Original Article

Running Title: LncRNA TMPO AS-1 expression level in breast cancer tissues
Received: December 22, 2024; Accepted: February 27, 2025

Evaluation of the LncRNA TMPO AS-1 expression level in breast cancer tissues in comparison with paired normal marginal tissues

Saman Niknam[*], MD, Habib Zarredar[*], PhD, Tooraj Asvadi[*], MD, Milad Asadi[**], PhD, Venus Zafari[**], PhD, Raheleh Aligholipour Maleki[*], MD, Dariush Shanehbandi[***], PhD, Shahryar Hashemzadeh[*♦], MD

[*]*Tuberculosis and Lung Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.*
[**]*Department of basic oncology of health institute of Ege University, Izmir, Turkey*
[***]*Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

♦Corresponding Author
Shahryar Hashemzadeh , MD
Tuberculosis and Lung Disease Research Center,
Tabriz University of Medical Sciences,
Tabriz, Iran.
Email Address: sh.hashemzadeh@gmail.com

Abstract

Background: Breast malignancy is a major public health problem and one of prevalent cancers, which leads to death in women all around the world. Long non-coding RNAs (lncRNAs) are widely studied in the pathogenesis of malignancies. Previous studies have confirmed the correlation of lncRNAs and the cellular development and growth of breast malignancy. The present study aimed to evaluate the expression level of long non-coding RNATMPO antisense RNA 1 (LncRNA TMPO AS-1) in breast cancer tissues and compare it with paired normal marginal tissues.

Methods: Fifty breast cancer patients were included in this case-control study, and tumor tissues and healthy marginal tissues were collected during surgery. Total RNAs were extracted from the normal and cancerous tissues, and after quality control cDNA was synthesized. The TMPO AS-1 expression level was determined by Quantitative polymerase chain reaction (qPCR). The gene expression level and their

relationships with the patient's clinicopathological characteristics were analyzed using appropriate statistical tests.

Results: TMPO Antisense RNA 1(TMPO-AS1) is significantly high expressed in breast cancer tissues compared with normal peripheral tissues (*P*-value <0.0001, fold change: 1.16). Among the 40 patients exposed to chemotherapy drugs 5-Fluorouracil (5FU) and doxorubicin, the TMPO-AS1 expression level was significantly decreased as compared with the 10 patients unexposed to chemotherapy (*P*<0.0001, fold change: 0.11). Lymph node metastatic patients (29 positive patients, 21 negative patients) showed high TMPO-AS1 expression level than negative lymph nodes metastasis patients (*P* = 0.0012, fold change = 1.161).

Conclusion: We demonstrated that TMPO-AS1 is highly expressed and its up-regulation correlates with the pathogenesis and tumorigenesis of breast cancer.

Keywords: Breast cancer, TMPO AS-1, Biomarkers, Prognoses

Introduction

Breast malignancy is a prevalent cancer that leads to death in women all around the world.[1] In 2018, the International Agency of Cancer Research reported that about 2,088,849 (11.6% of all malignancy) new cases of breast cancer were detected and 626,679 (6.6% of all cancers) patients died through this year.[2] Breast tumor, as the most diagnosed malignancy in women, is classified into heterogeneous groups of tumors and consists of specific subgroups related to different clinical manifestations.[3, 4] About 70%–80% of breast tumors will finally be a member of two main histopathological classes, not otherwise specified and invasive ductal carcinomas.[5] Breast cancer subtypes were classified based on immunohistochemical analysis of estrogen receptor progesterone receptor, and human epidermal growth factor receptor 2 (HER2) status. Recently, lncRNAs containing 200 or more nucleotides with no or limited protein-coding potential, have been identified as a main factor in controlling cellular activities and biological functions. Previous studies also recommend that lncRNAs are involved in various malignancies like breast malignancy.[6, 7] Thymopoietin antisense transcript 1, an antisense lncRNA, is encoded differently from the TMPO gene and located on the 12th chromosome. TMPO AS-1 protein is placed in the cell's nucleus and involved in the formation of the nuclear lamina and maintenance of the structure of the nuclear membrane.[8] Thus, the TMPO gene plays an essential role in the cellular processes and inhibits the depolymerization of nuclear laminas over the induction of the mitosis axis. TMPO-AS1 gene is the antisense of the TMPO gene, and may be important in cellular processes, which leads to

depolymerization of the nuclear laminas and mitosis pathway induction.[9] The construction between TMPO-AS1 and nuclear lamina maintenance has been extended to highlight its potential role in nuclear structural integrity, chromatin organization, and gene regulation in breast malignancy. New research recommends that dysregulation of TMPO-AS1 may lead to abnormal nuclear architecture and chromatin remodeling, thereby inducing carcinogenesis. Definitely, the abnormal expression of TMPO-AS1 has been linked to changed nuclear envelope integrity, increased nuclear deformability, and promoted cancer cell metastases and invasion.[10, 11] Previous research have revealed that lncRNA TMPO-AS1 has an oncogenic role in non-small cell lung cancer, osteosarcoma, cervical, and colorectal malignancies.[12] Also, TMPO-AS1 has an oncogenic function and is involved in the growth and proliferation of the breast tumor.[13] Previous studies indicated that the TMPO-AS1 gene induced migration, cell cycle progression, development, proliferation, and decreased apoptosis in prostate cancer.[14] Also, previous research has shown that TMPO- AS1 significantly correlated with cell growth and development factors, including proliferating cell nuclear antigen (PCNA) and MKi- 67.[15] In the present study, we evaluated the TMPO-AS1 mRNA expression level in breast cancer, and its potential as a prognostic and diagnostic biomarker.

Methods

Bioinformatics analysis

We used the Cancer Genome Atlas (TCGA) datasets for bioinformatics evaluation of the TMPO-AS1 expression level in breast tumors. After that, we used the Xena Functional Genomics Explorer (https://xena.ucsc.edu/) expression data to retrieve TMPO-AS1 from TCGA breast Cancer datasets, and then analyzed the data.

Research Population and Method for Clinical Sampling

We have registered all clinical trials reported in manuscripts in Academic Committee on Research Ethics (Human Studies Subjects) in university of medical sciences (trial Registration Number: IR.TBZMED.REC.1399.932). This case-control study was conducted between 2019 and 2021 by collecting tumor and margin healthy tissues from 50 breast cancer patients during surgery. The patients were recognized by surgical oncologists and confirmed by a pathologist at Imam Reza Hospital. Female patients diagnosed with breast malignancy, validated by pathology specialist and preparing of paired tumor and healthy marginal tissue from the same patient and comprehensive clinical and treatment history records were included in this study. Patients with previous cancer or concurrent malignancy, patients who exposed chemotherapy drug (pre-surgical chemotherapy) before sample collection, cases with low-quality RNA that could not be extracted from tissue samples were excluded from the study. The patients who received

chemotherapy drug had completed chemotherapy before surgery and sample collection. The clinicopathological characteristics of the patients are shown in Table 1. Tumor and paired healthy marginal tissues were collected from patients during surgical resection before any systemic treatment was initiated, except for patients in the chemotherapy-exposed group, who had completed chemotherapy prior to surgery. For patients who received chemotherapy drugs, tissue samples were gained after the completion of chemotherapy. In contrast, for patients unexposed to chemotherapy-, samples were collected prior to any systemic therapy, ensuring that the TMPO-AS1 expression was measured in tumors without being exposed to chemotherapy drugs. This distinction enables the comparison of TMPO-AS1 expression in both treated and untreated conditions, providing insight into its potential role in chemotherapy response. The chemotherapy regimens mainly included 5-FU, and doxorubicin. The number of chemotherapy cycles varied among patients, with an average of 4 to 6 cycles administered before surgery. After surgery, we put the tissue samples in the RNase inhibitor solution (Qiagen, Hilden, Germany) and transferred them to the laboratory. The sample size of 50 patients was evaluated based on initial data and statistical calculations. Using G Power software, we assessed that a minimum of 45–50 paired samples would be required to detect statistically significant differences.

Total RNA isolation, Reverse transcription and Quantitative Poly Chain Raction (qPCR)

Total RNA extraction from tissues was performed in accordance with the manufacturer's protocol using RiboEx reagent (Gene All biotechnology, Seoul, Korea). Next, the Nanodrop tool was used for checking the quantity of RNA (Thermo Fisher Scientific, USA). The samples were then kept at -80 ° C until the next step. For cDNA synthesis, the stem-loop method and 2x Real-time polymerase chain reaction (RT-PCR) (Taq) pre-mixture kit (BioFACT, Seoul) were used. Gene expression level was evaluated by SYBR Green Master Mix (Takara, Korea) Step-one and qPCR device (Applied Biosystems, USA). In addition, the housekeeping gene of this study was GAPDH. Formula 2 -ΔCT was used to calculate the relative LncRNA expression (Table 2).

Statistical analyses

Statistical analyses were evaluated using GraphPad Prism 6. A paired t-test was used for comparisons among malignant tissues and healthy marginal tissues from the same patient. For comparisons of independent group, like chemotherapy-exposed with unexposed patients and metastatic with non-metastatic cases, an unpaired t-test was applied. The normality of the data was evaluated via Kolmogorov-Smirnov test before performing t-tests to ensure appropriate application (P-value < 0.05, mean ± SD). Also, Benjamini-Hochberg FDR correction was used to

adjust p-values when performing multiple comparisons.

Results

TMPO-AS1 overexpressed in TCGA breast tumor samples

For the pre-estimation of TMPO-AS1 dysregulation in breast tumor, we firstly analyzed TCGA datasets for breast cancer. As shown in Figure 1, the TMPO-AS1 expression level was significantly increased in breast cancer samples compared with healthy breast samples (P<0.0001, fold change: 1.128). These results showed that the TMPO-AS1 could be associated with the pathogenesis of breast malignancy.

TMPO-AS1 expression status in clinical sample

To compare the obtained results from the TCGA database with the clinical sample, the lncRNA expression level of the TMPO-AS1 genes was evaluated in 50 breast tumor tissues samples compared with healthy margin tissues and then was statistically analyzed. Furthermore, qRT-PCR results and clinicopathological features of breast cancer cases, like age, tumor stage, and lymph node metastasis were analyzed. The data showed that TMPO-AS1 was significantly overexpressed in the tumor samples compared with the healthy margin tissues (P<0.0001, fold change: 1.16). Moreover, in breast cancer patients (10 patients negative and 40 patients positive), who were exposed to chemotherapy drugs (5-FU), significant lower expression level of TMPO-AS1 was observed as compared with patients unexposed to chemotherapy (P<0.0001, fold change: 0.11). Also, in patients with lymph nodes metastasis (29 patient positive, 21 patients negative) the TMPO-AS1 expression level was up-regulated as compared with negative lymph nodes metastasis patients (P = 0.0012, fold change = 1.161) (Figure 1).

Discussion

Our study shows that the TMPO-AS1 expression level is significantly high expressed in breast cancer tissues, and may play critical roles in breast tumorigenesis. Also, we found that the TMPO-AS1 high expression is correlated with cancer development and poor clinical result. We also analyzed the TCGA breast cancer dataset and showed that TMPO-AS1 expression level considerably high expressed in breast malignancy samples compared to breast healthy tissue samples which are similar to our results. We also found that in patients treated with chemotherapy drugs (5-FU and doxorubicin), the TMPO-AS1 expression level significantly reduced as compared with patients untreated with chemotherapy. Also, we showed that the expression level of TMPO-AS1 was highly expressed in lymph node metastatic patients than negative lymph nodes metastasis patients. According to the Roc curve analysis, we found that the expression level of TMPO-AS1in breast cancer samples could be served as a prognostic and diagnostic marker in breast malignancy

(Figure 2). Also, we carefully considered several clinicopathological variables that may affect TMPO-AS1 expression. These factors can affect gene expression profiles and potentially confuse the observed results. To control the influence of these factor, we performed subgroup analyses to evaluate the TMPO-AS1 expression across different tumor grades (low vs. high), and lymph node metastasis status (positive vs. negative) and patients exposed to chemotherapy drugs vs. patients unexposed to chemotherapy drugs. This method let us consider the potential relationship between TMPO-AS1 and these clinicopathological features. Change in lncRNAs expression levels has been widely proved to be associated with malignancies development and could be used as an effective marker for early detection in several human malignancies.[16] Previous studies indicated that the suppression of the TMPO-AS1 expression prevents the metastasis and growth of Triple-negative breast malignancy (TNBC). Mitobe et al. recommended that TMPO-AS1 have a central role in the pathophysiology of TNBC and might be used as a therapeutic target.[13] TMPO-AS1 that placed on the opposite strand of gene TMPO, is highly expressed in lung adenocarcinoma (LUAD) and related to the LUAD patients prognosis.[17] Peng et al., by analyzing microarray datasets of LUAD, indicated that TMPO-AS1 was up-regulated in LUAD cases and could be served as a prognostic factor.[18] In the prostate tumor, Huang et al. demonstrated that TMPO-AS1 could be used as a prognostic and diagnostic factor and its up-regulation induced cell progression, growth, and metastasis.[14] New reports showed that TMPO-AS1 has an oncogene function in various malignancies. For example, TMPO-AS1 induces growth, invasion, and metastasis in cervical malignancy cells via promoting Ras-Related Protein Rab-14 (RAB14) by miR-143 sponging.[19] In hepatocellular carcinoma, Guo et al. confirmed that TMPO-AS1 activate tumor cells' growth, invasion, and metastasis via miR-329-3p sponging by the promotion of the AKT-mammalian target of rapamycin (AKT-mTOR) axis.[20] Cui et al. indicated that TMPO-AS1 has a ceRNA function to induce osteosarcoma carcinogenesis via sponging the miR-199a-5p-Wnt Family Member axis. They reported that TMPO-AS1 is significantly up-regulated in osteosarcoma. Additionally, suppression of the TMPO-AS1 expression prevents the promotion of the Wnt/β-catenin axis and cell progression, and induces apoptosis.[21] Peng et al. indicated that TMPO-AS1 has an oncogenic role and could be used as a therapeutic target prognostic, and diagnostic marker in prostate cancer.[18] Ning et al. showed that silencing of TMPO-AS1 increases the docetaxel sensitivity via direct inhibition of miR-1179 in breast cancer (acts as a tumor suppressor).[22] Zhu et al.indicated that the expression of TMPO-AS1 is significantly up-regulated in breast tumors compared with adjacent healthy tissues. In vivo and in vitro studies have established that suppression of the TMPO-AS1 expression significantly reduces breast tumor cell survival, migration, and

invasion compared with the control group. Their outcomes recommended that TMPO-AS1 could be used as therapeutic target in breast malignancy.[23] Yu et al.indicated that TMPO-AS1 induces the progression, migration, and growth of non-small cell lung cancer through sponging miR-204-3p, which leads to ERBB2 up-regulating.[24, 25] Also, Luo et al.established that TMPO-AS1 induces the progression and metastasis of bladder tumor cells by sponging miR-98-5p.[26] Xing et al. indicated that suppression of the TMPO-AS1 expression prevents the proliferation, progression, and colony formation capabilities of nasopharyngeal carcinoma cells in vivo and in vitro.[27] Mitobe et al. indicated that TMPO- AS1 overexpression induces ER-positive breast tumor cell progression and growth.[13] Peng et al. demonstrated that TMPO-AS1 expression level is abnormally increased in retinoblastoma cells, which leads to promoting the progression, growth, and metastasis of retinoblastoma cells through sponging miR-199a-5p and HIF-1α high expression.[28] TMPO-AS1 as a potential biomarker has been extended to provide a more detailed comparison with other well-known breast cancer biomarkers as well as Ki-67 (a proliferation marker) [29], proliferating cell nuclear antigen (PCNA) (DNA replication and cell cycle regulation marker) [30], and HER2 (a key predictive marker for targeted therapy response).[31] Although Ki-67 and PCNA are broadly used to evaluate tumor proliferation, TMPO-AS1 may provide additional molecular insights into breast cancer development, mostly in its association with nuclear structural integrity and chromatin organization. Unlike HER2, which serves as a therapeutic target, the potential role of TMPO-AS1 seems to be more associated with cancer development and migration potential. The main limitations of the present study include the relatively small sample size, which may affect the generalizability of the results, absence of longitudinal follow-up data, which restricts the ability to assess the prognostic value of TMPO-AS1 over time and restrictions to evaluate other potential genes. Our study demonstrates that TMPO-AS1 is highly expressed in breast cancer and its up-regulation correlates to the pathogenesis and tumorigenesis of breast cancer.

Conclusion

The present study indicates that TMPO-AS1 is highly expressed in breast cancer, and its up-regulation is related to the pathogenesis and tumorigenesis of breast cancer.

Acknowledgements: Not applicable

Authors' Contribution

Sh.H: Study design, data analysis and interpretation, reviewing the manuscript; H.Z: Study design, writing the draft, reviewing the manuscript; S.N: Study design, data collection, processing and reviewing the manuscript; M.A: Data collection, writing the draft, data analysis and interpretation; T.A: Data collection and

processing; D.Sh: Writing the draft, data collection; V.Z: Data collection and writing the draft; R.A.M: Data collection, writing the draft. All authors have read and approved the final manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Funding: This work was funded as a research grant, by the Deputy of Research and Technology, Ministry of Health and Medical Education, Iran(grant number: 700/98,2015.03.14. Also, this study was supported by the Hematology and Oncology Research Center, Tabriz University of Medical Sciences (ID: 60156).

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Azamjah N, Soltan-Zadeh Y, Zayeri F. Global Trend of Breast Cancer Mortality Rate: A 25-Year Study. Asian Pac J Cancer Prev. 2019 20(7):2015-2020. doi: 10.31557/APJCP.2019.20.7.2015.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018 68(6):394-424. doi: 10.3322/caac.21492. Epub 2018 Sep 12.
3. Eliyatkın N, Yalçın E, Zengel B, Aktaş S, Vardar E. Molecular classification of breast carcinoma: from traditional, old-fashioned way to a new age, and a new way. The journal of breast health. 2015 11(2):59. doi: 10.5152/tjbh.2015.1669.
4. Shakhristova EV, Stepovaya EA, Ryazantseva NV, Nosareva OL, Yakushina VD, Ivanov VV, Novitsky VV. The Role of Protein Oxidative Modification and the Cellular Redox Status in Realization of Apoptosis of MCF-7 Breast Adenocarcmoma Cells. Izvestiia Akademii nauk Seriia biologicheskaia. 2016 (5):453-458.
5. Viale G. The current state of breast cancer classification. Annals of Oncology. 2012 23:x207-x210. doi: 10.1093/annonc/mds326.
6. Aftabi Y, Ansarin K, Shanehbandi D, Khalili M, Seyedrezazadeh E, Rahbarnia L, Asadi M, Amiri-Sadeghan A, Zafari V, Eyvazi S. Long non-coding RNAs as potential biomarkers in the prognosis and diagnosis of lung cancer: A review and target analysis. IUBMB life. 2021 73(2):307-327. doi: 10.1002/iub.2430. Epub 2020 Dec 24.
7. Wang Q, Shao X, Zhang Y, Zhu M, Wang FXC, Mu J, Li J, Yao H, Chen K. Role of tumor microenvironment in cancer progression and therapeutic strategy. Cancer Med. 2023 12(10):11149-11165. doi: 10.1002/cam4.5698.
8. Zhang L, Wang G, Chen S, Ding J, Ju S, Cao H, Tian H. Depletion of thymopoietin inhibits proliferation

and induces cell cycle arrest/apoptosis in glioblastoma cells. World journal of surgical oncology. 2016 14(1):1-8. doi: 10.1186/s12957-016-1018-y.
9. Brachner A, Foisner R. Lamina-associated polypeptide (LAP) 2α and other LEM proteins in cancer biology. Cancer Biology and the Nuclear Envelope. 2014:143-163. doi: 10.1007/978-1-4899-8032-8_7.
10. Peric-Hupkes D, van Steensel B. Role of the nuclear lamina in genome organization and gene expression. Cold Spring Harbor symposia on quantitative biology. 2010 75:517-524. doi: 10.1101/sqb.2010.75.014. Epub 2011 Jan 5.
11. Capo-chichi CD, Cai KQ, Smedberg J, Ganjei-Azar P, Godwin AK, Xu XX. Loss of A-type lamin expression compromises nuclear envelope integrity in breast cancer. Chinese journal of cancer. 2011 30(6):415-425. doi: 10.5732/cjc.010.10566.
12. Liu G, Yang H, Cao L, Han K, Li G. LncRNA TMPO-AS1 Promotes Proliferation and Invasion by Sponging miR-383-5p in Glioma Cells. Cancer Management and Research. 2020 12:12001. doi: 10.2147/CMAR.S282539. eCollection 2020.
13. Mitobe Y, Ikeda K, Sato W, Kodama Y, Naito M, Gotoh N, Miyata K, Kataoka K, Sasaki H, Horie-Inoue K. Proliferation-associated long noncoding RNA, TMPO-AS1, is a potential therapeutic target for triple-negative breast cancer. Cancer science. 2020 111(7):2440. doi: 10.1111/cas.14498. Epub 2020 Jun 13.
14. Huang W, Su X, Yan W, Kong Z, Wang D, Huang Y, Zhai Q, Zhang X, Wu H, Li Y. Overexpression of AR-regulated lncRNA TMPO-AS1 correlates with tumor progression and poor prognosis in prostate cancer. The Prostate. 2018 78(16):1248-1261. doi: 10.1002/pros.23700. Epub 2018 Aug 13.
15. Mitobe Y, Ikeda K, Suzuki T, Takagi K, Kawabata H, Horie-Inoue K, Inoue S. ESR1-stabilizing long noncoding RNA TMPO-AS1 promotes hormone-refractory breast cancer progression. Molecular and cellular biology. 2019 39(23). doi: 10.1128/MCB.00261-19.
16. Osielska MA, Jagodziński PP. Long non-coding RNA as potential biomarkers in non-small-cell lung cancer: What do we know so far? Biomedicine & Pharmacotherapy. 2018 101:322-333. doi: 10.1016/j.biopha.2018.02.099.
17. Li D, Ainiwaer J, Sheyhiding I, Zhang Z, Zhang L. Identification of key long non-coding RNAs as competing endogenous RNAs for miRNA-mRNA in lung adenocarcinoma. Eur Rev Med Pharmacol Sci. 2016 20(11):2285-2295.
18. Peng F, Wang R, Zhang Y, Zhao Z, Zhou W, Chang Z, Liang H, Zhao W, Qi L, Guo Z. Differential expression analysis at the individual level reveals a lncRNA prognostic signature for lung adenocarcinoma. Molecular cancer. 2017 16(1):1-12. doi: 10.1186/s12943-017-0666-z.
19. Gang X, Yuan M, Zhang J. Long non-coding RNA TMPO-AS1 promotes cervical cancer cell proliferation, migration, and invasion by regulating miR-143-3p/ZEB1 axis. Cancer management and

research. 2020 12:1587-1599. doi: 10.2147/CMAR.S226409.
20. Guo X, Wang Y. LncRNA TMPO-AS1 promotes hepatocellular carcinoma cell proliferation, migration and invasion through sponging miR-329-3p to stimulate FOXK1-mediated AKT/mTOR signaling pathway. Cancer Medicine. 2020 9(14):5235-5246. doi: 10.1002/cam4.3046.
21. Cui H, Zhao J. LncRNA TMPO-AS1 serves as a ceRNA to promote osteosarcoma tumorigenesis by regulating miR-199a-5p/WNT7B axis. Journal of cellular biochemistry. 2020 121(3):2284-2293. doi: 10.1002/jcb.29451.
22. Ning X, Zhao J, He F, Yuan Y, Li B, Ruan J. Long non‐coding RNA TMPO‐AS1 facilitates chemoresistance and invasion in breast cancer by modulating the miR‐1179/TRIM37 axis. Oncology Letters. 2021 22(1):1-9. doi: 10.3892/ol.2021.12761.
23. Zhu D, Lv W, Zhou X, He Y, Yao H, Yu Y, Zhang G, Zhang Q. Long non‐coding RNA TMPO‐AS1 promotes tumor progression via sponging miR‐140‐5p in breast cancer. Experimental and Therapeutic Medicine. 2021 21(1):1-1. doi: 10.3892/etm.2020.9449.
24. Yu X, Lin Q, Liu F, Yang F, Mao J, Chen X. LncRNA TMPO-AS1 facilitates the proliferation and metastasis of NSCLC cells by up-regulating ERBB2 via sponging miR-204-3p. International Journal of Immunopathology and Pharmacology. 2020 34:2058738420958947. doi: 10.1177/2058738420958947
25. Zarredar H, Ansarin K, Baradaran B, Ahdi Khosroshahi S, Farajnia S. Potential molecular targets in the treatment of lung cancer using siRNA technology. Cancer investigation. 2018 36(1):37-58. doi: 10.1080/07357907.2017.
26. Luo H, Yang L, Liu C, Wang X, Dong Q, Liu L, Wei Q. TMPO-AS1/miR-98-5p/EBF1 feedback loop contributes to the progression of bladder cancer. The international journal of biochemistry & cell biology. 2020 122:105702. doi: 10.1016/j.biocel.2020.105702.
27. Xing B, Qiao X-F, Qiu Y-H, Li X. TMPO-AS1 Regulates the Aggressiveness-Associated Traits of Nasopharyngeal Carcinoma Cells Through Sponging miR-320a. Cancer Management and Research. 2021 13:415. doi: 10.2147/CMAR.S285113.
28. Peng X, Yan J, Cheng F. LncRNA TMPO-AS1 up-regulates the expression of HIF-1α and promotes the malignant phenotypes of retinoblastoma cells via sponging miR-199a-5p. Pathology-Research and Practice. 2020 216(4):152853. doi: 10.1016/j.prp.2020.152853.
29. Davey MG, Hynes SO, Kerin MJ, Miller N, Lowery AJ. Ki-67 as a Prognostic Biomarker in Invasive Breast Cancer. Cancers (Basel). 2021 13(17). doi: 10.3390/cancers13174455.
30. Malkas LH, Herbert BS, Abdel-Aziz W, Dobrolecki LE, Liu Y, Agarwal B, Hoelz D, Badve S, Schnaper L, Arnold RJ *et al*. A cancer-associated PCNA expressed in breast cancer has implications as a potential biomarker. Proceedings of the National Academy of Sciences of the United States of America. 2006

103(51):19472-19477. doi: 10.1073/pnas.0604614103.
31. Ahn SK, Jung SY. Current Biomarkers for Precision Medicine in Breast Cancer. Advances in experimental medicine and biology. 2021 1187:363-379. doi: 10.1007/978-981-32-9620-6_18.

Table 1. Clinicopathological characteristics of study population with breast cancer

Number of Samples	50	
Mean age 56.7	21 sample<55	29 sample>55
Stage	7 stage 4 19 stage 2 24 stage 3	
Lymphnod metastasis	21 neg 29 positive	
Chemotherapy history	10 sample negative 40 sample pos	

Table 2.	Primer sequencing for target genes	Annealing temp (°C)
TMPO-AS1-	Forward: 5′-AGACGCCGATAAGGGACAG-3′ Reverse: 5′-AGCCAAGGGTCCTCACA-3′	58.8
GAPDH	Forward: 5′-CAAGATCATCAGCAATGCCTCC-3′ Reverse: 5′-GCCATCACGCCACAGTTTCC-3′	59

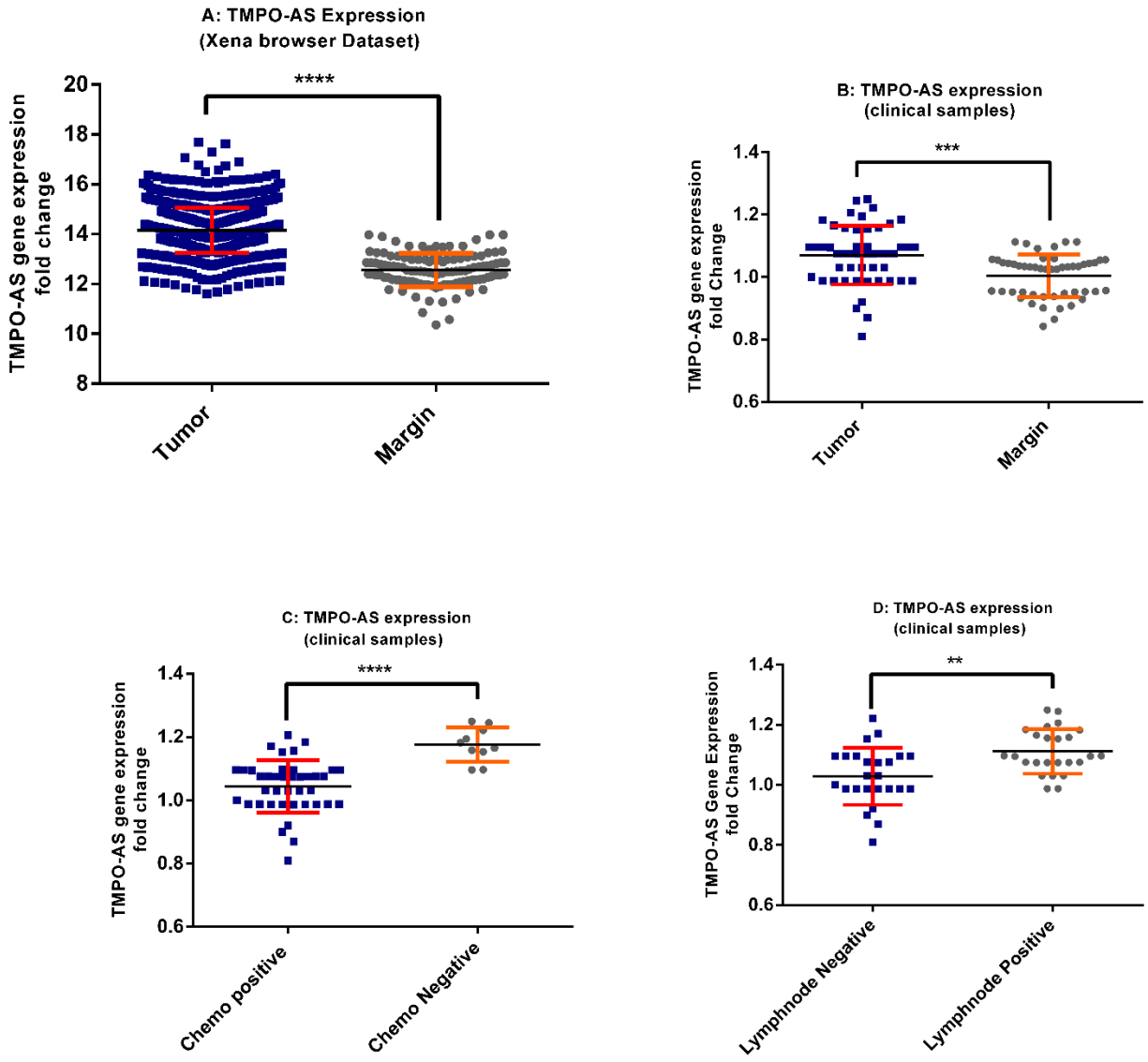

Figure 1. Expression levels of TMPO-AS1 across different breast cancer groups. This figure illustrates the differential expression of TMPO-AS1 in various breast cancer-related groups. (A) TMPO-AS1 expression levels derived from TCGA dataset using the Xena Functional Genomics Browser, comparing breast cancer samples to normal tissue samples. (B) Quantitative expression analysis of TMPO-AS1 in clinical samples, comparing breast cancer tissues with their paired normal marginal tissues. (C) Comparison of TMPO-AS1 expression levels between patients who received chemotherapy (5-FU) and those who did not undergo chemotherapy. (D) TMPO-AS1 expression levels in patients with lymph node metastasis compared with those without metastasis. Statistical significance is indicated by *P<0.1, **P<0.01, ***P<0.001, and ****P<0.00001. (5-fluorouracil; 5-fu, The Cancer Genome Atlas: TCGA).

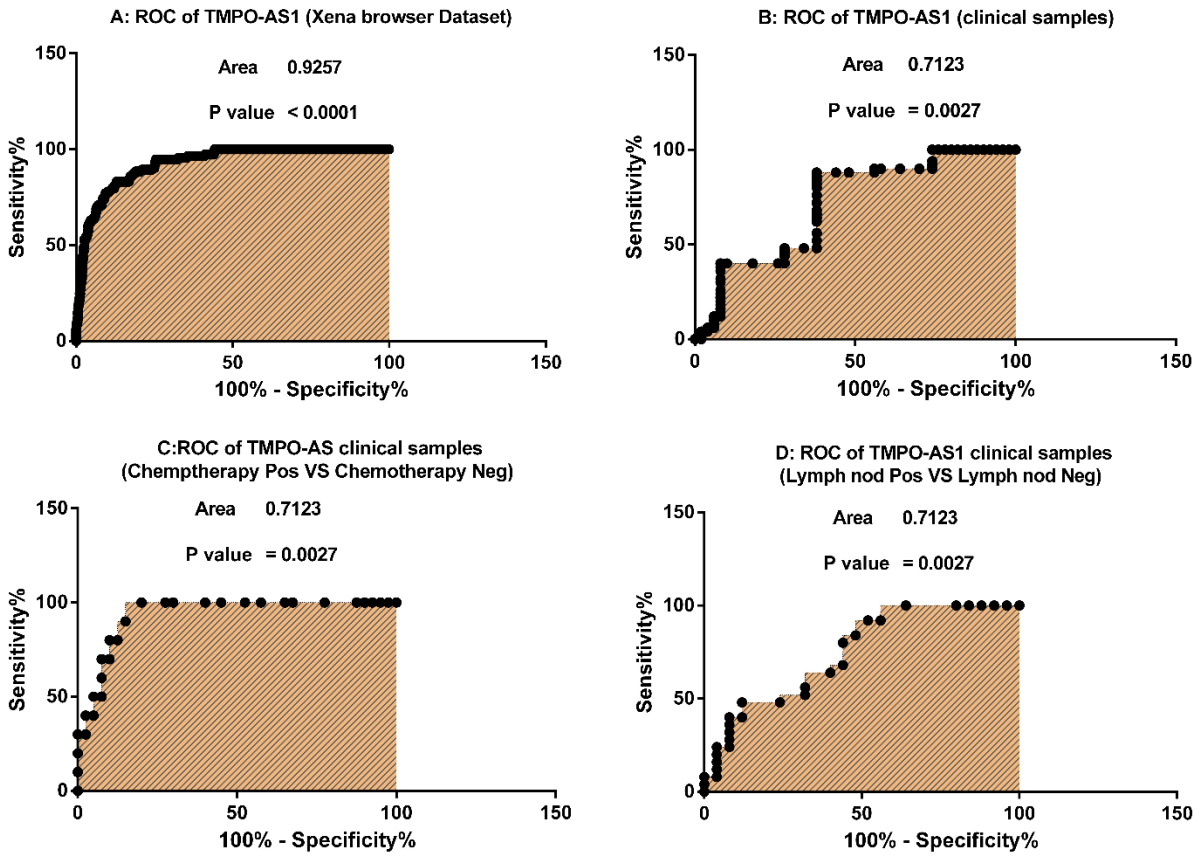

Figure 2. ROC curve analysis of TMPO-AS1 for diagnostic potential in breast cancer. This figure illustrates the ROC curve used to evaluate the diagnostic performance of TMPO-AS1 expression in distinguishing breast cancer tissues from normal marginal tissues. AUC value represents the diagnostic accuracy, with higher values indicating better discriminatory ability. The curve demonstrates the sensitivity and specificity of TMPO-AS1 expression as a potential biomarker for breast cancer detection. (Receiver Operating Characteristic: ROC, Area under the curve: AUC)