

Integrated transcriptomic and functional analysis identifies NEK2 as a miR-486-5p-regulated mitotic driver in ovarian cancer

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Integrated transcriptomic and functional analysis identifies NEK2 as a miR-486-5p-regulated mitotic driver in ovarian cancer

Running title: miR-486-5p/NEK2 axis in ovarian cancer

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Abstract:**Background:**

Dysregulation of mitotic kinases such as NEK2 is a key driver of centrosome amplification, mitotic spindle defects, and chromosomal instability in cancer. However, the upstream post-transcriptional regulation of NEK2 in ovarian cancer remains poorly characterized. MicroRNA-486-5p (miR-486-5p) has been identified as a tumor-suppressive miRNA and a direct negative regulator of NEK2 in multiple solid tumors.

Methods:

NEK2 expression was evaluated in two independent ovarian cancer microarray datasets and further validated using RNA-seq data from TCGA-OV and GTEx normal ovarian tissues. Potential upstream miRNAs targeting NEK2 were systematically predicted using miRWalk, TargetScan, and miRDB, then prioritized by integrating miRNA expression profiles from GSE131790 and AGO-CLIP-seq-supported interactions from starBase. Functional validation was performed in SKOV-3 ovarian cancer cells transfected with miR-486-5p mimics or negative control. miR-486-5p and NEK2 mRNA levels were quantified by qRT-PCR, NEK2 protein expression by Western blotting, and apoptosis by Annexin V-FITC/PI flow cytometry.

Results:

NEK2 was consistently and significantly overexpressed in ovarian tumors compared with normal ovarian tissue across all cohorts examined. Multi-layered integrative analysis identified miR-486-5p as a high-confidence upstream regulator of NEK2, supported by conserved 3'UTR binding sites, consistent downregulation in ovarian cancer, and AGO-CLIP-seq validation. Transfection with miR-486-5p mimics markedly suppressed both NEK2 mRNA and protein levels in SKOV-3 cells. Functionally, restoration of miR-486-5p significantly increased the proportion of apoptotic cells.

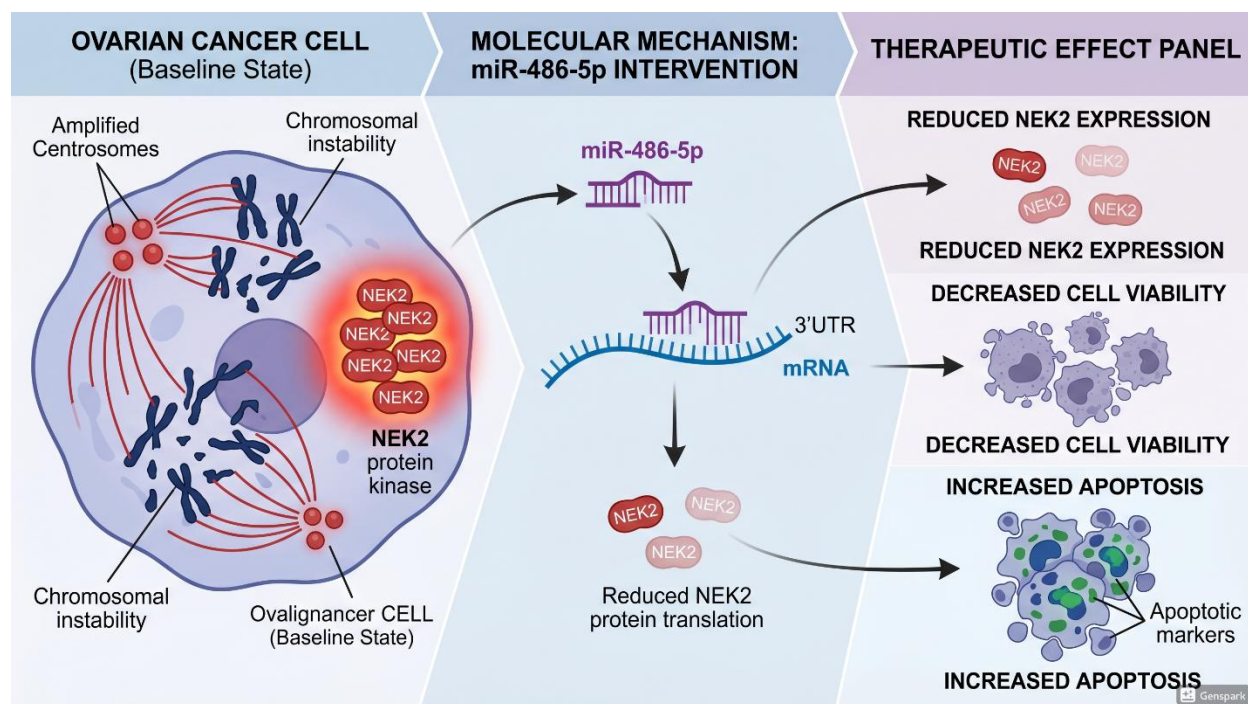
Conclusion:

These findings demonstrate that restoration of miR-486-5p attenuates NEK2 expression, inhibits proliferation, and promotes apoptosis in ovarian cancer cells. The miR-486-5p/NEK2 axis therefore represents a promising molecular target for therapeutic intervention in ovarian cancer.

Keywords:

Ovarian cancer; NEK2; miR-486-5p; RNA-seq; protein–protein interaction network; microRNA; apoptosis

Graphical abstract



Introduction:

Ovarian cancer remains the most lethal gynecological malignancy, often termed the "silent killer" due to its asymptomatic progression and frequent diagnosis at advanced stages (III or IV) (1). With a five-year survival rate of less than 50%, there is an urgent clinical necessity to identify novel diagnostic and prognostic biomarkers to overcome the limitations of current imaging and CA-125 testing(2). The pathogenesis of this disease is driven by a complex interplay of genetic mutations—such as those in *BRCA1* and *BRCA2*—and epigenetic modifications that disrupt regulatory control over cell growth and division. Despite standard interventions involving surgical resection and chemotherapy, significant challenges persist, particularly recurrence and acquired drug resistance, underscoring the need for more effective molecularly targeted strategies(3). At the cellular level, the centrosome serves as the primary microtubule-organizing center, essential for maintaining cellular architecture and ensuring genomic stability during division. Aberrations in centrosomal function are closely linked to tumorigenesis, leading to cell cycle dysregulation and impaired motility. A critical mediator in this process is NEK2 (Never in Mitosis Gene A-related kinase 2), a centrosomal kinase that is overexpressed in various human malignancies, including prostate, colorectal, and ovarian cancers(4). In ovarian cancer specifically, NEK2 mRNA and protein levels are significantly upregulated, often showing a 2- to 5-fold increase in malignant cell lines(5).

NEK2 drives cancer progression by localizing to the centrosomes to regulate separation and spindle formation; its overexpression leads to the accumulation of supernumerary centrosomes and multinucleated cells, contributing to the chromosomal instability hallmark of ovarian tumors. Furthermore, NEK2 expression is markedly higher in drug-resistant ovarian cancer cells compared to sensitive counterparts, making it a primary driver of treatment failure. Given its role in inhibiting cellular senescence and activating oncogenic pathways like NIK/NF- κ B, NEK2 has emerged as a promising target for therapeutic intervention. Recent advancements in RNA interference (RNAi) have demonstrated that targeting NEK2 can impede tumor growth and sensitize cancer cells to conventional therapies. Among these strategies, the use of synthetic microRNAs (miRNAs) represents a precise approach for gene silencing. miRNAs are small non-coding RNAs that bind to complementary sequences on target mRNAs, preventing their translation into functional proteins(6). While several miRNAs have been explored, miR-486-5p has been identified as a potent tumor suppressor that is frequently downregulated in malignant tissues. Therefore, this study aims to investigate the regulatory effect of the miR-486-5p/NEK2 axis in the SKOV-3 cell line, exploring how the restoration of miR-486-5p influences NEK2 protein levels and subsequent cellular apoptosis.

Materials and Methods

1. Transcriptomic Data Acquisition and Integration

Raw transcriptomic data were retrieved from the Gene Expression Omnibus (GEO) for two independent ovarian cancer cohorts: GSE14407 (12 normal, 12 tumor) and GSE54388 (6 normal, 16 tumor). Differential Gene Expression (DGE) analysis was performed using GEO2R (limma package), with significance thresholds defined as an adjusted P-value < 0.05 and $|\log_2FC| > 1$. To ensure clinical robustness beyond microarray platforms, we cross-validated our findings using an integrated large-scale cohort from The Cancer Genome Atlas (TCGA-OV) and the Genotype-Tissue Expression (GTEx) project.

2. Network Topology and Regulatory Bottleneck Identification

To decipher the functional interactome of ovarian cancer, a Protein-Protein Interaction (PPI) network was constructed using the STRING database (v11.5) with a high-confidence interaction

score of >0.7. The network was visualized in Cytoscape (v3.10). To pinpoint strategic regulatory nodes rather than general hubs, we employed the CytoHubba plugin using the Eccentricity algorithm. NEK2 was identified as a critical bottleneck node (Ranking: 16th), suggesting its role as a strategic coordinator in mitotic progression.

3. Multi-Omic miRNA-Target Validation

A multi-layered strategy was employed to identify high-confidence upstream regulators of NEK2. First, candidate miRNAs were screened using the miRWalk, TargetScan, and miRDB databases, with initial prioritization based on the conservation of predicted binding sites within the 3' untranslated region (3'UTR). Candidate miRNAs were then further ranked according to their predicted binding sites in the 3'UTRs of target genes and the evolutionary conservation of these sites across different species. To refine the list, we specifically selected miRNAs that were downregulated in ovarian cancer in the GSE131790 dataset. Subsequently, starBase analysis indicated that the predicted miR-486-5p binding site within the NEK2 3'UTR is supported by AGO-CLIP-seq reads in two independent datasets, providing experimental evidence for a physical miRNA–mRNA interaction. Ultimately, miR-486-5p was identified as the high-confidence upstream regulator of NEK2.

Cell Line Culture and Transfection

Ovarian cancer cell lines (SKOV-3) were obtained from the Pasteur Institute of Iran (Tehran, Iran). This cell lines divided into three groups: control, negative control (NC), and miRNA-treated and maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin/streptomycin, under standard cell culture conditions (37°C, 5% CO₂). For experimental assays, cells were seeded at appropriate densities in multi-well plates or culture dishes, allowing for 24-hour attachment prior to any intervention.

Transfection Protocol

Transfection experiments were carried out using transfection reagent (MCE corporation) according to the manufacturer's instructions. Briefly, cells were seeded in 6-well plates to achieve 70-80% confluence at the time of transfection. MiRNA mimics (concentration of 50 nM) were

complexed with the transfection reagent in serum-free medium for 15 minutes at room temperature. The miRNA-lipid complexes were then added dropwise to the cells, and incubation proceeded for 24 hours before subsequent analyses. Transfection efficiency was monitored by flowcytometry.

qRT-PCR Analysis

Total RNA was extracted from transfected and control cells using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. RNA concentration and purity were determined using a NanoDrop spectrophotometer. Complementary DNA (cDNA) synthesis was carried out using a high-capacity cDNA reverse transcription kit (Parstous co.) along with specific primers for the miRNA of interest and appropriate endogenous controls like U6 snRNA for miRNA and GAPDH for mRNA. Quantitative real-time polymerase chain reaction (qRT-PCR) was performed on a Corbett rotor gene using SYBR Green Master Mix (Takara). The relative expression levels of NEK2 mRNA and the inhibitory miRNA were calculated using the $2^{-\Delta\Delta C_t}$ method, normalizing to endogenous controls and comparing to control groups. Each sample was run in triplicate.

Apoptosis Assay

Apoptosis was quantified using the Annexin V-FITC/Propidium Iodide (PI) double staining kit (BD Biosciences). Transfected cells were harvested 48 hours post-transfection, washed twice with cold phosphate-buffered saline (PBS), and then resuspended in 1X binding buffer. Cells were stained with Annexin V-FITC and PI according to the manufacturer's instructions, typically by incubating for 15 minutes at room temperature in the dark. Samples were then analyzed immediately using a BD FACSCanto II flow cytometer. Data analysis was performed using FlowJo software, distinguishing between viable cells (Annexin V-/PI-), early apoptotic cells (Annexin V+/PI-), late apoptotic cells (Annexin V+/PI+), and necrotic cells (Annexin V-/PI+). The percentage of apoptotic cells (early + late apoptotic) was reported.

Western blot analysis:

Tissue was lysed with RIPA buffer. The lysates were removed by centrifugation at 14,000 rpm for 20 min at 4°C. Protein concentration was determined by the Bradford Protein Quantification kit

(DB0017, DNABioTech, Iran) according to manufacturer's instructions. The tissue lysates were mixed with equal volume of 2X Laemmli sample buffer. Lysates (20 µg) were then subjected to SDS-PAGE after a 5 min boiling and subsequently transferred to a 0.2 µm immune-Blot™ polyvinylidene difluoride (PVDF) membrane (Cat No: 162-017777; Bio-Rad Laboratories, CA, USA). The membranes were then blocked with 5% BSA (Cat No: A-7888; Sigma Aldrich, MO, USA) in 0.1% Tween 20 for 1 h. Then, the membranes were incubated with Anti-NEK2 (1/1000, Cat No: 37637, Abcam) and anti-β actin-loading control antibodies (1/2500, Cat No: ab8227, Abcam) for 1 h at room temperature. Subsequently, membranes were washed thrice with TBST, and incubated with goat anti-rabbit IgG H&L (HRP) (1/10000, Cat No: ab6721; Abcam) secondary antibody. The membranes were then incubated with enhanced chemiluminescence (ECL) for 1–2 min. Protein expression was normalized to β-actin.

Statistical analysis:

Statistical analyses were performed using GraphPad Prism version 10.0 (GraphPad Software, San Diego, CA, USA). All quantitative data are presented as mean ± standard deviation (SD), and for comparisons between three or more independent experimental groups, one-way analysis of variance (ANOVA) was employed under parametric assumptions. All statistical tests were two-tailed, and a p-value threshold of < 0.05 was adopted to denote statistical significance.

Results:

Transcriptomic profiling identifies a robust ovarian cancer gene signature and NEK2 overexpression

Differential expression analysis of GSE14407 and GSE54388 using limma-based workflows (GEO2R) revealed extensive transcriptional reprogramming between ovarian tumor and normal ovarian tissues, consistent with prior RNA-seq and microarray studies in ovarian cancer. In GSE14407, 5,664 DEGs were identified at adjusted ($p < 0.05$) and ($|\log_2FC| > 1$), including 3,504 up-regulated and 2160 down-regulated genes in tumor samples relative to normal tissue (Figure 1), whereas GSE54388 yielded 3,3096 DEGs (1,837 up-regulated and 1,259 down-regulated) under the same thresholds (Figure 2). The intersection of the two DEG lists comprised 1,747 overlapping genes, indicating a stable transcriptional signature that is robust to

cohort and platform differences and in line with previous integrative transcriptomic analyses in ovarian carcinoma (Figure 3).

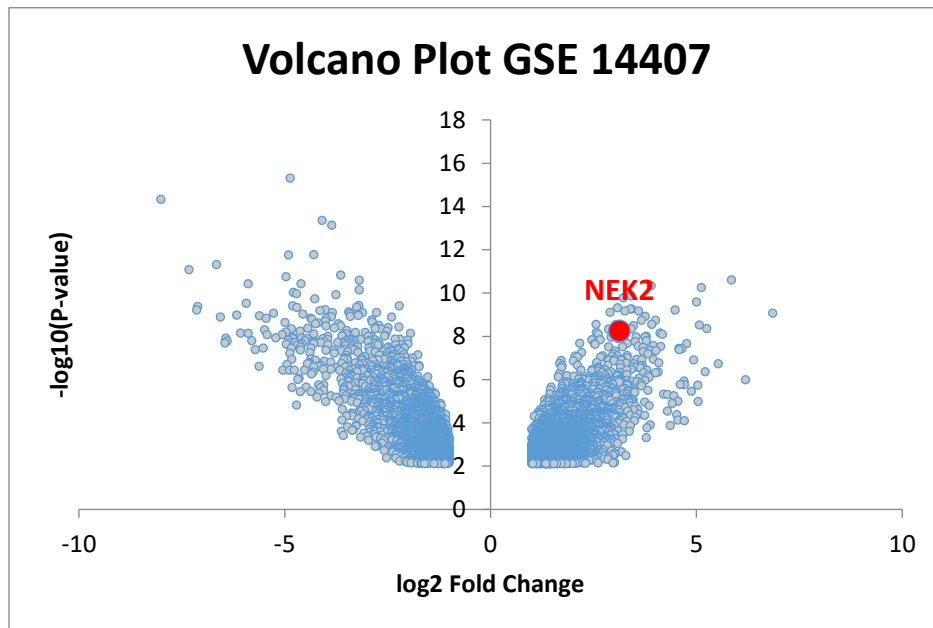


Figure 1. Volcano plot of differentially expressed genes in the GSE14407 ovarian cancer cohort. Each point represents a single gene, plotted as log₂ fold change (tumor vs. normal) on the x-axis and $-\log_{10}(\text{adjusted } P\text{-value})$ on the y-axis. NEK2 is highlighted as one of the most significantly upregulated genes in ovarian tumor samples.

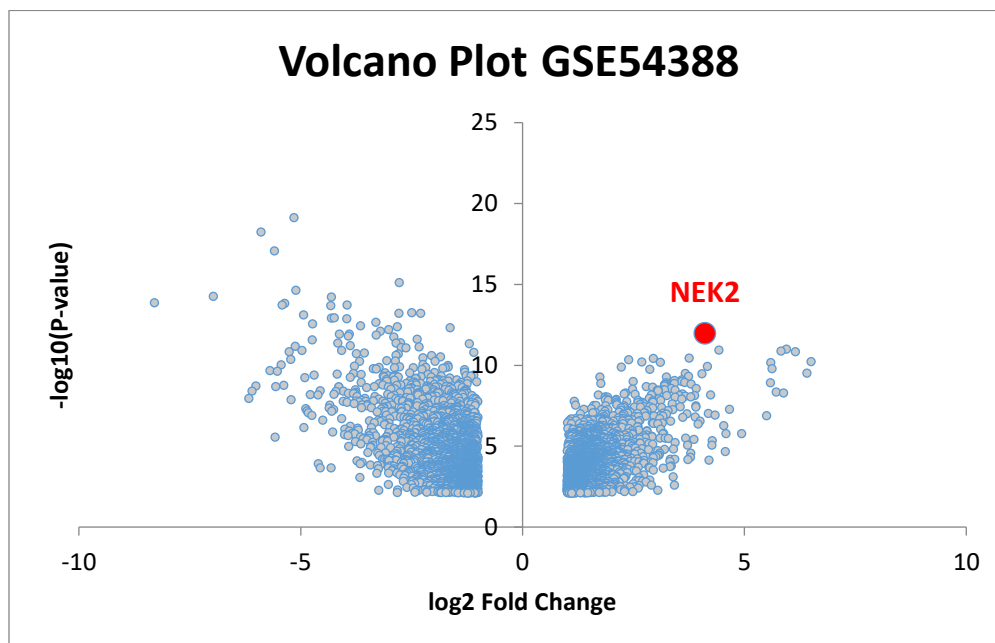


Figure 2. Volcano plot of differentially expressed genes in the GSE54388 ovarian cancer cohort. The x-axis shows log2 fold change (tumor vs. normal) and the y-axis shows $-\log_{10}(\text{adjusted } P\text{-value})$. NEK2 is again among the top upregulated candidates, supporting its consistent overexpression across independent cohorts.

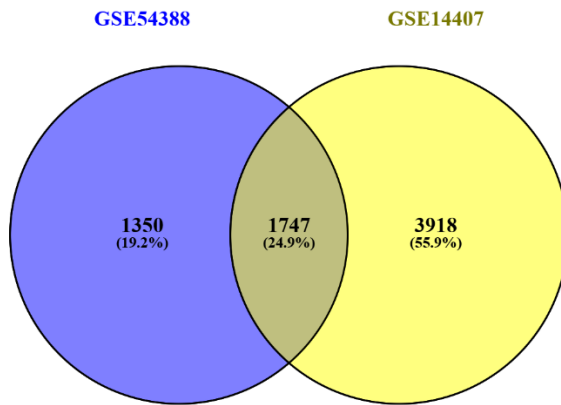


Figure 3. Overlap of differentially expressed genes between GSE14407 and GSE54388. Venn diagram depicting the number of up- and downregulated genes unique to each dataset and the 1,747 shared differentially expressed genes.

To validate NEK2 at the transcriptomic level in an independent, RNA-seq-based setting, we examined NEK2 expression in TCGA-OV tumors ($n \approx 426$) versus normal ovarian tissues from GTEx ($n \approx 88$). NEK2 was significantly overexpressed in tumor samples, confirming that NEK2 overexpression in ovarian cancer is consistent across independent cohorts and technologies (Figure 4). This pattern is concordant with previous reports identifying NEK2 up-regulation and adverse prognostic impact in ovarian and other solid tumors.

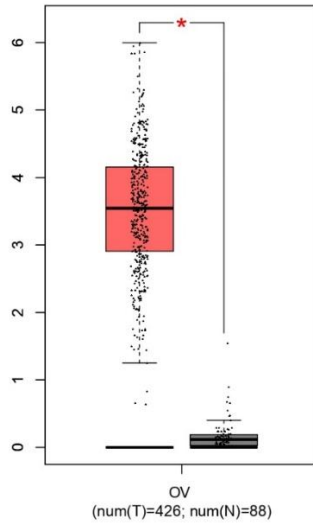


Figure 4. NEK2 mRNA expression in ovarian cancer tumors and normal ovarian tissue. Box-and-whisker plots depict NEK2 transcript levels derived from RNA-seq data in TCGA-OV tumor samples ($n \approx 426$) and normal ovarian tissues from the GTEx cohort ($n \approx 88$). NEK2 expression is significantly higher in tumor samples than in normal ovary, confirming that NEK2 overexpression in ovarian cancer is consistent across independent cohorts and measurement platforms.

PPI network analysis highlights NEK2 as a hub and bottleneck in mitotic regulatory circuits

After removal of isolated nodes, the resulting network comprised 1525 nodes and 17296 edges, forming a dense interactome with an average degree of approximately 22.68, indicative of substantial interconnectivity among dysregulated proteins in ovarian cancer. Among the hub genes, NEK2 has emerged as a top candidate for functional validation based on three convergent lines of evidence. Firstly, NEK2 showed significant overexpression in tumor tissues across both datasets, with a $\log_2FC$ of 3.13 and an adjusted p-value of 3.2×10^{-6} in GSE14407, and a $\log_2FC$ of 4.11 and an adjusted p-value of 2.17×10^{-9} in GSE54388. Secondly, NEK2 was ranked as a key bottleneck using Eccentricity as a centrality metric, holding the 16th position among all nodes and indicating its presence on numerous shortest paths connecting different regions of the network. Thirdly, NEK2 displayed notable degree centrality in the PPI network, where it interacts with key cell cycle effectors like CCNB1, CDK1, BUB1B, AURKA, and KIF14, positioning it as a critical regulatory node governing mitotic progression (Figure 5).

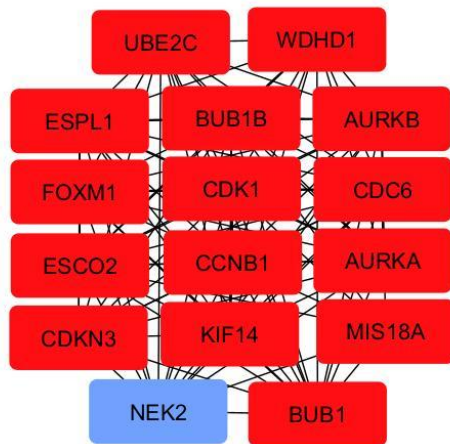


Figure 5. Protein–protein interaction network of overlapping differentially expressed genes and central position of NEK2. NEK2 appears as a highly connected hub interacting with key mitotic regulators such as CCNB1, CDK1, BUB1B, AURKA, and KIF14, highlighting its role in cell-cycle control.

Pathway enrichment analysis (KEGG) of hub genes showed significant enrichment of “Cell cycle”, all of which are critical to ovarian carcinogenesis and chemoresistance. NEK2 was represented in several of these pathways, further reinforcing its role in checkpoint override and chromosomal instability in ovarian cancer (Figure 6).

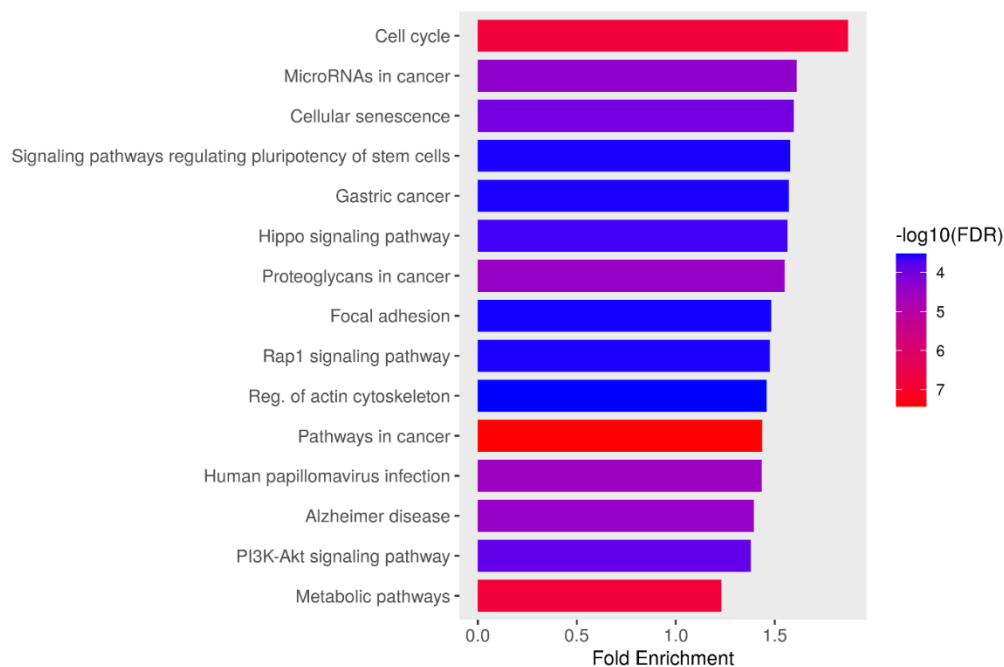


Figure 6. KEGG pathway enrichment analysis of hub genes in the PPI network. Bar plot showing significantly enriched pathways, including “Cell cycle”(adjusted $P < 0.05$). The presence of NEK2 in multiple cell-cycle-related pathways underscores its contribution to checkpoint dysregulation and chromosomal instability in ovarian cancer.

In silico identification of miR-486-5p as an upstream regulator of NEK2

A multi-layered strategy was employed to identify high-confidence upstream regulators of NEK2. First, candidate miRNAs were predicted using miRWalk, TargetScan, and miRDB, and the intersecting miRNAs were obtained using the VENNY online Venn diagram tool. Candidates were prioritized based on conserved binding sites within the NEK2 3'UTR, multiple predicted binding sites, and evolutionary conservation across vertebrate species. To refine this list, we interrogated the GSE131790 miRNA expression dataset, which includes profiles from high-grade serous ovarian cancer tissues and normal ovarian samples, and retained only those miRNAs that were downregulated in ovarian cancer. Finally, interactions between these miRNAs and NEK2 were evaluated using starBase (v2.0), and only candidates with AGO-CLIP-seq-supported binding sites within the NEK2 3'UTR were kept for further consideration. Through this integrative filtering approach, miR-486-5p emerged as the most robust candidate and was selected for functional validation.

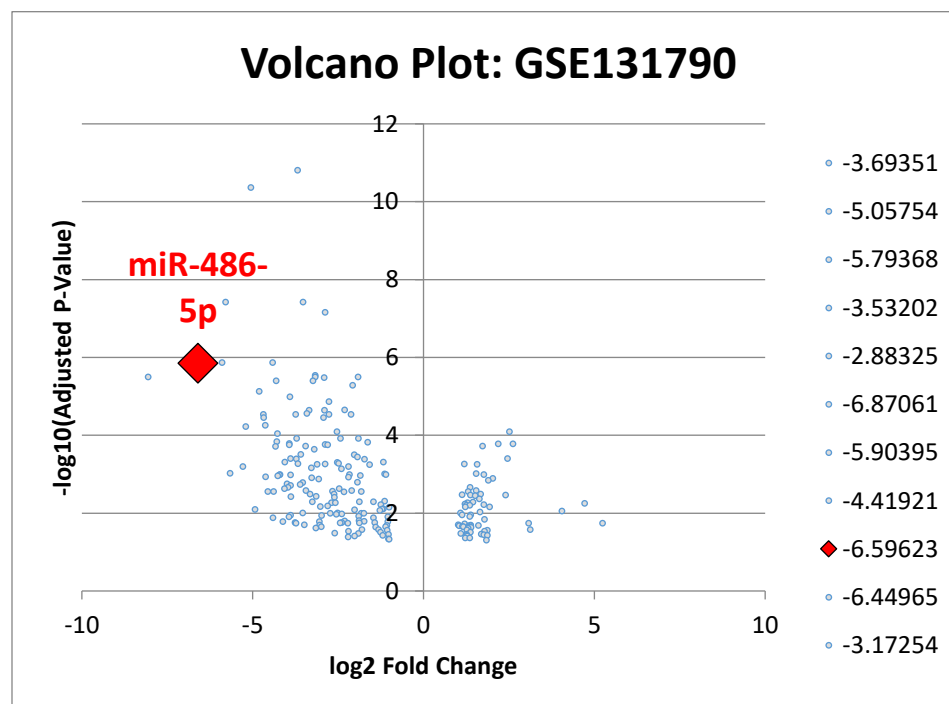


Figure 7. Volcano plot of differentially expressed genes in the GSE131790 comprising high-grade serous papillary ovarian tumors and normal ovarian epithelial samples. The x-axis shows log₂ fold change (tumor vs. normal) and the y-axis shows $-\log_{10}(\text{adjusted } P\text{-value})$. MiR-486-5p is again among the top downregulated candidates, supporting its consistent overexpression across independent cohorts.

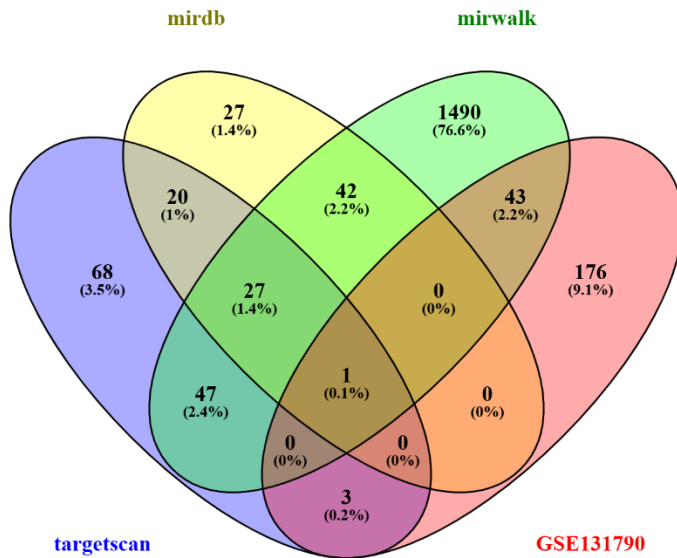


Figure 8. In silico prediction and expression-based prioritization of candidate NEK2-targeting miRNAs. Candidate miRNAs were first identified using three independent target-prediction algorithms (TargetScan, miRWalk, and miRDB), focusing on conserved binding sites within the NEK2 3'UTR and high prediction scores. The intersecting miRNAs were then filtered using the GSE131790 dataset, which profiles miRNA expression in 4 ovarian cancer tissues and 3 normal ovarian tissues, to retain only those that were downregulated in ovarian cancer.

Experimental Validation of Transfection Efficiency

To ensure the reliability of the miR-486-5p/NEK2 regulatory axis validation, we first confirmed high transfection efficiency in the SKOV-3 cell line. Qualitative assessment using a fluorescently labeled scrambled control (NC) revealed a robust and uniform uptake of the oligonucleotides

across the cell population (Figure 9). Quantitative analysis confirmed that over 96% of SKOV-3 cells Figure 10. These results demonstrate that the transfection process was highly efficient and consistent, providing a solid foundation for the subsequent functional assays.

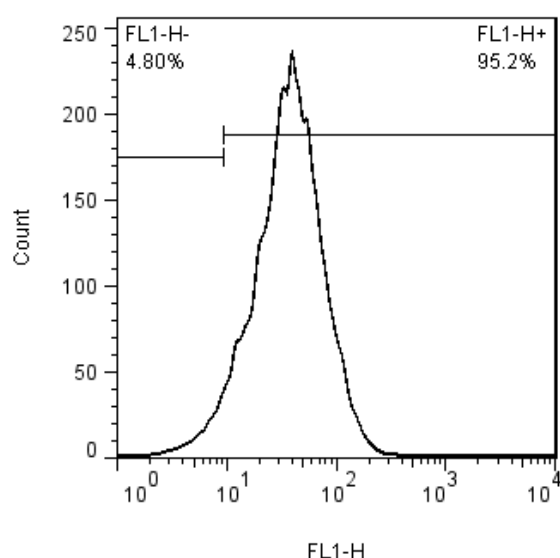
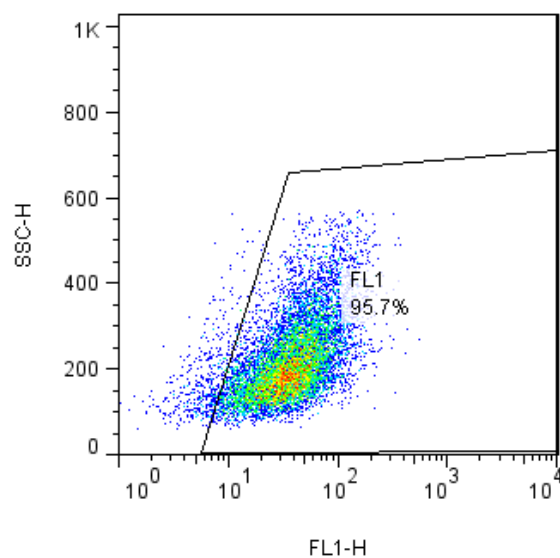
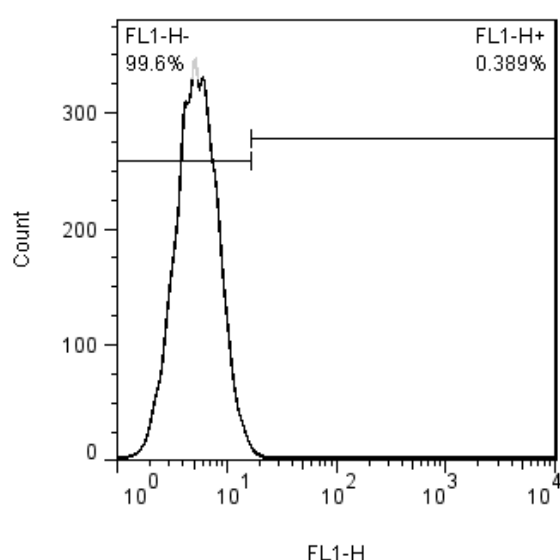
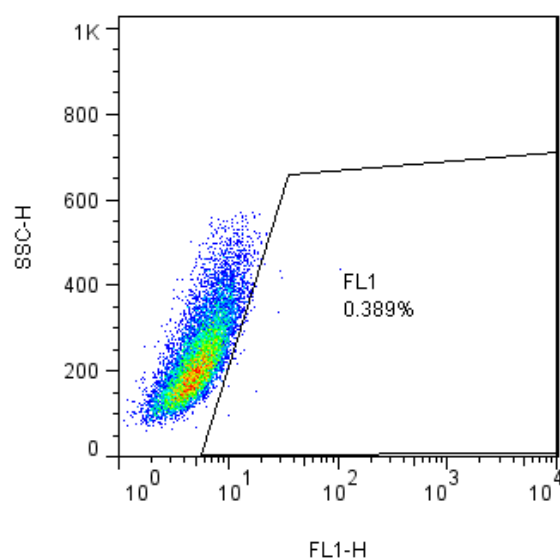


Figure 9. Quantitative transfection efficiency of miRNA mimics in SKOV-3 cells. Flow cytometry analysis demonstrating that more than 95% of SKOV-3 cells are positive for fluorescent miRNA after transfection, confirming high and reproducible transfection efficiency across biological replicates.

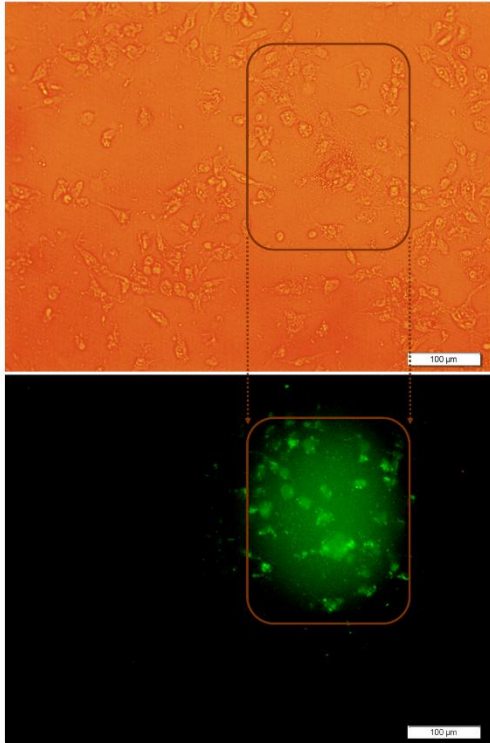


Figure 10 Qualitative assessment of transfection efficiency in SKOV-3 cells. Fluorescence microscopy images showing robust uptake of fluorescently labeled control miRNA in SKOV-3 cells, indicating efficient delivery of oligonucleotides.

qRT-PCR Analysis

qRT-PCR analyses confirmed NEK2 mRNA levels were significantly reduced in miR-486-5p–treated cells, showing a significant decrease relative to NC, whereas NEK2 expression remained

unchanged in NC-transfected cells, indicating a specific effect of miR-486-5p on NEK2 transcript abundance (Figure 9).

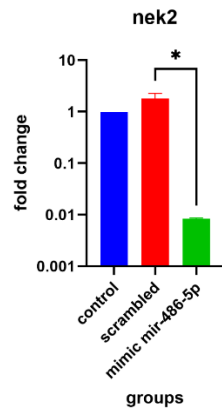


Figure 11. qRT-PCR validation of miR-486-5p overexpression in SKOV-3 cells. Bar graphs showing relative expression levels of miR-486-5p and NEK2 mRNA in control, negative control (NC), and miR-486-5p mimic-treated groups. miR-486-5p expression is markedly increased in the mimic group, while NEK2 mRNA levels are significantly reduced compared with NC, indicating effective and specific miRNA-mediated repression. Data are presented as mean $\pm$ SD; $P < 0.05$.

Western blot analysis

Western blot analysis further substantiated the transcript-level findings: NEK2 protein expression (normalized to β -actin) was markedly reduced in the miR-486-5p mimic-treated group compared with the negative control, whereas no significant difference was observed between the negative control and the untreated control group. In this assay, group 1 corresponded to the untreated control, group 2 to the negative control (NC), and group 3 to cells transfected with the miR-486-5p mimic. Taken together, these results indicate that miR-486-5p suppresses NEK2 expression at both the mRNA and protein levels in ovarian cancer cells, mirroring the direct regulatory relationship previously reported in hepatocellular carcinoma and supporting the conservation of this axis across tumor types (Figure 12).

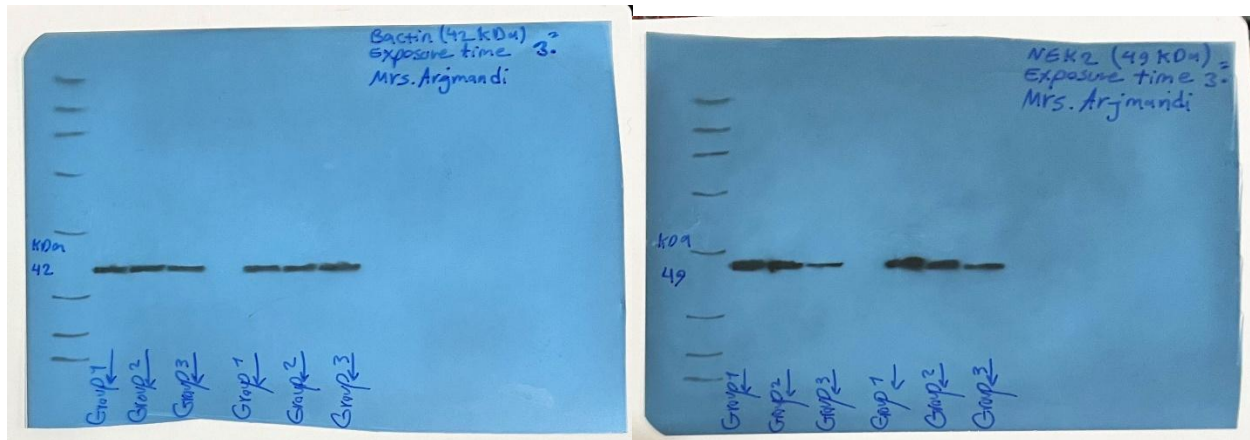


Figure 12. Western blot analysis of NEK2 protein expression in SKOV-3 cells following miR-486-5p overexpression. (A) Representative immunoblot showing NEK2 and β -actin (loading control) in three experimental groups: group 1, untreated control; group 2, negative control (NC); and group 3, miR-486-5p mimic-treated cells. (B) Densitometric quantification of NEK2 protein levels normalized to β -actin demonstrates a marked reduction in NEK2 expression in the miR-486-5p mimic group compared with NC, whereas no significant difference is observed between the NC and untreated control groups.

miR-486-5p promotes apoptosis in SKOV-3 cells via NEK2 suppression

Apoptosis was quantified 48 h after transfection using Annexin V-FITC/PI staining and flow cytometry. Baseline apoptotic rates in untreated control cells were approximately 85%, and NC-transfected cells showed similar values (~77%), with no statistically significant difference, indicating that the transfection procedure and NC oligonucleotide do not induce appreciable apoptosis. In contrast, miR-486-5p-transfected cells promoted apoptosis fraction reaching approximately 29%.

Notably, the majority of this increase was attributable to early apoptotic cells (Annexin V⁺/PI⁻), indicating activation of programmed cell death rather than nonspecific membrane damage or necrosis. These findings align with the known pro-apoptotic consequences of cell-cycle checkpoint failure and mitotic catastrophe induced by perturbation of centrosome function and spindle integrity—processes in which NEK2 plays a pivotal role. They are also consistent with prior reports showing that NEK2 inhibition or knockdown can trigger apoptosis and enhance chemosensitivity in ovarian and other cancer models (Figure 11).

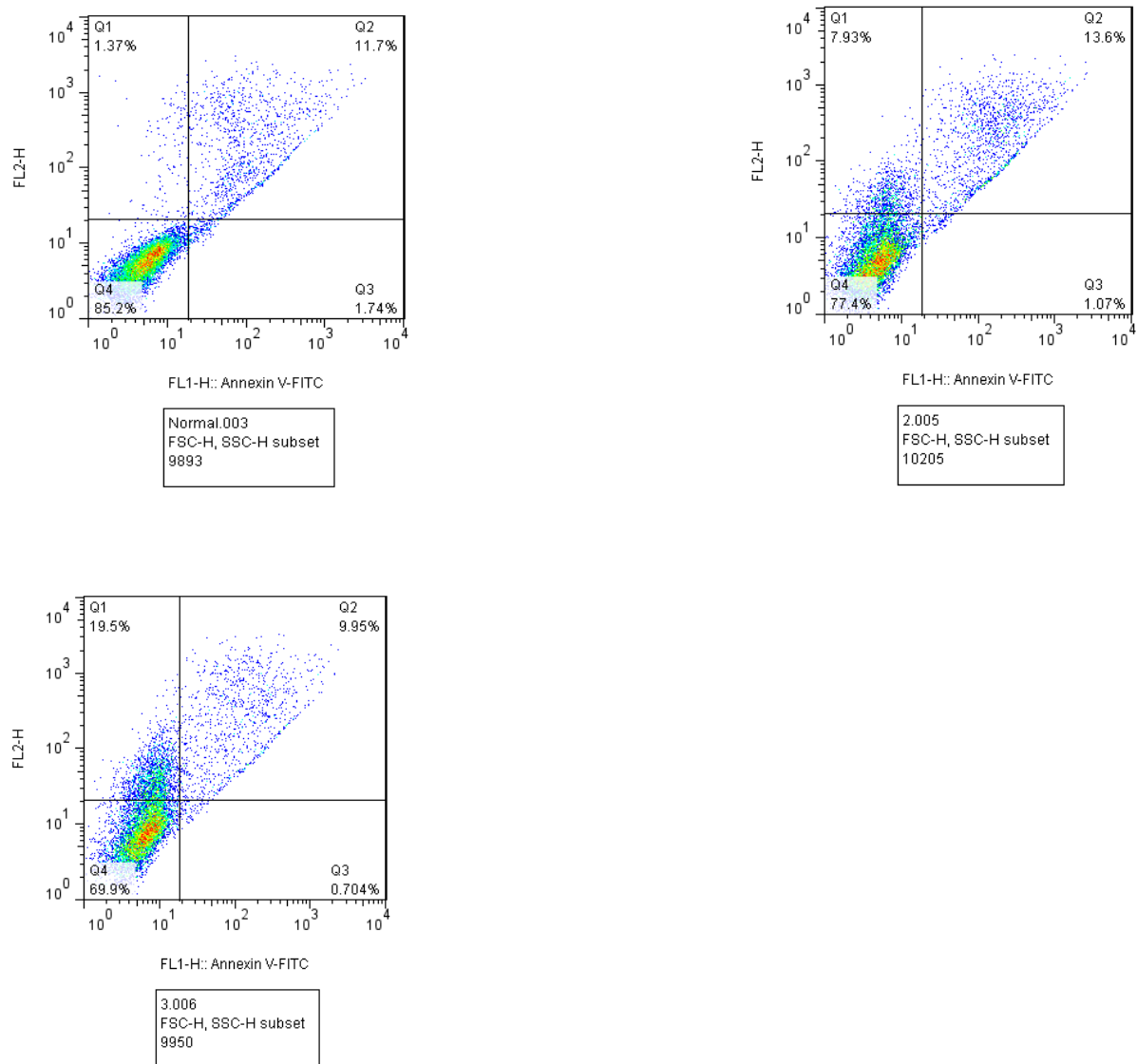


Figure 13. miR-486-5p induces apoptosis in SKOV-3 ovarian cancer cells. Representative Annexin V-FITC/PI flow-cytometry dot plots depicting viable, early apoptotic, late apoptotic, and necrotic cell populations 48 h post-transfection. Experimental conditions are: (A) untreated baseline control, (B) negative control (NC)–transfected cells, and (C) cells transfected with the miR-486-5p mimic. Quantitative analysis of viable cells shows a stepwise reduction in cell viability from 85% in the baseline control to 77% in the NC group and 69% in the miR-486-5p mimic group. Total apoptotic cells (early + late apoptosis) increase from 20% in the NC group to 29% in the miR-486-5p mimic group, while the NC group does not differ significantly from baseline. Together, these findings indicate that miR-486-5p overexpression reduces cell viability and promotes apoptosis in SKOV-3 ovarian cancer cells.

Discussion:

Ovarian cancer remains one of the most lethal gynecologic malignancies, largely due to late-stage diagnosis, extensive genomic instability, and the rapid emergence of chemoresistance despite initial responses to platinum–taxane–based regimens(7). The clinical management of epithelial ovarian cancer has been transformed by the incorporation of PARP inhibitors and anti-angiogenic agents into standard regimens, particularly for patients with BRCA mutations or homologous recombination deficiency, yet overall survival remains suboptimal and resistance to both chemotherapy and targeted agents is common. There is a pressing need for new molecular targets that can be leveraged to enhance chemosensitivity and overcome resistance(8, 9).

Given its role in regulating centrosome separation, spindle integrity, and cell-cycle checkpoints, NEK2 has emerged as an attractive target for small-molecule inhibition, with preclinical studies in various solid tumors demonstrating anti-proliferative effects and chemosensitization upon NEK2 blockade. In ovarian cancer specifically, NEK2 overexpression has been linked to platinum and taxane resistance, and its knockdown restores sensitivity, positioning NEK2 at the intersection(10). NEK2's oncogenic functions are not restricted to ovarian cancer and extend across multiple tumor types. Xing et al. (2022) demonstrated that NEK2 is highly expressed in several breast cancer cell lines, and that NEK2 knockdown in MDA-MB-231 and MCF-7 cells significantly reduced cell survival, migration, invasion, and colony formation, while increasing apoptosis and inducing G0/G1 arrest. These effects were accompanied by decreased Bcl-2, Cyclin B1, and Cyclin D1, increased Bax and p27, and reduced ERK and p38 phosphorylation, with NEK2 silencing almost completely reversing IGF-induced ERK activation (4). Similarly, Zhang et al. (2021) identified NEK2 as a regulator of PD-L1 expression in pancreatic cancer cells; NEK2 inhibition reduced PD-L1 levels and enhanced immune cell infiltration, thereby augmenting anti-tumor immune responses (11). A systematic review and meta-analysis further established high NEK2 expression as an adverse prognostic factor in human solid tumors, underscoring its potential as both biomarker and drug target (12).

In this study, we applied an integrated multi-step strategy—combining transcriptomic profiling, protein–protein interaction (PPI) network analysis, and functional in vitro validation—to interrogate the role of NEK2 and its upstream microRNA regulator miR-486-5p in ovarian carcinoma. Our transcriptomic analysis of two independent microarray cohorts (GSE14407 and

GSE54388) identified 1,747 overlapping differentially expressed genes (DEGs) between ovarian tumors and normal ovarian tissues, providing a robust, platform-independent molecular signature. Within the high-confidence PPI network derived from these DEGs, NEK2 emerged as one of the most highly connected hub genes and was also ranked as a topological bottleneck, clustering with canonical mitotic regulators that are recurrently implicated in cell-cycle dysregulation and aneuploidy. This network-level centrality strongly supports a gatekeeper role for NEK2 in coordinating mitotic progression in ovarian cancer cells. Consistent with this, Uddin et al. reported that NEK2 is significantly overexpressed in ovarian tumor tissues and cell lines compared with normal ovarian counterparts, and that NEK2 expression is higher in cisplatin-resistant A2780CP cells than in cisplatin-sensitive A2780 cells; NEK2 silencing restored cisplatin sensitivity, directly implicating NEK2 in platinum resistance (13). Our independent validation using TCGA-OV RNA-seq data and normal ovarian samples from GTEx confirms that NEK2 is consistently and significantly upregulated in ovarian tumors at the transcriptomic level, reinforcing its oncogenic relevance in this disease.

Accumulating evidence highlights microRNAs as key regulators of ovarian cancer biology. Multiple miRNAs, including the miR-200 family, miR-214, and miR-93-5p, have been linked to epithelial–mesenchymal transition (EMT), invasion, and chemoresistance, emphasizing the centrality of miRNA networks in ovarian tumor progression (14). Among these, miR-486-5p has been repeatedly characterized as a predominantly tumor-suppressive miRNA in lung, esophageal, breast, thyroid, and liver cancers, where it targets oncogenic components of PI3K/AKT, FOXO, and other signaling pathways to restrain proliferation, invasion, and metastasis (14-17). In papillary thyroid carcinoma, using miRNA microarray analysis, miRNA sequencing, and target-gene prediction, authors proposed that miR-486-5p functions as a tumor-suppressive miRNA and regulates a network of key genes involved in thyroid cancer(18).

Xu et al. (2019) explored the effects of miR-138 on the proliferation, differentiation, and apoptosis of chondrocytes through the WNT/ β -catenin signaling pathway in mice with osteoarthritis (OA). Their results showed a significant reduction in miR-138 expression in OA mice, while NEK2 expression was notably increased. Chondrocytes treated with miR-138 mimic and siRNA-NEK2 exhibited that reduced NEK2 levels were associated with increased chondrocyte proliferation and decreased apoptosis. The study suggested that miR-138 might regulate OA chondrocyte growth

and proliferation through positive modulation of NEK2 via the WNT/ β -catenin pathway(19). Ye et al. (2018) examined the impact of miR-128 on apoptosis in glioma cells, with a focus on its relationship with NEK2. They measured miR-128 and NEK2 expression levels in glioma and normal brain tissues, finding that miR-128 was significantly downregulated in glioma tissues, while NEK2 was upregulated. Luciferase reporter assays confirmed that miR-128 directly interacts with NEK2. The study suggested that miR-128 plays a role in controlling glioma growth and progression through positive regulation of NEK2(20).

Crucially, Fu et al. demonstrated in hepatocellular carcinoma that miR-486-5p directly binds the 3'UTR of NEK2, represses its expression, and consequently inhibits tumor growth, with an inverse correlation between miR-486-5p and NEK2 levels in patient samples. This study established NEK2 as a bona fide direct target of miR-486-5p and suggested that diminished miR-486-5p may underlie NEK2 overexpression in at least some tumor contexts(21).

Our multi-omic in silico screening—which combined TargetScan, miRWalk, miRDB, and starBase—converged on miR-486-5p as a high-confidence upstream regulator of NEK2 in ovarian cancer. miR-486-5p was consistently predicted to target conserved binding sites within the NEK2 3'UTR by multiple algorithms, is downregulated in ovarian and other cancers according to curated differential expression databases, and displays AGO-CLIP-seq-supported binding to NEK2 in starBase. Together with the prior functional data from hepatocellular carcinoma, these observations provided a strong rationale to prioritize miR-486-5p for experimental validation in ovarian cancer.

In SKOV-3 cells, forced overexpression of miR-486-5p markedly increased intracellular miR-486-5p levels and induced a significant reduction in both NEK2 mRNA and protein compared with negative controls, demonstrating functional repression of NEK2 by miR-486-5p in an ovarian cancer context. At the functional level, miR-486-5p overexpression promotes apoptosis, as determined by Annexin V-FITC/PI assays. This phenotype is consistent with the central role of NEK2 in promoting mitotic progression and cell survival: NEK2 overactivity promotes mitotic spindle integrity and may allow cells to override spindle assembly checkpoints, facilitating survival despite chromosomal missegregation(22). These interactions suggest that NEK2 is a crucial player in the development of drug resistance through its impact on these molecular pathways(23). This aligns with broader evidence that miR-486-5p restrains oncogenic pathways

and cell-cycle progression across multiple malignancies, and adds ovarian carcinoma to the spectrum of cancers in which miR-486-5p exerts tumor-suppressive effects(24). It is important to acknowledge that miR-486-5p likely has additional targets in ovarian cancer beyond NEK2, as suggested by multi-target predictions and pathway analyses in other tumor types. However, the consistent suppression of NEK2 at both mRNA and protein levels, together with the well-established oncogenic functions of NEK2, make it highly plausible that NEK2 is a major functional effector of miR-486-5p in this system.

Limitations and future directions

This study has several limitations that warrant consideration. First, functional validation was performed in a single ovarian cancer cell line (SKOV-3). Given the molecular and histologic heterogeneity of ovarian carcinoma, extending these analyses to additional lines (e.g., OVCAR-3, A2780, platinum-resistant derivatives) and to patient-derived primary cultures or organoids will be essential to assess the generalizability of the miR-486-5p/NEK2 axis. Second, we did not evaluate the impact of miR-486-5p-mediated NEK2 suppression in *in vivo* models. Xenograft or patient-derived xenograft (PDX) studies will be critical to determine whether miR-486-5p restoration can inhibit tumor growth, impair metastatic dissemination, and enhance sensitivity to chemotherapy in a physiologic microenvironment. Third, although we focused on NEK2 as a primary target, miR-486-5p undoubtedly regulates additional genes in ovarian cancer. Systematic transcriptome-wide profiling (e.g., RNA-seq) following miR-486-5p overexpression, coupled with integrative pathway analysis, would help delineate the broader network effects and distinguish direct from indirect targets. Finally, while we used multiple public datasets (GEO, TCGA, GTEx) and stringent criteria to derive a robust DEG and network signature, intrinsic heterogeneity in sample quality, technical platforms, and clinical annotations may introduce bias. Future integrative analyses combining bulk and single-cell RNA-seq, spatial transcriptomics, and multi-omics data may provide a more nuanced view of NEK2-centered networks and miR-486-5p regulation in specific cellular compartments of the tumor microenvironment.

Conclusions

In summary, our integrative study demonstrates that NEK2 is a centrally positioned, consistently overexpressed mitotic kinase in ovarian cancer that functions as a key hub and bottleneck in cell-cycle-related interaction networks. We further identify miR-486-5p as a tumor-suppressive

miRNA that negatively regulates NEK2, and show that restoration of miR-486-5p in ovarian cancer cells downregulates NEK2 expression, suppresses cell viability, and promotes apoptosis. These findings support the miR-486-5p/NEK2 axis as a critical regulatory pathway in ovarian carcinogenesis and chemoresistance, and highlight it as a promising candidate target for future therapeutic and biomarker development in ovarian cancer.

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Conflict of Interest

The authors declare no competing interests that may have influenced in this manuscript.

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References:

1. Cabasag CJ, Fagan PJ, Ferlay J, Vignat J, Laversanne M, Liu L, *et al.* Ovarian cancer today and tomorrow: A global assessment by world region and Human Development Index using GLOBOCAN 2020. *International journal of cancer* 2022; 151:1535-1541.
2. Burges A, Schmalfeldt B. Ovarian cancer: diagnosis and treatment. *Deutsches Ärzteblatt International* 2011; 108:635.
3. Rosen DG, Yang G, Liu G, Mercado-Uribe I, Chang B, Xiao XS, *et al.* Ovarian cancer: pathology, biology, and disease models. *Frontiers in bioscience: a journal and virtual library* 2009; 14:2089.
4. Xing Z, Zhang M, Wang X, Liu J, Liu G, Feng K, *et al.* Silencing of Nek2 suppresses the proliferation, migration and invasion and induces apoptosis of breast cancer cells by regulating ERK/MAPK signaling. *Journal of molecular histology* 2021; 52:809-821.
5. Hayward DG, Clarke RB, Faragher AJ, Pillai MR, Hagan IM, Fry AM. The centrosomal kinase Nek2 displays elevated levels of protein expression in human breast cancer. *Cancer Res* 2004; 64:7370-7376.
6. Fang Y, Zhang X. Targeting NEK2 as a promising therapeutic approach for cancer treatment. *Cell Cycle* 2016; 15:895-907.

7. Ratz L, Baek S, Panier S. Revisiting Genomic Instability, Tumor Microenvironment and Immune Response in High-Grade Serous Ovarian Cancer. *Geburtshilfe Frauenheilkd* 2025; 85:694-709.
8. Guan LY, Lu Y. New developments in molecular targeted therapy of ovarian cancer. *Discov Med* 2018; 26:219-229.
9. Loizzi V, Ranieri G, Laforgia M, Gadaleta CD, Gargano G, Kardhashi A, *et al.* PARP inhibitors and epithelial ovarian cancer: Molecular mechanisms, clinical development and future prospective. *Oncol Lett* 2020; 20:90.
10. White MC, Wong JP, Damania B. Inhibition of NEK2 Promotes Chemosensitivity and Reduces KSHV-positive Primary Effusion Lymphoma Burden. *Cancer Res Commun* 2024; 4:1024-1040.
11. Zhang X, Huang X, Xu J, Li E, Lao M, Tang T, *et al.* NEK2 inhibition triggers anti-pancreatic cancer immunity by targeting PD-L1. *Nature communications* 2021; 12:4536.
12. Wang X, Chen K, Liu H, Huang Z, Chen X, Yin L. Prognostic significance of NEK2 in human solid tumors: a systematic review and meta-analysis. *Biosci Rep* 2019; 39.
13. Uddin MH, Kim B, Cho U, Azmi AS, Song YS. Association of ALDH1A1-NEK-2 axis in cisplatin resistance in ovarian cancer cells. *Heliyon* 2020; 6:e05442.
14. Choi PW, Ng SW. The Functions of MicroRNA-200 Family in Ovarian Cancer: Beyond Epithelial-Mesenchymal Transition. *Int J Mol Sci* 2017; 18.
15. Tomioka Y, Suetsugu T, Seki N, Tanigawa K, Hagihara Y, Shinmura M, *et al.* The Molecular Pathogenesis of Tumor-Suppressive miR-486-5p and miR-486-3p Target Genes: GINS4 Facilitates Aggressiveness in Lung Adenocarcinoma. *Cells* 2023; 12.
16. Yi Y, Lu X, Chen J, Jiao C, Zhong J, Song Z, *et al.* Downregulated miR-486-5p acts as a tumor suppressor in esophageal squamous cell carcinoma. *Exp Ther Med* 2016; 12:3411-3416.
17. Ding L, Tian W, Zhang H, Li W, Ji C, Wang Y, *et al.* MicroRNA-486-5p Suppresses Lung Cancer via Downregulating mTOR Signaling In Vitro and In Vivo. *Front Oncol* 2021; 11:655236.
18. Wen DY, Pan DH, Lin P, Mo QY, Wei YP, Luo YH, *et al.* Downregulation of miR-486-5p in papillary thyroid carcinoma tissue: A study based on microarray and miRNA sequencing. *Mol Med Rep* 2018; 18:2631-2642.
19. Xu W, Gao P, Zhang Y, Piao L, Dong D. microRNA-138 induces cell survival and reduces WNT/ β -catenin signaling of osteoarthritis chondrocytes through NEK2. *IUBMB life* 2019; 71:1355-1366.
20. Ye Y, Zhi F, Peng Y, Yang C-C. MiR-128 promotes the apoptosis of glioma cells via binding to NEK2. *European Review for Medical & Pharmacological Sciences* 2018; 22.
21. Fu SJ, Chen J, Ji F, Ju WQ, Zhao Q, Chen MG, *et al.* MiR-486-5p negatively regulates oncogenic NEK2 in hepatocellular carcinoma. *Oncotarget* 2017; 8:52948-52959.
22. Rivera-Rivera Y, Marina M, Jusino S, Lee M, Velázquez JV, Chardón-Colón C, *et al.* The Nek2 centrosome-mitotic kinase contributes to the mesenchymal state, cell invasion, and migration of triple-negative breast cancer cells. *Sci Rep* 2021; 11:9016.
23. Liu X, Gao Y, Lu Y, Zhang J, Li L, Yin F. Upregulation of NEK2 is associated with drug resistance in ovarian cancer. *Oncology Reports* 2014; 31:745-754.
24. ElKhoully AM, Youness RA, Gad MZ. MicroRNA-486-5p and microRNA-486-3p: Multifaceted pleiotropic mediators in oncological and non-oncological conditions. *Noncoding RNA Res* 2020; 5:11-21.