*Gene Expression* **2025** vol. 000(000) | 000–000 Epub
DOI: 10.14218/GE.2025.00021

**Original Article**

# Microarray Analysis of Differential Expression of Long Non-coding RNAs in Peripheral Blood Mononuclear Cells in Luminal A Breast Cancer

Varvara I. Minina[1,2], Ruslan A. Titov[1,2], Vladislav Yu. Buslaev[1*], Renata R. Savchenko[3], Alexey A. Sleptcov[3], Natalia A. Gavrineva[2], Marina L. Bakanova[1,2], Yana A. Zakharova[1,2] and Andrey N. Glushkov[1]

*[1]The Federal Research Center of Coal and Coal Chemistry of Siberian Branch of the Russian Academy of Sciences, Kemerovo, Russia; [2]Kemerovo State University, Kemerovo, Russia; [3]Research Institute of Medical Genetics, Tomsk National Research Medical Center of the Russian Academy of Science, Tomsk, Russia*

**Received:** March 03, 2025 | **Revised:** June 12, 2025 | **Accepted:** July 02, 2025 | **Published online:** August 13, 2025

## Abstract

**Background and objectives:** In the post-genomic era, long non-coding RNAs (lncRNAs) have emerged as critical regulators in various cancers and hold potential as minimally invasive diagnostic biomarkers. This study aimed to perform microarray analysis of the peripheral blood mononuclear cell (PBMC) transcriptome to evaluate differential lncRNA expression in women with luminal A breast cancer.

**Methods:** A one-color microarray analysis was conducted using SurePrint G3 Human Unrestricted 8×60K arrays and a SureScan Microarray Scanner (Agilent Technologies, USA). The study cohort comprised 16 participants: eight patients diagnosed with luminal A breast cancer and eight healthy controls. Bioinformatic analysis was performed using the "limma" and "tidyverse" packages in the R statistical environment. Functional enrichment analysis was conducted to identify significantly differentially expressed gene clusters. The false discovery rate-adjusted *p*-value ($p_{adj}$) was applied to ensure methodological rigor. Associations between lncRNAs and disease progression were explored using the LncRNADisease 2.0 database.

**Results:** Differential expression was observed for long intergenic non-coding (LINC), LOC, and antisense RNA genes. Notably, LINC RNA 974 (LINC00974) exhibited significant differential expression (log fold change > |1.5|, $p_{adj} < 0.05$) after multiple comparison correction. Analysis using the LncRNADisease 2.0 database revealed associations between LINC and antisense RNAs and other oncological disorders.

**Conclusions:** This study is the first to demonstrate differential lncRNA expression in PBMCs of patients with luminal A breast cancer. Despite the limited sample size, the study demonstrates statistically significant differences between groups, highlighting the potential of PBMC-derived lncRNAs as minimally invasive biomarkers. These findings enhance our understanding of the utility of PBMC-derived lncRNAs as biomarkers for breast cancer.

## Introduction

Breast cancer (BC) is the most prevalent oncological disorder in women, posing a significant health threat. In 2022, approximately 2.3 million cases and 670,000 deaths were recorded worldwide. BC arises and progresses due to the interplay of internal and external factors. It has been established that 5–10% of cases are attributable to genetic mutations and family history, while 20–30% are linked to lifestyle factors (including alcohol and tobacco use, obesity, and physical inactivity), environmental exposures, and occupational activities.[1,2] The influence of pollutants, such as those generated by industrial processes and smoking, has also been identified as a contributing factor.[3] Reproductive factors, hormonal influences, and socio-psychological factors are under active investigation. Approximately 5% of BC cases occur in individuals with specific risk

**Keywords:** Long non-coding RNA; Differential expression; Breast cancer; Transcriptome; Microarray analysis; Mononuclears of peripheral blood.
***Correspondence to:** Vladislav Yu. Buslaev, The Federal Research Center of Coal and Coal Chemistry of Siberian Branch of the Russian Academy of Sciences, 18 Sovietskiy Str., Kemerovo 650065, Russia. ORCID: https://orcid.org/0000-0001-5566-5323. Tel: +7-770-89134369668, E-mail: vladislasbus2358@yandex.ru
**How to cite this article:** Minina VI, Titov RA, Buslaev VY, Savchenko RR, Sleptcov AA, Gavrineva NA, *et al*. Microarray Analysis of Differential Expression of Long Non-coding RNAs in Peripheral Blood Mononuclear Cells in Luminal A Breast Cancer. *Gene Expr* 2025;000(000):000–000. doi: 10.14218/GE.2025.00021.

factors for BC, beyond female sex and age over 40.

Notably, BC is a heterogeneous disease comprising distinct subtypes with unique pathological characteristics and clinical manifestations. Based on the expression of estrogen receptor (ER), progesterone receptor (PR), HER2 (ERBB2) amplification, and the Ki67 proliferation index, BC is classified into four clinical subtypes: luminal A (ER+/PR+/HER2−, low Ki67 proliferation), luminal B (ER+/PR±/HER2±, Ki67 ≥ 14%), HER2-positive (ER−/PR−/HER2+, variable Ki67 proliferation), and triple-negative BC (ER−/PR−/HER2−, variable Ki67 proliferation). Luminal A is the most prevalent subtype, whereas triple-negative BC is the most aggressive.

Furthermore, available diagnostic tests include those for *BRCA1*, *BRCA2*, *PIK3CA*, and topoisomerase II alpha genes, as well as evaluations of HER2, programmed death-ligand 1, and Ki67 status, and gene expression profiling. Messenger RNA (mRNA) analysis offers advantages over DNA analysis, as demonstrated by studies showing that gene expression profiles in malignant tissues (e.g., Oncotype DX, Prosigna, EndoPredict) can detect signaling pathway alterations with greater precision and predict recurrence more accurately than DNA-based analyses.[4]

However, effective cancer screening relies on detecting subtle systemic alterations, making minimally invasive, cost-effective peripheral blood analysis a valuable approach. This method enables the detection of clinically significant biomarkers, including circulating tumor cells, cell-free DNA, extracellular vesicles, cell-free microRNAs, and methylation markers, among others.[5,6]

In this context, research on triple-negative BC has highlighted the potential role of non-coding RNAs in its pathogenesis.[7] These molecules play a critical role in regulating gene expression, and their involvement has been investigated across various cancers. However, limited data exist regarding their expression profiles in peripheral blood mononuclear cells (PBMCs).

To address this gap, this study aimed to investigate the expression profiles of long non-coding RNAs (lncRNAs) in the PBMCs of BC patients with the luminal A subtype.

## Material and methods

This study was conducted at the Laboratory of Cytogenetics, Institute of Human Ecology, Federal Research Centre of Coal and Coal Chemistry, Siberian Branch of the Russian Academy of Sciences, the Laboratory of Genome Medicine, Kemerovo State University (Kemerovo, Russian Federation), and the "Medical Genomics" Center for Collective Use, Tomsk National Research Medical Center, Russian Academy of Sciences (Tomsk, Russian Federation).

### *Study participants*

Based on power calculations, 16 participants were included in the study. Peripheral blood samples from eight women diagnosed with BC and eight healthy controls were collected and analyzed in 2024. All participants were postmenopausal, confirmed by physician evaluation based on clinical symptoms. Inclusion criteria for BC patients were: women aged 50–60 years (mean age 55), Caucasian, non-smokers, and residents of the Kemerovo region. Patients had stage 1 or 2 luminal A BC confirmed by biopsy, with subsequent histological and immunohistochemical analysis (ER, PR, HER2, and Ki-67) performed at the Regional Clinical Oncological Hospital. Exclusion criteria included prior BC treatment, use of hormonal medications, hereditary BC, and metastases to lymph nodes or distant organs. Control samples were obtained from Caucasian women of the same age (mean 55), postmenopausal, residing in the same region, non-smokers, and with no evidence of oncological disorders, as confirmed by a comprehensive medical evaluation by qualified professionals.

### *Sample collection*

Peripheral blood samples were collected before treatment by qualified medical staff under sterile conditions at the hospital. Blood was collected in tubes containing ethylenediaminetetraacetic acid and immediately transported to the laboratory. RNA stabilization was performed using RNAlater/RNA Stabilization Reagent (Qiagen, Germany).

### *RNA extraction*

Mononuclear cells were isolated using a Ficoll density gradient (density 1.077 g/cm$^3$, PanEco, Russia). Total RNA was extracted using TRIzol reagent (Thermo Fisher Scientific, USA, Cat. No. 15596026) in accordance with the manufacturer's protocol. After incubation for 5 m at room temperature, 0.2 mL of chloroform was added, the tube was sealed, vigorously shaken, and incubated for 15 m at room temperature. The mixture was centrifuged at 12,000 × g for 15 m at 4°C, resulting in three phases: a red organic phase (containing proteins), an interphase (DNA), and a colorless aqueous phase. The aqueous phase was transferred to a new tube, and 0.5 mL of isopropyl alcohol was added. After a 10-m incubation at room temperature, the mixture was centrifuged at 10,000 × g for 10 m at 4°C. The supernatant was carefully removed without disturbing the precipitate (the RNA forms a white pellet at the bottom of the tube). The RNA was washed with 1 mL of 75% ethanol. The mixture was centrifuged at 10,000 rpm for 5 m. The ethanol was removed, and the pellet was air-dried for 3–5 m. The RNA was dissolved in 100 µL of purified water. RNA quality was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, USA), with the RNA integrity number as the key parameter. Only samples with RNA integrity number ≥7 were used for microarray analysis. All samples met this criterion.

### *cDNA synthesis*

A cDNA Master Mix was prepared, containing per sample: 2 µL of 5× First Strand Buffer, 1 µL of 0.1 M dithiothreitol, 0.5 µL of 10 mM deoxynucleotide triphosphates Mix, and 1.2 µL of AffinityScript RNase Block Mix (enzymes added last). A volume of 4.7 µL of cDNA Master Mix was added to each tube to achieve a final volume of 10 µL, gently mixed using a vortex centrifuge, and incubated in a thermal cycler for 2 h at 40°C, followed by 15 m at 70°C. Samples were then placed on ice.

### *Complementary RNA (cRNA) synthesis*

A Transcription Master Mix was prepared for cRNA synthesis from cDNA templates, containing per sample: 0.75 µL of nuclease-free water, 3.2 µL of 5× Transcription Buffer, 0.6 µL of 0.1 M dithiothreitol, 1 µL of nucleotide triphosphates Mix, 0.21 µL of T7 RNA Polymerase Blend (enzymes added last), and 0.24 µL of Cyanine 3-CTP. A volume of 6 µL of the Transcription Master Mix was added to each tube to achieve a final volume of 16 µL. Samples were incubated in a thermal cycler for 2 h at 40°C.

### *Microarray analysis*

One-color microarray analysis was performed at the "Medical Genomics" Center for Collective Use, Tomsk National Research Medical Center, Russian Academy of Sciences. SurePrint G3 Human Unrestricted 8×60K microarrays (Agilent Technologies, USA) were used. Labeling and hybridization were conducted ac-

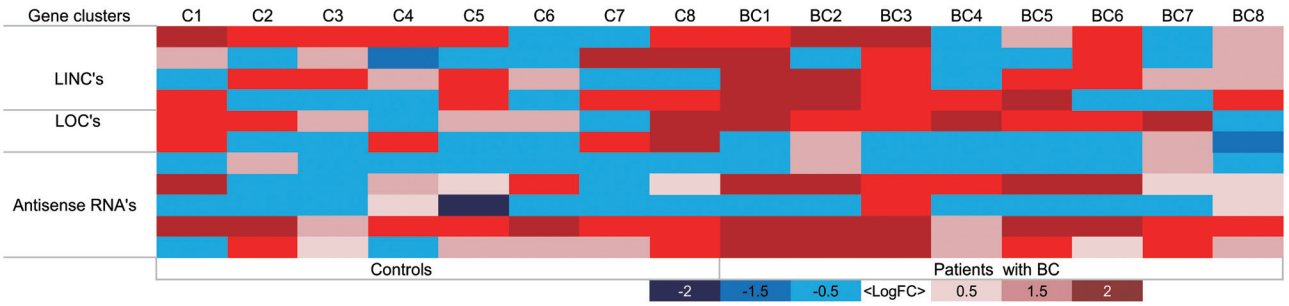


**Fig. 1. Heatmap of differentially expressed lncRNA gene clusters.** Color intensity in Figure 1 represents the LogFC parameter. Vinous (LogFC ≥ 2), red (LogFC ≥1.5), light red (LogFC≥1.0), grey (LogFC = 1.0), blue (LogFC < 1.0). BC, breast cancer; lncRNA, long non-coding RNA.

cording to the manufacturer's protocol (version 6.9.1). Signal processing was performed using Feature Extraction software, version 10.7.3.1 (Agilent Technologies, USA).

### *Bioinformatics*

Microarray data were analyzed using the "limma" and "tidyverse" packages in R (version 4.4.1). Statistical analysis was conducted, with p-values corrected for multiple testing using the false discovery rate (FDR) method. Following gene set enrichment analysis (GSEA) guidelines to ensure statistical rigor, thresholds of |logFC| > 1.5 and adjusted *p*-value ($p_{adj}$) < 0.05 were applied. Functional gene enrichment analysis was used to identify significantly differentially expressed gene clusters. Associations were explored using data from the LncRNADisease 2.0 database, investigating relationships between lncRNAs and other diseases with keywords: breast cancer, non-coding RNAs, LINC RNAs, LOC RNAs, and antisense RNAs.

## Results

### *Transcriptome study*

Whole-transcriptome microarray analysis identified 4,176 unique differentially expressed gene clusters with a fold change greater than 1.5. Functional gene enrichment analysis revealed significant clusters of lncRNA genes (log fold change [LogFC] > |1.5|, $p < 0.05$). Among these were long intergenic non-coding (LINC) RNAs, uncharacterized LOC transcripts, and antisense RNAs (Fig. 1).

At the first stage of functional enrichment analysis in BC patients, genes with increased expression were identified (Table 1). Among them, the long intergenic non-protein-coding RNA 974 gene (LINC00974) passed FDR correction (LogFC = 1.80; padj = 0.03).

The second stage of functional enrichment analysis was performed to identify non-coding RNAs with decreased expression (Table 2). The group of LINC RNAs was the most represented in this case.

### *Analysis with LncRNADisease 2.0 database*

Analysis using the LncRNADisease 2.0 database revealed associations between the lncRNAs identified in the microarray analysis and the development of various diseases. Specific criteria were chosen for consideration of non-coding RNA features, including associated diseases, expression patterns, methods of analysis, and sample sizes. Initially, information was extracted for specific LINC RNAs (Table 3). LINC RNAs showed low expression mainly in

**Table 1. Genes with increased expression in breast cancer patients**

| LINC | LOC | AntisenseRNA |
|---|---|---|
| *LINC01303, LINC01226, LINC01982, LINC01877, LINC02375, LINC02003, LINC00174, LINC02530, LINC00974, LINC00701, LINC02691, LINC01655, LINC00886, LINC01097, LINC00423, LINC01617, LINC00671, LINC02324, LINC02451, LINC01049* | *LOC101928943, LOC401357, LOC100133331, LOC105369595, LOC101927159, LOC102723385, LOC102546294, LOC105370954, LOC105373764, LOC106699570, LOC100131472, LOC101927751, LOC101929470, LOC100507516, LOC643406, LOC101927851, LOC105370401* | *TMLHE-AS1, RPS6KA2-AS1, ID2-AS1, NDST1-AS1, TEX26-AS1, SACS-AS1, VPS13A-AS1, GRIK1-AS2, LRP1-AS, SVIL-AS1, ANKRD34C-AS1, PGM5P3-AS1, TMEM92-AS1, OSMR-AS1, CERS6-AS1, ELMO1-AS1, JMJD1C-AS1, HTR3E-AS1, CELF2-AS1, ZNF385D-AS2, PLBD1-AS1* |

Genes with logFC > 1.5 were selected. LINC, long intergenic non-coding.

**Table 2. Genes with decreased expression in BC patients**

| LINC | LOC | AntisenseRNA |
|---|---|---|
| *LINC01530, LINC02233, LINC01281, LINC02561, LINC01101, LINC01056, LINC01933, LINC02696, LINC00159, LINC01819, LINC00673, LINC01734, LINC01424, LINC00515, LINC01644, LINC02190, LINC01342, LINC01711, LINC01128, LINC00663, LINC00412* | *LOC100129203, LOC105370941, LOC100506476, LOC101927497, LOC101927824, LOC101929341, LOC100996671, LOC100130992, LOC106660606, LOC102724958, LOC100996671, LOC728752, LOC101926935* | *NPHP3-AS1, PHKA2-AS1, ACVR2B-AS1, LEF1-AS1, MEF2C-AS1, PRKCZ-AS1, CARS-AS1, ZNF582-AS1, LSAMP-AS1, IDI2-AS1, ARHGAP5-AS1, SRP14-AS1, ADIPOQ-AS1, RASGRF2-AS1, LYPLAL1-AS1, STAM-AS1* |

Genes with logFC > 1.5 were selected. BC, breast cancer; LINC, long intergenic non-coding.

**Table 3. Analysis of LINC RNAs via LncRNADisease 2.0 database**

| ncRNA symbol | Species | Disease name | Sample | Dysfunction pattern | Validated method | PMID | Sample size |
|---|---|---|---|---|---|---|---|
| LINC01303 | Homo sapiens | Astrocytoma | Brain | Regulation [down-regulated] | Microarray | 26252651 | 130 |
| LINC02375 | Homo sapiens | Astrocytoma | Brain | Regulation [down-regulated] | Microarray | 26252651 | 130 |
| LINC00974 | Homo sapiens | Hepatocellular carcinoma | HCC tissue, blood (plasma) | Regulation [up-regulated] | qPCR, RIP | 25476897 | 150 |
| LINC01097 | Homo sapiens | Stomach cancer | Gastric cancer tissues, large GC cohorts | Regulation [down-regulated] | qPCR, Microarray | 27647437 | 300 |
| LINC01617 | Homo sapiens | Colorectal cancer | N/A | Expression | qPCR, Microarray | 26328256 | 290 |
| LINC01101 | Homo sapiens | Cervical cancer | HPV-induced neoplasia | Expression [high expression] | qRT-PCR, Microarray | 28767188 | 110 |

EDTA, ethylenediaminetetraacetic acid; HCC, hepatocellular carcinoma; HPV, human papillomavirus; LINC, long intergenic non-coding; lncRNA, long non-coding RNA; ncRNA, non-coding RNA; qPCR, quantitative polymerase chain reaction; qRT-PCR, quantitative reverse transcription polymerase chain reaction; RIP, receptor interacting protein.

astrocytoma, as well as in oncological disorders such as stomach and cervical cancer. LINC00974, which previously passed FDR correction, was represented as a highly expressed factor in hepatocellular carcinoma samples.

A similar analysis was performed for antisense RNAs (Table 4). For astrocytoma, both up-regulated and down-regulated antisense RNAs were reported. Antisense RNAs were also involved in neurodegenerative disorders (e.g., Alzheimer's disease), non-cancerous conditions (e.g., nasal polyps), and certain types of cancer (e.g., pancreatic and lung cancer).

## Discussion

In this study, we focused on the most prevalent type of BC, luminal A. However, rare BC subtypes also warrant consideration. For instance, micropapillary carcinoma of the breast, which accounts for 2–8% of all BC cases, exhibits a unique histological pattern that is both elusive and aggressive.[8] A 75% micropapillary component is proposed as the threshold for classifying a carcinoma as pure invasive micropapillary carcinoma. Conversely, the presence of a micropapillary component within no-special-type tumors is more common, and its clinical significance has been widely debated. Multiple studies have reported that the presence of a micropapillary element within a tumor is associated with worse prognosis and a lymphotropic pattern. Diagnostic strategies, such as using an indocyanine green tracer for sentinel lymph node mapping, have been developed.[9] HER2-positive cases are often associated with adverse clinicopathological parameters and increased recurrence

**Table 4. Analysis of antisense RNAs via LncRNADisease 2.0 database**

| ncRNA symbol | Species | Disease name | Sample | Dysfunction pattern | Validated method | PMID | Sample size |
|---|---|---|---|---|---|---|---|
| LRP1-AS | Homo sapiens | Alzheimer's disease | Brain | Regulation [up-regulated] | Luciferase reporter gene assay//qRT-PCR//Western blot | 25937287 | |
| PGM5P3-AS1 | Homo sapiens | Astrocytoma | Brain | Regulation [up-regulated] | Microarray | 26252651 | 130 |
| OSMR | Homo sapiens | Nasal Polyps | Nasal polyp tissue | Regulation [up-regulated] | RNA sequencing | 25476897 | 150 |
| znf385d | Homo sapiens | Pancreatic cancer | Pancreatic tissue | Regulation [up-regulated] | qRT-PCR | 22078386 | 38 |
| NPHP3-AS1 | Homo sapiens | Astrocytoma | Brain | Regulation [down-regulated] | Microarray | 26252651 | 130 |
| LEF1-AS1 | Homo sapiens | Astrocytoma | Brain | Regulation [up-regulated] | Microarray | 26252651 | 130 |
| PRKCZ-AS1 | Homo sapiens | Lung adenocarcinoma | N/A | N/A | qRT-PCR | 26857641 | 33 |
| ZNF582-AS1 | Homo sapiens | Astrocytoma | Brain | Regulation [up-regulated] | Microarray | 26252651 | 130 |

lncRNA, long non-coding RNA; ncRNA, non-coding RNA; qRT-PCR, quantitative reverse transcription polymerase chain reaction.

rates.

Our study included postmenopausal women, confirmed by medical evaluation. It is critical to consider the specific characteristics of menopausal stages, which are marked by a significant decrease in estrogen and progesterone levels, reducing the influence of hormone-driven mutagenic processes. Two primary menopausal phases are distinguished: premenopause/perimenopause and postmenopause. Perimenopause refers to the transitional years before the final menstrual period, during which estrogen levels are, on average, approximately 30% higher than in premenopause. In postmenopause, follicle-stimulating hormone levels continue to rise, while estradiol levels decline. Studies have shown that the most frequently mutated genes in BC, including *PIK3CA*, *CDH1*, *MUC16*, *PTEN*, *FAT3*, *FAT1*, *SPEN*, *ARID1A*, *LRP1B*, and *RUNX1*, exhibit differential expression between premenopausal and postmenopausal periods.[10]

mRNA-based biomarkers derived from peripheral blood cells are of significant interest. For example, a previous transcriptomic analysis of peripheral blood from BC patients, conducted using microarrays in a large cohort of Norwegian women (Norwegian Women and Cancer study), identified enrichment of 345 genes associated with processes such as apoptosis, RNA binding, RNA metabolism, splicing, protein synthesis, transcriptional regulation, cell cycle, metabolism, and signal transduction.[11] Current diagnostic methods for BC focus on detecting alterations in DNA sequences, analyzing RNA profiles, measuring protein expression, classifying BC subtypes, and assessing cancer spread, prognosis, and recurrence risk.[12]

It has been established that circulating malignant cells in BC patients overexpress, compared to non-transformed PBMCs, genes involved in proteolytic degradation of the extracellular matrix and epithelial-mesenchymal transition. Gene expression profiling of PBMCs in BC patients, compared to healthy donors, has revealed significantly reduced expression of *CD24*, *HDAC2*, *mTOR*, *MYC*, *PARP*, and *TP53* genes, whereas *MRP4* was overexpressed.[13] Some studies have reported transcriptional signatures in blood associated with triple-negative BC, typically linked to mild inflammation and altered immunological signaling. These signatures can distinguish triple-negative BC from other BC subtypes, facilitating diagnosis.[14,15]

Non-coding RNAs have received significant attention in cancer research. LINC RNAs possess unique features that distinguish them from protein-coding mRNA genes, contributing to processes such as chromatin and genome architecture remodeling, RNA stabilization, and transcriptional regulation, including enhancer activity.[16] LINC RNAs can enable precise regulation of neighboring genes with tissue specificity. Dysregulation of lncRNAs has been observed in numerous cancers, highlighting their potential role in cancer therapy.[17] Based on their genomic localization and origin, lncRNAs can be classified into five categories: sense, antisense, intronic, bidirectional, and intergenic RNAs. LINC RNAs are located between protein-coding regions in the genome, while antisense RNAs are transcribed from the antisense strand of protein-coding genes. Alongside these, LOC transcripts represent a relatively novel class of non-coding RNAs. In primates, LOC transcripts are highly conserved, lack coding potential, and are involved in various regulatory processes. In the context of BC, LOC transcripts may regulate tumor microenvironment dynamics by interacting with immune-related pathways, as suggested by their differential expression in our study. Oncogenic properties of intergenic RNAs have been investigated in triple-negative BC.[18] Numerous studies have demonstrated that lncRNAs regulate cancer cell biology by binding to target microRNAs, thereby preventing their interaction with mRNA.[19]

The most significant finding in our study was the differential expression of the long intergenic non-protein-coding RNA 974 gene (LINC00974). Previous studies have reported increased LINC00974 expression in hepatocellular carcinoma, gastric cancer, and oral squamous cell carcinoma.[20–22] LINC00974 exerts regulatory functions by interacting with miRNAs, for example, by sponging miR-33a to promote cell proliferation, invasion, and epithelial-mesenchymal transition in ovarian cancer through HMGB2 upregulation.[23] Biologically, LINC00974 acts as a competing endogenous RNA, modulating oncogenic pathways by sequestering miRNAs and preventing their suppressive effects on target oncogenes. These mechanisms suggest that LINC00974 may contribute to BC pathogenesis by dysregulating pathways involved in cell proliferation, metastasis, and immune evasion. From a clinical perspective, LINC00974 overexpression in peripheral blood, as observed in our study, positions it as a potential minimally invasive biomarker for early BC detection, particularly for the luminal A subtype. Its consistent dysregulation across multiple cancers underscores its potential as a diagnostic or prognostic marker, and its detection in PBMCs could facilitate non-invasive monitoring of disease progression or therapeutic response. Furthermore, targeting LINC00974–miRNA interactions may offer novel therapeutic strategies, such as RNA-based therapies to inhibit its oncogenic activity. In our study, LOC transcripts were also identified as an important group of regulatory RNAs modulating tumorigenesis. Antisense RNAs were identified as significant regulatory molecules as well. Literature data confirm the involvement of certain RNAs in BC pathogenesis.[24] The clinical predictive value of LINC RNAs can be compared to other BC markers, such as *CEA* and *CA15–3*, with promising results reported when combining these markers.[25]

This study is limited by its small sample size, lack of validation in independent cohorts, and absence of functional experiments, which may affect the generalizability of the findings. Additionally, the study population was restricted to Russian Caucasian women, limiting its applicability to other groups. RNA sequencing should be employed in future studies due to its higher sensitivity compared to microarray analysis. Despite these limitations, the high-throughput microarray-based transcriptomic analysis provided valuable insights, guiding future research directions.

## Conclusions

This study is the first to demonstrate alterations in the expression levels of lncRNAs in PBMCs of BC patients at early stages (Stage 1–2), supporting their potential as diagnostic markers. The long intergenic non-protein-coding RNA 974 gene (LINC00974) passed FDR correction (LogFC = 1.80; FDR-adjusted *p*-value [$p_{adj}$] = 0.03) and may be considered a sensitive marker for BC risk. Given its role in regulating oncogenic pathways through miRNA sponging and its detectability in peripheral blood, LINC00974 holds promise as a minimally invasive biomarker for early BC diagnosis and potentially as a therapeutic target. These findings are consistent with established concepts of lncRNA functionality, including their roles in gene expression regulation, chromatin remodeling, RNA stabilization, cell proliferation, tumor migration, and metastasis.

The next phase of this work will focus on expanding the sample size and functionally validating the identified lncRNAs. It is essential to assess reproducibility in larger patient cohorts using

alternative methods, such as quantitative polymerase chain reaction, to evaluate specific gene expression. Investigating lncRNA expression profiles in PBMCs may provide an informative and minimally invasive cknowlegments approach for studying prevalent BC subtypes.

## Acknowledgments

We want to thank Kemerovo Regional Clinical Oncological Hospital for providing research material and "Medical Genomics" Center for Collective Use for help with the experimental part.

## Funding

This study was supported by the state task on project "Immune and hormonal interactions in breast cancer" for 2024–2025, № of state registration 124041100077-1.

## Conflict of interest

The authors declare no conflicts of interest related to the publication of this article.

## Author contributions

Research design (VIM, ANG), discussion of the results, editing, revision (VIM), RNA extraction (RAT), article writing (VYB, NAG), microarray analysis, bioinformatics data analysis (RRS, AAS), and literature collection and review (MLB, YAZ). All authors have approved the final version and publication of the manuscript.

## Ethical statement

This study was approved by the Kemerovo State University Committee on Bioethics (*6 Krasnaya* St., *Kemerovo*), protocol №14, dated 28.02.2025. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2024). All participants were informed about the study's objectives and procedures and provided written consent.

## Data sharing statement

The data that support the finding of this study is publicly available.

## References

[1] Obeagu EI, Obeagu GU. Breast cancer: A review of risk factors and diagnosis. Medicine (Baltimore) 2024;103(3):e36905. doi:10.1097/MD.0000000000036905, PMID:38241592.

[2] Sun YS, Zhao Z, Yang ZN, Xu F, Lu HJ, Zhu ZY, *et al*. Risk Factors and Preventions of Breast Cancer. Int J Biol Sci 2017;13(11):1387–1397. doi:10.7150/ijbs.21635, PMID:29209143.

[3] Korsh J, Shen A, Aliano K, Davenport T. Polycyclic Aromatic Hydrocarbons and Breast Cancer: A Review of the Literature. Breast Care (Basel) 2015;10(5):316–318. doi:10.1159/000436956, PMID:26688678.

[4] Buus R, Szijgyarto Z, Schuster EF, Xiao H, Haynes BP, Sestak I, *et al*. Development and validation for research assessment of Oncotype DX® Breast Recurrence Score, EndoPredict® and Prosigna®. NPJ Breast Cancer 2021;7(1):15. doi:10.1038/s41523-021-00216-w, PMID:33579961.

[5] Markou A, Tzanikou E, Lianidou E. The potential of liquid biopsy in the management of cancer patients. Semin Cancer Biol 2022;84:69–79. doi:10.1016/j.semcancer.2022.03.013, PMID:35331850.

[6] Li J, Guan X, Fan Z, Ching LM, Li Y, Wang X, *et al*. Non-Invasive Biomarkers for Early Detection of Breast Cancer. Cancers (Basel) 2020;12(10):2767. doi:10.3390/cancers12102767, PMID:32992445.

[7] Liu YR, Jiang YZ, Xu XE, Yu KD, Jin X, Hu X, *et al*. Comprehensive transcriptome analysis identifies novel molecular subtypes and subtype-specific RNAs of triple-negative breast cancer. Breast Cancer Res 2016;18(1):33. doi:10.1186/s13058-016-0690-8, PMID:26975198.

[8] Yuen S, Monzawa S, Gose A, Yanai S, Yata Y, Matsumoto H, *et al*. Impact of background parenchymal enhancement levels on the diagnosis of contrast-enhanced digital mammography in evaluations of breast cancer: comparison with contrast-enhanced breast MRI. Breast Cancer 2022;29(4):677–687. doi:10.1007/s12282-022-01345-1, PMID:35220511.

[9] Hidar S, Alimi A, Khlifi A, Chachia S, Kaabia O, Bouguizane S, *et al*. Indocyanine Green Fluorescence-Guided Sentinel Node Biopsy in Breast Cancer Within a North African Population: A Retrospective Study. Eur J Breast Health 2021;17(4):352–355. doi:10.4274/ejbh.galenos.2021.2021-4-11, PMID:34651114.

[10] Berkel C, Cacan E. Half of most frequently mutated genes in breast cancer are expressed differentially between premenopausal and postmenopausal breast cancer patients. Cancer Genet 2024;286-287:11–17. doi:10.1016/j.cancergen.2024.06.001, PMID:38879914.

[11] Dumeaux V, Ursini-Siegel J, Flatberg A, Fjosne HE, Frantzen JO, Holmen MM, *et al*. Peripheral blood cells inform on the presence of breast cancer: a population-based case-control study. Int J Cancer 2015;136(3):656–667. doi:10.1002/ijc.29030, PMID:24931809.

[12] Čelešnik H, Potočnik U. Blood-Based mRNA Tests as Emerging Diagnostic Tools for Personalised Medicine in Breast Cancer. Cancers (Basel) 2023;15(4):1087. doi:10.3390/cancers15041087, PMID:36831426.

[13] Hensler M, Vančurová I, Becht E, Palata O, Strnad P, Tesařová P, *et al*. Gene expression profiling of circulating tumor cells and peripheral blood mononuclear cells from breast cancer patients. Oncoimmunology 2016;5(4):e1102827. doi:10.1080/2162402X.2015.1102827, PMID:27141386.

[14] Čelešnik H, Potočnik U. Peripheral Blood Transcriptome in Breast Cancer Patients as a Source of Less Invasive Immune Biomarkers for Personalized Medicine, and Implications for Triple Negative Breast Cancer. Cancers (Basel) 2022;14(3):591. doi:10.3390/cancers14030591, PMID:35158858.

[15] Foulds GA, Vadakekolathu J, Abdel-Fatah TMA, Nagarajan D, Reeder S, Johnson C, *et al*. Immune-Phenotyping and Transcriptomic Profiling of Peripheral Blood Mononuclear Cells From Patients With Breast Cancer: Identification of a 3 Gene Signature Which Predicts Relapse of Triple Negative Breast Cancer. Front Immunol 2018;9:2028. doi:10.3389/fimmu.2018.02028, PMID:30254632.

[16] Ransohoff JD, Wei Y, Khavari PA. The functions and unique features of long intergenic non-coding RNA. Nat Rev Mol Cell Biol 2018;19(3):143–157. doi:10.1038/nrm.2017.104, PMID:29138516.

[17] Quinn JJ, Chang HY. Unique features of long non-coding RNA biogenesis and function. Nat Rev Genet 2016;17(1):47–62. doi:10.1038/nrg.2015.10, PMID:26666209.

[18] Singh DD, Lee HJ, Yadav DK. Recent Clinical Advances on Long Non-Coding RNAs in Triple-Negative Breast Cancer. Cells 2023;12(4):674. doi:10.3390/cells12040674, PMID:36831341.

[19] Zhou X, Zhang W, Jin M, Chen J, Xu W, Kong X. lncRNA MIAT functions as a competing endogenous RNA to upregulate DAPK2 by sponging miR-22-3p in diabetic cardiomyopathy. Cell Death Dis 2017;8(7):e2929. doi:10.1038/cddis.2017.321, PMID:28703801.

[20] Tang J, Zhuo H, Zhang X, Jiang R, Ji J, Deng L, *et al*. A novel biomarker Linc00974 interacting with KRT19 promotes proliferation and metastasis in hepatocellular carcinoma. Cell Death Dis 2014;5(12):e1549. doi:10.1038/cddis.2014.518, PMID:25476897.

[21] Gao H, Yin Y, Qian A, Guo R, Qi J. LncRNA LINC00974 Upregulates CDK6 to Promote Cell Cycle Progression in Gastric Carcinoma. Cancer Biother Radiopharm 2019;34(10):666–670. doi:10.1089/cbr.2019.2904, PMID:31596614.

[22] Tian Y, Zhong L, Gao S, Yu Y, Sun D, Liu X, *et al*. LncRNA LINC00974 Downregulates miR-122 to Upregulate RhoA in Oral Squamous

Cell Carcinoma. Cancer Biother Radiopharm 2021;36(1):18–22. doi:10.1089/cbr.2019.2907, PMID:31702382.
[23] Liu W, Cheng J. LINC00974 sponges miR-33a to facilitate cell proliferation, invasion, and EMT of ovarian cancer through HMGB2 upregulation. Genet Mol Biol 2022;45(1):e20210224. doi:10.1590/1678-4685-GMB-2021-0224, PMID:35129574.
[24] Jadaliha M, Gholamalamdari O, Tang W, Zhang Y, Petracovici A, Hao Q, *et al*. A natural antisense lncRNA controls breast cancer progression by promoting tumor suppressor gene mRNA stability. PLoS Genet 2018;14(11):e1007802. doi:10.1371/journal.pgen.1007802, PMID:30496290.
[25] Zaleski M, Kobilay M, Schroeder L, Debald M, Semaan A, Hettwer K, *et al*. Improved sensitivity for detection of breast cancer by combination of miR-34a and tumor markers CA 15-3 or CEA. Oncotarget 2018;9(32):22523–22536. doi:10.18632/oncotarget.25077, PMID:29854296.