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ORIGINAL RESEARCH ARTICLE

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The long noncoding RNA sONE represses triple-negative breast cancer aggressiveness through inducing the expression of miR-34a, miR-15a, miR-16, and let-7a

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Abstract

Triple-negative breast cancer (TNBC) represents an aggressive breast cancer subtype. Among young females, TNBC is the leading cause of cancer-related mortalities. Recently, long noncoding RNAs (lncRNAs) are representing a promising pool of regulators for tuning the aggressiveness of several solid malignancies. However, this still needs further investigations in TNBC. The main aim of this study is to unravel the expression pattern of sONE lncRNA and its mechanistic role in TNBC. Results showed that sONE is restrictedly expressed in TNBC patients; its expression level is inversely correlated with the aggressiveness of the disease. sONE acts as a posttranscriptional regulator to endothelial nitric oxide synthase (eNOS) and thus affecting eNOS-induced nitric oxide (NO) production from TNBC cells measured by Greiss reagent. Mechanistically, sONE is a potential tumor suppressor lncRNA in TNBC cells; repressing cellular viability, proliferation, colony-forming ability, migration, and invasion capacities of MDA-MB-231. Furthermore, sONE effects were found to be extended to affect the maestro tumor suppressor TP53 and the oncogenic transcription factor c-Myc. Knocking down of sONE resulted in a marked decrease in TP53 and increase in c-Myc and consequently altering the expression status of their downstream tumor suppressor microRNAs (miRNAs) such as miR-34a, miR-15, miR-16, and let-7a. In conclusion, this study highlights sONE as a downregulated tumor suppressor lncRNA in TNBC cells acting through repressing eNOS-induced NO production, affecting TP53 and c-Myc proteins levels and finally altering the levels of a panel of tumor suppressor miRNAs downstream TP53/c-Myc proteins.

KEYWORDS

eNOS, long noncoding RNAs, microRNAs, nitric oxide, sONE, triple-negative breast cancer

1 | INTRODUCTION

Despite the current advances witnessed in the treatment of breast cancer (BC), BC statistics are still enormously high and competes with heart disease for the leading cause of death worldwide (Jemal et al., 2006; McGee et al., 2018). Triple-negative breast cancer (TNBC) represents an aggressive subtype that is candidate for neither endocrine nor human epidermal growth factor receptor 2 (HER-2) directed therapies, leaving chemotherapeutic agents solely as the mainstay of treatment in both early and advanced stages of the disease (Bader, Brown, Stoudemire, & Lammers, 2011; Bianchini, Balko, Mayer, Sanders, & Gianni, 2016). Nonetheless, TNBC patients are the least fortunate in terms of diagnosis and prognosis due to lack of early specific diagnostic and prognostic biomarkers (Dong,

Mu, Zhao, & Sun, 2018). It is also worth noting that robust evidence suggests that among young women (<40 years old), TNBC is undoubtedly the leading cause of cancer-related mortalities, thus representing a significant burden in developing countries in particular (Anastasiadi, Lianos, Ignatiadou, Harissis, & Mitsis, 2017). It is even more terrifying to know that about one in every 300 women will be diagnosed with BC before the age of 40 and a significant number of young women lose their lives every year because of such deadly disease (Narod, 2012).

Sequencing technologies have recently reached a breakthrough in terms of understanding the multifaceted human genome (S. Zhou, He, et al., 2018). Long noncoding RNAs (lncRNAs) are among the relatively recent discoveries of such studies; lncRNAs as their name annotates are nonprotein coding transcripts with length >200 nucleotides (S. Zhou, He, et al., 2018). Myriad studies reported that lncRNAs expression pattern is deregulated in different malignancies thus suggesting its possible contribution in the process of carcinogenesis (Huarte, 2015). However, there is still a lot of missing information about their diagnostic, prognostic potentials, and their mechanistic role in BC (Qi & Du, 2013).

Recent studies have reported that there is a fascinating reciprocal regulation between lncRNAs and other classes of ncRNAs especially the microRNAs (miRNAs; Kumar, Williams, Sur, Wang, & Jo, 2018; Yin, Feng, Shen, & Ju, 2018). However, this crosstalk has been rarely investigated in the context of BC.

sONE is a newly discovered lncRNA that acts as a natural antisense transcript binding to the endothelial nitric oxide synthase (eNOS) 3′-untranslated region (3′-UTR; R. A. Youness, Assal, Abdel Motaal, & Gad, 2018; Zhang et al., 2015). We and others have validated sONE as a direct posttranscriptional regulator of eNOS-induced nitric oxide (NO) production in cultured as well as primary endothelial cells and TNBC cell lines (Fish et al., 2007; Robb et al., 2004; R. A. Youness, Assal, Abdel Motaal, et al., 2018). However, it was clear in the literature that neither the expression status nor the functional characterization of sONE has been characterized in any type of malignancy including BC. Therefore, this inspired us to unravel the anonymity of sONE as a novel lncRNA in BC and to further unveil its functional role in TNBC cells.

Several decades of literature highly supports the involvement NO as a pleiotropic onco-regulatory gasotransmitter in several types of cancers including BC (Shafran et al., 2017; Yarlagadda, Hassani, Foote, & Markowitz, 2017). Recently, eNOS-induced NO production has been highly implicated in determining the aggressiveness of TNBC tumors (Cranford et al., 2017; Trimmer et al., 2011; R. A. Youness, Assal, Abdel Motaal, et al., 2018). Yet, the exact underlying mechanism has been highly controversial. Recently, a causal relationship has been validated between several gasotransmitters and vital cell cycle regulators such as TP53 and c-Myc (R. A. Youness, Assal, Abdel Motaal, et al., 2018).

The tumor suppressor TP53 and the oncogenic transcription factor c-Myc are paradoxically acting regulatory proteins in BC, yet their mechanisms of actions are highly inter-connected (Ho, Ma, Mao, & Benchimol, 2005; Phesse et al., 2014). Recently, we and others have found that they could share a novel arm of intrinsic epigenetic regulatory mechanism affecting the expression levels of an array of tumor suppressor miRNAs such as miR-34a (Abdi, Rastgoo, Li, Chen, & Chang, 2017; Xiao, Gu, Wang, & Chen, 2018; R. A. Youness, Assal, Ezzat, Gad, & Abdel Motaal, 2018), miR-15a (Jackstadt & Hermeking, 2015; Rampazzo et al., 2017; R. A. Youness, Assal, Ezzat, et al., 2018), miR-16 (Issler & Mombach, 2017; R. A. Youness, Assal, Ezzat, et al., 2018) and let-7a(Luu et al., 2013; Lyu et al., 2014). However, this has been rarely examined in TNBC cells.

Therefore, the main aim of this study is to unravel the expression status of the lncRNA sONE, to investigate its impact on TNBC hallmarks and finally to investigate its exact mechanistic role in TNBC through unveiling its impact on several cell cycle regulatory proteins and their downstream miRNAs.

2 | MATERIALS AND METHODS

2.1 | Study patients

Tumor tissues and healthy control counterparts were resected from 80 BC patients. All tumor and control samples were morphologically confirmed by a pathologist. Immunohistochemical and pathological profiles of all patients were provided (Table 1). Out of all BC patients, 25 were TNBC, their detailed clinical details provided in Table 2. All experiments were performed in compliance with the guidelines of the Institutional Review Board of Kasr El Aini Medical School, Cairo University, and German University in Cairo and in accordance with the ethical standards of the declaration of Helsinki. All participants gave their written informed consent.

2.2 | Cell culture and treatment

MDA-MB-231 and MCF-7 BC cells were used in the study. They were maintained in Dulbecco's modified Eagle's medium (DMEM; Lonza, Basel, Switzerland) supplemented with 4.5 g/L glucose, 4 mmol/L L-glutamine, 10% fetal bovine serum (FBS) and MycoZap (1:500; Lonza) at 37°C in 5% CO_2 atmosphere. The cells were passaged upon reaching 70–80% confluency as previously described in (R. A. Youness, Assal, Abdel Motaal, et al., 2018). Stock solution of NO donor (DETA/NO) was prepared using free DMEM. Furthermore, serial dilutions were prepared. Treatment of TNBC cells using DETA/NO in 96-well or 24-well plates was performed based on the experimental setup as previously described in R. A. Youness, Assal, Abdel Motaal et al. (2018).

2.3 | Transfection of MDA-MB-231 using different oligonucleotides

MDA-MB-231 cells were transfected using sONE or scrambled small interfering RNA (Scr-siRNAs) or miR-34a or miR-15a or miR-16 or let-7a oligonucleotides (Qiagen, Hilden, Germany). All transfection experiments were carried out in triplicates using HiPerfect Transfection Reagent (Qiagen) and fast forward protocol. Experiments were repeated at least three times as previously described in R. A. Youness,

TABLE 1 Characteristic features of BC patients

BC patients	Percentage
Age (years)	
≥40 (59/80)	73.75
<40 (21/80)	26.25
Grade	
I (3/80)	3.75
II (62/80)	77.5
III (15/80)	18.75
Histological type	
Ductal (77/80)	96.25
Lobular (1/80)	1.25
Both (2/80)	2.5
Molecular subtype	
Luminal A (18/80)	22.5
Luminal B (35/80)	43.75
HER-2 enriched (2/80)	2.5
TNBC (25/80)	31.25
ER status	
Positive (53/80)	66.25
Negative (27/80)	33.75
PR status	
Positive (53/80)	66.25
Negative (27/80)	33.75
HER-2 status	
Positive(21/80)	26.25
Negative (59/80)	73.75
Lymphatic involvement	
Yes (53/80)	66.25
No (27/80)	33.75
Proliferative index (Ki-67)	
High (≥14%, 56/80)	70
Low (<14%, 24/80)	30

Note. BC: breast cancer; ER: estrogen receptor; HER-2: human epidermal growth factor receptor 2; PR: progesterone receptor; TNBC: triple-negative breast cancer.

Assal, Abdel Motaal, et al. (2018); R. A. Youness, El-Tayebi, et al. (2016); and R. A. Youness, Rahmoon, et al., (2016). In case of cotreatment experiments, transfection was performed and 48 hr later cotreatment with DETA/NO was performed as previously described in (R. A. Youness, Assal, Ezzat, et al., 2018). While in case of cotransfection experiments, transfection using sONE siRNAs was performed and 48 hr later cotreatment with miR-34a or miR-15a or miR-16a or let-7a mimics or collectively were performed also as previously described in R. A. Youness, Assal, Ezzat, et al. (2018). The detailed experimental setup is tabulated in Figure 5.

2.4 | Total RNA, lncRNAs, and miRNAs extraction from BC tissues and cell lines

Total RNA, lncRNAs, and miRNAs were extracted from BC patients and cell lines using Biazol reagent. RNA yield was quantified with a spectrophotometer, and RNA integrity was tested by 18s rRNA bands detection on 1% agarose gel electrophoresis. RNA samples with optical density 260/280 more than two were excluded from the study (R. A. Youness, El-Tayebi, et al., 2016; R. A. Youness, Rahmoon, et al., 2016).

2.5 | Quantitative real-time polymerase chain reaction (PCR) analysis

sONE, eNOS, and 18s rRNA messenger RNAs were reverse-transcribed into complementary DNA (cDNA) using the High-capacity cDNA Reverse Transcription Kit (ABI, Foster City, CA) according to the manufacturer's instruction. However, the extracted miRNAs were reverse-transcribed into single-stranded cDNA using TaqMan_MicroRNA Reverse Transcription Kit (ABI) and specific primers for hsa-miR-15a, hsa-miR-16, hsa-miR-34a, and hsa-let-7a and RNU6B. Relative expression of sONE, eNOS, and 18s rRNA (for normalization) and miR-15a, miR-16, miR-34a, let-7a, and RNU6B (for normalization) was quantified using TaqMan Real-Time q-PCR (ABI Assay IDs: Hs01377254_m1, Hs01574665_m1, 4319413E, Hs04231417_s1, Hs00234713_m1, Hs00418522_m1, 002406, 001093, respectively) measured on StepOne™ Systems (ABI). Relative expression was calculated using the $2^{-\Delta\Delta C_t}$ method. All PCR reactions were run in triplicates and repeated at least three times (R. A. Youness, El-Tayebi, et al., 2016; R. A. Youness, Rahmoon, et al., 2016).

2.6 | Western blot analysis

MDA-MB-231 cells were lysed. BCA Protein Assay Kit (Pierce Biotechnology, Rockford, IL) was used to determine the concentration of protein lysates. Forty micrograms of each lysate were separated by 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis gels and then blotted onto polyvinylidene difluoride (PVDF) membrane. PVDF membrane was then blocked using 5% fat-free dry milk and incubated over-night with primary antibodies. Primary antibodies against eNOS (ab5589; Abcam, Cambridge, UK) and β-actin which was used as a loading dye (ab8229; Abcam) that were kindly provided by Prof. Dr. Laila Rashed, Faculty of Medicine, Cairo University were used for detecting respective proteins by the standard protocol (R. A. Youness, Assal, Abdel Motaal, et al., 2018).

2.7 | Quantification of NO production

Nitric oxide production was determined using Griess Reagent Assay (Promega) as previously described in (Dong et al., 2018). Briefly, 50 µl of cell culture supernatant were dispensed with 50 µl of the sulfanilamide solution. Ten minutes later, 50 µl of *N*-1-naphthylethylenediamine dihydrochloride solution were added to each well. Absorbance was measured at 540 nm using Wallace 1420 Victor2 Multi-label Counter (Perkin Elmer, Waltham, MA). All experiments were done in triplicates and repeated at least three times (R. A. Youness, Assal, Abdel Motaal et al., 2018).

TABLE 2 Clinical data for individual TNBC patients

Patient Nos	Age	Histology	Grade	Lymphnode metastasis	ER	PR	HER-2	Tumor size (cm)	Ki-67	Menopause
Patient 1	37	IDC	II	No	No	No	No	2.1×2.4	Low 10%	Pre
Patient 2	37	IDC	II	Yes	No	No	No	1.5×2.8	Low 12%	Pre
Patient 3	38	IDC	II	Yes	No	No	No	4×6	High 85%	Pre
Patient 4	61	IDC	II	Yes	No	No	No	Multifocal	High 50%	Post
Patient 5	35	IDC	II	No	No	No	No	1.1×2	High 50%	Pre
Patient 6	42	IDC	II	No	No	No	No	4.5×6	High 45%	Pre
Patient 7	34	IDC	II	Yes	No	No	No	1.9×2	High 45%	Pre
Patient 8	35	IDC	II	Yes	No	No	No	1.2×2	High 40%	Pre
Patient 9	63	IDC	II	Yes	No	No	No	3.5×2.0	High 60%	Post
Patient 10	24	IDC	II	Yes	No	No	No	0.73×0.24	High 50%	Pre
Patient 11	28	IDC	II	No	No	No	No	0.26×0.14	Low 12%	Pre
Patient 12	20	IDC	II	Yes	No	No	No	2.5×1.3	Low 12%	Pre
Patient 13	50	IDC	III	Yes	No	No	No	4×4	High 23%	Post
Patient 14	41	IDC	II	Yes	No	No	No	1.2×0.9	High 50%	Pre
Patient 15	44	IDC	III	Yes	No	No	No	3.4×2.8	High 45%	Post
Patient 16	28	IDC	II	Yes	No	No	No	3.5×3.2	High 35%	Pre
Patient 17	42	IDC	II	Yes	No	No	No	2.8×1.6	High 23%	Pre
Patient 18	31	IDC	II	Yes	No	No	No	3.2×2.9	High 23%	Pre
Patient 19	51	IDC	III	No	No	No	No	5×5	High 22%	Post
Patient 20	34	IDC	II	Yes	No	No	No	4.4×3.6	High 50%	Pre
Patient 21	28	IDC	II	Yes	No	No	No	4×4	High 45%	Pre
Patient 22	26	IDC	II	No	No	No	No	3.6×2.6	High 28%	Pre
Patient 23	41	IDC	III	Yes	No	No	No	4.5×3.6	High 50%	Pre
Patient 24	32	IDC	III	Yes	No	No	No	5×4	High 30%	Pre
Patient 25	53	IDC	III	No	No	No	No	Multifocal	High 23%	Post

Note. ER: estrogen receptor; HER-2: human epidermal growth factor receptor 2; PR: progesterone receptor; TNBC: triple-negative breast cancer.

2.8 | Cellular viability and proliferation experiments

For the cellular viability experiments, 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used. In a 96-well plate, 10,000 MDA-MB-231 cells were seeded in 200 µl media. Forty-eight hours posttransfection, media was removed and 20 µl working solution was added to each well. After 6 hr, the formed formazan crystals were solubilized in 200 µl lysis buffer and absorbance was measured as previously described in R. A. Youness, Assal, Abdel Motaal, et al. (2018); R. A. Youness, El-Tayebi, et al. (2016); and R. A. Youness, Rahmoon, et al. (2016). For the proliferation experiments, bromodeoxyuridine (BrdU) incorporation assay was used. TNBC cells were seeded into black 96-well plates with an initial cell count of 5×10^4 cells/well. Posttransfection, cells were labeled with BrdU labeling reagent for 4 hr using the Cell Proliferation ELISA Kit (Roche Applied Science, Penzberg, Germany). The cells were then fixed using Fix-Denate for 30 min and incubated with Anti-BrdU POD for 90 min (R. A. Youness, Assal, Abdel Motaal, et al., 2018; R. A. Youness, El-Tayebi, et al., 2016; R. A. Youness, Rahmoon et al., 2016). All experiments were performed in triplicates and repeated at least three times.

2.9 | Cellular migration and invasion

Scratch assay was performed to assess MDA-MB-231 cellular migration, cells were transfected and left to grow till reaching 90–95% confluency in 24-well plates. Posttransfection; 3 scratches/well were made using 10-µl pipette tip. Detached cells were washed out using PBS and replaced with new low-serum media (1% FBS). Twenty-four hours postscratching, migration was documented, and wound closure was quantified with Zen2012 (ZEISS Microscopy, Jena, Germany) software by measuring the surface area of the scratch (R. A. Youness, Assal, Abdel Motaal, et al., 2018; R. A. Youness, El-Tayebi, et al., 2016). While for assessing the *in vitro* invasion capacity, the Boyden-chamber assay (BD Bioscience, Bedford, MA) was used in 24-well plates. MDA-MB-231 was transfected with different oligonucleotides. Posttransfection, 6×10^4 treated cells were resuspended in 200 µl low-serum media (1% FBS) and seeded in the upper compartment while the lower compartment of the membrane contained high-serum media (20% FBS). Eight hours postseeding, cells were washed from the upper surface using a cotton swab; the invaded cells were fixed and stained using crystal violet (Sigma Chemical Co., St. Louis, MO) and counted under an inverted light microscope. All experiments

were performed in triplicate and repeated at least three times (R. A. Youness, Assal, Abdel Motaal, et al., 2018).

2.10 | Colony-forming assay

Forty-eight hrs posttransfection, cells were trypsinized, suspended, counted, and seeded in six-well plate with an initial count of 1,000 cells/well. Cells were kept to colonize in full DMEM under normal conditions (37°C and 5% CO_2) for 2–3 weeks. Colonies were fixed using 6% glutaraldehyde and stained using 0.5% crystal violet and then manual counting was performed (R. A. Youness, Assal, Abdel Motaal, et al., 2018).

2.11 | Cell cycle analysis

Different expression vectors containing response elements for vital cell cycle proteins upstream a luciferase reporter gene were used. The vital cell cycle proteins assayed were p53 (pp53-TA-Luc), c-Myc (pMyc-TA-Luc), RB (pRB-TA-Luc), E2F (pE2F-TA-Luc; Clontech, St-Germain-en-Laye, France). MDA-MB-231 cells were also transfected with pLuc vector containing an unspecific binding site (Clontech). MDA-MB-231 cells were seeded and transfected with each vector using Superfect Transfection Reagent (Qiagen) according to the manufacturer's protocol. Twenty-four hours posttransfection of the plasmid DNA, cells were transfected using sONE siRNAs. Cells were then lysed and assayed for luciferase expression after 72 hr using Steady-GLO Luciferase Kit (Promega, Mannheim, Germany) according to the manufacturer's instructions. Luminescence was measured and plotted as % luciferase activity relative to cells transfected with the vector alone. Unspecific luminescence detected by the reagents and the empty pTA-Luc vector (baseline luminescence) was subtracted from all values before plotting as previously illustrated (R. A. Youness, Assal, Abdel Motaal, et al., 2018).

2.12 | Statistical analysis

All data are presented as mean ± standard error of the mean. Nonparametric unpaired Student's *t* test was performed to compare between every two independent groups. Statistical significance was considered as $p < 0.05$. One-way analysis of variance with post hoc analysis was used for multiple comparisons. Correlation analysis was performed by Spearman analysis. Data were statistically analyzed using GraphPad Prism 5.00 software (GraphPad Software Inc., San Diego CA).

3 | RESULTS

3.1 | Reciprocal expression pattern of sONE/eNOS axis in TNBC patients

sONE was found to be restrictedly expressed in TNBC patients and cell lines. However, it was found to be undetectable in Luminal A/B and Her-2-enriched BC patients and in hormone receptor (HR+) cell lines. sONE is significantly downregulated in TNBC tumor tissues compared with its normal counterparts ($p < 0.0001$; Figure 1a). A reciprocal expression pattern is reported for the first time in TNBC patients between sONE and its antisense transcript eNOS ($p = 0.0002$; Figure 1a). Using Spearman's correlation analysis, a significant inverse correlation between sONE and eNOS in 25 TNBC patients was observed with correlation coefficient of Spearman's $r = 0.8642$ ($p < 0.0001$; Figure 1b). It is also worth mentioning that sONE level is inversely correlated with the aggressiveness of the disease as manifested in TNBC patients with larger tumor size ($p < 0.0001$) and lymphnode metastasis ($p = 0.0016$; Figure 1c). Furthermore,sONE is also inversely correlated with the age of TNBC patients as sONE was found to be almost diminished in tumor tissues obtained from young (<40 years old) aggressive TNBC patients when compared with older TNBC patients (≥40 years old; $p < 0.0001$; Figure 1d), thus highlighting its pivotal impact in determining the aggressiveness of the disease.

3.2 | sONE modulates eNOS-induced NO production in MDA-MB-231 cell line

Efficient knocking down of sONE in MDA-MB-231 was confirmed ($p = 0.0023$; Figure 2a). In concordance to sONE repression, a dramatic elevation of eNOS transcript ($p = 0.0002$; Figure 2b) and protein levels ($p < 0.0001$; Figure 2d) were observed. Additionally, a significant increase in NO production correlated to eNOS-induced NO production (nM range) was witnessed compared with mock and scr-siRNAs transfected wells ($p < 0.0001$; Figure 2c).

3.3 | Impact of sONE siRNAs on MDA-MB-231 oncological profile

Inspired by the novelty of sONE, being a validated upstream posttranscriptional regulator of eNOS-induced NO production in TNBC cells and its differential expression pattern among differently aggressive TNBC patients, functional analysis of sONE was performed. Knocking down of sONE in MDA-MB-231 cells led to a significant increase in cellular viability ($p = 0.0018$), proliferation ($p < 0.0001$), migration ($p = 0.0022$), invasion ($p = 0.0498$), and clonogenicity ($p = 0.0038$) compared with mock cells and Scr-siRNAs transfected cells (Figure 3) thus highlighting its tumor suppressor activity in TNBC cells.

3.4 | Impact of sONE siRNAs on vital cell cycle proteins in MDA-MB-231 cell line

Thus after validating sONE as a tumor suppressor lncRNA in TNBC cells, the aim of this study was extended to unveil the mechanism by which sONE siRNAs promotes TNBC progression, vital cell cycle proteins were screened such as the tumor suppressor TP53, the oncogenic protein c-Myc, the RB/E2F complex proteins. The results showed that sONE siRNAs led to a significant elevation in TP53 protein levels ($p = 0.0023$) and a marked repression in c-Myc activity. However, it had no effect on RB/E2F complex activity (Figure 4a–d).

3.5 | Impact of sONE siRNAs on miRNAs downstream TP53 and c-Myc transcription factors

Next it was interesting to investigate the impact sONE siRNAs on miRNAs downstream TP53 and c-MYC transcription factors especially

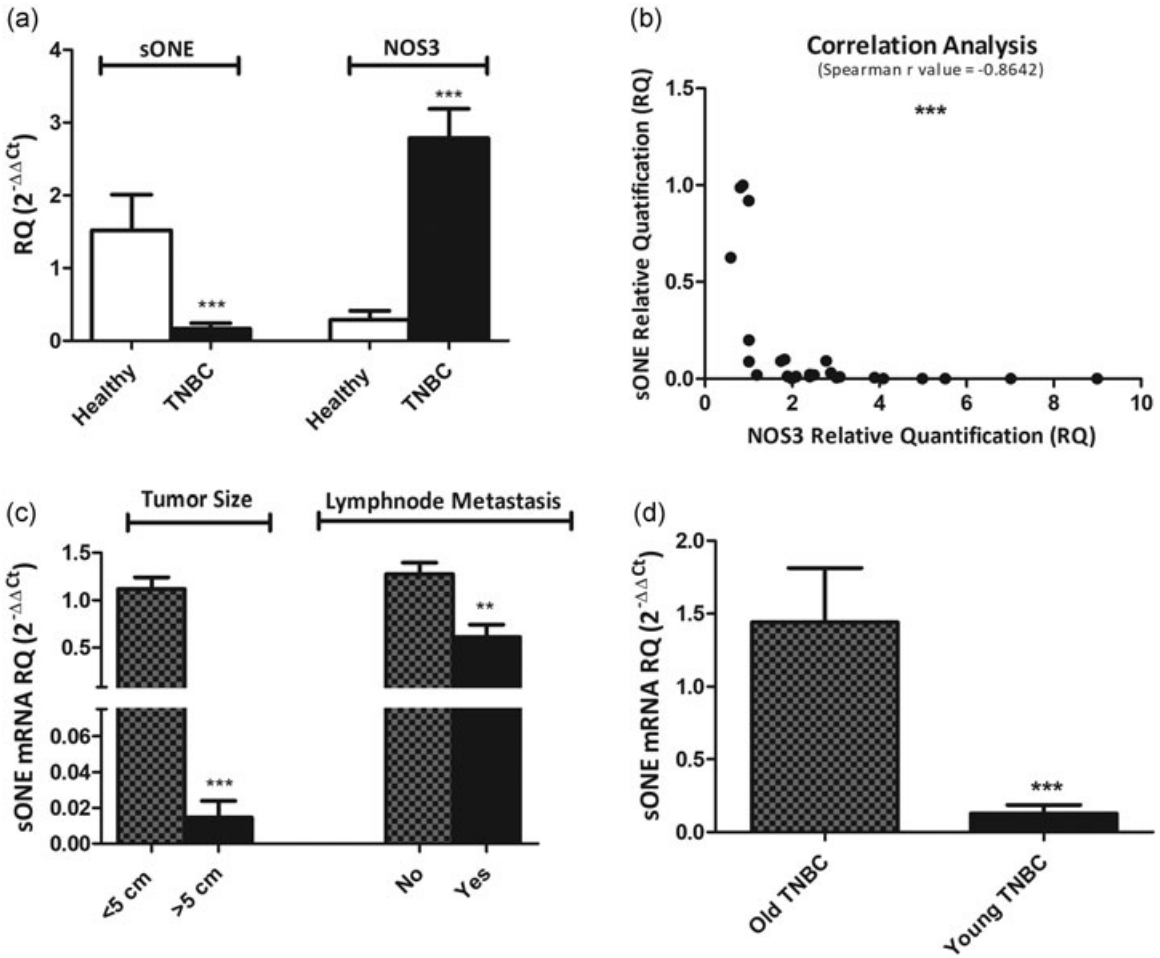


FIGURE 1 Screening of sONE/eNOS signaling axis in TNBC patients. sONE expression level was quantified in 25 TNBC tissues and its normal counterparts using qRT-PCR and normalized to 18s rRNA as an internal control. (a) sONE was found to be significantly downregulated in TNBC tissues. On the contrary, eNOS was found to be reciprocally upregulated in the same group of patients. (b) A significant inverse correlation was found between sONE and eNOS with correlation coefficient of Spearman's $r = -0.8642$. (c) sONE levels is markedly repressed in TNBC patients with tumor size (>5 cm) and lymphnode metastasis. (d) sONE is significantly downregulated in young TNBC patients (<40 years old) compared with older TNBC patients (≥40 years old). The Student *t* test was performed. Data are presented as mean ± *SEM* of three independent experiments. $^{***}p < 0.001$, $^{**}p < 0.01$ compared with control group. eNOS: endothelial nitric oxide synthase; qRT-PCR: quantitative real-time polymerase chain reaction; rRNA: ribosomal RNA; *SEM*: standard error of the mean; TNBC: triple-negative breast cancer

that a potential crosstalk between lncRNAs and miRNAs has been recently ruling in TNBC (Yuan et al., 2017). The results showed a significant repression of tumor suppressor miRNAs, miR-34a ($p = 0.0011$), miR-15a ($p = 0.0012$), miR-16 ($p = 0.0003$), and let-7a ($p = 0.0095$) which are known to act as validated downstream miRNAs to TP53 and c-Myc confirmed by our research group as well as other investigators (Fabbri et al., 2011; Kawano, Tanaka, Itonaga, Iwasaki, & Tsumura, 2015; Okada et al., 2014; R. A. Youness, Assal, Ezzat, et al., 2018). Finally, to validate the functional dependence of the sONE siRNAs on the aforementioned tumor suppressor miRNAs, several cotransfection experiments were performed as described in Figure 5. A partial abrogation of the sONE siRNAs tumor-promoting effects were observed upon cotransfecting sONE siRNAs with either miR-34a or miR-15 or miR-16 or let-7a mimics separately. However, upon cotransfection of all the tumor suppressor miRNAs with sONE siRNAs a total abrogation of the tumor-promoting results were obtained. This was confirmed on the level of cellular viability and colony-forming assay (Figure 5a,b).

4 | DISCUSSION

BC represents a highly prevalent heterogeneous malignancy comprising several subtypes. It is widely accepted that TNBC is one of the most hostile subtypes, often associated with poor patient outcomes (Neophytou, Boutsikos, & Papageorgis, 2018). The lack of effective targeted therapies for TNBC patients as well as reliable diagnostic and prognostic markers has fostered significant research efforts during the past few years to identify molecular "drivers" of this lethal subtype (Neophytou et al., 2018). LncRNAs have been recently casted as potential regulators of the malignant transformation process (Fan, Ma, & Liu, 2018; Hou et al., 2018; Wang et al., 2018). However, the exact mechanism by which

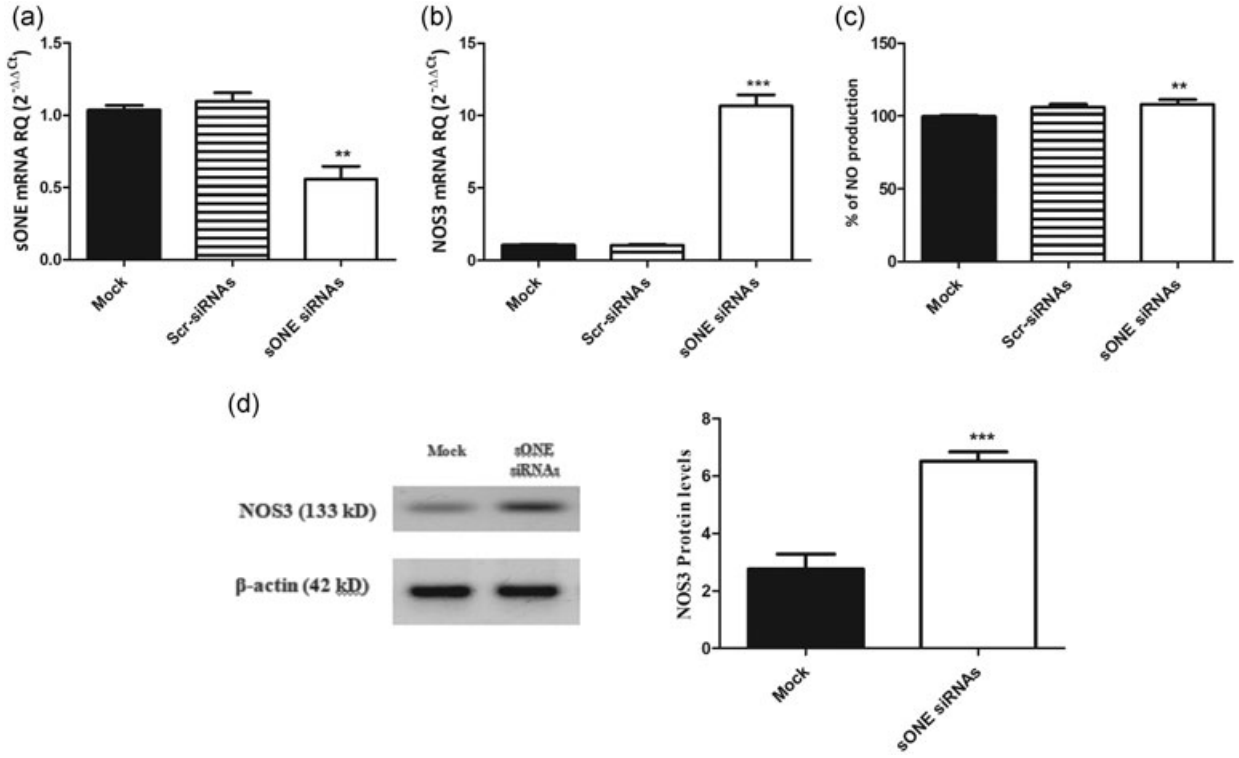


FIGURE 2 sONE/eNOS/NO signaling cascade in MDA-MB-231 cells. (a) Efficient silencing of sONE transcript levels was assessed. sONE siRNAs resulted in a marked induction of eNOS transcript levels (b) and protein levels (d). (c) NO production measured by Greiss reagent assay. The Student *t* test was performed. Data are presented as mean ± *SEM* of three independent experiments; ***$p < 0.001$, **$p < 0.01$ compared with Mock cells. eNOS: endothelial nitric oxide synthase; NO: nitric oxide; *SEM*: standard error of the mean; siRNA: small interfering RNA

lncRNAs affect TNBC progression is largely unexplored. This study was mainly focused on deciphering the mechanistic role of a novel lncRNA known as sONE in BC. Up to our knowledge, sONE has never been investigated in the field of oncology and in BC in particular. Thus, it was essential first to evaluate its expression pattern in correlation with its downstream target eNOS in BC patients and cell lines. Surprisingly, sONE was found to be restrictedly expressed in TNBC tissues and cell lines where it was found to be significantly downregulated with an inverse correlation with its aberrantly upregulated target (eNOS). Thus validating a reciprocal expression pattern between sONE and eNOS in TNBC tissues and MDA-MB-231 cell lines similar to endothelial and HUVEC cells (Fish et al., 2007; Robb et al., 2004).

Nonetheless, sONE expression level was found to be negatively correlated with the aggressiveness of the disease in TNBC patients where sONE transcript level was found to diminished in TNBC patients with tumor size (>5 cm) and TNBC patients with lymphnode metastasis. In a similar pattern to GAS5 (Mourtada-Maarabouni, Pickard, Hedge, Farzaneh, & Williams, 2009), EPB41L4A-AS2 (Xu et al., 2016), and NBAT-1 (Hu et al., 2015) that were reported to act as tumor suppressor lncRNAs in TNBC patients and their expression patterns were highly correlated with poor prognosis of the disease. This sheds light onto a high prognostic and diagnostic values of sONE among TNBC patients yet this merits more detailed studies.

As previously mentioned, among all the BC subtypes TNBC is associated with the worst prognosis (Ovcaricek, Frkovic, Matos, Mozina, & Borstnar, 2011). Yet, among the TNBC patients, young women represent the major challenge and the worst prognosis if compared with older TNBC patients (Bauer, Brown, Cress, Parise, & Caggiano, 2007). Moreover, recent evidence suggests that young age at diagnosis of TNBC represent an independent demolishing prognostic factor of survival (Han et al., 2004). For that reason, TNBC patients in this study were sorted according to their age into young TNBC patients (<40 years) and old TNBC patient (≥40 years). Interestingly, our results showed a strikingly marked repression of sONE lncRNA in young TNBC patients (<40 years) when compared with older patients. Up to our knowledge, sONE was the first reported lncRNA that was found to be differentially expressed among different age groups of TNBC patients (R. Youness, Assal, Hafez, Abdel Motaal, & Gad, 2018) until recently the tumor suppressor GAS5 was also found to be significantly repressed in TNBC patients <45 years together with SRA, and NEAT1 lncRNAs (Arshi et al., 2018).

On the functional level, knocking down of sONE resulted in a marked elevation of different hallmarks of TNBC such as cellular viability, proliferation, colony-forming ability, migration and invasion which might rank sONE lncRNA as a novel tumor suppressor lncRNA in TNBC *in vitro*. Therefore, this study highly proposes sONE to be added to the recently growing list of tumor suppressor lncRNAs in BC together with GAS5 (Mourtada-Maarabouni et al., 2009) and EPB41L4A-AS2 (Xu et al., 2016) lncRNAs.

Mechanistically, knocking down of the sONE lncRNA in the TNBC cell line, MDA-MB-231, showed a significant repression of

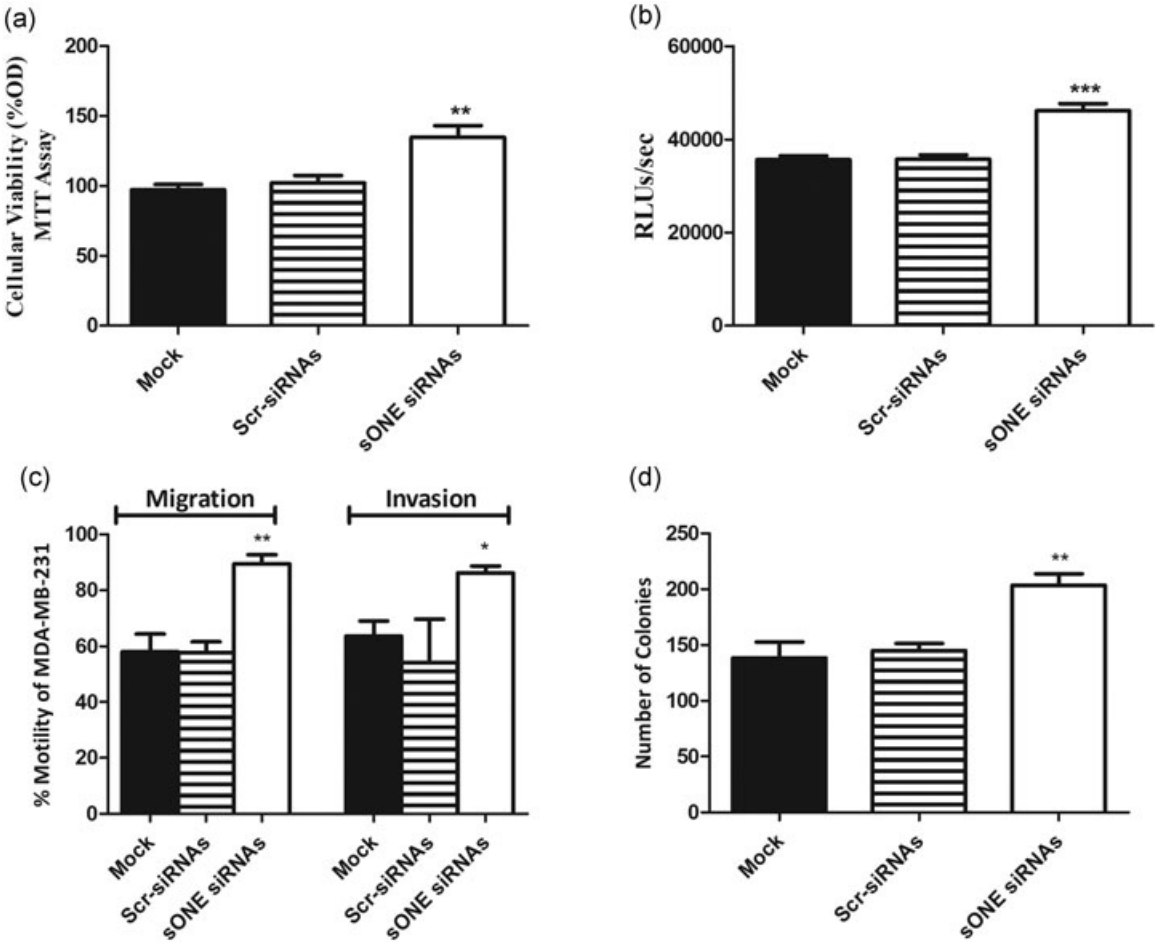


FIGURE 3 Impact of sONE siRNAs on MDA-MB-231 TNBC hallmarks. (a) Cellular viability was assessed using MTT assay, sONE siRNAs resulted in a significant increase in cellular viability compared with mock and Scr-siRNAs transfected cells. (b) Cellular proliferation rate was assessed using BrdU incorporation assay. sONE siRNAs resulted in a marked increase in MDA-MB-231 cellular proliferation rate compared with mock and Scr-siRNAs transfected cells. (c) Cellular migration was assessed using wound healing assay while invasion assay was performed using Boyden-chamber assay, sONE siRNAs resulted in a marked increase in migration and invasion capacities of MDA-MB-231 compared with mock and Scr-siRNAs cells. (d) sONE siRNAs resulted in a marked increase in clonogenicity of MDA-MB-231 cells assessed using colony-forming assay. One-way analysis of variance was performed. Data are presented as mean ± *SEM* of three independent experiments; $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$. MTT: 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; *SEM*: standard error of the mean; siRNA: small interfering RNA; TNBC: triple-negative breast cancer

eNOS-induced NO production and thus further validating sONE as an upstream regulator of eNOS transcript in TNBC cells as previously reported by our lab and others in case of cultured and primary endothelial cells (Fish et al., 2007; Robb et al., 2004; R. A. Youness, Assal, Abdel Motaal, et al., 2018).

Recently, our research group has highlighted a direct correlation between gasotransmitters (such as NO and H_2S) and the transcriptional activity of vital regulatory proteins affecting cell cycle progression in TNBC cells (R. A. Youness, Assal, Abdel Motaal, et al., 2018). Thus, digging deeper to understand the mechanism by which sONE might act as a tumor suppressor lncRNA in TNBC cells, the impact of sONE siRNAs on vital cell cycle regulatory proteins was assessed. The results showed that upon knocking down of the sONE lncRNA, a marked repression of eNOS-induced NO production was observed resulted in a repression of TP53 and elevation of c-Myc protein levels. However, it had no effect on RB/E2F complex activity. These results go in line with previous reports highlighting TP53 (Mizuno et al., 2004) and c-Myc (Zhan et al., 2018) as functional regulatory proteins downstream NO in different cellular contexts. Moreover, this also goes in line with a recent study reported by our group highlighting indirect alterations to the eNOS-induced NO production using NaHS resulted in similar effects on the transcriptional activity of TP53 and c-Myc, yet it showed no activity on the RB/E2F complex (R. A. Youness, Assal, Abdel Motaal, et al., 2018).

Finally, to draw the full picture regarding the mechanism by which sONE acts in TNBC cells, literature was screened to unravel a recent intrinsic miRNA-related activity downstream TP53 and c-Myc. It was evident in literature that TP53 and c-Myc transcription factors act as pivotal regulators for the expression of several miRNAs with growth suppression properties such as miR-34a, miR-15a, miR-16, and let-7a (Fabbri et al., 2011; Kawano et al., 2015; Okada et al., 2014; R. A. Youness, Assal, Ezzat, et al., 2018; R. A. Youness, Assal, Gad, & Abdel

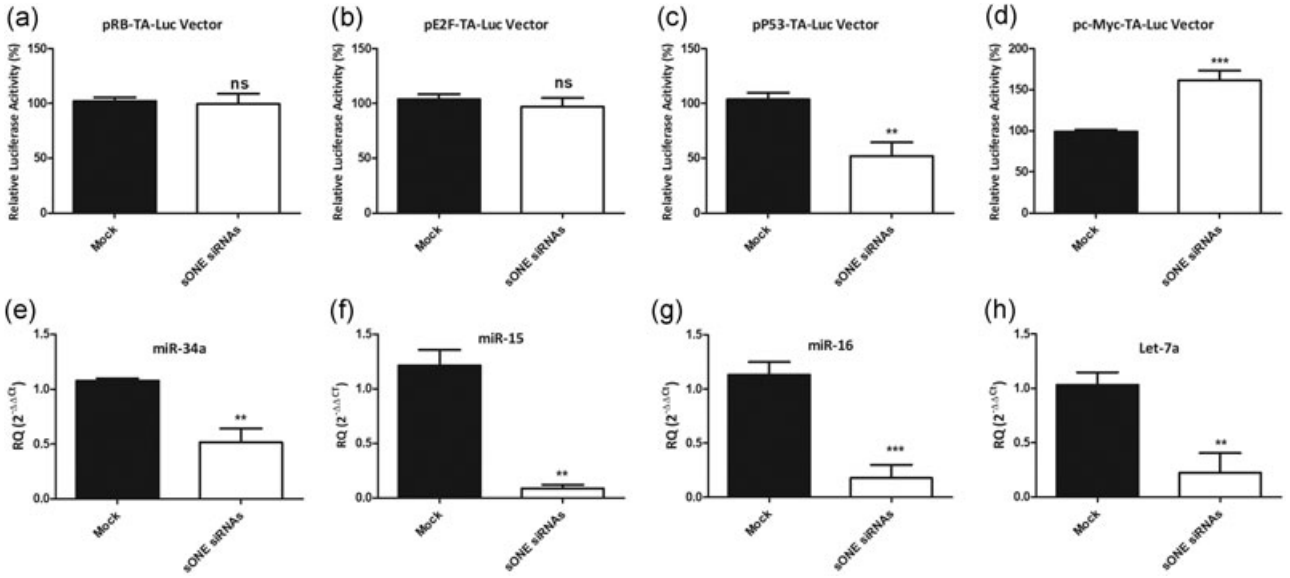
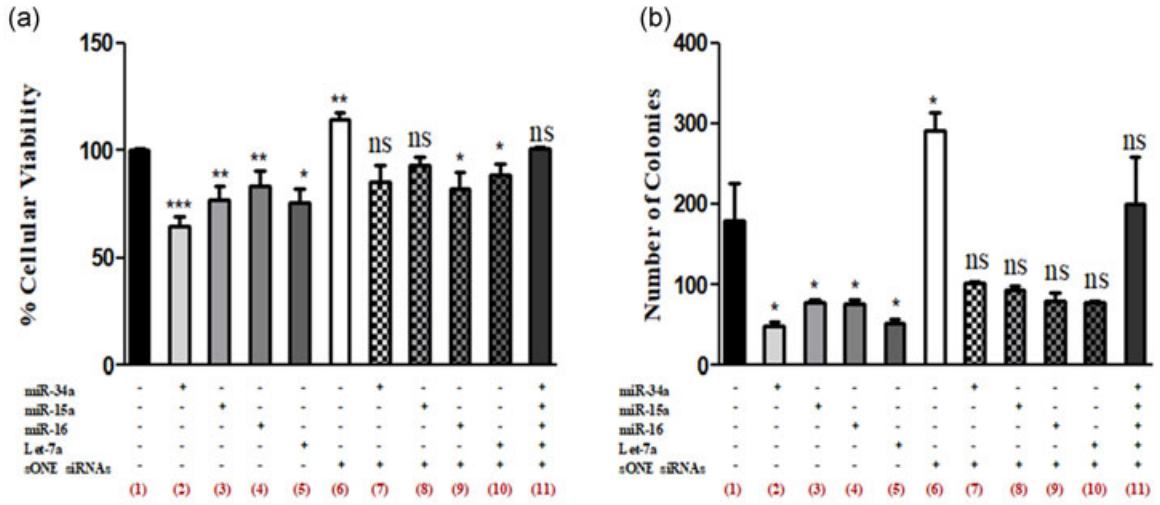


FIGURE 4 Impact of sONE siRNAs on vital cell cycle regulators and their downstream miRNAs. sONE siRNAs resulted in (a) no significant effect on RB luciferase activity and (b) E2F luciferase activity. However, it had a significant inducing effect on (c) TP53 activity compared with mock cells. (d) However, sONE siRNAs resulted in a significant repression of c-Myc activity. MDA-MB-231 cells transfected with sONE siRNAs resulted in a marked repression of (e) miR-34a, (f) miR-15a, (g) miR-16, and (h) let-7a expression levels. Data are presented as mean ± *SEM* of three independent experiments; ***$p < 0.001$, **$p < 0.01$ compared with mock cells. miRNA: microRNA; *SEM*: standard error of the mean; siRNA: small interfering RNA



(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
Mock cells	Transfected with miR-34a mimics	Transfected with miR-15a mimics	Transfected with miR-16a mimics	Transfected with let-7a mimics	Transfected with sONE siRNAs	Transfected with miR-34a mimics + sONE siRNAs	Transfected with miR-15a mimics + sONE siRNAs	Transfected with miR-16a mimics + sONE siRNAs	Transfected with let-7a mimics + sONE siRNAs	Transfected with miR (-15a,-16a,-34a and let-7a) mimics + sONE siRNAs

FIGURE 5 Impact of cotransfecting TP53/c-MYC downstream miRNAs with sONE siRNAs. Cellular viability and colony-forming ability of MDA-MB-231 cells were assessed posttransfection with sONE siRNAs, miR-34a mimics, miR-15a mimics, miR-16 mimics, and let-7a mimics and upon cotransfection of sONE siRNAs and the respective miRNAs mimics. (a) MTT assay and (b) colony-forming assay were performed, sONE siRNAs resulted in a significant increase in cellular viability and colony-forming ability of MDA-MB-231 while the tumor suppressor miRNAs (miR-34a, -15a, -16, and let-7a) mimics resulted in a marked repression of cellular viability and colony-forming ability. Upon cotransfection of sONE siRNAs with each tumor suppressor miRNA solely, a partial abrogation of the sONE siRNAs effects were shown. However, upon cotransfecting sONE siRNAs with all the altered tumor suppressor miRNAs collectively, a total abrogation of sONE siRNAs effects was shown. Data are presented as mean ± *SEM* of three independent experiments; ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ compared with mock cells. MTT: 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; *SEM*: standard error of the mean; siRNA: small interfering RNA [Color figure can be viewed at wileyonlinelibrary.com]

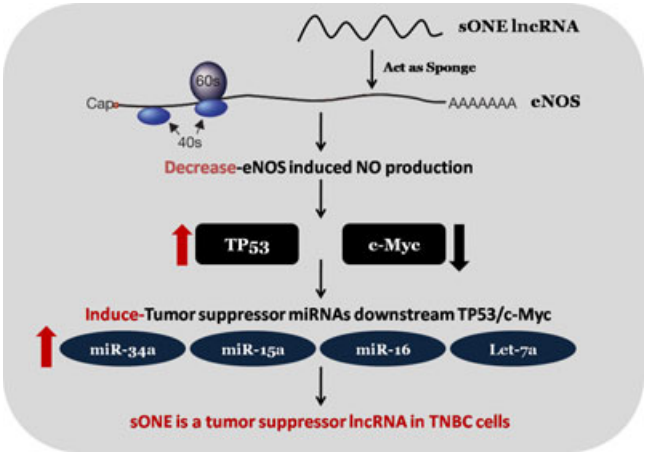


FIGURE 6 Mechanistic role of sONE in MDA-MB-231. sONE lncRNA acts as a direct posttranscriptional regulator to eNOS transcript leading to a marked decrease in eNOS-induced NO production in MDA-MB-231 TNBC cells. This results in a significant increase in TP53 and repression of c-Myc protein levels and thus affecting their downstream tumor suppressor miRNAs (miR-34a, miR-15a, miR-16, and let-7a). Therefore, this shows that sONE acts as a tumor suppressor lncRNA in TNBC cells partially through affecting a panel of growth suppressive miRNAs downstream TP53/c-Myc proteins. eNOS: endothelial nitric oxide synthase; lncRNA: long noncoding RNA; miRNA: microRNA; NO: nitric oxide; TNBC: triple-negative breast cancer [Color figure can be viewed at wileyonlinelibrary.com]

Motaal, 2018). Thus the impact of sONE siRNAs on tumor suppressor miRNAs downstream TP53 and c-Myc was evaluated. sONE siRNAs resulted in a significant repression of the tumor suppressor miRNAs (miR-34a,-15a,-16, and let-7a) and upon cotransfecting a respective miRNA mimic with sONE siRNAs a partial abrogation of the tumor-promoting effects induced by sONE siRNAs were observed while upon collectively cotransfecting miR-34a,-15a,-16, and let-7a mimics with sONE siRNAs a total abrogation of the sONE siRNAs results was shown. Thus highlighting a functional dependence activity of sONE siRNAs on tumor suppressor miRNAs downstream TP53 and c-Myc. This mechanism of interaction and functional dependence relationship between lncRNAs and miRNAs is highly supported by the recent crosstalk and interactive relationship reported between lncRNAs and miRNAs in different solid malignancies including TNBC (Chang, Hu, Zhou, & Zhang, 2018; Yuan et al., 2017; Y. Zhou, Meng, et al., 2018). However, this study represents the first in TNBC to highlight the involvement of gasotransmitters (NO), TP53 and c-Myc transcription factors to orchestrate the crosstalk between lncRNAs and miRNAs.

In conclusion, this study crystallizes sONE as a novel tumor suppressor lncRNA that is restrictedly expressed in TNBC patients and cell lines. sONE expression level was found to be inversely correlated with the aggressiveness of the disease and showed diminished expression in poorly prognosed young TNBC patients in particular. Mechanistically, sONE siRNAs were found to induce eNOS-induced NO production, thus affecting the protein expression of TP53 and c-Myc transcription factors and finally resulting in a marked alterations in the tumor suppressor miRNAs (miR-34a, -15, -16, and let-7a) drawn downstream TP53 and c-Myc transcription factors as shown in Figure 6.

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AUTHOR CONTRIBUTIONS

R. A. Y.: experimental design, running the practical experiments, interpretation of data, and writing the manuscript; H. M. H.: providing clinical samples, following ethical approvals, and aid in interpretation of clinical data; R. A. A.: aid in practical work and interpretation of data; A. A. M.: contribute in the study design, supervision of experimental work and revising the manuscript; M. Z. G.: contribute in the study design, supervision of experimental work and revising the manuscript.

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REFERENCES

Abdi, J., Rastgoo, N., Li, L., Chen, W., & Chang, H. (2017). Role of tumor suppressor p53 and micro-RNA interplay in multiple myeloma pathogenesis. *Journal of Hematology & Oncology*, *10*(1), 169. https://doi.org/10.1186/s13045-017-0538-4

Anastasiadi, Z., Lianos, G. D., Ignatiadou, E., Harissis, H. V., & Mitsis, M. (2017). Breast cancer in young women: An overview. *Updates in Surgery*, *69*(3), 313–317. https://doi.org/10.1007/s13304-017-0424-1

Arshi, A., Sharifi, F. S., Khorramian Ghahfarokhi, M., Faghih, Z., Doosti, A., Ostovari, S., … Ghahramani Seno, M. M. (2018). Expression analysis of MALAT1, GAS5, SRA, and NEAT1 lncRNAs in breast cancer tissues from young women and women over 45 years of age. *Molecular Therapy. Nucleic Acids*, *12*, 751–757. https://doi.org/10.1016/j.omtn.2018.07.014

Bader, A. G., Brown, D., Stoudemire, J., & Lammers, P. (2011). Developing therapeutic microRNAs for cancer. *Gene Therapy*, *18*(12), 1121–1126. https://doi.org/10.1038/gt.2011.79

Bauer, K. R., Brown, M., Cress, R. D., Parise, C. A., & Caggiano, V. (2007). Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: A population-based study from the California Cancer Registry. *Cancer*, *109*(9), 1721–1728. https://doi.org/10.1002/cncr.22618

Bianchini, G., Balko, J. M., Mayer, I. A., Sanders, M. E., & Gianni, L. (2016). Triple-negative breast cancer: Challenges and opportunities of a heterogeneous disease. *Nature Reviews Clinical Oncology*, *13*(11), 674–690. https://doi.org/10.1038/nrclinonc.2016.66

Chang, L., Hu, Z., Zhou, Z., & Zhang, H. (2018). Linc00518 contributes to multidrug resistance through regulating the MiR-199a/MRP1 axis in breast cancer. *Cellular Physiology and Biochemistry*, *48*(1), 16–28. https://doi.org/10.1159/000491659

Cranford, T. L., Velazquez, K. T., Enos, R. T., Bader, J. E., Carson, M. S., Chatzistamou, I., … Murphy, E. A. (2017). Loss of monocyte chemoattractant protein-1 expression delays mammary tumorigenesis and reduces localized inflammation in the C3(1)/SV40Tag triple

negative breast cancer model. *Cancer Biology & Therapy*, *18*(2), 85–93. https://doi.org/10.1080/15384047.2016.1276135

Dong, D., Mu, Z., Zhao, C., & Sun, M. (2018). ZFAS1: A novel tumor-related long non-coding RNA. *Cancer Cell International*, *18*, 125. https://doi.org/10.1186/s12935-018-0623-y

Fabbri, M., Bottoni, A., Shimizu, M., Spizzo, R., Nicoloso, M. S., Rossi, S., … Croce, C. M. (2011). Association of a microRNA/TP53 feedback circuitry with pathogenesis and outcome of B-cell chronic lymphocytic leukemia. *Journal of the American Medical Association*, *305*(1), 59–67. https://doi.org/10.1001/jama.2010.1919

Fan, C. N., Ma, L., & Liu, N. (2018). Systematic analysis of lncRNA-miRNA-mRNA competing endogenous RNA network identifies four-lncRNA signature as a prognostic biomarker for breast cancer. *Journal of Translational Medicine*, *16*(1), 264. https://doi.org/10.1186/s12967-018-1640-2

Fish, J. E., Matouk, C. C., Yeboah, E., Bevan, S. C., Khan, M., Patil, K., … Marsden, P. A. (2007). Hypoxia-inducible expression of a natural cis-antisense transcript inhibits endothelial nitric-oxide synthase. *Journal of Biological Chemistry*, *282*(21), 15652–15666. https://doi.org/10.1074/jbc.M608318200

Han, W., Kim, S. W., Park, I. A., Kang, D., Kim, S. W., Youn, Y. K., … Noh, D. Y. (2004). Young age: An independent risk factor for disease-free survival in women with operable breast cancer. *BMC Cancer*, *4*, 82. https://doi.org/10.1186/1471-2407-4-82

Ho, J. S., Ma, W., Mao, D. Y., & Benchimol, S. (2005). p53-dependent transcriptional repression of c-myc is required for G1 cell cycle arrest. *Molecular and Cellular Biology*, *25*(17), 7423–7431. https://doi.org/10.1128/MCB.25.17.7423-7431.2005

Hou, L., Tu, J., Cheng, F., Yang, H., Yu, F., Wang, M., … Zhou, G. (2018). Long noncoding RNA ROR promotes breast cancer by regulating the TGF-beta pathway. *Cancer Cell International*, *18*, 142. https://doi.org/10.1186/s12935-018-0638-4

Hu, P., Chu, J., Wu, Y., Sun, L., Lv, X., Zhu, Y., … Su, S. (2015). NBAT1 suppresses breast cancer metastasis by regulating DKK1 via PRC2. *Oncotarget*, *6*(32), 32410–32425. https://doi.org/10.18632/oncotarget.5609

Huarte, M. (2015). The emerging role of lncRNAs in cancer. *Nature Medicine*, *21*(11), 1253–1261. https://doi.org/10.1038/nm.3981

Issler, M. V. C., & Mombach, J. C. M. (2017). MicroRNA-16 feedback loop with p53 and Wip1 can regulate cell fate determination between apoptosis and senescence in DNA damage response. *PLOS One*, *12*(10), e0185794. https://doi.org/10.1371/journal.pone.0185794

Jackstadt, R., & Hermeking, H. (2015). MicroRNAs as regulators and mediators of c-MYC function. *Biochimica et Biophysica Acta/General Subjects*, *1849*(5), 544–553. https://doi.org/10.1016/j.bbagrm.2014.04.003

Jemal, A., Siegel, R., Ward, E., Murray, T., Xu, J., Smigal, C., & Thun, M. J. (2006). Cancer statistics, 2006. *CA: A Cancer Journal for Clinicians*, *56*(2), 106–130.

Kawano, M., Tanaka, K., Itonaga, I., Iwasaki, T., & Tsumura, H. (2015). c-Myc Represses Tumor-Suppressive microRNAs, let-7a, miR-16 and miR-29b, and Induces Cyclin D2-Mediated Cell Proliferation in Ewing's Sarcoma Cell Line. *PLOS One*, *10*(9), e0138560. https://doi.org/10.1371/journal.pone.0138560

Kumar, S., Williams, D., Sur, S., Wang, J. Y., & Jo, H. (2018). Role of flow-sensitive microRNAs and long noncoding RNAs in vascular dysfunction and atherosclerosis. *Vascular Pharmacology*, *114*, 76–92. https://doi.org/10.1016/j.vph.2018.10.001

Luu, C., Heinrich, E. L., Duldulao, M., Arrington, A. K., Fakih, M., Garcia-Aguilar, J., & Kim, J. (2013). TP53 and let-7a micro-RNA regulate K-Ras activity in HCT116 colorectal cancer cells. *PLOS One*, *8*(8), e70604. https://doi.org/10.1371/journal.pone.0070604

Lyu, S., Yu, Q., Ying, G., Wang, S., Wang, Y., Zhang, J., & Niu, Y. (2014). Androgen receptor decreases CMYC and KRAS expression by upregulating let-7a expression in ER-, PR-, AR+ breast cancer. *International Journal of Oncology*, *44*(1), 229–237. https://doi.org/10.3892/ijo.2013.2151

McGee, S. F., Mazzarello, S., Caudrelier, J. M., Lima, M. A. G., Hutton, B., Sienkiewicz, M., … Clemons, M. (2018). Optimal sequence of adjuvant endocrine and radiation therapy in early-stage breast cancer—A systematic review. *Cancer Treatment Reviews*, *69*, 132–142. https://doi.org/10.1016/j.ctrv.2018.06.015

Mizuno, S., Kadowaki, M., Demura, Y., Ameshima, S., Miyamori, I., & Ishizaki, T. (2004). p42/44 Mitogen-activated protein kinase regulated by p53 and nitric oxide in human pulmonary arterial smooth muscle cells. *American Journal of Respiratory Cell and Molecular Biology*, *31*(2), 184–192. https://doi.org/10.1165/rcmb.2003-0397OC

Mourtada-Maarabouni, M., Pickard, M. R., Hedge, V. L., Farzaneh, F., & Williams, G. T. (2009). GAS5, a non-protein-coding RNA, controls apoptosis and is downregulated in breast cancer. *Oncogene*, *28*(2), 195–208. https://doi.org/10.1038/onc.2008.373

Narod, S. A. (2012). Breast cancer in young women. *Nature Reviews Clinical Oncology*, *9*(8), 460–470. https://doi.org/10.1038/nrclinonc.2012.102

Neophytou, C., Boutsikos, P., & Papageorgis, P. (2018). Molecular mechanisms and emerging therapeutic targets of triple-negative breast cancer metastasis. *Frontiers in Oncology*, *8*, 31. https://doi.org/10.3389/fonc.2018.00031

Okada, N., Lin, C. P., Ribeiro, M. C., Biton, A., Lai, G., He, X., … He, L. (2014). A positive feedback between p53 and miR-34 miRNAs mediates tumor suppression. *Genes and Development*, *28*(5), 438–450. https://doi.org/10.1101/gad.233585.113

Ovcaricek, T., Frkovic, S. G., Matos, E., Mozina, B., & Borstnar, S. (2011). Triple negative breast cancer—Prognostic factors and survival. *Radiology and Oncology*, *45*(1), 46–52. https://doi.org/10.2478/v10019-010-0054-4

Phesse, T. J., Myant, K. B., Cole, A. M., Ridgway, R. A., Pearson, H., Muncan, V., … Sansom, O. J. (2014). Endogenous c-Myc is essential for p53-induced apoptosis in response to DNA damage in vivo. *Cell Death and Differentiation*, *21*(6), 956–966. https://doi.org/10.1038/cdd.2014.15

Qi, P., & Du, X. (2013). The long non-coding RNAs, a new cancer diagnostic and therapeutic gold mine. *Modern Pathology*, *26*(2), 155–165. https://doi.org/10.1038/modpathol.2012.160

Rampazzo, E., Bojnik, E., Trentin, L., Bonaldi, L., Del Bianco, P., Frezzato, F., … De Rossi, A. (2017). Role of miR-15a/miR-16-1 and the TP53 axis in regulating telomerase expression in chronic lymphocytic leukemia. *Haematologica*, *102*(7), e253–e256. https://doi.org/10.3324/haematol.2016.157669

Robb, G. B., Carson, A. R., Tai, S. C., Fish, J. E., Singh, S., Yamada, T., … Marsden, P. A. (2004). Post-transcriptional regulation of endothelial nitric-oxide synthase by an overlapping antisense mRNA transcript. *Journal of Biological Chemistry*, *279*(36), 37982–37996. https://doi.org/10.1074/jbc.M400271200

Shafran, Y., Zurgil, N., Ravid-Hermesh, O., Sobolev, M., Afrimzon, E., Hakuk, Y., … Deutsch, M. (2017). Nitric oxide is cytoprotective to breast cancer spheroids vulnerable to estrogen-induced apoptosis. *Oncotarget*, *8*(65), 108890–108911. https://doi.org/10.18632/oncotarget.21610

Trimmer, C., Sotgia, F., Whitaker-Menezes, D., Balliet, R. M., Eaton, G., Martinez-Outschoorn, U. E., … Lisanti, M. P. (2011). Caveolin-1 and mitochondrial SOD2 (MnSOD) function as tumor suppressors in the stromal microenvironment: A new genetically tractable model for human cancer associated fibroblasts. *Cancer Biology & Therapy*, *11*(4), 383–394.

Wang, G., Chen, X., Liang, Y., Wang, W., Fang, Y., & Shen, K. (2018). Long noncoding RNA signature and disease outcome in estrogen receptor-positive breast cancer patients treated with tamoxifen. *Journal of Breast Cancer*, *21*(3), 277–287. https://doi.org/10.4048/jbc.2018.21.e39

Xiao, X., Gu, Y., Wang, G., & Chen, S. (2018). c-Myc, RMRP, and miR-34a-5p form a positive-feedback loop to regulate cell proliferation and apoptosis in multiple myeloma. *International Journal of Biological Macromolecules*, *122*, 526–537. https://doi.org/10.1016/j.ijbiomac.2018.10.207

Xu, S., Wang, P., You, Z., Meng, H., Mu, G., Bai, X., … Pang, D. (2016). The long non-coding RNA EPB41L4A-AS2 inhibits tumor proliferation and is associated with favorable prognoses in breast cancer and other solid tumors. *Oncotarget*, *7*(15), 20704–20717. https://doi.org/10.18632/oncotarget.8007

Yarlagadda, K., Hassani, J., Foote, I. P., & Markowitz, J. (2017). The role of nitric oxide in melanoma. *Biochimica et Biophysica Acta, Reviews on Cancer*, *1868*(2), 500–509. https://doi.org/10.1016/j.bbcan.2017.09.005

Yin, Q., Feng, W., Shen, X., & Ju, S. (2018). Regulatory effects of lncRNAs and miRNAs on autophagy in malignant tumorigenesis. *Bioscience Reports*, *38*(5), BSR20180516. https://doi.org/10.1042/BSR20180516

Youness, R., Assal, R., Gad, M.Z., & Motaal, A. A. (2018). PO-352 Hijacking hepatocellular carcinoma (HCC) tumour progression through restoring TP53/miR-15a/miR-16 tumour suppressor axis by a novel quercetin glycoside. *ESMO Open*, *3*(Suppl 2), A160–A160. https://doi.org/10.1136/esmoopen-2018-EACR25.382

Youness, R., Assal, R., Hafez, M., Abdel Motaal, A., & Gad, M.Z. (2018). PO-347 sONE, a novel tumour suppressor lncRNA, with diminished expression level in young triple negative breast cancer (TNBC) patients with lymphnode metastasis and large tumour size. *ESMO Open*, *3*(Suppl 2), A364–A365. https://doi.org/10.1136/esmoopen-2018-EACR25.859

Youness, R. A., Assal, R. A., Abdel Motaal, A., & Gad, M. Z. (2018). A novel role of sONE/NOS3/NO signaling cascade in mediating hydrogen sulphide bilateral effects on triple negative breast cancer progression. *Nitric oxide*, *80*, 12–23. https://doi.org/10.1016/j.niox.2018.07.004

Youness, R. A., Assal, R. A., Ezzat, S. M., Gad, M. Z., & Abdel Motaal, A. (2018). A methoxylated quercetin glycoside harnesses HCC tumor progression in a TP53/miR-15/miR-16 dependent manner. *Natural Product Research*, *11*, 1–6. https://doi.org/10.1080/14786419.2018.1509326

Youness, R. A., El-Tayebi, H. M., Assal, R. A., Hosny, K., Esmat, G., & Abdelaziz, A. I. (2016). MicroRNA-486-5p enhances hepatocellular carcinoma tumor suppression through repression of IGF-1R and its downstream mTOR, STAT3 and c-Myc. *Oncology Letters*, *12*(4), 2567–2573. https://doi.org/10.3892/ol.2016.4914

Youness, R. A., Rahmoon, M. A., Assal, R. A., Gomaa, A. I., Hamza, M. T., Waked, I., … Abdelaziz, A. I. (2016). Contradicting interplay between insulin-like growth factor-1 and miR-486-5p in primary NK cells and hepatoma cell lines with a contemporary inhibitory impact on HCC tumor progression. *Growth Factors*, *34*(3-4), 128–140. https://doi.org/10.1080/08977194.2016.1200571

Yuan, N., Zhang, G., Bie, F., Ma, M., Ma, Y., Jiang, X., … Hao, X. (2017). Integrative analysis of lncRNAs and miRNAs with coding RNAs associated with ceRNA crosstalk network in triple negative breast cancer. *OncoTargets and Therapy*, *10*, 5883–5897. https://doi.org/10.2147/OTT.S149308

Zhan, R., Wang, F., Wu, Y., Wang, Y., Qian, W., Liu, M., … Luo, G. (2018). Nitric oxide promotes epidermal stem cell proliferation via FOXG1-c-Myc signalling. *Nitric oxide*, *73*, 1–8. https://doi.org/10.1016/j.niox.2017.12.002

Zhang, X., Yang, X., Lin, Y., Suo, M., Gong, L., Chen, J., & Hui, R. (2015). Anti-hypertensive effect of *Lycium barbarum* L. with down-regulated expression of renal endothelial lncRNA sONE in a rat model of salt-sensitive hypertension. *International Journal of Clinical and Experimental Pathology*, *8*(6), 6981–6987.

Zhou, S., He, Y., Yang, S., Hu, J., Zhang, Q., Chen, W., … Tang, J. (2018). The regulatory roles of lncRNAs in the process of breast cancer invasion and metastasis. *Bioscience Reports*, *38*(5), BSR20180772. https://doi.org/10.1042/BSR20180772

Zhou, Y., Meng, X., Chen, S., Li, W., Li, D., Singer, R., & Gu, W. (2018). IMP1 regulates UCA1-mediated cell invasion through facilitating UCA1 decay and decreasing the sponge effect of UCA1 for miR-122-5p. *Breast Cancer Research*, *20*(1), 32. https://doi.org/10.1186/s13058-018-0959-1

SUPPORTING INFORMATION

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

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