

ORIGINAL ARTICLE

Iran J Allergy Asthma Immunol

December 2024; 23(6):738-745.

DOI: 10.18502/ijaa.v23i6.17382

Bioinformatics-driven Identification of lncRNA LINC02381 in Mediating Cisplatin Resistance via IL-12 Induced Wnt/TCF7 Signaling in Ovarian Cancer

Zeinab Karbalaee Pazoki¹, Mostafa Hosseini², Shiva Irani³, and Bahram Mohammad Soltani⁴

¹ Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran

² Chemical Injuries Research Center, System Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran

³ Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran

⁴ Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran

Received: 15 October 2024; Received in revised form: 26 October 2024; Accepted: 28 October 2024

ABSTRACT

Cisplatin resistance presents a considerable hurdle in the treatment of ovarian cancer, significantly impacting patient outcomes and limiting the effectiveness of chemotherapy. This study employs advanced bioinformatics techniques-including RNA sequencing (RNA-seq), DNA sequencing (DNA-seq), and chromatin immunoprecipitation sequencing (ChIP-seq)-to elucidate the molecular mechanisms underlying this resistance, with a particular focus on the long non-coding RNA (lncRNA) *LINC02381*.

Our findings reveal that *LINC02381* is significantly upregulated in ovarian cancer cells exhibiting resistance to cisplatin, suggesting its pivotal role in mediating this phenomenon. We further demonstrate that cytokines, particularly interleukin-12 (*IL-12*), secreted by immune cells within the tumor microenvironment, activate the Wnt signaling pathway. This activation leads to the binding of the transcription factor *TCF7* to the promoter region of *LINC02381*, resulting in enhanced expression of this lncRNA.

Notably, this interaction establishes a positive feedback loop in which *LINC02381* not only promotes its own expression but also amplifies Wnt signaling activity. This cascade ultimately drives the upregulation of ATP-binding cassette (*ABC*) transporters, which are crucial for the efflux of cisplatin from cancer cells. Thus, the drug's intracellular concentration is reduced, and cell survival under chemotherapy pressure is facilitated. These insights uncover a novel mechanism of cisplatin resistance driven by the *IL-12/Wnt/TCF7/LINC02381* axis, highlighting the complex interplay between immune signaling and drug resistance in ovarian cancer.

Our findings suggest that targeting this regulatory pathway may offer promising therapeutic strategies to overcome chemotherapy resistance, paving the way for improved treatment outcomes in patients with ovarian cancer. Future research should focus on validating these mechanisms and exploring potential interventions that disrupt this feedback loop.

Keywords: Cisplatin resistance; *IL12*; *LINC02381*; *TCF7*; Ovarian cancer; Wnt signaling

Corresponding Author: Mostafa Hosseini, PhD;
Chemical Injuries Research Center, System Biology and Poisonings
Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran.

Tel: (+98 912) 2343 564, Fax: (+98 21) 8804 0060 Email:
mostafa.hosseini@modares.ac.ir

INTRODUCTION

Ovarian cancer is one of the most lethal forms of gynecological cancer,¹ primarily due to its propensity to develop resistance to chemotherapy, especially cisplatin, which is the standard first-line treatment.² Cisplatin functions by inducing DNA damage, ultimately triggering apoptosis in cancer cells.³ Unfortunately, many patients develop resistance to this treatment, significantly diminishing its effectiveness and leading to relapse. Therefore, understanding the underlying molecular mechanisms that contribute to cisplatin resistance is crucial for informing the development of new therapeutic strategies.

Long non-coding RNAs (lncRNAs), which are transcripts longer than 200 nucleotides that do not code for proteins,⁴ have been implicated in regulating drug resistance across various cancers, including ovarian cancer.⁵ These lncRNAs play roles in modulating cellular pathways associated with drug transport, apoptosis, and immune responses.⁶ Recently, *LINC02381* has been identified as a potential regulator of cancer progression; however, its specific involvement in ovarian cancer chemoresistance remains largely unexplored.⁷

In this study, we aimed to elucidate the contribution of *LINC02381* to cisplatin resistance through advanced bioinformatics analyses. By examining RNA-seq, DNA-seq, and ChIP-seq datasets, we hypothesized that IL-12, a cytokine released within the tumor microenvironment, activates the Wnt signaling pathway,⁸ which subsequently activates *TCF7*, this transcription factor that binds to the promoter of *LINC02381* and upregulates its expression.⁹ In turn, *LINC02381* amplifies Wnt signaling, establishing a positive feedback loop that enhances the expression of ATP-binding cassette (*ABC*) transporters. These transporters actively expel cisplatin from cells, thus contributing to drug resistance. Our study proposes a novel regulatory pathway that underlies cisplatin resistance in ovarian cancer.

MATERIALS AND METHODS

Data Sources

We conducted our bioinformatics analysis using publicly available RNA-sequencing (RNA-seq) and chromatin immunoprecipitation sequencing (ChIP-seq) datasets sourced from repositories the Gene Expression Omnibus (GEO) and specifically, we analyzed data from both cisplatin-sensitive (A2780) and cisplatin-resistant

(A2780-CP) ovarian cancer cell lines (GSE176218& GSE172510). To enhance transparency in our data selection process, we provided detailed criteria for choosing the publicly available RNA-seq, DNA-seq, and ChIP-seq datasets used in this study. Our selection focused on datasets representing cisplatin-resistant and cisplatin-sensitive ovarian cancer cell lines, with particular attention to quality indicators such as read depth, alignment efficiency, and consistency across biological replicates. These criteria were crucial for ensuring that our bioinformatics analyses were reliable and meaningful with respect to our study objectives.

RNA-seq Analysis

To preprocess the RNA-seq datasets, we employed FastQC for quality control. Next, we used HISAT2 to align the reads to the human genome (GRCh38). StringTie was utilized for transcript assembly and quantification, while DESeq2 was applied to identify differentially expressed genes, focusing particularly on *LINC02381* and other genes involved in the Wnt signaling pathway.

DNA-seq Analysis

For DNA-seq analysis, we followed the Genome Analysis Toolkit (GATK) pipeline for variant calling. Our focus was on identifying mutations within genes associated with drug resistance and the Wnt signaling pathway. ANNOVAR was used for annotating the variants and assessing their potential functional impacts.

ChIP-seq Analysis

The ChIP-seq datasets were analyzed using Bowtie2 for read alignment and Model-based Analysis of ChIP-Seq (MACS2) for peak calling. We identified *TCF7* binding sites within the promoter region of *LINC02381*, alongside H3K27ac peaks, which indicate transcriptionally active regions.

Pathway Enrichment and Network Analysis

We performed gene and pathway enrichment analyses using the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) databases to understand the role of *LINC02381* in regulating drug resistance pathways. The STRING database was utilized to construct a protein-protein interaction network, elucidating the connections between *LINC02381*, Wnt signaling components, and *ABC* transporters.

Predictive Modeling

Using Cytoscape software, we developed a bioinformatics model to simulate the feedback loop involving IL-12, Wnt signaling, *TCF7*, and *LINC02381*. This model predicts how these interactions affect the expression of ABC transporters and contribute to the progression of cisplatin-resistant ovarian cancer

RESULTS

LINC02381 is Upregulated in Cisplatin-Resistant Ovarian Cancer Cells

RNA sequencing (RNA-seq) analysis demonstrated a significant upregulation of *LINC02381* in A2780-CP cells, which are resistant to cisplatin, compared to their cisplatin-sensitive counterparts, A2780 cells. The differential expression analysis revealed that *LINC02381* is not an isolated factor but part of a larger gene network implicated in the Wnt signaling pathway and drug transport mechanisms. This suggests that *LINC02381* may play a critical role in mediating resistance to cisplatin by influencing multiple cellular processes (Figure 1).

IL-12 Induces Activation of the Wnt Signaling Pathway in Cisplatin-Resistant Ovarian Cancer Cells

To investigate the role of IL-12 in modulating the Wnt signaling pathway in cisplatin-resistant ovarian cancer cells, we performed differential gene expression analysis using RNA-seq data from A2780-CP cells treated with IL-12. The heatmap (Figure 2A) demonstrates significant upregulation of key Wnt pathway components, including *WNT3A*, *CTNNB1* (β -catenin), and *TCF7*, following IL-12 stimulation. These results suggest that IL-12 may contribute to the activation of the Wnt pathway, which is known to be associated with drug resistance mechanisms.

Quantitative analysis via bar plots (Figure 2B) confirmed the increased expression of *WNT3A* and *CTNNB1*, which were markedly higher in IL-12-treated cells compared to untreated controls. This upregulation correlates with the increased activation of downstream Wnt signaling targets, suggesting that IL-12 enhances the transcriptional activity of the pathway.

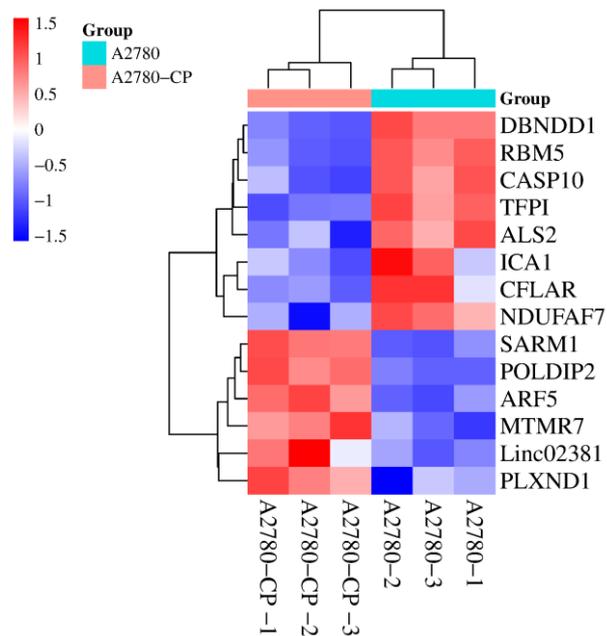


Figure 1. Differential Expression of LINC02381 in Cisplatin-Resistant vs. Sensitive Ovarian Cancer Cells. The heatmap illustrating the differential expression of LINC02381 and related genes in A2780 (cisplatin-sensitive) and A2780-CP (cisplatin-resistant) ovarian cancer cells based on RNA-sequencing data.

lncRNA LINC02381 in Cisplatin Resistance Ovarian Cancer

Additionally, volcano plot analysis (Figure 2C) identified several genes within the Wnt signaling cascade that were significantly upregulated following IL-12 exposure ($p < 0.05$, fold change > 2). Among these, *CTNNB1* and *TCF7* were the most prominent, suggesting a potential mechanism through which IL-12 may drive cisplatin resistance.

Finally, the scatter plot (Figure 2D) comparing

overall gene expression between IL-12-treated and untreated cisplatin-resistant cells revealed widespread transcriptional changes, with a clear shift in genes associated with the Wnt pathway. This global analysis underscores the importance of IL-12 in reprogramming cellular pathways that contribute to drug resistance, with the Wnt signaling axis playing a central role.

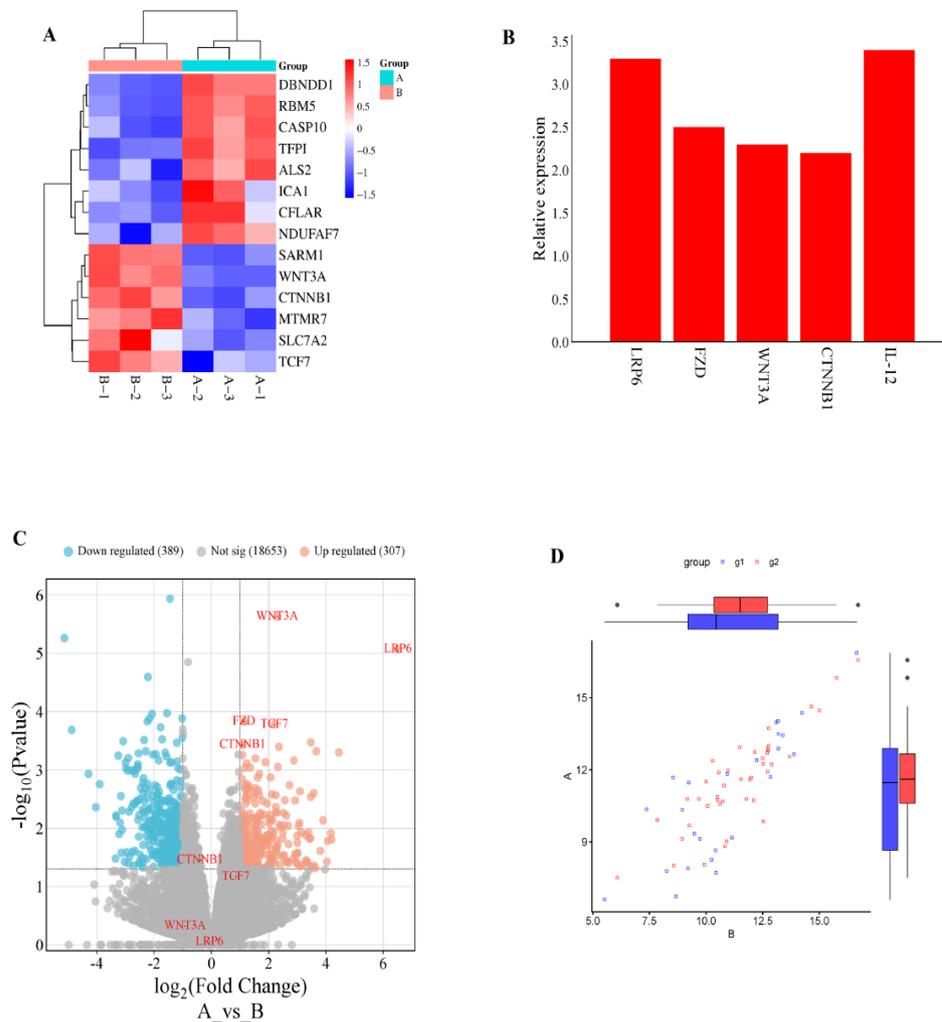


Figure 2. Bioinformatics Analysis of IL-12-Induced Wnt Signaling Activation in Cisplatin-Resistant Ovarian Cancer Cells. (A) Heatmap illustrating the differential expression of *WNT3A*, *CTNNB1* (β -catenin), and *TCF7* in cisplatin-resistant ovarian cancer cells before and after IL-12 stimulation based on RNA-seq data. **(B)** Bar plot quantifying the relative expression levels of key Wnt pathway genes. **(C)** Volcano plot highlighting statistically significant up regulation of Wnt-related genes following IL-12 stimulation. **(D)** Scatter plot comparing global gene expression changes between resistant and sensitive cells, demonstrating prominent activation of Wnt pathway.

ChIP-seq Confirms *TCF7* Binding at the *LINC02381* Promoter

Chromatin immunoprecipitation sequencing (ChIP-seq) data provided direct evidence of *TCF7* binding to the promoter region of *LINC02381*. The presence of H3K27ac marks at this region further supports the notion that it is transcriptionally active, particularly in cisplatin-resistant cells. This indicates that *TCF7* may regulate *LINC02381* expression, contributing to the resistance mechanism (Figure 3).

LINC02381 Activates *ABC* Transporters to Promote Drug Efflux

Results demonstrate a significant upregulation of *LINC02381* in A2780 cells treated with cisplatin compared to untreated controls. This increase indicates that *LINC02381* may play a crucial role in modulating drug resistance pathways. Additionally, the expression of *ABCB1* and *ABCB2* also shows a corresponding rise, particularly in the A2780-cp and A2780-cp+cisplatin groups, highlighting the relationship between *LINC02381* expression and the activation of these efflux pumps.

Overall, the data supports the hypothesis that *LINC02381* contributes to the development of cisplatin resistance in ovarian cancer by upregulating *ABC* transporters, thereby facilitating greater drug efflux and reducing intracellular drug accumulation. This underscores the importance of targeting *LINC02381* as a potential therapeutic strategy to overcome drug resistance in cancer treatment (Figure 4).

Bioinformatics Model of the IL-12/Wnt/*TCF7*/*LINC02381* Feedback Loop

To further elucidate the interactions within this signaling network, a bioinformatics model was constructed. This model illustrates a feedback loop wherein IL-12 activates Wnt signaling, leading to *TCF7* binding at the *LINC02381* promoter. The subsequent upregulation of *LINC02381* enhances Wnt pathway activity, creating a positive feedback mechanism that further stimulates the expression of *ABC* transporters. This complex interplay highlights the potential of targeting this feedback loop in therapeutic strategies aimed at overcoming cisplatin resistance in ovarian cancer (Figure 5).

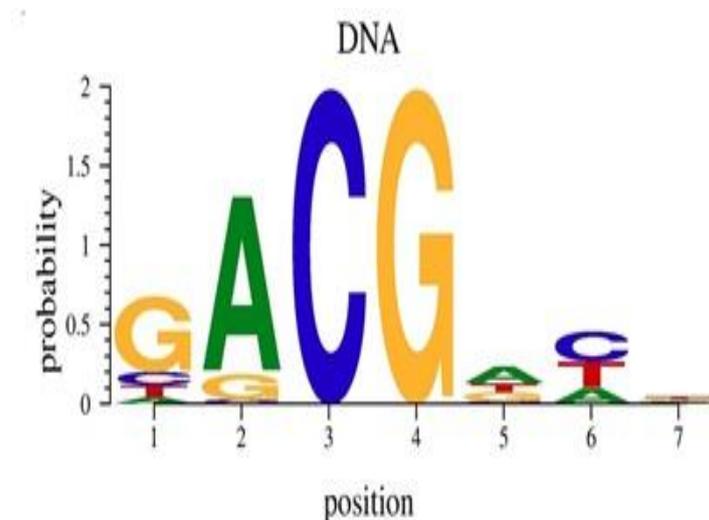


Figure 3. Chromatin immunoprecipitation sequencing (ChIP-seq) Analysis of *TCF7* Binding to the *LINC02381* Promoter Region ChIP-seq analysis showing *TCF7* binding peaks at the promoter region of the *LINC02381* gene. The x-axis represents genomic coordinates, while the y-axis indicates the signal intensity of *TCF7* binding. Overlapping peaks of H3K27ac histone modification marks are observed, indicating transcriptional activation. The region surrounding the promoter of *LINC02381* is highlighted, demonstrating co-localization of *TCF7* binding and H3K27ac signals, suggesting a regulatory role of *TCF7* in *LINC02381* expression.

lncRNA LINC02381 in Cisplatin Resistance Ovarian Cancer

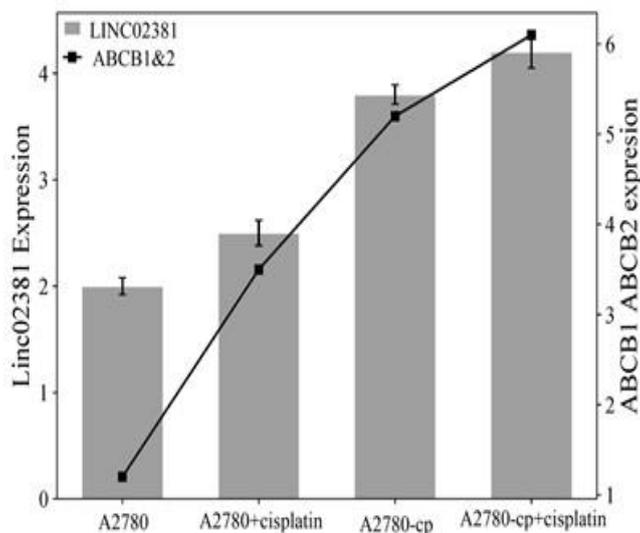


Figure 4. Impact of *LINC02381* on ABC Transporter Expression in Response to Cisplatin Treatment. This figure illustrates the expression levels of *LINC02381* and ABC transporters (*ABCB1* and *ABCB2*) in A2780 ovarian cancer cells under different treatment conditions. The bars represent *LINC02381* expression, while the line indicates the combined expression of *ABCB1* and *ABCB2*. The data shows that *LINC02381* expression increases in response to cisplatin treatment (A2780+cisplatin) and is further elevated in cells treated with cisplatin in combination with the chemo resistant variant (A2780-cp). This suggests that *LINC02381* may play a crucial role in enhancing the expression of ABC transporters, which are involved in drug efflux and resistance mechanisms.

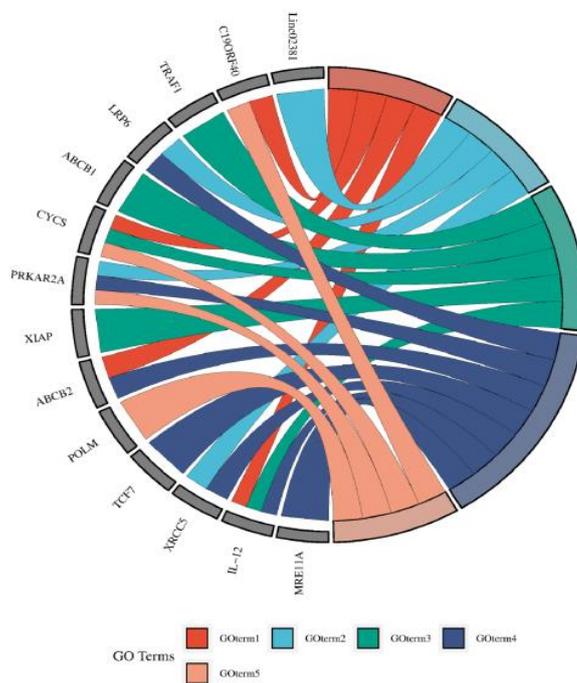


Figure 5. Bioinformatics Model of the IL-12/Wnt/TCF7/LINC02381 Feedback Loop Description: A bioinformatics model illustrating the positive feedback loop whereby IL-12 activates Wnt signaling, which triggers *TCF7* to bind and upregulate *LINC02381*. This lncRNA then enhances Wnt signaling, promoting the expression of ABC transporters.

DISCUSSION

Our bioinformatics analyses uncover a novel mechanism in which *LINC02381* mediates cisplatin resistance through the activation of Wnt signaling by IL-12.¹⁰ This finding highlights the intricate interplay between the tumor microenvironment and cancer cell behavior.¹¹ Specifically, IL-12, a cytokine known for its role in immune responses, is secreted within the tumor milieu and activates key components of the Wnt signaling pathway, namely *WNT3A* and *CTNNB1* (β -catenin).¹² The activation of these components leads to the subsequent activation of the transcription factor *TCF7*, which is crucial for various cellular processes, including proliferation and differentiation.¹³

ChIP-seq analysis provides compelling evidence that *TCF7* directly binds to the *LINC02381* promoter, thereby enhancing its expression in cisplatin-resistant cells. This direct interaction suggests that *TCF7* acts as a transcriptional regulator of *LINC02381*, linking Wnt signaling to the expression of this long non-coding RNA (lncRNA). The upregulation of *LINC02381* creates a scenario where Wnt signaling is further amplified, establishing a positive feedback loop that not only sustains but enhances the expression of ABC transporters.¹⁴ Transporters such as *ABCB1* and *ABCG2* play a critical role in the efflux of cisplatin from cancer cells,¹⁵ leading to reduced intracellular drug concentrations and ultimately facilitating the survival of ovarian cancer cells under chemotherapy pressure.

The role of cytokines, particularly IL-12, in modulating tumor behavior and drug resistance (is a critical aspect of this study).¹⁶ By demonstrating that cytokine-mediated activation of Wnt signaling and *LINC02381* plays a significant role in drug resistance, we introduce an immune component to the regulation of chemotherapy response. This suggests that strategies aimed at modulating immune responses- either through the inhibition of IL-12 or the manipulation of Wnt signaling- could be viable approaches to overcome cisplatin resistance.¹⁷

Importantly, targeting components of the Wnt signaling pathway, particularly the *LINC02381-TCF7* axis, may provide novel therapeutic opportunities. Inhibiting *TCF7* or disrupting its interaction with *LINC02381* could potentially restore sensitivity to cisplatin in resistant ovarian cancer cells. However, further experimental validation is essential to confirm these bioinformatics predictions. In vitro and in vivo

studies will be necessary to assess the feasibility and efficacy of these targets in clinical settings.

The identification of *LINC02381* as a key player in the resistance mechanism opens new avenues for research into lncRNAs as potential biomarkers for predicting treatment responses in ovarian cancer.¹⁸ Understanding the broader implications of *LINC02381* in other cancer types and treatment modalities could further enhance its therapeutic potential.

Future research could involve in vitro CRISPR knockdown studies to inhibit *LINC02381* expression and analyze its impact on Wnt signaling and drug resistance. Additionally, in vivo studies using ovarian cancer models could confirm the relevance of this pathway in physiological conditions. These experimental directions aim to establish a framework for future research and therapeutic exploration based on our findings.

In summary, our research underscores the complexity of drug resistance mechanisms in ovarian cancer and highlights the significance of the IL-12/Wnt/TCF7/LINC02381 axis in mediating cisplatin resistance. Future studies focused on unraveling the detailed molecular interactions within this pathway will be essential for developing effective therapeutic strategies to combat chemotherapy resistance.

This study identifies *LINC02381* as a key mediator of cisplatin resistance in ovarian cancer, based on comprehensive bioinformatics analysis. The IL-12/Wnt/TCF7/LINC02381 axis forms a crucial feedback loop that drives drug resistance by upregulating ABC transporters and facilitating cisplatin efflux from ovarian cancer cells. This novel mechanism presents potential therapeutic targets, particularly in interrupting the Wnt signaling cascade or inhibiting *LINC02381* expression, to overcome chemotherapy resistance.

Our findings open avenues for future research, where experimental validation and in vivo studies will be necessary to confirm the clinical relevance of this bioinformatics-driven model and to translate these findings into therapeutic interventions.

STATEMENT OF ETHICS

The authors have no relevant financial or non-financial interests to disclose.

FUNDING

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

The authors gratefully thank Dr. Amir Reza Javanmard, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran

Data Availability

Data are available from the corresponding author upon reasonable request.

AI Assistance Disclosure

Not Applicable.

REFERENCES

1. Stewart C, Ralyea C, Lockwood S, editors. Ovarian cancer: an integrated review. *Seminars in oncology nursing*; 2019: Elsevier.
2. Zoń A, Bednarek I. Cisplatin in ovarian cancer treatment-known limitations in therapy force new solutions. *Int J Mol Sci*. 2023;24(8):7585.
3. Zhu S, Pabla N, Tang C, He L, Dong Z. DNA damage response in cisplatin-induced nephrotoxicity. *Arch Toxicol*. 2015;89:2197-205.
4. Hombach S, Kretz M. Non-coding RNAs: classification, biology and functioning. Non-coding RNAs in colorectal cancer. *Adv Exp Med Biol*. 2016:3-17.
5. Barth DA, Juracek J, Slaby O, Pichler M, Calin GA. lncRNA and mechanisms of drug resistance in cancers of the genitourinary system. *Cancers*. 2020;12(8):2148.
6. Miraghel SA, Ebrahimi N, Khani L, Mansouri A, Jafarzadeh A, Ahmadi A, et al. Crosstalk between non-coding RNAs expression profile, drug resistance and immune response in breast cancer. *Pharmacoll Res*. 2022;176:106041.
7. Zhang J, Wu Y, Mu J, Xin D, Wang L, Fan Y, et al. Glycosyltransferase-related long non-coding RNA signature predicts the prognosis of colon adenocarcinoma. *Front Oncol*. 2022;12:954226.
8. Li X, Xiang Y, Li F, Yin C, Li B, Ke X. WNT/ β -catenin signaling pathway regulating T cell-inflammation in the tumor microenvironment. *Front Immunol*. 2019;10:2293.
9. Hrculak D, Kolar M, Strnad H, Korinek V. TCF/LEF transcription factors: an update from the internet resources. *Cancers*. 2016;8(7):70.
10. Wang J, Zhao Q. Linc02381 exacerbates rheumatoid arthritis through adsorbing miR-590-5p and activating the mitogen-activated protein kinase signaling pathway in rheumatoid arthritis-fibroblast-like synoviocytes. *Cell Transplant*. 2020;29:0963689720938023.
11. Hass R, von der Ohe J, Ungefroren H. Impact of the tumor microenvironment on tumor heterogeneity and consequences for cancer cell plasticity and stemness. *Cancers*. 2020;12(12):3716.
12. Goldsberry WN, Londoño A, Randall TD, Norian LA, Arend RC. A review of the role of Wnt in cancer immunomodulation. *Cancers*. 2019;11(6):771.
13. Kim C, Jin J, Weyand CM, Goronzy JJ. The transcription factor TCF1 in T cell differentiation and aging. *Int J Mol Sci*. 2020;21(18):6497.
14. Dréan A, Rosenberg S, Lejeune F-X, Goli L, Nadaradjane AA, Guehenec J, et al. ATP binding cassette (ABC) transporters: expression and clinical value in glioblastoma. *J Neurooncol*. 2018;138:479-86.
15. Vesel M, Rapp J, Feller D, Kiss E, Jaromi L, Meggyes M, et al. ABCB1 and ABCG2 drug transporters are differentially expressed in non-small cell lung cancers (NSCLC) and expression is modified by cisplatin treatment via altered Wnt signaling. *Resp Res*. 2017;18:1-11.
16. Mirlekar B, Pylayeva-Gupta Y. IL-12 family cytokines in cancer and immunotherapy. *Cancers*. 2021;13(2):167.
17. Zhu S, Zhang T, Zheng L, Liu H, Song W, Liu D, et al. Combination strategies to maximize the benefits of cancer immunotherapy. *J Hematol Oncol*. 2021;14(1):156.
18. Shan C, Liang Y, Wang K, Li P. Noncoding RNAs in cancer ferroptosis: from biology to clinical opportunity. *Biomed Pharmacother*. 2023;165:115053.