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Original Article

Expression profiles and regulatory crosstalk of lncRNA HOTAIR and miR-29b-3p across breast cancer subtypes: Implications for tumor progression and therapy resistance

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ABSTRACT

Objectives: This study explores the interaction between HOX transcript antisense RNA (HOTAIR) and miR-29b-3p across three different breast cancer subtypes, aiming to identify new potential therapeutic targets.

Materials and methods: Quantitative polymerase chain reaction was used to analyze the expression levels of HOTAIR and miR-29b-3p in three breast cancer cell lines: MCF-7 (estrogen receptor-positive: ER^+), MDA-MB-453 (human epidermal growth factor receptor 2-positive: HER2+), and MDA-MB-231 (triple-negative breast cancer: TNBC). MCF-10A cells were used as the normal control. GAPDH and U6 served as housekeeping genes. Gene expression was calculated using the 2-ΔΔCt method, with statistical significance defined as $p<0.05$.

Results: In MCF-7 cells, both HOTAIR (0.48-fold) and miR-29b-3p (0.18-fold) were significantly downregulated ($p<0.05$). In MDA-MB-231 cells, HOTAIR expression was moderately reduced (0.82-fold), while miR-29b-3p was increased (1.87-fold), although not statistically significant ($p>0.05$). In HER2+ MDA-MB-453 cells, HOTAIR was slightly elevated (1.06-fold) and miR-29b-3p modestly decreased (0.96-fold), also without statistical significance. These findings highlight subtype-specific differences in the HOTAIR/miR-29b-3p axis.

Conclusion: Our study reveals differential regulation of the HOTAIR/miR-29b-3p axis across breast cancer subtypes, suggesting its potential role in shaping tumor progression and therapy resistance. Findings reveal variable regulation of the HOTAIR/miR-29b-3p axis in breast cancer, highlighting its potential for biomarker and targeted therapy development in TNBC. Further validation with clinical datasets is recommended.

Keywords: Breast cancer, ceRNA, EMT, HOTAIR, miR-29b-3p.

Globally, breast cancer ranks as the leading cancer affecting women, accounting for 11.6% of total cases-just ahead of lung cancer at 11.4%. With around 2.3 million new diagnoses every year, it continues to be a serious health concern.[1] Despite advancements in diagnostic tools and treatments, breast cancer remains a major global health issue, especially in subtypes like triple-negative breast cancer (TNBC), which is linked to poor prognosis, high recurrence rates, and limited treatment options.[2,3] Molecular profiling is capable of identifying four primary breast cancer subtypes, which are designated as luminal A, luminal B, human epidermal growth factor receptor 2 (HER2)-enriched, and triple-negative, respectively. Each of these subtypes has been found to be associated with specific biological traits and clinical results.[3] Researchers typically use specific cell lines to study these subtypes: MCF-7 for hormone-sensitive breast cancer, MDA-MB-453 for HER2+ tumors, and MDA-MB-231 for TNBC due to its invasive and metastatic properties (Table 1).[4,5] Non-coding RNAs (ncRNAs) are assuming an increasingly pivotal function in the regulation of cancerous development and advancement, with a mounting emphasis on long non-coding RNAs (lncRNAs) and microRNAs (miRNAs).[6,7] Through competing endogenous RNA (ceRNA) interactions, lncRNA molecules regulate critical pathways involved in cell growth, metastasis, and immune suppression.[8,9] Among ncRNAs, HOX transcript antisense RNA (HOTAIR) has become one of the most studied lncRNAs, recognized as a powerful oncogene that

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Table 1. Breast cancer molecular subtypes and representative cell lines used in this study

Molecular subtype	Cell line	Receptor status	Key characteristics
Luminal A/B (ER^+)	MCF-7 (ATCC® HTB-22™)	ER^+ / PR^+, $HER2^-$	Hormone-sensitive, low invasiveness, widely used in endocrine therapy studies
HER2-enriched	MDA-MB-453 (ATCC® HTB-131™)	ER^- / PR^-, $HER2^+$	HER2 amplification, aggressive growth, model for anti-HER2 therapies
Triple-negative (TNBC)	MDA-MB-231 (ATCC® HTB-26™)	ER^- / PR^- / $HER2^-$	Highly invasive and metastatic, model for aggressive and therapy-resistant tumors

ER^+: Estrogen receptor-positive: HER2: Human epidermal growth factor receptor 2.

promotes epithelial-mesenchymal transition (EMT), invasion, and metastasis, and epigenetic silencing of tumor suppressor genes.[10,11] Notably, the HOTAIR/miR-29b-3p axis has recently been identified as a key driver of breast cancer progression by influencing EMT and metastasis-related signaling pathways. Higher HOTAIR levels, along with lower miR-29b-3p expression, have been associated with poorer clinical outcomes in breast cancer patients.[11,12]

The present study aims to elucidate the regulatory relationships between HOTAIR, miR-29b-3p, and the various breast cancer subtypes. The objective is to identify new therapeutic targets for more effective, subtype-specific and personalized treatment, and to facilitate the development of new drugs.

MATERIALS AND METHODS

In vitro experiments

All studies were conducted at the Demiroğlu Science University Research Laboratory. MCF-7 (HTB-22™), MDA-MB-453 (HTB-131™), and MDA-MB-231 (CRM-HTB-26™) breast cancer cell lines were sourced from ATCC, Manassas, USA. Cells were maintained in RPMI-1640 (Sigma-Aldrich, Darmstadt, Germany) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C in 5% CO_2 for 48 h. Cells were seeded at 5×10^5 per well in six-well plates and collected at 80-90% confluence for RNA extraction. Additionally, MCF-10A served as the normal epithelial control (CRL-10317, ATCC, Manassas, USA).

RNA isolation and complementary DNA synthesis

Total RNA was isolated using the miRNeasy Advanced Kit (Qiagen, Hilden, Germany), and its quality was confirmed by evaluating absorbance ratios. Complementary DNA synthesis for both lncRNA and miRNA was carried out using Qiagen kits (Qiagen, Hilden, Germany), following the manufacturer's protocols.

Quantitative real-time polymerase chain reaction (PCR)

Gene expression analysis was performed using SYBR Green-based quantitative polymerase chain reaction (qPCR) (Qiagen, Hilden, Germany), with reactions conducted on a Rotor-Gene Q system (Qiagen, Hilden, Germany) using the cycling conditions summarized in Table 2. GAPDH (GeneGlobe ID: PPH00150F, Catalog Number: 330001) and U6 (GeneGlobe ID: YP02119464, Catalog Number: 339306) served as internal reference

Table 2. Real-time polymerase chain reaction cycling conditions

Step	Temperature (°C)	Time	Cycles
Initial denaturation	95	10 min	1
Denaturation	95	15 sec	40
Annealing/extension	60	60 sec	
Melt curve analysis*	60 → 95	Incremental	1

* Melt curve analysis was performed to verify product specificity.

genes. To ensure the reliability of the results, both technical and biological replicates were run in triplicate.

Statistical analysis

Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method. A fold change of ≥2 or ≤0.5 was considered biologically significant. Statistical differences were assessed using Student's t-test.

RESULTS

Differential regulation of miR-29b-3p and lncRNA HOTAIR in breast cancer subtypes

We examined the expression levels of miR-29b-3p and lncRNA HOTAIR in MCF-7, MDA-MB-453, and MDA-MB-231 cell lines using qPCR. In MCF-7 cells, both lncRNA HOTAIR (0.48) and miR-29b-3p (0.18) showed significantly decreased expression, as shown in Figure 1 ($p<0.05$). In the triple-negative breast cancer cell line, HOTAIR expression (0.82) was reduced while miR-29b-3p (1.87) was increased, although the changes were not statistically significant (Figure 1, $p>0.05$). In the HER2+ cell line, HOTAIR expression (1.06) was slightly elevated, and miR-29b-3p expression (0.96) was decreased, but again without statistical significance (Figure 1, $p>0.05$). All expression changes reported in this section are based on the MCF-10A control group, unless stated otherwise. Upregulation means an increase compared to MCF-10A cells, while downregulation means a decrease compared to MCF-10A. These findings suggest that the HOTAIR–miR-29b-3p regulatory axis may operate differently across breast cancer subtypes, providing important insights into lncRNA-driven mechanisms that affect drug sensitivity and resistance, especially in triple-negative breast cancer. Error bars indicate standard deviation.

DISCUSSION

Our research sheds light on the intricate regulation of the HOTAIR/miR-29b-3p axis in various breast cancer subtypes, highlighting its influence on disease progression and treatment outcomes. HOTAIR, a well-established oncogenic lncRNA, plays its role through chromatin remodeling, ceRNA interactions, and epigenetic modifications.[10,13] Elevated HOTAIR expression has been consistently associated with worse prognosis, resistance to therapy, and increased metastatic potential in breast cancer cases.[14,15]

Conversely, miR-29b-3p functions as a tumor suppressor, controlling key genes tied to extracellular matrix remodeling, DNA methylation, and EMT.[12,16] The inverse dynamic between HOTAIR and miR-29b-3p points to a regulatory pathway that could affect crucial oncogenic mechanisms, such as DNA methyltransferase 3 beta-mediated epigenetic silencing and matrix metalloproteinase-driven cellular invasion. This interplay is especially critical in TNBC, a form that currently lacks targeted therapeutic options.[17]

From a clinical standpoint, the HOTAIR/miR-29b-3p axis has strong potential as both a biomarker and a treatment target. Preclinical models show that knocking down HOTAIR

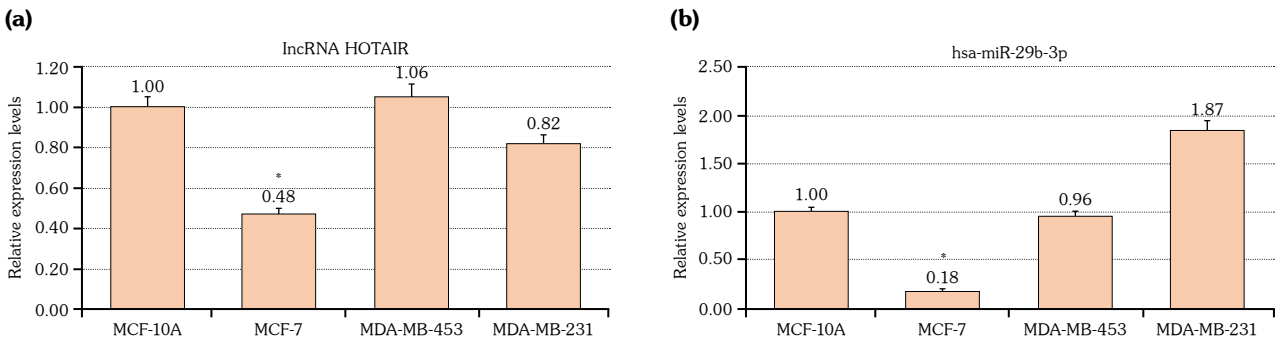

Figure 1. (a) Relative expression levels of lncRNA HOTAIR and **(b)** hsa-miR-29b-3p in breast cell lines.
* $p<0.05$.

reactivates miR-29b, curbs tumor growth, and boosts the effectiveness of endocrine therapies.[15,18] Moreover, nanoparticle-based delivery systems for miRNA mimics help overcome challenges like molecular instability and targeting precision, pushing RNA therapies closer to clinical application.[19] As lncRNA-focused treatments gain momentum in precision medicine, targeting this axis becomes increasingly relevant.[20]

Importantly, this axis also contributes to resistance against chemotherapy and hormone therapies. High HOTAIR levels disrupt ceRNA networks and chromatin configurations, complicating treatment efforts.[10,13] Integrating lncRNA and miRNA profiles into diagnostic routines may refine patient classification and personalize therapeutic strategies.[20]

However, this study's reliance solely on established cell lines and its limited subtype representation reduces its immediate clinical applicability. Broader subtype inclusion and patient-derived samples are needed to deepen our understanding.

In conclusion, the HOTAIR/miR-29b-3p axis represents a key molecular intersection connecting epigenetic control, EMT, and treatment resistance across breast cancer types. Future research should prioritize clinical validation and development of axis-targeted therapies, with the aim of improving outcomes, especially for TNBC patients.

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Author Contributions: Idea/concept, design, control/supervision, data collection and/or processing, analysis and/or interpretation, literature review, writing the article, references and fundings, materials Ş.A., Ş.D.K., critical review Ş.A.

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