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Clinical and Bioinformatic Characterization of the Mitochondrial-Encoded Circular RNA hsa_circ_0002363 in Triple-Negative Breast Cancer

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Abstract
Background: Mitochondrial-encoded circular RNAs have recently emerged as novel regulators of mitochondrial homeostasis and cancer progression. However, the expression pattern and biological significance of hsa_circ_0002363 in triple-negative breast cancer (TNBC) remain unknown. The present study aimed to evaluate the expression and potential clinical and biological significance of the hsa_circ_0002363 in TNBC.
Method: In a case-control study, expression of hsa_circ_0002363 was evaluated by real-time quantitative reverse transcription polymerase chain reaction in 20 paired TNBC and adjacent normal tissues. Associations with clinicopathological characteristics were assessed using Wilcoxon, Mann–Whitney, Kruskal–Wallis, and Spearman's correlation tests. Diagnostic performance was evaluated using receiver operating characteristic curve analysis. Statistical analyses were performed using IBM SPSS Statistics version 21, with $P < 0.05$ considered

statistically significant. Bioinformatic analyses were performed to construct a competing endogenous RNA (ceRNA) network and identify enriched pathways and hub genes.
Results: hsa_circ_0002363 was significantly upregulated in TNBC tissues ($P = 0.001$), positively correlated with tumor size ($r = 0.466$, $P = 0.038$), and exhibited good diagnostic performance (area under the curve = 0.890). Bioinformatic analyses identified hsa-miR-384 and hsa-miR-668 as interacting miRNAs and highlighted HSPA5, YY1, and AGO1 as key hub genes. Enrichment analysis suggested the involvement of cancer-related pathways, including breast cancer, mTOR signaling, and RNA degradation.
Conclusion: Our study provides the first evidence that the mecciRNA hsa_circ_0002363 is aberrantly expressed in TNBC and may participate in tumor progression through a putative ceRNA regulatory network. These findings introduce hsa_circ_0002363 as a promising biomarker and provide a basis for future mechanistic investigations.

Keywords: RNA, Circular, Mitochondria, MicroRNAs, Triple negative breast neoplasms, Biomarkers

Introduction

Breast cancer (BC) is one of the most common malignancies in women and one of the leading causes of cancer death, worldwide.[1] Triple-negative breast cancer (TNBC), one of the subgroups of BC, accounts for approximately 10%–20% of BC cases. TNBC does not express estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), which is associated with rapid tumor growth, high metastasis rates, higher likelihood of recurrence, and poorer survival than other molecular subtypes.[2, 3] Thus, identifying novel molecular biomarkers contributing to early diagnosis, prognostic assessment, and the development of targeted therapies for TNBC has become a key priority in cancer research.[4]

In recent years, increasing evidence has shown that metabolic alterations and mitochondrial dysfunction are essential features of TNBC cells and play important roles in regulating energy production, reactive oxygen species, drug resistance, metabolic rearrangement, and metastatic ability. Therefore, regulatory molecules related to mitochondrial function have been considered as potential targets for identifying novel biomarkers and designing novel therapeutic strategies.[5, 6]

Recent evidence has shown that the mitochondrial genome is capable of producing a diverse range of non-coding RNAs (ncRNAs) involved in the regulation of mitochondrial function and communication between the nucleus and mitochondria.[7] Among these molecules, mitochondria-encoded circular RNAs (circRNAs) or Mitochondrial-encoded circular RNAs (mecciRNAs) have been introduced as a new group of regulatory RNAs that may play a role in the transport of proteins into mitochondria, maintenance of mitochondrial homeostasis, and cellular response to stress.[8] Preliminary studies have also shown that impaired expression of mecciRNAs is associated with various diseases, including cancer; however, the function of many of these RNAs and their role in BC are still not fully understood.[9, 10]

Despite significant advances in the identification of ncRNAs and their role in cancer, the available information on mecciRNAs, especially in TNBC, remains limited. Based on data available in bioinformatics databases, hsa_circ_0002363 is known as a mitochondrial-origin circRNA; however, its expression pattern, clinical

significance, and potential role in the pathogenesis of TNBC have not been investigated so far. Therefore, the present study aimed to evaluate the expression of hsa_circ_0002363 in tissue samples from patients with TNBC and to investigate its association with clinicopathological features. Also, in order to gain a better understanding of the possible molecular mechanisms of this mecciRNA, bioinformatics analyses were performed to predict circRNA–miRNA–mRNA interactions and identify related biological pathways.

Material and Method

Study design and patient recruitment

This case–control study was conducted on tissue samples from women with TNBC who had not received any chemotherapy or neoadjuvant therapy prior to surgery. A total of 20 female patients with a pathological diagnosis of TNBC who underwent surgery at Shahid Faghihi Hospital in Shiraz were included in the study. One sample of tumor tissue and one sample of adjacent normal tissue were collected from each patient.[11, 12] The sample size was determined with reference to previous exploratory studies evaluating circRNA expression in paired TNBC and adjacent normal tissues using real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR), which have employed comparable sample sizes. Accordingly, 20 paired TNBC and adjacent normal tissue samples were included in the present study.[13] All tissue samples were immediately verified by a pathologist after collection and stored under appropriate conditions until RNA extraction. Clinicopathological information of patients, including age, tumor size, histopathological grade, lymph node status, and disease stage, were also collected from medical records to assess their association with hsa_circ_0002363 expression (Table 1).

This study was conducted in accordance with the ethical principles set forth in the Declaration of Helsinki. The research protocol was approved by the Ethics Committee of Isfahan University of Medical Sciences with the ethics code IR.MUI.MED.REC.1403.363, and written informed consent was obtained from all participants before patients were enrolled in the study.

RNA extraction, cDNA synthesis, and primer design

Total RNA was extracted from tumor tissue samples and adjacent normal tissues using TRIzol® reagent (Invitrogen, Thermo Fisher Scientific, USA) according to the manufacturer's instructions. The concentration and purity of the extracted RNA were assessed using a NanoDrop device based on the ratio of optical absorption at wavelengths of 260 and 280 nm. Also, the integrity of the RNA was checked using agarose gel electrophoresis, and only samples of appropriate quality and purity were used for the next stages of the study. Complementary DNA (cDNA) synthesis was performed using the PrimeScript™ RT Reagent Kit (BioFact™, Cat. No. BR441096, Korea) according to the manufacturer's instructions. In this step, a combination of Random Hexamer primers and specific primers were used to increase reverse transcription efficiency. We used RealQ Plus 2× Master Mix Green with High ROX (Ampliqon, Cat. No. A325402-25) and divergent primers targeting the back-splice junction to specifically measure hsa_circ_0002363 expression and avoid linear transcript amplification by qRT-PCR. The cycling conditions consisted of an initial activation at 95°C for 15 min, followed by 45 cycles of 95°C for 20 s and 60°C for 30 s. One internal control that was used was ACTB. The sequences of the primers used in this study are presented in Table 2. Primer amplification efficiency was evaluated using

standard curves generated from serial dilutions of cDNA. The amplification efficiencies were 96.24% (R^2 = 0.992) for hsa_circ_0002363 and 94.13% (R^2 = 0.998) for ACTB.

Statistical analysis

The relative expression level of hsa_circ_0002363 was calculated as fold change ($2^{\wedge-\Delta\Delta Ct}$) and all statistical analyses were performed based on fold change values. Data analysis was performed using IBM SPSS Statistics version 21 software and graphs were drawn using GraphPad Prism version 8. Given the exploratory nature of the clinicopathological association analyses and the multiple comparisons performed, *P*-values were adjusted using the Benjamini–Hochberg procedure to control the false discovery rate (FDR). Adjusted *P*-values < 0.05 were considered statistically significant.

Due to the non-normal distribution of the data, non-parametric tests were used for statistical analysis. The difference in hsa_circ_0002363 expression levels between tumor tissues and adjacent normal tissues was evaluated using the Wilcoxon test. Also, the relationship between the expression levels of this circRNA and the demographic and clinicopathological characteristics of the patients was examined using the Mann–Whitney and Kruskal–Wallis tests. The correlation between hsa_circ_0002363 expression levels and tumor size was evaluated using Spearman's rank correlation coefficient. In order to investigate the diagnostic value of hsa_circ_0002363, the receiver operating characteristic (ROC) curve was drawn based on the fold change values, and the diagnostic performance of this marker was evaluated by calculating the area under the curve.

Bioinformatics Analysis

Annotation of* hsa_circ_0002363 *and prediction of interacting miRNAs

To determine the genomic origin of hsa_circ_0002363 and identify miRNAs with potential binding sites, the circInteractome database was used.[14] Accordingly, hsa_circ_0002363 was annotated as a mitochondrial-derived circular RNA (mecciRNA). Then, miRNAs with potential binding to this circRNA were extracted and selected for further analysis. hsa-miR-384 and hsa-miR-668 were selected because they were the two candidate miRNAs identified by circInteractome for hsa_circ_0002363.

Identification of target mRNAs

The target genes of each of the selected miRNAs were independently predicted using the TargetScan[15] and miRTargetLink databases.[16] In order to increase the reliability of the results, common genes between the two databases were extracted for each miRNA. Subsequently, the common genes obtained from the two miRNAs were also compared with each other and the final set of target genes was selected for further analysis.

Functional enrichment analysis

In order to investigate the biological functions and molecular pathways associated with the target genes, functional enrichment analysis was performed using the Enrichr.[17] Gene ontology (GO) analysis was performed in three sections: Biological process (BP), molecular function (MF), and cellular component (CC). Also, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was used to identify signaling pathways associated with the target genes.[18]

Protein–protein interaction (PPI) network construction and Identification of hub genes

In order to investigate the functional interactions between proteins encoded by the target genes, a PPI network was created using the STRING database.[19] The initial network included the 24 predicted target proteins. To identify additional functionally associated proteins, the network was expanded by allowing up to 10 interactors in each of the first and second interaction shells. The full STRING network was used with evidence-

based edges and a minimum required interaction score of 0.400 (medium confidence). All available interaction sources, including text mining, experiments, databases, co-expression, neighborhood, gene fusion, and co-occurrence, were considered. This procedure resulted in the addition of 20 proteins, generating a final network of 44 nodes and 123 edges. The expanded network was transferred to Cytoscape (version 3.9.1) for topological analysis.[20] To identify key genes in the PPI network, topological indices including Degree centrality, Betweenness centrality, and Closeness centrality were calculated in Cytoscape. Then, the top 20 genes from each of these three indices were extracted and the common genes among them were selected as Hub genes.

Construction of the circRNA–miRNA–mRNA regulatory network

To visualize the potential regulatory relationships between hsa_circ_0002363, the predicted miRNAs, and the target genes, a circRNA–miRNA–mRNA competing endogenous RNA (ceRNA) network was constructed using Cytoscape software.[20] This network was constructed based on the predicted interactions between hsa_circ_0002363, the two related miRNAs, and their common target genes and was used to visualize the potential regulatory relationships.

Results

Clinicopathological characteristics of the study population

A total of 20 patients with histopathologically confirmed TNBC were enrolled in this study. Among the participants, 11 (55%) were aged ≥50 years, while 9 (45%) were younger than 50 years. 14 patients (70%) had tumors measuring ≥2.5 cm, and lymph node metastasis was observed in 14 cases (70%). Histopathological evaluation revealed grade 1, grade 2, and grade 3 tumors in 6 (30%), 9 (45%), and 5 (25%) patients, respectively. The majority of tumors were classified as invasive ductal carcinoma (95%), whereas one patient (5%) had invasive lobular carcinoma. Detailed demographic and clinicopathological characteristics are summarized in Table 1.

Relative expression of* hsa_circ_0002363 *and association between its expression and clinicopathological characteristics

Hsa_circ_0002363 expression was significantly upregulated in tumor tissues compared with matched adjacent normal tissues (Median fold change: 5.850 vs. 1.685, $P = 0.001$) (Figure 1).

The association between hsa_circ_0002363 expression and clinicopathological characteristics was investigated using non-parametric tests. Although an association between hsa_circ_0002363 expression and oral contraceptive pill consumption was observed in the unadjusted analysis ($P = 0.045$, Figure 2A), this association did not remain statistically significant after adjustment for multiple comparisons (FDR-adjusted P-value = 0.319).

Further, Spearman's rank correlation analysis was performed using tumor size as a continuous variable. A moderate positive correlation was identified between tumor size and hsa_circ_0002363 expression (Correlation coefficient = 0.466, $P = 0.038$), indicating that higher expression levels were associated with larger tumor size (Figure 2B).

Diagnostic performance of hsa_circ_0002363

ROC curve analysis was performed to evaluate the diagnostic value of hsa_circ_0002363 in distinguishing TNBC tissues from adjacent normal tissues. hsa_circ_0002363 demonstrated good diagnostic performance, with an area under the curve of 0.890 (95% CI: 0.786–0.994, $P < 0.001$) (Figure 3), suggesting a high discriminatory ability for differentiating tumor from normal breast tissue.

Bioinformatics Analysis

Prediction of interacting miRNAs and mRNAs

Based on the circInteractome database,[14] two miRNAs, hsa-miR-384 and hsa-miR-668, were identified as having potential binding sites for hsa_circ_0002363. Subsequently, the target genes for each miRNA were retrieved using the TargetScan[15] and miRTargetLink[16] databases. After removing duplicates and selecting common genes, a final set of 24 target genes was obtained for further analysis.

Functional enrichment analysis

GO analysis revealed that the target genes were primarily enriched in BPs related to cellular response to starvation, positive regulation of transcription by RNA polymerase II, regulation of organelle organization, response to epidermal growth factor, and regulation of angiogenesis (Adjusted $P < 0.05$) (Figure 4A). In the CC category, the most enriched components included the Hrd1p ubiquitin ligase complex, RNA-induced silencing complex (RISC)-loading complex, Golgi medial cisterna, RISC complex, and voltage-gated sodium channel complex; however, none reached statistical significance after multiple testing correction (Figure 4B). Similarly, in the MF category, miRNA binding, single-stranded RNA binding, regulatory RNA binding, protein kinase inhibitor activity, and phosphatase binding were among the enriched MFs; yet, none achieved statistical significance following multiple testing correction (Figure 4C).

KEGG pathway enrichment analysis identified several pathways potentially associated with the predicted target genes of the hsa_circ_0002363 regulatory network. The top enriched pathways included RNA degradation, Melanogenesis, Sulfur relay system, signaling pathways regulating pluripotency of stem cells, Gastric cancer, BC, and Protein processing in endoplasmic reticulum. However, none of the KEGG pathways reached statistical significance after multiple testing correction (adjusted *P*-value > 0.05, Figure 4D). Therefore, these findings should be considered exploratory and hypothesis-generating rather than statistically supported pathway associations.

PPI network and hub gene identification

To investigate functional interactions among the target genes, a PPI network was constructed using the STRING database and expanded via the network expansion function. The expanded network was then imported into Cytoscape for topological analysis. Based on Degree, Betweenness, and Closeness centrality metrics, 12 genes, Heat Shock Protein Family A (Hsp70) Member 5 (HSPA5; also known as GRP78), Yin Yang 1 (YY1), Ubiquitin-Conjugating Enzyme E2 J1 (UBE2J1), Argonaute RISC Catalytic Component 1 (AGO1), Nuclear Receptor Coactivator 3 (NCOA3), Retinoid X Receptor Alpha (RXRA), Cyclin-Dependent Kinase 19 (CDK19), Specificity Protein 1 (SP1), Autocrine Motility Factor Receptor (AMFR), Derlin 2 (DERL2), Endoplasmic Reticulum Lectin 1 (ERLEC1), and ER Degradation Enhancing Alpha-Mannosidase Like Protein 1 (EDEM1) were identified as hub genes.

Construction of the circRNA–miRNA–mRNA regulatory network

To visualize the potential regulatory relationships between hsa_circ_0002363, the predicted miRNAs, and the target genes, a circRNA–miRNA–mRNA regulatory network was constructed using Cytoscape software (Figure 5).

Discussion

In the present study, we demonstrated for the first time that the mitochondria-encoded circular RNA, hsa_circ_0002363 was significantly upregulated in TNBC tissues compared with matched adjacent normal breast tissues. Furthermore, higher

hsa_circ_0002363 expression was positively correlated with tumor size and exhibited good diagnostic performance for discriminating TNBC tissues from non-tumorous adjacent tissues, suggesting its potential clinical relevance as a diagnostic biomarker. To gain insight into the potential biological function of hsa_circ_0002363, we further constructed a putative circRNA–miRNA–mRNA regulatory network comprising two candidate miRNAs (hsa-miR-384 and hsa-miR-668) and 24 downstream target genes, followed by functional enrichment and PPI interaction analyses. Interestingly, integrative topological analysis identified HSPA5, YY1, and AGO1 as the only hub genes that were also present among the initially predicted target genes, highlighting them as the most biologically plausible downstream effectors of the proposed hsa_circ_0002363-mediated regulatory network. Taken together, these findings suggest that hsa_circ_0002363 may participate in the molecular pathogenesis of TNBC through mitochondria-associated post-transcriptional regulatory mechanisms and support its potential as a promising biomarker deserving further functional investigation.

TNBC is characterized by remarkable molecular heterogeneity and aggressive biological behavior, highlighting the need for reliable biomarkers that may improve disease characterization and provide insights into its underlying molecular mechanisms.[21, 22] Unlike the vast majority of previously reported circRNAs in BC, hsa_circ_0002363 is a mitochondria-encoded circular RNA (mecciRNA), making the biological interpretation of our findings particularly intriguing.[8, 14] MecciRNAs represent a recently identified subclass of circRNAs transcribed from the mitochondrial genome, and accumulating evidence indicates that they participate in mitochondrial–nuclear communication, mitochondrial protein import, and cellular adaptation to physiological and pathological stress.[8, 23] Mitochondria are now recognized as central regulators of cancer cell metabolism, redox homeostasis, apoptosis, and cellular stress adaptation rather than merely ATP-producing organelles.[24] These mitochondrial functions are particularly relevant in TNBC, a BC subtype characterized by profound metabolic plasticity, mitochondrial remodeling, and increased dependence on adaptive stress-response pathways to sustain tumor progression and therapeutic resistance.[25, 26]

Previous studies have demonstrated that mecciRNAs are dynamically regulated under different stress conditions and facilitate the mitochondrial import of selected nuclear-encoded proteins through interactions with components of the mitochondrial import machinery, thereby contributing to mitochondrial homeostasis.[8] Therefore, the upregulation of hsa_circ_0002363 observed in our TNBC cohort may reflect an adaptive mitochondrial response to the metabolic and oxidative stress associated with this aggressive BC subtype. Although this hypothesis requires experimental validation.

Interestingly, hsa_circ_0002363 has received limited attention in the literature. It has been identified as a mitochondria-derived circRNA and reported among the upregulated mecciRNAs in patients with chronic lymphocytic leukemia,[9] as well as in experimental models of sepsis, where altered mecciRNA expression has been observed during systemic inflammatory stress.[27] However, to the best of our knowledge, no previous study has investigated the expression pattern or potential biological significance of hsa_circ_0002363 in TNBC.

A growing body of evidence suggests that many circRNAs exert their biological functions by acting as ceRNAs, thereby sequestering miRNAs and indirectly modulating the expression of their

downstream target genes.[12, 28, 29]. Based on circInteractome prediction, hsa-miR-384 and hsa-miR-668 were identified as the two most likely miRNAs interacting with hsa_circ_0002363. Among the predicted miRNAs, hsa-miR-384 has been reported as a tumor suppressor in BC. Wang et al. demonstrated that miR-384 is downregulated in TNBC and that its restoration suppresses cell proliferation and migration by targeting ACVR1 and inhibiting the Wnt/β-catenin signaling pathway.[30] Therefore, the predicted interaction between hsa_circ_0002363 and miR-384 suggests a potential regulatory mechanism that warrants further experimental investigation.

Unlike miR-384, miR-668 has been reported to exert oncogenic effects in BC. Zhang et al. demonstrated that miR-668 promotes radio resistance by directly targeting IκBα, thereby activating the NF-κB signaling pathway and enhancing BC cell survival following irradiation.[31] Thus, the predicted interaction between hsa_circ_0002363 and miR-668 may represent another potential mechanism through which this mecciRNA contributes to TNBC progression, although further experimental validation such as dual-luciferase reporter analysis, RNA immunoprecipitation, or pull-down experiments, is required.

Functional enrichment analysis provided exploratory insights into the potential biological context of the hsa_circ_0002363-associated network. KEGG analysis identified several nominally enriched pathways, including BC, mTOR signaling pathway, Protein processing in the endoplasmic reticulum, and RNA degradation. However, none of these pathways remained statistically significant after multiple-testing correction and should therefore be considered hypothesis-generating observations rather than statistically supported pathway associations. The mTOR pathway is a key regulator of cell growth, metabolism, and survival in TNBC.[32] In addition, enrichment of the RNA degradation pathway is in agreement with the predicted ceRNA mechanism and the identification of AGO1, a core component of the RNA-induced silencing complex, supporting a potential role of hsa_circ_0002363 in post-transcriptional gene regulation.[33] Nevertheless, these interpretations remain speculative and require validation in larger datasets and functional experiments.

A notable finding of this study was that HSPA5, YY1, and AGO1 were the only genes shared between the initially predicted target genes and the final hub gene set, suggesting that they may represent key downstream components of the hsa_circ_0002363 regulatory network.

HSPA5 is a central regulator of endoplasmic reticulum stress and the unfolded protein response, both of which contribute to TNBC progression and therapeutic resistance.[34] YY1 is a multifunctional transcription factor implicated in cell proliferation, epithelial–mesenchymal transition, and BC progression.[35] In addition, AGO1, a core component of the RISC, plays an essential role in miRNA-mediated gene silencing, further supporting the predicted ceRNA mechanism of hsa_circ_0002363.[33]

Collectively, these findings suggest that hsa_circ_0002363 may contribute to TNBC through coordinated regulation of cellular stress responses, transcriptional control, and post-transcriptional gene regulation. However, experimental validation is required to confirm these predicted interactions.

The strength of the present study is the integrated assessment of hsa_circ_0002363 expression and its potential regulatory network using paired clinical samples and complementary bioinformatic analyses. The present study has certain limitations. First, the relatively small clinical cohort (20 paired TNBC and adjacent normal tissue samples)

limits the generalizability of the findings and may reduce the statistical power of clinicopathological association analyses. In addition, the lack of follow-up data, including survival and recurrence information, precluded assessment of the prognostic value of hsa_circ_0002363. Second, the proposed hsa_circ_0002363–miRNA–mRNA network is based entirely on computational predictions and was not supported by expression correlation analyses, subcellular localization or RNA abundance assessments, or experimental validation of the predicted interactions. Therefore, the proposed regulatory relationships should be considered hypothesis-generating rather than the evidence of a functional ceRNA mechanism. Validation in larger independent cohorts and dedicated functional studies will be required to confirm these observations and clarify the biological role of hsa_circ_0002363 in TNBC.

Conclusion

Our study demonstrates that the mitochondrial-encoded circular RNA hsa_circ_0002363 is significantly upregulated in TNBC and positively associated with tumor size. Integrated bioinformatic analyses suggest that hsa_circ_0002363 may participate in TNBC progression through a putative ceRNA network involving hsa-miR-384, hsa-miR-668, and key regulatory genes including HSPA5, YY1, and AGO1; however, these predicted interactions and their potential biological implications should be considered hypothesis-generating. Collectively, these findings identify hsa_circ_0002363 as a potential candidate biomarker and provide novel insights into the potential contribution of mitochondria-derived circRNAs to the molecular pathogenesis of TNBC. Further experimental and clinical studies are required to validate these observations and elucidate the underlying molecular mechanisms.

Declaration of Generative AI in Scientific Writing

During the preparation of this manuscript, the authors used ChatGPT to improve the readability, language, and grammatical quality of the text. All AI-assisted content was carefully reviewed, revised, and verified by the authors, who take full responsibility for the accuracy and integrity of the final manuscript.

Availability of Data and Materials

The datasets generated and/or analyzed during the present study are available from the corresponding author upon reasonable request.

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Authors' Contribution

ZF, AS, and BM collected clinical samples and performed experimental procedures. ZF, AS, MS, AD, BM, YM, and RS contributed to data interpretation and manuscript preparation. ZF and AS performed statistical and bioinformatic analyses. RS and YM critically revised the manuscript. RS conceived and supervised the study.

All authors read and approved the final version of the manuscript.

Conflict of Interest

None declared.

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Table 1. Demographic and clinicopathological characteristics of patients with TNBC and their association with hsa_circ_0002363 expression in paired TNBC tumor and adjacent normal breast tissues

		Relative expression of hsa_circ_0002363					
		Valid N	Mean	Standard deviation	Median	*P*-value	FDR-adjusted *P*-value
Age	<50	9	5.74	2.25	6.11	0.732	0.860
	≥50	11	6.02	2.43	5.58		
BMI	≤25	13	5.26	2.45	5.43	0.205	0.451
	25-29	5	6.72	1.58	6.59		
	≥30	2	7.91	1.19	7.91		
Age at menarche	<14	14	6.04	2.65	6.37	0.409	0.684
	≥14	6	5.54	1.27	5.34		
Menarche status	No	8	6.16	2.61	7.14	0.537	0.738
	Yes	12	5.72	2.16	5.51		
Menopausal status	pre	7	5.63	2.51	6.11	0.782	0.860
	post	13	6.04	2.26	5.58		
Abortion history	No	14	5.86	2.55	6.13	0.869	0.869
	Yes	6	5.96	1.78	5.51		
OCP consumption	No	8	4.76	1.95	5.15	0.045	0.319
	Yes	12	6.65	2.27	6.61		
Tumor size	<2.5	6	4.53	1.94	5.23	0.058	0.319
	≥2.5	14	6.47	2.25	6.61		
Histologic grade	1	6	4.59	1.97	5.32	0.159	0.437
	2	9	6.01	2.31	6.11		
	3	5	7.23	2.18	7.57		
Lymph node metastasis	Yes	14	6.45	2.26	6.61	0.099	0.363
	No	6	4.59	1.97	5.32		
Type of invasive carcinoma	ILC	1	5.21	.	5.21	0.435	0.684
	IDC	19	5.93	2.35	6.11		

N: Number of participants in each group; BMI: Body mass index; OCP: Oral contraceptive pill; IDC: Invasive ductal carcinoma; ILC: Invasive lobular carcinoma; TNBC: Triple-negative breast cancer; FDR: False discovery rate

Table 2. Primer sequences used for real-time quantitative reverse transcription PCR analysis

Genes	Forward (5′–3′)	Reverse (5′–3′)
hsa_circ_0002363	GGGCTGTGAGTTTTAGGTAGTT	AGGACTTCTAACAGCCCTAGA
ACTB (housekeeping)	TGGAACGGTGAAGGTGACAG	CTGTAACAACGCATCTCATATTTGG

PCR: Polymerase chain reaction, ACTB: Beta-actin

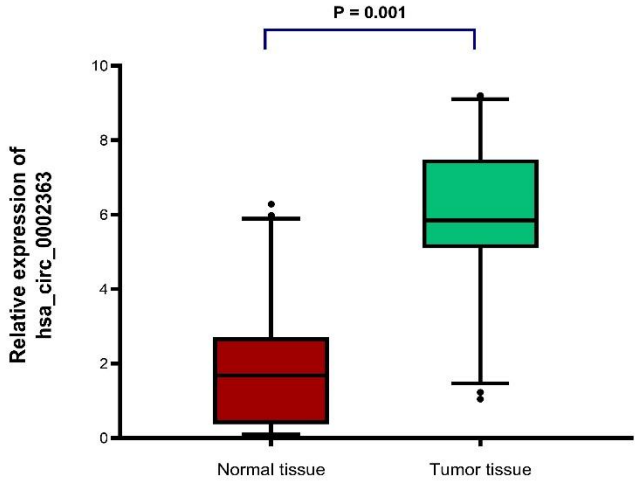


Figure 1. This figure shows the relative expression of hsa_circ_0002363 in TNBC tumor and paired adjacent normal breast tissues. The expression level of hsa_circ_0002363 was significantly higher in TNBC tumor tissues than in adjacent normal tissues.

TNBC: Triple-negative breast cancer

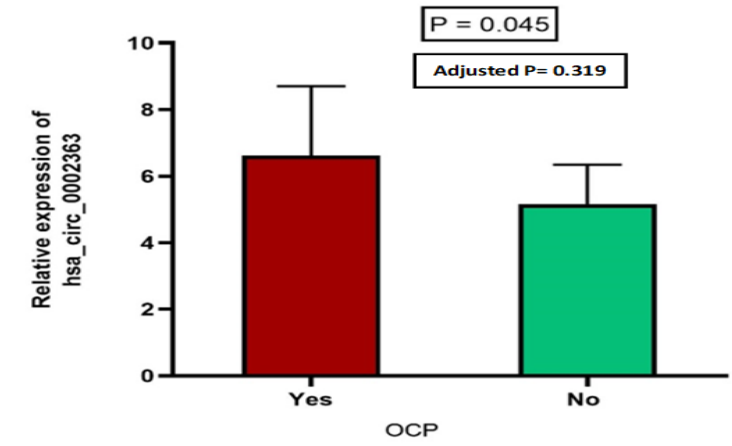


Figure 2A

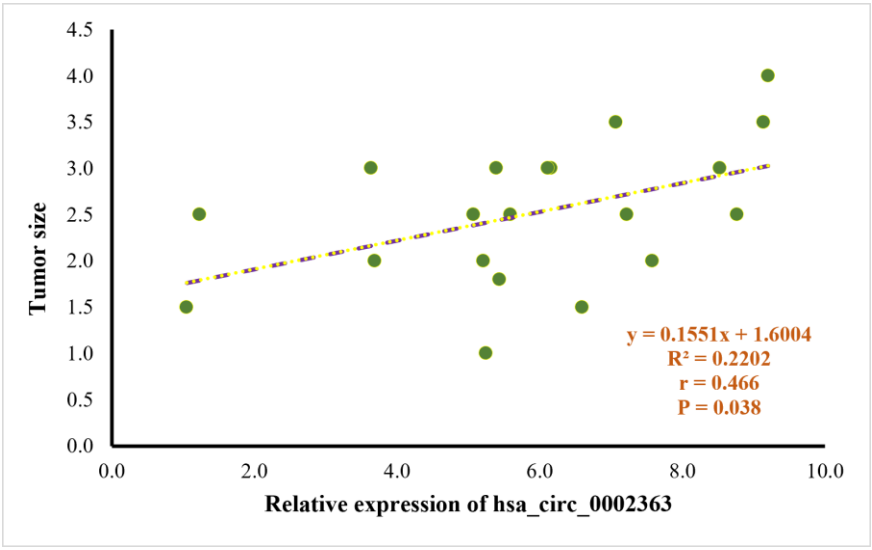


Figure 2B

Figure 2. This figure shows the association of hsa_circ_0002363 expression with clinicopathological features of TNBC. (A) Comparison of hsa_circ_0002363 expression between patients with and without a history of OCP use. This association did not remain statistically significant after adjustment for multiple comparisons (B) Correlation between hsa_circ_0002363 expression and tumor size based on Spearman's rank correlation analysis.

TNBC: Triple-negative breast cancer; OCP: Oral contraceptive pill

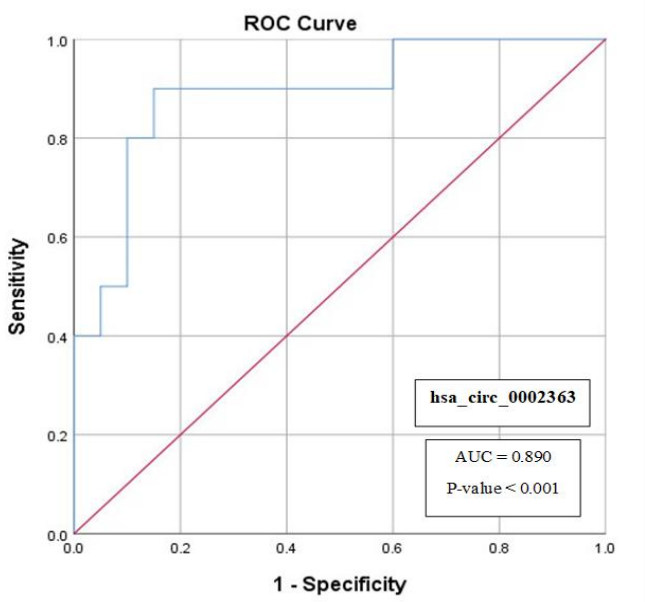


Figure 3. This figure shows the ROC curve for hsa_circ_0002363 expression in distinguishing TNBC tumor tissues from paired adjacent normal tissues. AUC indicates the discriminatory performance of hsa_circ_0002363 expression.

ROC: Receiver operating characteristic; AUC: Area under the curve; TNBC: Triple-negative breast cancer

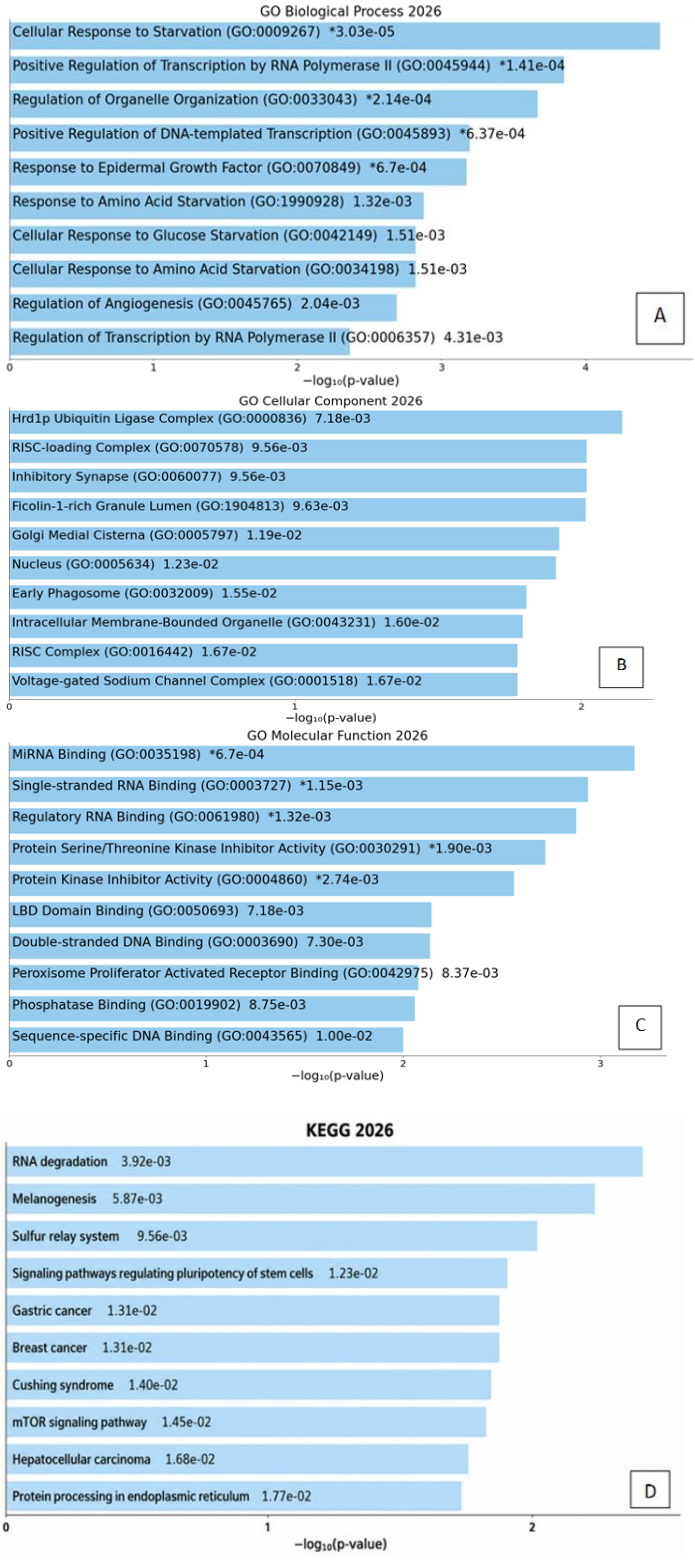


Figure 4. This figure shows the functional enrichment analysis of the predicted target genes associated with hsa_circ_0002363. (A) BP, (B) CC, (C) MF, and (D) KEGG pathway enrichment. The enriched terms are presented according to their statistical significance.

BP: Biological process; CC: Cellular component; MF: Molecular function; KEGG: Kyoto Encyclopedia of Genes and Genomes

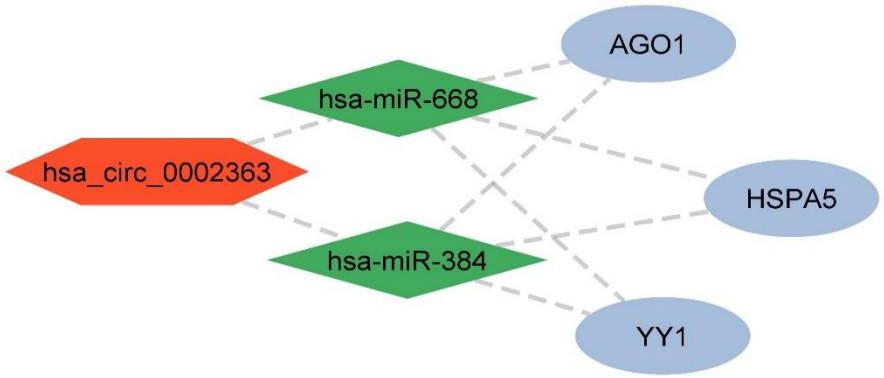


Figure 5. This figure shows the integrated hsa_circ_0002363–miRNA–mRNA regulatory network based on computational predictions. The network illustrates the predicted interactions between hsa_circ_0002363, miRNAs, and their target mRNAs. The red hexagon represents the circRNA, green diamonds represent miRNAs, and blue ellipses represent mRNAs.