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Research Article

Functional and clinical analysis of the HOTAIR–mir34a–CCND1 axis in BRCA1-mutated ovarian cancer

BRCA1 mutasyonlu over kanserinde HOTAIR –mir34a– CCND1 ekseninin fonksiyonel ve klinik analizi

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Abstract

Aim: BRCA1-mutated ovarian cancer is characterized by impaired DNA double-strand break repair and homologous recombination deficiency, leading to distinct tumor biology and therapeutic responses. Competing endogenous RNA networks have emerged as important regulatory mechanisms in cancer progression. This study aimed to investigate the functional and clinical significance of the HOTAIR–miR34a–CCND1 axis in BRCA1-mutated ovarian cancer.

Material and Methods: Transcriptomic and clinical data of high-grade serous ovarian cancer patients were obtained from the TCGA-OV cohort via the GDC portal. Differential gene expression analysis was performed using edgeR, while pathway enrichment analysis was conducted with clusterProfiler and KEGG. Survival analyses were evaluated using Kaplan–Meier curves.

Results: The HOTAIR–miR34a–CCND1 axis displayed distinct expression profiles in BRCA1-mutated ovarian cancer tissues compared to wild-type cases. Kaplan–Meier survival analysis revealed no statistically significant differences in overall survival based on the expression levels of these genes ($p > 0.05$). However, KEGG pathway enrichment analysis demonstrated that these genes are involved in cancer-associated microRNA pathways, suggesting their potential role in tumor progression despite the lack of direct survival association.

Conclusion: The HOTAIR–miR-34a–CCND1 axis may represent a potential prognostic biomarker and therapeutic target in BRCA1-mutated ovarian cancer, supporting the development of novel strategies such as HOTAIR inhibition, miR-34a replacement, or combination with PARP and CDK4/6 inhibitors.

Keywords: BRCA1 mutation, ovarian neoplasms, HOTAIR, microRNAs, CCND1

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Öz

Amaç: BRCA1 mutasyonlu over kanseri, DNA çift sarmal kırıklarının onarımında ve homolog rekombinasyon mekanizmasında bozulma ile karakterizedir; bu durum tümör biyolojisi ve tedavi yanıtlarında belirgin farklılıklara yol açar. Rekabetçi endojen RNA (ceRNA) ağlarının kanser progresyonunda önemli düzenleyici mekanizmalar olduğu gösterilmiştir. Bu çalışma, BRCA1 mutasyonlu over kanserinde HOTAIR–miR-34a–CCND1 ekseninin fonksiyonel ve klinik önemini araştırmayı amaçlamıştır.

Gereç ve Yöntemler: Yüksek dereceli seröz over kanseri (HGSOC) hastalarına ait transkriptomik ve klinik veriler, TCGA-OV kohortundan GDC portalı aracılığıyla elde edilmiştir. Diferansiyel gen ekspresyon analizi edgeR paketiyle, yol zenginleştirme analizi ise clusterProfiler ve KEGG veritabanları kullanılarak gerçekleştirilmiştir. Sağkalım analizleri Kaplan–Meier eğrileriyle değerlendirilmiştir.

Bulgular: HOTAIR–miR-34a–CCND1 ekseni, BRCA1 mutasyonlu over kanseri dokularında vahşi tip olgulara göre farklı ekspresyon profilleri sergilemiştir. Kaplan–Meier sağkalım analizleri, bu genlerin ekspresyon düzeyleriyle genel sağkalım arasında istatistiksel olarak anlamlı bir fark göstermemiştir ($p > 0.05$). Bununla birlikte, KEGG yol zenginleştirme analizi, bu genlerin kanserle ilişkili mikroRNA yollarında yer aldığını ortaya koymuştur; bu da doğrudan sağkalım ilişkisi olmamasına rağmen tümör progresyonunda olası rollerini desteklemektedir.

Sonuç: HOTAIR–miR-34a–CCND1 ekseni, BRCA1 mutasyonlu over kanserinde potansiyel bir prognostik biyobelirteç ve terapötik hedef olabilir. Bu bulgular, HOTAIR inhibisyonu, miR-34a replasman tedavisi veya PARP ve CDK4/6 inhibitörleriyle kombinasyon stratejileri gibi yeni tedavi yaklaşımlarının geliştirilmesini desteklemektedir.

Anahtar Kelimeler: BRCA1 mutasyonu, over neoplazmları, HOTAIR, MikroRNA'lar, CCND1

Introduction

Ovarian cancer remains one of the leading causes of gynecological cancer-related mortality worldwide, with high-grade serous ovarian carcinoma (HGSOC) being the most common and aggressive histological subtype [1]. Mutations in the BRCA1 (Breast Cancer) gene play a critical role in ovarian carcinogenesis, as they impair homologous recombination repair of DNA (Deoxyribonucleic acid) double-strand breaks, thereby contributing to genomic instability and altered therapeutic responses [2,3]. Significantly, BRCA1 mutation carriers often benefit from targeted therapies, such as PARP (Poly(ADP-ribose) polymerase) inhibitors, yet variability in prognosis and treatment outcomes suggests additional molecular regulators may be involved [4]. In recent years, competing endogenous RNA (ceRNA) networks, based on the interactions among long non-coding RNAs (lncRNAs), microRNAs (miRNAs), and messenger RNAs (mRNA), have emerged as key post-transcriptional regulatory mechanisms in cancer biology [5]. Dysregulation of ceRNA axes has been implicated in tumor initiation, progression, and chemoresistance across multiple cancer types, including ovarian cancer [6]. Among these, the HOTAIR–miR-34a–D1 (Cyclin D1) axis has drawn particular attention. HOTAIR, a lncRNA known for its oncogenic function, promotes proliferation, invasion, and metastasis in several malignancies [7].

This study aimed to investigate the functional and clinical significance of the HOTAIR–miR-34a–CCND1 competing endogenous RNA axis in BRCA1-mutated ovarian cancer by analyzing transcriptomic and clinical data from the TCGA-OV (The Cancer Genome Atlas - Ovarian Serous Cystadenocarcinoma) cohort.

Material And Methods

This study was conducted at the Departments of Obstetrics and Gynecology and Medical Biology, Faculty of Medicine, Selçuk University, as a retrospective data analysis using TCGA datasets.

This retrospective study was conducted using transcriptomic and clinical data from patients with high-grade serous ovarian carcinoma included in The Cancer Genome Atlas (TCGA) - Ovarian Serous Cystadenocarcinoma cohort. Although TCGA data are publicly available and de-identified, the study protocol was reviewed and approved by the Selçuk University Institutional Ethics Committee (Ethics Approval No: E.1103897). The study was performed in accordance with the principles of the Declaration of Helsinki, and all procedures involving human participants were conducted in compliance with relevant institutional guidelines and regulations.

A total of 429 patients with high-grade serous ovarian cancer were initially retrieved from the TCGA-OV cohort.

Patients diagnosed with high-grade serous ovarian carcinoma were retrospectively evaluated for inclusion in the study. The inclusion criteria required a confirmed BRCA1 mutation status and corresponding RNA-seq expression profiles. Patients were excluded based on the following criteria: lack of confirmed BRCA1 mutation status, incomplete or missing clinical data, insufficient RNA sequencing quality, or inadequate follow-up survival information. After a rigorous screening process, a total of 412 patients met the criteria and were included in the final analysis, comprising 37 BRCA1-mutant (n = 37) and 375 BRCA1 wild-type (n = 375) cases.

Bioinformatics Tools and Statistical Analysis

All bioinformatics and statistical analyses were performed using R software (version 4.3.1; R Foundation for Statistical Computing, Vienna, Austria). Transcriptomic data and related clinical information were obtained from The Cancer Genome Atlas (TCGA) database via the TCGAbiolinks package (v2.28.3). Raw count data were preprocessed by filtering out genes showing low expression and normalizing library sizes to ensure inter-sample comparability.

Differential gene expression analysis was performed using the edgeR package (v3.42.4). Gene expression data were modeled under the assumption of a negative binomial distribution, and dispersion estimates were calculated prior to statistical testing. To control for errors arising from multiple comparisons, p-values were corrected using the Benjamini–Hochberg false discovery rate (FDR) method. Genes with an adjusted p-value < 0.05 and an absolute $\log_2$ fold change > 1 were considered differentially expressed genes (DEG).

Functional enrichment analyses were performed using the clusterProfiler package (v4.10.0) to reveal the relationship between differentially expressed genes and biological processes and signaling pathways. In this context, pathway analyses from the Kyoto Encyclopedia of Genes and Genomes (KEGG) were applied to identify significantly enriched biological pathways. Survival analyses were performed using the survival package (v3.5-7) and the survminer package (v0.4.9). The relationship between clinical outcomes and gene expression levels was analyzed using Kaplan–Meier survival curves, and the log-rank test was used to evaluate differences between groups. Data manipulation, statistical summarization, and visualization were performed using the dplyr (v1.1.4) and ggplot2 (v3.5.1) packages. The resulting graphs and tables were prepared to facilitate the interpretation of the analysis results.

Results

The expression levels of HOTAIR, miR-34a, and CCND1 were compared between BRCA1-mutant and wild-type ovarian cancer patients. Statistical analysis revealed no significant differences between the two groups (CCND1, p=0.970; HOTAIR, p=0.454). The expression of miR-34a was detected at very low levels across the cohort and was therefore considered unsuitable for grouping and excluded from further analysis (Table 1).

Table 1. Gene parameters and their relationship.

Gen	p-value	comment
CCND1	0.970	There is no difference in expression
HOTAIR	0.454	The difference in expression is not significant
MIR34A	NaN	Test failed (values are probably fixed/null)

Functional analysis using KEGG pathway enrichment demonstrated that HOTAIR, miR-34a, and CCND1 were significantly enriched in the "MicroRNAs in cancer" pathway ($p < 0.05$). Additional enrichment was observed in pathways related to thyroid cancer, bladder cancer, and endometrial cancer.

The distribution of gene expression levels in BRCA1-mutant and wild-type groups was visualized using boxplot graphs '(Figure 1)'. Kaplan–Meier survival analysis showed no significant difference in overall survival between high- and low-expression groups for HOTAIR (p=0.95) and CCND1 (p=0.7). Survival analysis for miR-34a could not be performed due to uniformly low expression levels.

The findings of this study are presented through gene expression comparisons '(Table 1)', KEGG pathway enrichment results, distribution plots '(Figure 1)' , and Kaplan–Meier survival curves '(Figures 2,3)'.

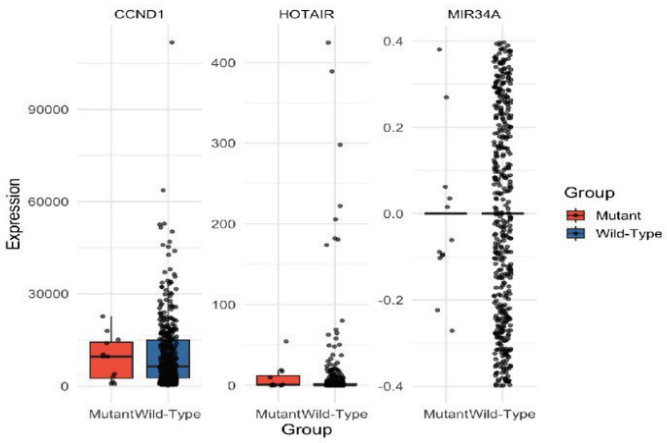

Figure 1. Gene expression analysis (BRCA1 Mutant vs Wild-Type)

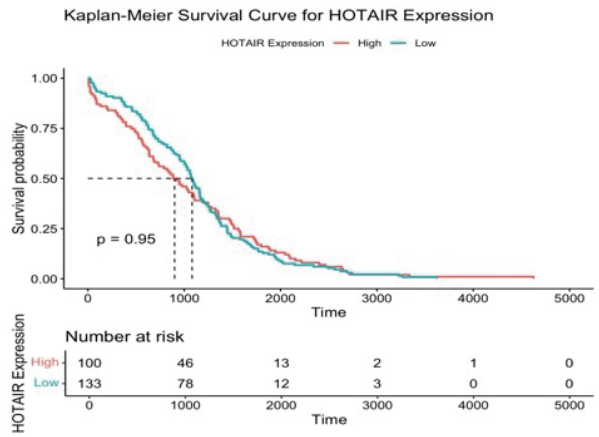

Figure 2. Kaplan-Meier Survival Analyses.

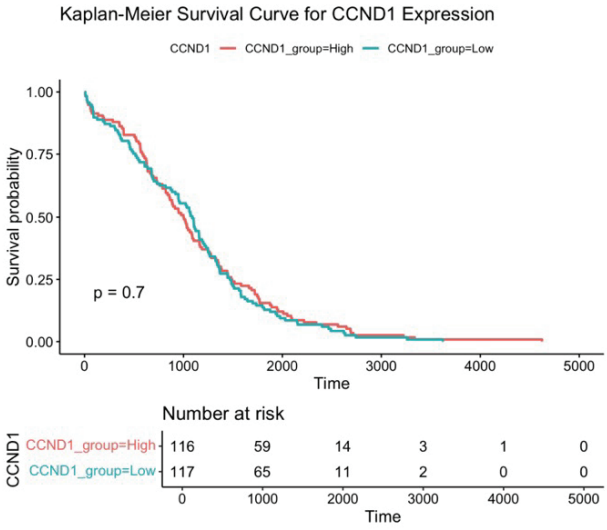

Figure 3. CCND1: Survival curves.

Discussion

BRCA1 mutations have long been known to cause flaws in DNA double-strand break repair, which has a significant impact on tumor biology and treatment outcomes [8,9]. BRCA1 deficiency has been linked in clinical practice to sensitivity to PARP inhibitors, namely in the subgroup of high-grade serous ovarian tumors; nonetheless, the variability of responses has underscored the need for additional biomarkers [10,11]. While these studies highlight the prognostic and therapeutic importance of cell cycle regulators in the context of BRCA1 mutations, the lack of a significant association between the expression levels of the HOTAIR–miR-34a–CCND1 axis and overall survival in BRCA1-mutant ovarian cancer in our study suggests that this axis may be effective at the level of early stages of tumor biology, signaling pathways, or microRNA-mediated regulatory mechanisms, rather than clinical outcomes. This reflects the molecular heterogeneity of BRCA1-mutant ovarian cancer and indicates that this axis, rather than being a prognostic marker alone, may gain clinical significance when evaluated in conjunction with targeted therapies such as PARP and CDK4/6 inhibitors.

Long non-coding RNAs (lncRNAs), microRNAs (miRNAs), and mRNAs form competing endogenous RNA (ceRNA) networks that have been demonstrated to play important regulatory roles in cancer progression, metastasis, and drug resistance in recent years. These networks offer a framework for the discovery of new molecular targets [12,13]. While these findings support the idea that HOTAIR has a strong biological effect in ovarian cancer, the lack of a significant association between the HOTAIR–miR-34a–CCND1 axis and overall survival in BRCA1-mutant ovarian cancer in our study suggests that the effects of this axis may manifest at the level of cellular signaling pathways and microRNA-mediated regulatory mechanisms rather than clinical outcomes. Furthermore, this difference can be explained by the molecular heterogeneity of BRCA1-mutant ovarian cancer and the ability of HOTAIR to exert context-dependent effects through different miRNA targets. Therefore, rather than contradicting the oncogenic functions of HOTAIR reported in the literature, our current findings suggest that HOTAIR may play a more complex and indirect regulatory role in BRCA1-mutated tumors.

In this study, we analyzed the HOTAIR–miR-34a–CCND1 axis in BRCA1-mutated ovarian cancer using TCGA-OV data. Although no significant differences in overall survival were observed, this axis showed distinct expression patterns and enrichment in cancer-related microRNA pathways, suggesting a possible biological role in tumor progression. HOTAIR has been reported as an oncogenic long non-coding RNA that promotes chromatin remodeling and metastasis, with overexpression associated with poor prognosis in breast, ovarian, and cervical cancers [14]. Similarly, CCND1 overexpression has been linked to uncontrolled proliferation and adverse outcomes in multiple cancer types. In contrast, miR-34a is known as a tumor suppressor involved in apoptosis and cell cycle control, often downregulated in malignancies [15]. The fact that the HOTAIR–miR-34a–CCND1 axis did not show a significant association with survival in BRCA1-mutant ovarian cancer in our study suggests that these molecules may play specific regulatory roles rather than prognostic indicators in BRCA1-mutated tumors, and that their effects may occur more at

the level of cellular signaling networks, and should be considered from this perspective. The strengths of this study include the use of a large, well-characterized public dataset and standardized bioinformatics methods, such as edgeR, clusterProfiler, and Kaplan–Meier analyses. However, limitations should be acknowledged. These include the retrospective design, reliance on a single cohort, lack of experimental validation, and the inability to evaluate survival for miR-34a due to uniformly low expression levels. Additionally, potential confounders such as treatment regimen and residual disease status could not be fully controlled . From a clinical perspective, although survival significance was not observed, the enrichment of this axis in oncogenic pathways highlights its potential as a biomarker. Targeting HOTAIR, restoring miR-34a, or inhibiting CCND1 with CDK4/6 inhibitors may provide therapeutic benefit, especially in combination with PARP inhibitors already used in BRCA1-mutated ovarian cancer . Future studies should validate these findings in independent cohorts and functional models. Experimental confirmation using qPCR, immunohistochemistry, or RNAscope, along with liquid biopsy approaches for circulating RNA detection, could clarify the clinical applicability of the HOTAIR–miR-34a–CCND1 axis.

Limitations of the study

This study has several limitations. First, the analyses were performed retrospectively based solely on the TCGA-OV cohort, and the lack of validation in independent and prospective patient groups limits generalizability. Second, the very low levels of miR-34a expression detected across the cohort prevented survival analyses for this molecule and restricted a comprehensive evaluation of the clinical effects of all components of the HOTAIR–miR-34a–CCND1 axis. Furthermore, potential confounding clinical variables such as treatment regimens, residual disease status, and tumor microenvironment could not be included in the analyses. Finally, the study's reliance solely on bioinformatics analyses and the lack of experimental validation (in vitro or in vivo) limit the interpretation of the findings at the level of functional mechanisms

Future studies should be conducted in independent cohorts and with prospective designs to more comprehensively elucidate the role of the HOTAIR–miR-34a–CCND1 axis in BRCA1-mutant ovarian cancer. Experimental validation of the biological effects of this axis using qPCR, immunohistochemistry, RNAscope, and functional cell culture models is particularly important. Furthermore, liquid biopsy-based approaches to miR-34a low expression and investigation of epigenetic regulatory mechanisms could further elucidate the clinical potential of this microRNA. From a clinical perspective, HOTAIR suppression, miR-34a replacement strategies, and the evaluation of CCND1/CDK4/6 inhibitors in combination with PARP inhibitors could contribute to the development of novel targeted therapeutic approaches for BRCA1-mutant ovarian cancer.

In conclusion, while this study reveals that the HOTAIR–miR-34a–CCND1 axis is not directly associated with survival in BRCA1-mutant ovarian cancer, considering the involvement of this molecular network in cancer-associated microRNA pathways and its reported oncogenic roles in the literature, it suggests that it may play an indirect but potentially clinically significant regulatory role in tumor biology and targeted therapy strategies in BRCA1-mutated tumors.

The differential expression profile of the HOTAIR–miR-34a–CCND1 axis in BRCA1-mutated ovarian cancer suggests that these pathways play a significant role in tumor biology. However, given the complex molecular structure of the disease, it is crucial to comprehensively investigate other signaling networks and regulatory mechanisms interacting with this pathway, rather than focusing solely on a single axis. Elucidating the entire biological network and inter-pathway interactions will contribute to a better understanding of the molecular map of the disease and provide a stronger scientific foundation for future targeted and combination-based therapeutic strategies.

Declaration of conflicting interests

The authors declare they have no conflicts of interest.

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Ethics approval

This study was approved by the Selçuk University Institutional Ethics Committee (Ethics Approval No: E.1103897).

Authors' contribution

BGÖ: Concept, Design, Analysis and/or Interpretation, Writing of the Article. AB: Materials, Data Collection and/or Processing, Analysis and/or Interpretation, Critical Review. ÇÇ: Supervision, Materials, Critical Review, Final Approval. HA: Methodology, Analysis and/or Interpretation, Data Collection and/or Processing.

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