditions. MCF7 cells transfected with hsa-miR-451a significantly decreased their migration abilities (Figure 4a), the results were coherent with the invasion assay results which means that cell mechanisms to migrate and invade were markedly decreased ($p = <0,0001$). Given that BT474 cells showed diminished slight change in their ability to migrate in the presence of the hsa-miR-451a, most likely due to alternative and active signaling pathways ($p = 0,01160.4017$), it can be hypothesized that the presence of some membrane receptors specific for cancer phenotypes and microRNAs upregulation, or downregulation, can affect cell behavior in a very specific signaling way. Figure 4b.

hsa-miR-451a affects invasion properties of breast cancer cells

MCF7 cells transfected with hsa-miR-451a and scramble, were evaluated for invasion capacities in vitro. We found that transfected cells with hsa-miR-451a significantly decreased their migratory properties and limiting them to invade. Statistical data results ($p = <0,0001$). (Figure 5A). The behavior of BT474 cells in the invasion assay was remarkably similar, with lower invasion properties in the transfected group compared to scramble. Our statistics analysis results support this behavior with a value of $p = 0,0001$ (Figure 5B).



(c)



(d)

Figure 4. Migration abilities of breast cancer cells. a) MCF7 cells in migration assay. Blue box is scramble compared to cell expression of hsa-miRNA-451a. b) BT474 cells in migration assay. Blue box is scramble compared to orange box transfected with hsa-miRNA-451a. c) MCF7 cancer cells dyed with Giemsa at 10X. Up: scramble; down: hsa-miR-451. d) BT474 cancer cells dyed with Giemsa at 10X Up: scramble; down: hsa-miR-451.

Note: Due to “patchy” growth of BT474 cell line, 10X image were obtained to assess the whole visual field.

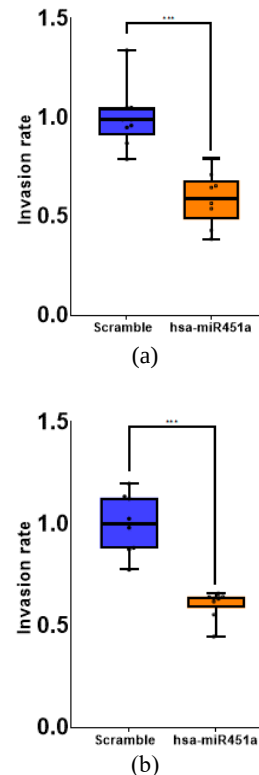


Figure 5. a) MCF7 cells in invasion assay. Transfected cells with hsa-miRNA-451a (orange box), showed decreased invasion properties. b) BT474 cells in invasion assay. Transfected cells (hsa-miR-451a) showed decreased invasion properties.

In-silico enrichment analysis for hsa-miR-451a targets

We used TarBase V.8 (Vlachos *et al.*, 2015), to identify and evaluate the target genes for hsa-miR-451a. 66 evidenced target gene interactions were obtained. After that, we selected the targets with high interaction score and relation with cell progression - cancer progression or cell viability - pathways. Based on those criteria, we selected the gene targets:

CAB39, AKT1 and BRAF (Figure 6), all of them were evaluated in vitro by qPCR to assess mRNA expression in transfected and scramble.

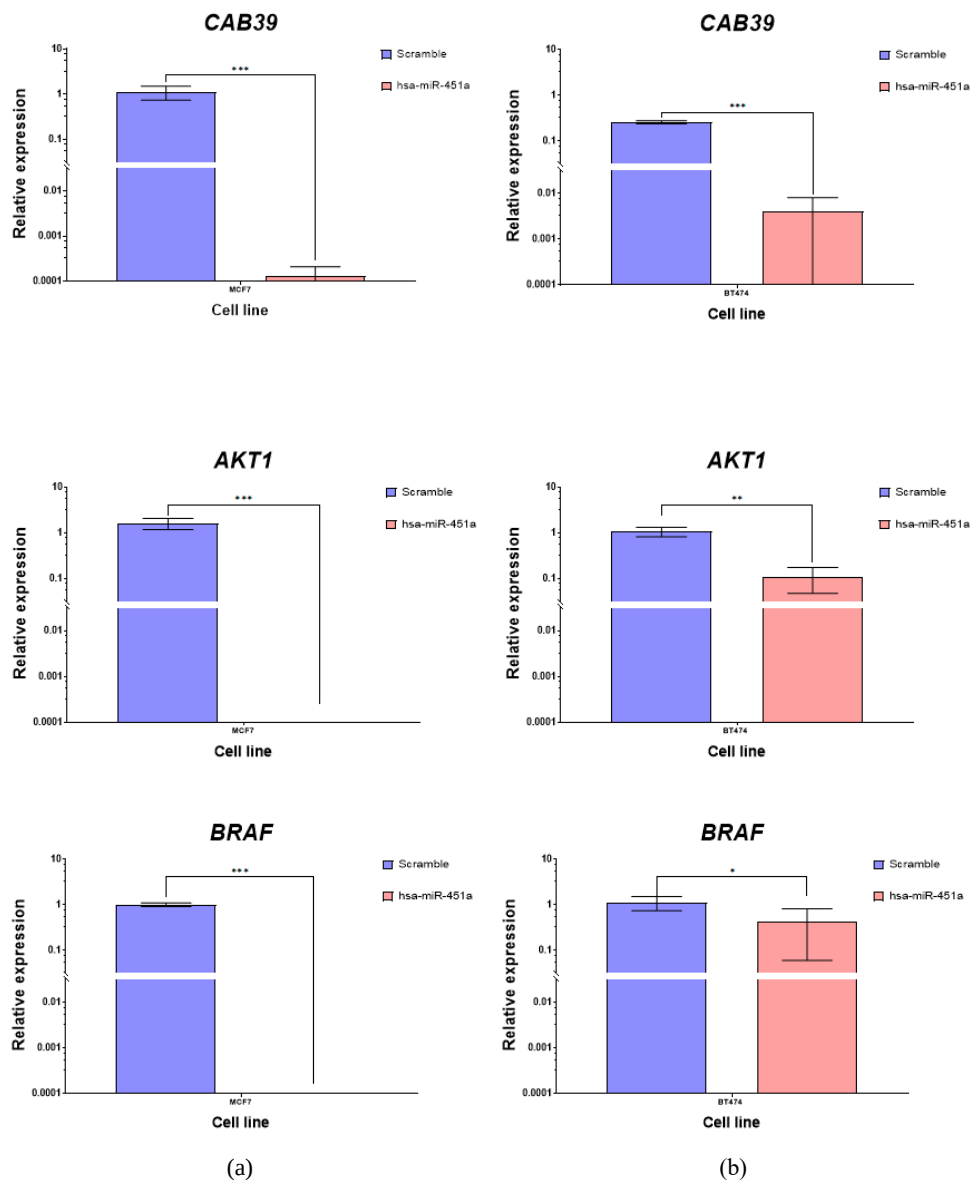


Figure 6. Relative expression of predicted genes by qPCR. a) Gene expression for MCF7 breast cancer cell line. b) Gene expression for BT474 breast cancer cell line.

Discussion

According to global statistics on cancer, there are 10.3 million cancer deaths and 19.3 million new cases per year. Notably, it has been observed that over 90% of breast cancer fatalities are linked to a successful metastatic process, and that 30% of individuals with breast cancer who have an early diagnosis undergo metastasis. (Riggio *et al.*, 2021). The biological, biochemical and biophysical mechanism of metastasis involves several reprogramming strategies which allows cancer cells to achieve a survival in their extravasation, and

finally the conformation of a metastatic niche with preference for some organs in a process called organotropism; for breast cancer, preference location are bone, brain and lungs (Wang *et al.*, 2021). The prognosis in breast cancer is associated with several variables and conditions, including tumor cell phenotype, tumor microenvironment and tumor heterogeneity, among others, emphasizing the relevance of heterogeneity in the treatment of cancer and the performance of molecules and proteins which are directed at therapeutic targets

(Tuasha and Petros, 2020). The phenotype of a cancer determines its course and the survival of tumor cells, including the degree to which they are intimately connected to microenvironmental elements. Cells that can invade nearby or distant tissues have a poor prognosis. The ability of colony cell formation, invasion, migration, and effective proliferation of cells, which ensure tumor maintenance and sustainability, mediates this pathologic mechanism. (Klein, 2020). When viewed in the context of placental development, which in healthy conditions is highly coordinated, provides a solid model to comprehend potential mechanisms of solid tumor progression in which cancer cells lose some characteristics of self-control but share molecular and biological mechanisms with placenta to achieve their growth. (Costanzo *et al.*, 2018) and leads us to review deeply the theory proposed by Beard since 1902 and reconfirmed by several researchers (Ross, 2018). In both contexts, genetic and epigenetic mechanisms related to cell proliferation, migration and invasion have been described under healthy and pathological scenarios, both of them consider the necessary participation of miRNAs as potent regulators (Suzuki *et al.*, 2015). We selected a high expressed microRNA in placenta tissue, the hsa-miR-451a as target for evaluating its role in tumor cell mechanisms associated with cancer progression.

First of all, cell division is ultimately promoted by cell proliferation, which is the response to growth factors and subsequent activation of signaling pathways that causes cells to decide to enter S phase. (Duronio and Xiong, 2013). According to our research, hsa-miR-451a significantly affects proliferative capacity in luminal A and B breast cancer cells, slowing their rate of proliferation. Lower proliferative capacities in the transfected group demonstrated the harmful influence of hsa-miR-451a on the luminal breast cancer subtype. We first conducted an in-silico analysis to determine the targets of hsa-miR-451a as a foundation for confirming the findings. (Huang *et al.*, 2020). We then examined a group of genes connected to biological processes associated to the development of cancer, including target score and linkage with signaling pathways in cancer. The genes identified were CAB39, BRAF, and AKT1, which are all directly connected to signaling pathways that control cell division, migration, and invasion. It is significant to note that in vitro studies confirmed the results from computational models and showed that hsa-miR-451a negatively regulated a subset of genes. Each gene's comparative expression between the scramble and transfected groups was determined by qPCR, and we found that the presence of hsa-miR-451a significantly reduced the degree of gene

expression in the transfected group. The interaction between hsa-miR-451a and the CAB39 gene has been validated in silico and experimentally in tumor models, in which its activity was reported as an initiator of the mTOR-mediated proliferation signaling pathway (Goldhirsch *et al.*, 2013; Peng *et al.*, 2020). The CAB39 protein is an important activator of the STRAD/STK11 complex, which subsequently and once stabilized activates AMPK and subsequently, through phosphorylation, AMPK regulates the activity the hyperactivation of mTORC (Sadria *et al.*, 2022). Activation of mTORC1 in this cell signaling pathway leads to activation of S6K, which is an important transcription factor that stimulates the expression of genes relevant to cell cycle progression and survival capacity (Artemenko *et al.*, 2022). Therefore, mTORC1 is known as the master of growth control, given its proto-oncogenic activity. The presence of hsa-miR-451a in the cellular environment will inhibit the expression of CAB39, thereby repressing the previously presented pathway, thus leading to a decrease in proliferation and survival mediated by the repression of mTORC1, among others.

Likewise, as previously described, it was found that hsa-miR-451a interacts with AKT isoform 1 (AKT1), which has been widely demonstrated and reported in various roles within which participation in cell proliferation stands out and the ability to survive. AKT1 (protein kinase B) activity is turned on by response to external stimuli, such as growth factors, insulin, cellular stress, and the participation of internal stimuli, as occurs with altered receptor tyrosine kinases, overexpression of Ras, Rac, and src among others.

With key roles in cell proliferation, survival, migration, and invasion, the physiologic and pathological activities of the PI3K/AKT signaling pathway and its interaction with mTORC have been reported in several tumor models (Porta *et al.*, 2014) under the following model:

In response to extracellular and intracellular stimuli, AKT activation occurs by phosphorylation, subsequently, the activation of AKT1 leads to the direct activation of YB-1 by phosphorylation, allowing its translocation to the cell nucleus. YB-1 in the nucleus binds to CPTS1, an important transcription factor for metalloproteinases, Nanog, and β -catenin, among others, which are recognized as potent inducers of cell migration and invasion (Delicato *et al.*, 2021). In addition, YB-1 directly binds tubulin and actin, allowing their assembly, thus regulating different components of the cytoskeleton, which is essential for cell migration (Evdokimova *et al.*, 2009).

AKT1 has been widely reported in cancer progression due to maintain its hyperactive state in

some tumor processes (Cho *et al.*, 2015), that leads to direct activation of cascade proteins that promote cell migration and invasion (Heron-Milhavet *et al.*, 2011).

Supporting our findings, we studied the role of the BRAF/ERK pathway, as part of the in-silico target prediction, BRAF is a direct target of hsa-miR-451a. The BRAF/ERK pathway is involved in cell processes which provides key mediators for successful tumor progression (Zhu *et al.*, 2020). The permanent and pathological activation of BRAF, proto-oncogene (Zaman *et al.*, 2019) by cytokines and growth factors drives the tumorigenesis, including the cascade of signals related to MAPK pathway and Myc protein. Downregulation of BRAF mediated by hsa-miR-451a, leads to an effective decrease of cancer cells proliferation and survival, due to the role of BRAF in the MEK/ERK axis (Liu and Zhou, 2020).

The Ras-RAF-MEK-ERK (MAPK) signaling pathway plays an important role in the migratory and invasive capacity of tumor cells (Hussain *et al.*, 2015). The RAF signaling intermediate has 3 isoforms (ARAF, BRAF, CRAF) each with a different activation mechanism. The participation of BRAF in tumor invasion has been widely studied and validated in various tumor models (Hoeflich *et al.*, 2006; Grisham *et al.*, 2013; Haling *et al.*, 2014).

The overexpression or hyperactivation of BRAF will lead the cell to a permanently active signaling pathway and the induction of expression of genes related to the cell cycle and finally proliferation, migration and invasion of an oncogenic type.

BT474 cell line has been described as adherent and “patchy” with growth morphology epithelial-like, its immunoprofile was reported: ER⁺, PR^{+/−}, HER2⁺ (Holliday and Speirs, 2011) with slow rate of growth in vitro.

After being transfected with hsa-miR-451a, the luminal B tumor subtype, whose phenotypic characteristics include the presence of the HER2 receptor (Creighton, 2012; Ades *et al.*, 2014), and its subsequent intracellular signaling pathway, which includes intermediates like PI3K and the Ras/Raf axis, showed unique characteristics like lesser response to hsa-miR-451a in migration assays. This phenomenon could be explained by the loss of function of retinoblastoma tumor suppressor in luminal B breast cancer, as reported by Perou *et al.* (Herschkowitz *et al.*, 2008) which indicates a significant variation that also affects how anticancer treatments respond.

The luminal B subtype has been clinically recognized as having a more aggressive phenotype and greater metastatic potential, Gray *et al.* (Sears

et al., 2018) reported the epigenomic inactivation of RasGAPs inducing the activation of Ras signaling in luminal B subtype and favoring migration and invasion capacities. It is also remarkable the biological function of protein kinase C (PKC) and its isoforms. PKC activity has been extensively shown in the luminal B subtype’s proliferation, invasion, and resistance to apoptosis (Albert *et al.*, 2022). PKC is crucial for tumor cell populations’ ability to change, resist treatment, and sustain a stem cell phenotype (Jain and Basu, 2014).

In conclusion, we discovered that hsa-miR-451a overexpression significantly diminished the proliferative, migratory, and invasive capacity of luminal A subtype breast carcinoma cells based on our in silico and experimental in vitro results. These strong results may be correlated to the overexpression of microRNA-451a that directly interact with the mRNA of the CAB39, BRAF, and AKT1 genes, which are implicated in intracellular signaling pathways such as mTOR and MEK/ERK, and prospective involvement of mediators such as YB-1. We discovered differences between the luminal A and luminal B subtypes based on the genotypic and phenotypic attributes of the cell population. Further research may be necessary to find out the purpose of unique membrane receptors for each subtype of cell and the activation mechanisms of cell signaling pathways other than the conventional ones that successfully enable breast cancer growth. The activity of microRNA-451a had a less substantial effect on the ability of luminal B to migrate, although this can be explained by the particular dynamics of cell proliferation in this cell population.

Conclusions

Our findings demonstrated that increased hsa-miRNA-451a expression decreases the growth, survival, migration, and invasion of luminal breast cancer cells by inhibiting the expression of CAB39, AKT1, and BRAF. These results showed that hsa-miRNA-451a has a functional role in important signaling pathways that support the growth of cancer cells. According to its results in other cancer models, hsa-miRNA-451a may be a promising target in controlling the development of tumors.

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Conflict of interest

None of the authors have conflicts of interest to declare.

Statements & Declarations

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Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Hernández Velandia Andrés, and Aristizabal-Pachón, Andres. Images and integrative designing were performed by García Fonseca, Angela. The first draft of the manuscript was written by Hernández Velandia Andrés and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Pontificia Universidad Javeriana (2018, Ethics and research committee). For placenta samples, approval was provided by Hospital Universitario San Ignacio and Instituto de Genética Humana.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Content to publish

The information provided in this manuscript can be published under journal criteria and policies. Authors approve the publication of all data provided.

Availability of data and materials

The authors confirm that the data supporting the findings of this study are available within the article.

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