EXPLORING SMALL NUCLEOLAR RNA HOST GENE 3 AS A THERAPEUTIC TARGET IN BREAST CANCER THROUGH METABOLIC REPROGRAMMING

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Abstract. One of the main causes of death worldwide at the moment is cancer. Breast cancer (BC) is the most common type of cancer in women. Triple-negative breast cancer (TNBC) accounts for at least 14.6-20.6% of all incidences of BC. This study was conducted to provide evidence supporting the therapeutic use of combinatorial regimens of CDK4/6 inhibitors for TNBC patients. Exosomes were used to characterize cancer-associated fibroblasts (CAFs) from BC patients. The MD-MBA-453 and MCF7 cells were transfected using the labelled exosomes. Cell viability, extracellular acidification rate (ECAR), and OCR were determined. The expression levels of small nucleolar RNA host gene 3 (SNHG3), pyruvate kinase M1/M2 (PKM), and miR330-5p in the transfected cells were measured. Stable cell lines and a BC mouse model were generated to investigate test DNA and RNA sequences. The results showed that exosomes produced from CAFs were able to reprogram metabolic pathways following their absorption by tumor cells. PKM could be targeted by miR330-5p as well as SNHG3 in BC cells. By upregulating expression linked to miR330-5p and downregulating PKM in tumor cells, SNHG3 inhibited the growth of cells. Exosomes released by breast CAFs reduced the OCR of MD-MBA-453 and MCF-7 cells. Furthermore, it was observed that exosomes secreted by CAFs altered the metabolic pathways regarding BC cells and decreased mitochondrial activity. SNHG3 inhibited miR330 expression in vitro by acting like a molecular sponge. The findings of this study suggest that, when treating cancer, focusing on exosome-mediated communication between cancer and stromal cells may have therapeutic potential.

Keywords: anti-HER2 therapy, triple-negative breast cancer, cancer, cancer-associated fibroblasts.

List of Abbreviations

GLOBOCAN – Global Cancer Observatory
BC – Breast cancer
TNBC – Triple-negative breast cancer
DEDD – Death effector domain-containing DNA-binding protein
HSC70 – Heat shock cognate protein 70
CDK 4/6 – Cyclin-dependent kinase 4/6
PKM – Pyruvate kinase M1/M2
CAF – Cancer-associated fibroblast
SNHG3 – Small nucleolar RNA host gene 3
miRNA – Micro RNA
snoRNA – small nucleolar RNAs
HOXA1 – Homeobox A1
TFAM – Transcription factor A mitochondrial
HIF-1 – Oxygen deprivation factor-1
ECAR – Extracellular acidification rate
TERT – Telomerase reverse transcriptase
ZEB1 – Zinc finger E-box binding homeobox 1
ATCC – American-type culture collection
FBS – Fetal bovine serum
anti-miR-con – AntagomiR control
TEM – Transmission electron microscopy
PTA – Phosphotungstic acid
DAPI – 4′,6-diamidino-2-phenylindole
CCK8 – Cell counting kit 8
SDS-PAGE – Sodium dodecyl sulphate polyacrylamide gel electrophoresis
shRNA – Short hairpin RNA
lncRNA – Long noncoding RNA
OCR – Oxygen Consumption Rate
MCF-7 – Michigan Cancer Foundation-7
MDA-MB453 – M.D.Anderson-Metastasis breast cancer
EGER – Epidermal growth factor receptor
HER2 – Human epidermal growth factor receptor 2.
QPCR – Quantitative polymerase chain reaction
Rb RB – Retinoblastoma tumor suppressor

Introduction

One of the main causes of death worldwide at the moment is cancer. Approximately 8.4 million people died in the year 2021, and approximately 14.5 million individuals received a new diagnosis annually, according to the Global Cancer Observatory (GLOBOCAN) (Sharma *et al*., 2019). The cancer may cause by genetic variability (Buniya *et al.*, 2018) or mutations in genes gastric cancer (Bresam *et al*., 2023a) and peptic ulcer (Bresam *et al*., 2023b) and others. Despite the great achievements and improvements in clinical operations, cancer is still a terrible disease with a poor prognosis and one of the substantial health burdens that threaten people's health globally (Nuevo-Tapioles *et al*., 2020). Cancer is a highly diverse disease in terms of both phenotype and genotype (Hanahan &Weinberg, 2000). The genetic programming regarding the cancer genome has a major impact on cancer progression, initiation, and metastasis. It is essential to investigate the intricate link behind the spread and onset of cancer (Ni *et al.*, 2019; Paull *et al*., 2021). The most frequent cancer type in women is breast cancer, as it is responsible for more than one in ten new cases annually. It represents the 2nd most frequent cancer-related mortality cause for women globally. Anatomically, the milk-producing breast glands are situated in front of the chest wall (Azkaban & Ferguson, 2021).

They rest on the pectoralis major muscle and are supported by ligaments that join the breast to the wall of the chest. There are fifteen to twenty lobes in the breast, which had been arranged in a circle. The fat that covers the lobes determines the shape and size of breasts. The glands that generate milk in response to the hormone stimulations are found in lobules, which make up each lobe (Alkabban & Ferguson, 2018). Breast cancer (BC) always progresses silently. Most individuals discover they have cancer through routine testing. Others may unintentionally discover breast lump, nipple discharge, or altered breast shape and size. On the other hand, mastalgia is one of the common conditions associated with breast cancer (Łukasiewicz *et al*., 2021). Tissue biopsy, imaging, especially mammography, and physical examination are required for breast cancer diagnosis. The rates of survival rise with earlier diagnoses. Tumors that spread hematologically and lymphatically tend to have poor prognoses and distant metastases. This makes sense and emphasizes the importance of breast cancer screening programs (TA Shaha and SS Guraya, 2017; Smolarz *et al*., 2022). Breast cancer is the most common type of malignant tumor in women to be identified worldwide, and it also ranks first in terms of cancer-related deaths. The incidence of BC is continuously increasing all over the world (Łukasiewicz *et al.*, 2021). Therefore, even with advances in treatment and diagnosis that lead to lower death rates, it is imperative to search for new therapeutic techniques and prognostic and predictive markers.

Treatment methods differ based on molecular subtype (Gonzalez-Angulo *et al.*, 2007). In addition to locoregional treatment (radiation therapy and surgery), the interdisciplinary approach to treating BC includes systemic therapy (Maughan *et al*., 2010). Chemotherapy, hormone therapy (hormone-positive diseases), anti-HER2 therapy (HER2-positive diseases), and, more recently, immuno-therapy are systemic treatment examples. Triple-negative breast cancer (TNBC) has been found responsible for no less than 14.60-20.60% of all of the instances of BC. It represents a therapeutic challenge and is hence of great interest for research because of its limited response to treatment and highly invasive nature. Based on cancer biology and early therapeutic responses, future therapeutic methods for BC aim towards care individualization, escalate when appropriate, and de-escalate when required (Trayes & Cokenakes, 2021; Waks & Winer, 2019).

Because it lacks targetable genetic drivers, TNBC represents the most therapeutically challenging BC sub-type. Studies have shown that a mitogen-independent G1/S cell cycle transition is triggered by death effector domain-containing DNA-binding protein (DEDD) cytoplasmic localization, which is over-expressed in over 60% of the TNBCs. Heat shock 71 kDa protein 8 interacts with the cytosolic DEDD to promote the production of cyclin D1 heat shock cognate protein 70 (HSC70). By interacting

with such proteins, DEDD promotes the degradation of the Rb family members by proteasomes. The overexpression of DEDD makes TNBCs more vulnerable to the inhibition of the cell cycle. TNBC patients have not been included in clinical trials including cyclin-dependent kinase 4/6 (CDK 4/6) inhibitors since it has been suggested that Rb-loss is widespread in these patients. The results of the studies have demonstrated that, regardless of Rb status, the TNBCs with the over-expression of DEDD are susceptible to combination therapy with the EGFR inhibitor and a CDK 4/6 inhibitor *in vitro* and *in vivo*. Consequently, the research provided support for the therapeutic application of CDK 4/6 inhibitor combination regimens for TNBC patients (Ni *et al*., 2019; Dong *et al*., 2019). The canonical roles of small nucleolar RNAs (snoRNAs) in RNA modification, as well as ribosome synthesis are well-established. SnoRNAs are endogenous sponges that regulate the synthesis of miRNAs. Accurate expression of the snoRNA is necessary for miRNA expression fine-tuning. SnoRNAs that were transformed into miRNA-like sequences are important in the process, much like miRNAs control the transcription of genes that code for proteins. Latest investigations have established a connection between dysregulated snoRNA and BC. Incorrect expression of snoRNA plays a role in BC pathogenesis through the facilitation of the development of cancerous traits in the breast cells. The levels of snoRNA could be employed for diagnosis and prognosis of BC since they reveal large variations in the expression between cancerous and normal situations. The role of snoRNA in BC pathogenesis is comprehensively summarized (Dsouza *et al.*, 2021). Because it lacks targetable genetic drivers, TNBC is possibly the most therapeutically challenging BC subtype. This study shows how mitogen-independent G1/S cell cycle transition is triggered by DEDD cytoplasmic localization, which might be over-expressed in over 60% of the TNBCs. Heat shock 71 kDa protein 8 interacts with cytosolic DEDD to promote the production of cyclin D1 (HSC70). By interacting with such proteins, DEDD promotes Rb family members' degradation by the proteasomes. The over-expression of DEDD makes TNBCs more vulnerable to the inhibition of the cell cycle. TNBC patients were excluded from treatment trials including CDK 4/6 antagonists due to the assumption that Rb-loss occurs frequently in TNBCs (Mourksi *et al.*, 2020; Su *et al*., 2013). For example, it is thought that increased snoRNA biogenesis, which necessitates increased rRNA expression, is the cause of the observed rise in protein output in BC. As a result, it was discovered that certain snoRNAs are linked to the prognostic value of BC and its etiology (Jiang *et al*., 2020). It was shown that SNHG1 promotes the proliferation and spread of BC cells *in vivo* as well as *in vitro*. It is significantly increased in female BC cell lines and tissues. The expression of homeobox A1 is activated by miR-193a-5p, which is subsequently sucked up by SNHG1 through a molecular method of action (Kang *et al*., 2021). It has been found that the expression regarding miR448 and miR-382 is regulated by the homeobox A1 (HOXA1) oncogene (Louca & Gkretsi, 2022). Furthermore, it was discovered that SNHG1 co-expresses with miR199a-3p, which controls its target gene transcription factor A mitochondrial (TFAM), upregulating MDA-MB-231 in hypoxic conditions in a way that is dependent on oxygen deprivation factor-1 (HIF-1) and lastly leading to the metastasis of BC cells (Louca & Gkretsi, 2022). Finally, it was discovered that miR-18b-5p, which regulates telomerase reverse transcriptase (TERT) synthesis, uses SNHG1 as a sponge. Furthermore, through the control of miR-186-5p and zinc finger E-box binding homeobox 1 (ZEB1) expression, SNHG-3 overexpression in MCF7 band MDA-MB231 cells resulted in BC cell proliferation, migration, EMT, and invasion (Kang *et al.*, 2021). The expression of SNHG3 was likewise significantly elevated in BC tissues and cells. Furthermore, SNHG-3 silencing *in vivo* and *in vitro* prevented the proliferation of BC cells (Jiang *et al*., 2020).

The present study aimed to explore the therapeutic use of combinatorial regimens of the CDK 4/6 inhibitors for patients with

TNBC. The role of small nucleolar RNA host gene 3 (SNHG3) and status in cancer-associated fibroblast (CAF) were also investigated.

Research questions

1. How does SNHG3 contribute to the proliferation of CAFs?
2. How does SNHG3 control the synthesis of possible microRNAs (miRNAs) and its role in BC?

Significance of the study

The TNBC represents the most therapeutically challenging breast cancer sub-type as a result of its lack of targetable genetic drivers. The study focused on the CAFs. This study is important because it shows how the DEDD is over-expressed in no less than 60% of the TNBCs and how it results in mitogen-independent transition regarding the G1/S cell cycle. As a result, the research presented potential therapeutic applications for CDK 4/6 inhibitor combinatorial regimens in TNBC patients.

Materials and Methods

Culture preparation and transfection

MD-MBA-453 and MCF7 BC cells were obtained from the American Type Culture Collection (ATCC; Maryland, US). MCF7 and MD-MBA453 cells were cultured by using the ATCC-formulated Leibovitz's L-15 medium, as well as ATCC-formulated Eagle's minimum essential medium, respectively, together with 10% fetal bovine serum (FBS; Gibco, Thermo) and antibiotics (100 g/ml streptomycin sulfate and 100 U/ml penicillin) (HyClone, US). Cells that had attained 75% confluence were used in each one of the experiments. Fibroblast cells that were derived from BC patients were cultured in Iscove's modified Dulbecco medium, containing 15% of the FBS. The CAFs were placed onto a 15 cm dish once they reached 75% confluence. The culture medium concerning CAFs was isolated from the exosomes secreted within 48 hours, and the exosomes were then added back to the medium to continue the BC cells' culture. Small interfering RNA (siRNA) were generated from Genscript: miR330-5p mimic (miR330), AntagomiR control (anti-miR-con), miR330-5p AntagomiR (anti-miR330), empty vector (Vector), pyruvate kinase M1/M2 (PKM), and SNHG3 (Nanjing, China). Lipofectamine 2000 (Invitrogen) was used for transfecting many plasmids or oligonucleotides. Cells were collected 48 hours post-transfection to facilitate further research.

Exosome extraction and size distribution

Cells were cultured in serum depleted of exosomes. FBS was centrifuged at 100,000 g for a period of 10 mins at 4 °C to create the serum. Following a 72-hour incubation period, the culture liquid was removed and centrifuged. Floating cells were removed from the medium by centrifuging at 400 g for 5 minutes at a temperature of 4 °C. After that, cell debris was extracted from supernatants by using centrifugation for 20 mins at 4 °C and 3000 g. Following the filtered culture supernatant through a 0.22 μm filter (Beckman), exosomes in the supernatant were recovered with the use of ultracentrifuge and centrifuged at 110,000 g for 4 hours at 4 °C. The exosomes were rinsed in PBS and then stored at 80 °C for further research. Exosome size distribution was assessed with a Brookhaven Nanobrook Omni. Exosomes were measured by adding 2 milliliters of exosome PBS to the Nanobrook Omni apparatus after they were resuspended and diluted in PBS.

Tumor microenvironment by transmission electron microscopy

The shape of the exosome samples was assessed with the use of transmission electron microscopy (TEM). First, exosomes were prepared, diluted in PBS, and after that placed on copper grids in a liquid state. Subsequently, the excess liquid was removed and the copper grids were allowed to dry. Following a 5-minute fixation with 2% glutaraldehyde, the samples were colored for 5 mins at room temperature using 2% phosphotungstic acid (PTA). After three PBS washes, exosomes were inspected under a TEM.

Exosome labeling and uptake by MD-MBA-453 and MCF7 cells

For the investigation of exosome uptake by cancer cells, MCF7 and MD-MBA453 cells were seeded into a confocal imaging chamber. Following a 24-hour culture period, the chamber was cleaned 3 times with PBS, and the cells were then stained using different media that either included exosomes tagged with PKH67 or had none at all. Following a further 48 hours of incubation, each confocal chamber was washed 3 times with PBS before the cells were fixed for 8 minutes with 4% PFA. DNA was stained with 4′,6-diamidino-2-phenylindole (DAPI) and following that, two further PBS washes. Lastly, images of the uptake of exosomes by tumor cells were obtained with the use of a confocal microscope LSM880 and a software, named Zen.

Viability assay

To assess the viability of cells, the cell counting kit 8 (CCK8) test was performed with the use of a CCK8 detection kit (Dojindo), following the manufacturer's instructions. For every condition, cells were plated in 96-well Corning plates. The absorbance at 450 nm was determined after the CCK8 test solution was incubated for two hours.

OCR and ECAR measurements

Extracellular acidification rate (ECAR) and OCR were determined with the use of XF metabolic analyzers (Seahorse, Agilent). Initially, 24-well Seahorse microplates were used for cell plating. When the cell density reached 70% confluence, each well received an application of the appropriate culture medium with its associated parameters. The plate was subsequently filled with 800 μL of assay medium following incubation at a temperature of 37 °C and 5% CO_2. OCR was determined following an extra hour of incubation at 37 °C without 5% CO_2.

Extraction of RNA, real-time qPCR, and western blot

RNA was isolated from MD-MBA453 cells using Trizol, and cDNA was prepared with the use of a reverse transcription kit. The internal control employed was 36B4 (human). Real-time qPCR was performed with the use of the SYBR Green Mix. Data were collected and assessed with the use of a Step-One-Plus Real-Time PCR System. Cell protein was extracted with the RIPA buffer (Thermo). Protein concentration was measured with BCA Protein Assay. The complete protein was separated using the sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) technique. The membrane was transferred to PVDF, and antibody probing was carried out using actin, antiPFKM, antiPKM, anti-IDH2, and anti-PDHB. A signal was discovered with the use of a chemiluminescence imaging technique.

Generation of stable cell lines by the lentivirus transduction

Cloning the short hairpin RNAs (shRNAs) matching sequences with SNHG3 (sh-SNHG3), anti-miR330-5p (anti-miR330), scrambled control (sh-control), and anti-miR-control (anti-miR-con) was done by using pCDH vectors with red fluorescence protein (mCherry) reporter. Those plasmids were transfected into HEK-293T cells using helper plasmids as vectors of packaging (GeneChem, Shanghai, China). MD-MBA453 cells were transduced by using lentivirus containing anti-miR330-5p and 5 mg/ml polybrene Genechem (Shanghai, China). Two days following lentivirus transfection, cells with stable miR330-5p knockdown were identified by FACS measurement of the mCherry signal. Comparably, lentiviruses expressing sh-SNHG3 were used for transducing BC patient-derived CAFs to produce stable SNHG-3 knockdown cells.

Luciferase reporter assay

SNHG3 fragments with both control and mutant miR330-5p binding sites were integrated into firefly luciferase backbone plasmids to produce a plasmid containing the luciferase reporter genes (pGL3, Promega). For generating pGL3-PKM 3′-UTR, the PKM 3′-UTR sequence was cloned into pGL3 plasmids. A similar number of MD-MBA-453 cells was co-transfected with the PKM3′-UTR and miR-con, miR330-5p, miR330-5p + plasmids, miR330-5p, anti-miR330-5p, or 250 ng of the SNHG3-

WT or SNHG3-mutant, 25 ng of the Renilla reporter plasmid, or mismatched controls. Cell lysates were collected and then diluted with the use of the dual-luciferase reporter assay kit.

Generation of breast cancer mouse model

Female BALB/c nude mice weighing 20 g were purchased. The nu/nu mice were given an injection of 5 106 MD-MBA-453 cells alone, in combination with 1:1 Matrigel (Thermo), or in combination with cells that expressed anti-miR330-5p stable (MD-MBA453/anti-miR330-5p or MD-MBA453/control). The animals were sorted into the appropriate groups at random once their average tumor volumes reached 50 mm^3. The intra-tumoral pH was measured with a pH meter.

Measurement of lactate and acetate levels

The levels of acetate and lactate in the metabolic profiling were analyzed using Thermo's acetate assay kit and lactate assay kit, both of which were utilized according to the manufacturer's instructions.

Statistical analysis

Data were expressed as mean ± S.E. of no less than 3 different experiments. Statistical analysis was conducted using the Prism software (GraphPad8). The unpaired Student's t-test and one-way analysis of variance (ANOVA) were employed to determine whether there were any significant differences between the two groups. *P < 0.050, **P < < 0.010, and ***P < 0.001 were deemed statistically significant.

Results

Table 1A shows the 40–100 nm particle size range of the exosomes produced by CAF. The expression of exosome marker CD-63 was confirmed by western blot. To determine whether the exosomes that were produced by CAF were consumed by the MD-MBA453 and MCF7 BC cells, the exosomes with the PKH green dye were first labeled and then exposed to the cells. With the use of MCF7 or MD-MBA453 cells, green fluorescent signals were observed with the use of confocal laser scanning microscopy. The ingestion of the exosomes that were generated by CAF by BC cells was further confirmed by flow cytometry. The results showed that the exosomes from CAFs were absorbed by BC cells, supporting the reception of exosomes by breast tumor cells. MD-MBA453 cell proliferation was stimulated by the exosomes produced by the CAF in a concentration-dependent manner. To find out whether CAF-secreted exosomes led to the induction of metabolic reprogramming in tumor cells (OCR), the MD-MBA453 cells were cultivated with such exosomes and the rate of oxygen consumption was monitored. The results (Table 1B) showed that the exosomes that were released by the breast CAF reduced the OCR of MDMBA-453 and MCF-7 cells. To verify if the internalization of exosomes was the reason for the reduction in OCR in tumor cells, exosome-release inhibitor GW-4869 was employed to stop the synthesis of exosomes in the CAFs. Maximal and reserve mitochondrial activity of tumor cells was considerably decreased after receiving exosome treatment, suggesting that the exosomes that were generated by CAF could have less capacity for mitochondrial respiration. The ECAR, of breast tumor cells, rose significantly in the treatment when they were co-cultured with exosomes generated by CAF. The existence of exosomes caused a significant increase in lactate levels in BC cells. All of these observations indicated that exosomes secreted by CAF altered the metabolic pathways of BC cells and decreased mitochondrial function. Exosomal SNHG3 was downregulated and promoted the proliferation of SNHG3.

It was then determined whether the impact depends on the exosomal long noncoding RNA (lncRNA) SNHG3, which is generated by CAFs and has the potential to change the metabolism and promote the proliferation of BC cells. SNHG3 expression levels in both intracellular and secretory CAFs were examined first. Breast cancer-derived CAFs produced SNHG3 at significantly higher levels than normal breast cells, MCF10A. Next, utilizing gain-of-function as well as loss-of-function experiments, the biological involvement of SNHG-3 during the formation of BC cells was examined.

Table 1A

Cancer-associated fibroblast-derived exosomes re-programmed breast cancer cell metabolism

Vesicle size (nm)	Intensity (%)
27	6
34	12
41	20
47	55
53	62
58	64
64	117
70	98
76	79
82	58
88	16
95	22
100	13
Exo (µg/ml)	**Relative viable cells**
0	1.10
22.70	1.40
46	1.80
92	2.30

Table 1B

Cancer-associated fibroblast (CAF)-derived exosomes re-programmed breast cancer cell metabolism

Breast Viability			
Normalized OCR ± OCR	**Control**	**CAF/ Exo**	**CAF/ GW 4869/ Exo**
MCF7	100.23 ± 19.80	15.46 ± 22.30	62.12 ± 42.34
MDA-MB453	100.25 ± 58.70	21.30 ± 63.40	58.50 ± 98.3
Basal OCR for the MD-MBA453 and MCF7 cells that have been cultured with the CAF exosomes			
Normalized ECAR ± ECAR			
MCF7	98.30 ± 15.30	160.54 ± 22.40	115.60 ± 43.50
MDA-MB453	99.40 ± 61.30	145.70 ± 78.50	103.40 ± 97.60
Relative lactate concentration ± lactate			
MCF7	0.90 ± 17.60	1.80 ± 21.40	1.30 ± 34.70
MDA-MB453	1.10 ± 58.50	1.40 ± 81.30	1.20 ± 96.50
Maximal and reserve OCR of the MCF7 and MD-MBA453 have been examined after CAF exosomes' uptake			
MCF7			
Reserve	98.20 ± 15.60	48.70 ± 21.40	89.30 ± 37.80
Maximal	99.20 ± 49.20	32.50 ± 53.50	87.60 ± 68.90
MDA-MB453			
Reserve	97.10 ± 14.70	36.30 ± 22.40	88.40 ± 34.50
Maximal	96.20 ± 48.70	39.20 ± 34.60	86.90 ± 67.35

Exosomes that were released from the CAFs transfected with the si-SNHG-3 or with SNHG-3 over-expression plasmid were tested with the use of the CCK8 to determine if they affected the rate of MDA-MB-453 cell growth (Table 2). The CCK8 assay demonstrated that exosomes that were generated from the CAFs transfected with the si-SNHG-3 significantly inhibited the growth of MDA-MB231 cells compared with the si-control group. Accordingly, MDA-MB-453 cells that were transfected directly with a plasmid for SNHG3 overexpression revealed much higher proliferation than the group under control. Furthermore, lactate production was significantly increased by overexpressing si-SNHG-3 in MDA-MB453 cells. However, the treatment regarding exosomes generated by CAFs transfected with si-SNHG-3 reversed lactate production elevation. Then, mitochondrial respiration in the BC cells that were transfected with si-SNHG3 was examined to determine whether or not exosomes were released from CAFs. After applying CAF-secreted exosomes, the OCR of MDA-MB-453 cells decreased. However, it could be restored in the presence of the exosomes from the CAFs that were transfected with si-SNHG-3 as presented in Table 2.

The overexpression of miR330 significantly reduced luciferase signals of SNHG-3-wildtype in the MD-MBA453 cells. On the other hand, SNHG-3-wildtype's luciferase signals were significantly improved by miR330 suppression in MD-MBA453 cells, but SNHG-3 mutation did not exhibit any positive luciferase signals. Furthermore, real-time qPCR showed that SNHG-3 overexpression significantly reduced the expression of SNHG-3, but SNHG3-mutant treatment of MD-MBA453 cells did not significantly alter SNHG3 expression. The release of exosomes upon si-SNHG-3 transfection into CAFs significantly increased the expression of miR330 in MD-MBA453 cells. The results demonstrated that SNHG-3 inhibited miR330 expression *in vitro* by acting like a molecular sponge (Table 3).

Table 2

Cancer-associated fibroblast (CAF)-derived exosomal SNHG-3 re-programmed the metabolism of breast cancer cells

Treatment	**MCF-10 A**	**CAFs**	
Relative SNHG-3 level ± secreted intracellular	0.8 ± 2.3	4.8 ± 3.1	
Relatively viable cells ± secreted intra-cellular	si- Control/ CAF- Exo	siSNHG-3/ CAF- Exo	
	0.90 ± 14.60	0.60 ± 21.20	
Relatively viable cells ± secreted intra-cellular	Control	SNHG-3	
	0.85 ± 12.30	4.20 ± 34.23	
Relative lactate concentration ± secreted intra-cellular	Control medium	Exo/ si-control	Exo/ si-SNHG-3
	0.95 ± 13.45	1.76 ± 23.40	1.32 ± 36.50
Relative lactate concentration ± secreted intracellular	Control	SNHG3	
	0.97 ± 22.4	1.65 ± 45.6	
Normalized OCR	Control medium	Exo/ si-control	Exo/ si-SNHG3
	101.50 ± 21.60	24.80 ± 34.50	76.89 ± 46.70
Normalized OCR	Control	SNHG-3	
	103.40 ± 34.50	8.79 ± 65.60	
Normalized OCR	Control medium	Exo/ si-control	Exo/ si-SNHG3

End of table. 2

Treatment	MCF-10 A	CAFs	
	98.60 ± 20.90	167.90 ± 34.20	115.70 v 58.90
Normalized ECAR	Control	SNHG3	
	98.70 ± 34.50	167.90 ± 56.90	

Table 3

Cancer-associated fibroblast (CAF)-derived exosomal SNHG-3 regulates miR330 expression in the cells of breast tumors

Relative luciferase activity	SNHG-3- WT	SNHG-3- MUT	
miR- con	98.65 ± 16.89	124.50 ± 54.70	
miR330	47.89 ± 23.89	127.56 ± 65.70	
Relative luciferase activity	SNHG 3- WT	SNHG 3- MUT	
Anti- miR- con	98.78 ± 15.67	67.80 ± 45.60	
Anti- miR330	173.60 ± 25.67	64.80 ± 54.78	
Relative miR330 expression	Vector	SNHG3	Mutant SNHG3
	0.98 ± 18.90	0.48 ± 34.68	0.89 ± 43.67

WT: Wildtype; MUT: Mutant

Discussion

The canonical roles of snoRNAs in ribosome synthesis and RNA modification are well-established. SnoRNAs are endogenous sponges that regulate the synthesis of miRNAs. Accurate expression of snoRNA is necessary for the fine-tuning of the miRNA expression. Conversion of snoRNAs to miRNA-like sequences is important in this process, just as miRNAs control expression regarding protein-coding genes. Recent investigations have established a connection between BC and dysregulated snoRNA. Inappropriate snoRNA expression contributes to the pathogenesis of BC by facilitating the development of cancerous traits in breast cells. Since snoRNA levels show a significant variation in expression between normal and malignant conditions, they could be employed for BC diagnosis and prognosis (Dsouza *et al.,* 2021). The biological function, regulation, signaling pathways, and therapeutic significance of aberrantly produced snoRNAs in BC have all been reported in greater depth (Dsouza *et al.,* 2021; Mourksi *et al.,* 2020).

In cancer, genomic rearrangements could mix sequences of 2 different genes. Research that has been conducted on these gene fusion events has primarily focused on detecting fusion proteins from the chimeric transcripts. In the present study, the potential effects of fusions on the synthesis of intronic micro-RNA genes were investigated, which are present in fusion gene partners. The scope of the study was broadened to include snoRNAs, which are present inside host genes that do not code for proteins. It has been discovered that the snoRNA hosts are preferentially enriched in the fusion transcripts, just like the miRNA host genes, and that such enrichment has been related to all of the classes of snoRNA.

The structural alterations might have functional implications for the cell, as shown by protein over-representation in the protein translation machinery amongst snoRNA host genes; a gene architecture that is assumed necessary for closely coordinated snoRNA production as well as the production of host proteins. The evidence suggests that cancer often disrupts this structure. Furthermore, it was observed that

several snoRNA genes that are involved in the fusion link with stronger promoters than the usual hosts, indicating a mechanism that supports the over-expression of snoRNA. Consequently, structural change has been highlighted that often occurs in cancer and impacts significant cellular physiological aspects (Persson *et al*., 2020). For instance, higher rRNA expression in BC is assumed to be the origin of the reported increase in protein synthesis, which necessitates increased snoRNA production. Consequently, it has been discovered that several snoRNAs are helpful prognostic indicators and play a role in BC etiology (Su *et al.,* 2013). It was shown that SNHG1 promotes the growth and spread of breast carcinoma cells *in vivo* and *in vitro*. Furthermore, it was discovered to be significantly elevated in specific cell lines and tissues from BC (Jiang *et al.,* 2020). Concerning the molecular mechanism of action, it has been shown that SNHG1 functions as a sponge for miR193a-5p, which in turn promotes the expression of oncogene HOXA-1. Nevertheless, it has been shown that SNHG1 functions by regulating the expression related to miR-448 and miR-382 (Kang *et al*., 2021).

Future studies must take into account the possibility that most snoRNAs and lincRNAs could serve as helpful diagnostic or prognostic biomarkers, as they are discovered in higher amounts in BC cells compared to healthy tissues. The complexity of their activities is further highlighted by the fact that the majority of them also function as ceRNA, which blocks different miRNAs involved in regulating the expression of different genes. As earlier reported (Louca & Gkretsi, 2022). The findings of the present study indicated that snoRNAs and lincRNAs may be novel bio-markers and/or therapeutic targets that inhibit BC cell metastasis. To address all of the open questions regarding such molecules and to raise the significance of the previously underappreciated non-coding DNA sections, further research is required.

Conclusion

The findings of the present study revealed that the expression level of SNHG-3 was significantly high in CAFs. The proliferation of BC cells can be inhibited by blocking the release of exosomal SNHG3 by CAFs. In terms of mechanism, miR330 may control PKM expression in BC cells by using SNHG3 as a molecular sponge. To the best of our knowledge, these findings present the first evidence for molecular regulation, as well as the biological function of CAF-secreted exosomal SNHG-3 in BC. Furthermore, PKM is essential for metabolic reprogramming and for controlling the death, proliferation, and metastases of cancer cells. This suggests possible therapeutic targets for the suppression of PKM in cancer treatment. The findings also demonstrated that by enhancing exosomal non-coding RNA, exosomes produced by CAFs might change the metabolism of cancer cells. More importantly, the findings of the present study point to the therapeutic potential of concentrating on exosome-mediated cross-talk between cancer and stromal cells when treating cancer.

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