Chapter 4:
Molecular Mechanisms of Kisspeptin-10 Mediated Attenuation of Ovarian Cancer Metastasis and Progression

INTRODUCTION

Ovarian cancer is one of the deadliest gynaecological cancers and continues to be a significant contributor to cancer-related death in women globally. High mortality rate of ovarian cancer is attributed to asymptomatic characteristics in early stages, no available effective screening methods and diagnosis at late stages with extensive intraperitoneal metastasis(Lee et al., 2018). Even though conventional therapeutic approaches, consisting of cytoreductive surgery and platinum- and taxane-based chemotherapy, have generally led to improved short-term response rates, most patients will ultimately have disease relapse and become chemo-resistant. These clinical problems highlight the need to identify molecular processes that mediate ovarian cancer progression, metastasis and treatment failure (Franjić, 2023).

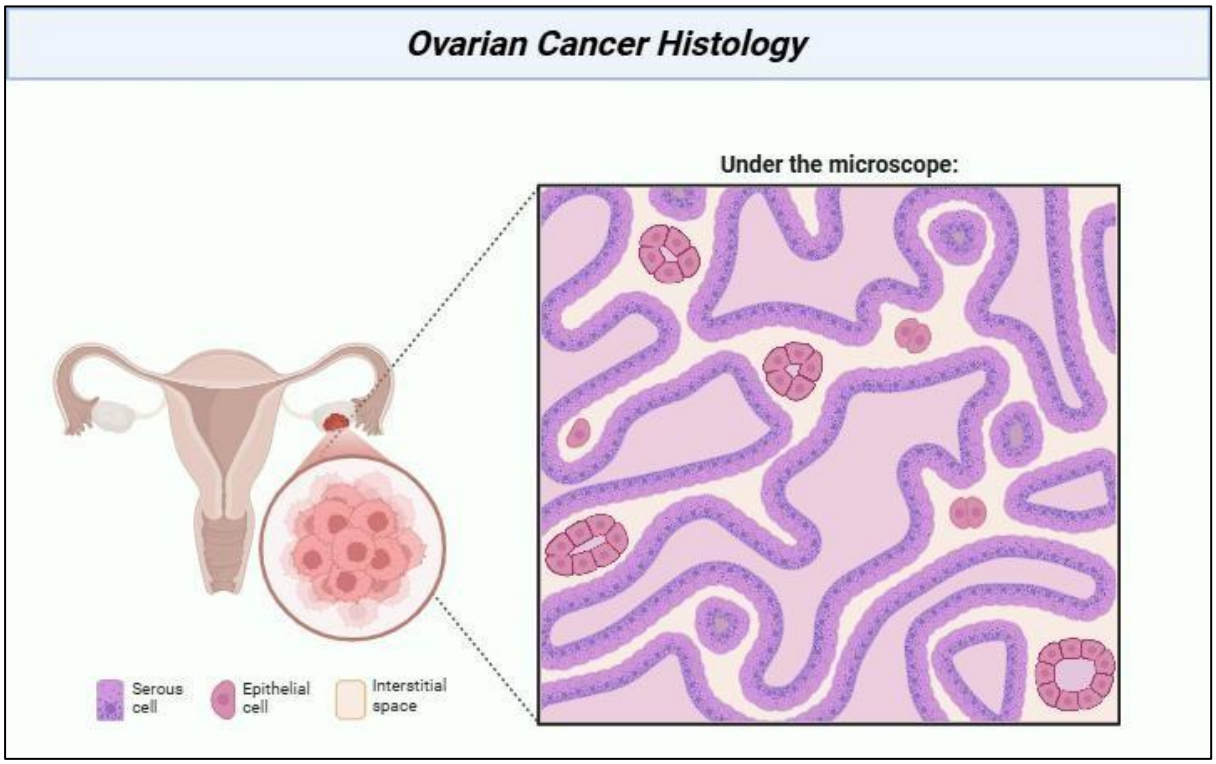


Figure 4.1: *Ovarian Cancer Histology consisting of Serous cells, epithelial cells and interstitial space.*

Ovarian cancer progression is not solely a consequence of uncontrolled cell proliferation but rather reflects a complex interplay of altered cell adhesion, enhanced migratory capacity, resistance to apoptosis, and reactivation of developmental signalling pathways(López-Reig & López-Guerrero, 2020). Among these, epithelial–mesenchymal transition (EMT), adhesion

remodelling, and telomerase activation represent interconnected biological processes that collectively enable tumour dissemination and long-term survival(Imran et al., 2021). In contrast to most solid tumours, which metastasise mainly through the blood or lymphatic system, ovarian cancer mainly disseminates via transcoelomic spread within the peritoneal cavity(Tan et al., 2006). This specialised route of metastasis forces tumour cells to detach from the primary tumour, survive in ascitic fluid as multicellular spheroids, and then attach and invade the mesothelial layer of peritoneal organs. All of these processes are tightly regulated by dynamic modifications in cell–cell and cell–extracellular matrix adhesion(Mitra, 2016).

E-cadherin, a calcium-dependent cell–cell adhesion molecule, is the principal component involved in the maintenance of epithelial integrity. E-cadherin expression loss or repression is a defining event of ovarian cancer progression and is tightly correlated with enhanced invasiveness and poor patient outcome(Rubtsova et al., 2022). Such cells, on the other hand, frequently display higher expression levels of mesenchymal adhesion molecules, like N-cadherin and vimentin, which bind stromal and mesothelial cells and promote migration. This cadherin switch is a key molecular event in bridging adhesion loss to EMT-mediated metastasis(De Wever et al., 2008).

EMT is a well-conserved developmental program that is pathologically reactivated in cancer cells to drive invasion, metastasis, and therapeutic resistance(Yeung & Yang, 2017). Epithelial cells lose their apical–basal polarity and stable intercellular junctions and gain mesenchymal characteristics, including increased motility and cytoskeletal plasticity during EMT. In ovarian cancer, EMT is crucial for spheroid formation and peritoneal implantation(Loret et al., 2019).

EMT is controlled by a panel of transcription factors, such as ZEB1, ZEB2, SNAIL, SLUG, and TWIST, that suppress epithelial gene expression and promote mesenchymal gene expression(Casas et al., 2011). Among them, ZEB1 exerts a dominant function in ovarian cancer via direct transcriptional repression of E-cadherin and induction of mesenchymal traits. High ZEB1 expression is associated with late tumour stage, high metastatic ability, and poor patient survival(WU et al., 2016). Multiple signalling pathways converge to regulate EMT, including TGF-β, Wnt/β-catenin, PI3K/AKT, MAPK, and PKA signalling(J. Zhang et al., 2016). Disruption of adherens junctions leads to the release of β-catenin from the cell membrane, allowing its translocation to the nucleus, where it activates Wnt-responsive genes that promote proliferation, stemness, and EMT. These signalling cascades establish feedback

loops that stabilise the mesenchymal state and enhance resistance to apoptosis(Xue et al., 2025).

Adhesion receptors and their associated signalling molecules are important regulators of the coordination of EMT and stem-like characteristics of ovarian cancer cells(Le Bras et al., 2012). The hyaluronan-binding cell surface receptor CD44 is a well-established cancer stem cell marker and is commonly overexpressed in aggressive ovarian tumours. CD44 receptor-mediated signalling leads to stimulation of downstream effectors such as PI3K/AKT and MAPK associated with survival, Epithelial to Mesenchymal Transition (EMT) and chemoresistance(Hassn Mesrati et al., 2021).

Cancer stem-like cells are more efficient in forming spheroids, self-renew, and initiate tumours and are considered responsible for disease relapse after chemotherapy(Phi et al., 2018). EMT and stemness are tightly coupled, and EMT-inducing transcription factors (e.g. ZEB1) have also been implicated in the maintenance of stem-like states(Drápela et al., 2020b). Therefore, adhesion remodelling and EMT signalling converge to promote both metastatic dissemination and tumour survival(Huang et al., 2022).

Transcription factors serve as critical regulators that integrate extracellular signals with gene expression programs governing cell fate decisions. In ovarian cancer, transcription factors such as SP1, CDX2, GATA2, FLI1, and ZEB1 play important roles in controlling EMT, adhesion, proliferation, and telomerase activation(Nameki et al., 2021). SP1 is a ubiquitously expressed transcription factor that binds to GC-rich promoter regions and controls several genes associated with cell cycle progression, apoptosis resistance, and EMT. SP1 has also been associated with resistance to chemotherapy in ovarian cancer by regulating oncogenic lncRNAs and growth factor signalling pathways(Wierstra, 2008). GATA2 is a transcriptional activator of genes involved in proliferation and survival, such as the telomerase reverse transcriptase (TERT) gene. FLI1, an ETS transcription factor family member, has also been reported to bind mutant TERT promoters and increase telomerase activity in cancer cells(Aktar et al., 2025).

On the other hand, CDX2 is linked to epithelial differentiation, tumour suppressive effects, and has been shown to inhibit EMT and invasive signalling. ZEB1 is considered a key EMT regulator that suppresses expression of epithelial adhesion genes and induces that of

mesenchymal genes. The balance of tumour suppression versus aggressive progression is, in the final analysis, dictated by the concerted regulation of these TFs(J. Yu et al., 2021).

Telomerase activation is a hallmark of cancer and enables unlimited replicative potential. The catalytic subunit of telomerase, telomerase reverse transcriptase (TERT), is transcriptionally regulated by a complex network of transcription factors that bind to the TERT promoter(Tornesello et al., 2023). Mutations within the TERT promoter generate de novo ETS binding sites, facilitating aberrant transcriptional activation by factors such as FLI1 and SP1(Stern et al., 2015). Upregulated TERT expression in ovarian cancer correlates with high-stage disease, high proliferation, and poor outcome(Dratwa et al., 2020). Emerging data indicate that EMT-associated signalling pathways (Wnt/β-catenin, PI3K/AKT) can also stimulate telomerase activity, thus connecting loss of adhesion and EMT to replicative immortality. Nevertheless, the interplay between metastasis suppressor pathways and the regulation of telomerase is still unclear(Behrooz & Syahir, 2021). The *KISS1* gene codes for kisspeptins, a group of peptides which signal via the G-protein-coupled receptor KISS1R (previously known as GPR54)(Mead et al., 2007a). Kisspeptins were first described as metastasis suppressors in melanoma, but have now been associated with several other cancers, including ovarian cancer. The activation of the KISS1/KISS1R pathway has been found to suppress migration, invasion, and metastasis and to induce apoptotic signalling(JI et al., 2013).

Kisspeptin-10, a biologically active peptide fragment, can modulate intracellular signalling MAPK, PI3K/AKT and PKA pathways. Significantly, kisspeptin signalling has been shown to restore epithelial phenotype through the upregulation of adhesion molecules and downregulation of EMT-inducer transcription factors(Shah, Mohan, et al., 2025b). However, the effector transcriptional and posttranscriptional pathways involved in these kisspeptin-dependent regulation of EMT, adhesion and telomerase activity in ovarian cancer are largely unknown(Stathaki et al., 2019).

miRNAs provide an important post-transcriptional level of regulation in molecular oncology. Among them, miR-145, miR-200c and miR-34a directly target transcription factors linked with EMT, adhesion molecules and telomerase regulators(Ding, 2014). Depletion of such tumour-suppressive miRNAs facilitates EMT, stemness, and invasive phenotypes, whereas their replenishment inhibits metastasis(Pan et al., 2021).

Emerging evidence suggests that kisspeptin signalling may influence miRNA expression profiles, thereby establishing transcription factor–miRNA feedback loops that reinforce metastasis suppression. Understanding these regulatory circuits is essential for delineating the full spectrum of kisspeptin's tumour-suppressive functions(Jabeen et al., 2016).

Given the central roles of adhesion remodelling, EMT signalling, transcriptional regulation, and telomerase activation in ovarian cancer progression, and the emerging evidence supporting kisspeptin as a metastasis suppressor, this study aimed to elucidate the molecular mechanisms underlying kisspeptin-10–mediated tumour suppression in ovarian cancer. Specifically, the present work investigates the effects of exogenous kisspeptin-10 on adhesion molecules, EMT signalling pathways, and a transcriptional network comprising SP1, CDX2, GATA2, FLI1, and ZEB1 in SKOV-3 ovarian cancer cells(Golzar & Javanmard, 2015).

By integrating in vitro gene expression analysis with in silico transcription factor binding and miRNA expression analysis, this chapter seeks to provide a comprehensive understanding of how kisspeptin signalling modulates transcriptional and post-transcriptional programs that converge on EMT suppression, telomerase regulation, and apoptosis induction.The findings from this work may contribute to the identification of novel therapeutic targets and strategies for the management of ovarian cancer.

HYPOTHESIS

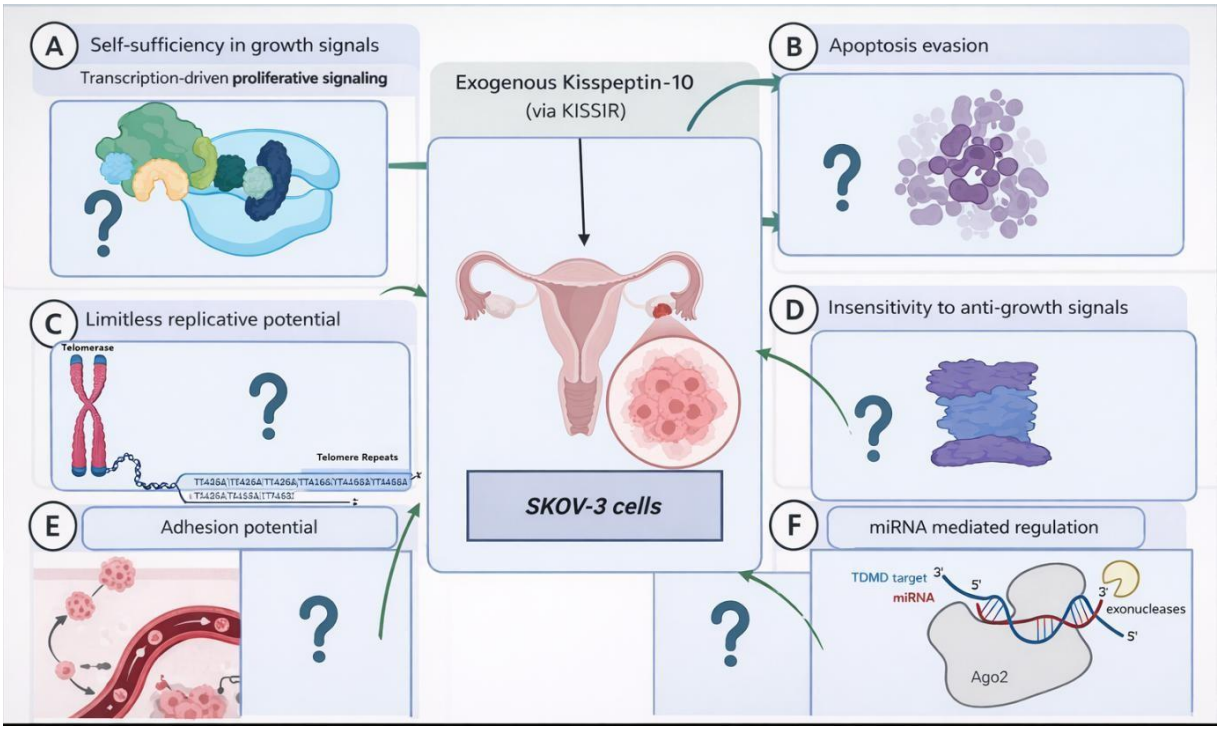


Figure 4.2: *Hypothesis on how Kisspeptin reprograms molecular pathway in Ovarian Cancer.*

Exogenous Kisspeptin-10 may reduce the aggressiveness of ovarian cancer SKOV-3 cells. This may act through by decreasing the overall cell growth, altering the candidate genes and miRNA expression. As a result, the EMT may get reduced, resulting in lower migration and invasion, and ultimately apoptosis.

4.3.1 Cell Culture and Treatment

Epithelial Ovarian Cancer cells SKOV-3 cells were cultured in McCoy's (HiMedia AL057a) media supplemented with 10% fetal bovine serum (FBS) 1% penicillin–streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were seeded at a density of 2×10^5 cells per well in six-well plates and allowed to adhere overnight.

Cell viability following Kisspeptin-10 treatment was evaluated using the MTT assay. SKOV3 cells were seeded into 96-well plates at a density of 5×10^3 cells per well and allowed to adhere overnight. Cells were then treated with increasing concentrations of Kisspeptin-10 ranging from **0, 10, 25, 50, 75, 100, 150, and 200 nM** for 24 h. After treatment, 20 µL of MTT reagent (HiMedia: TC191) (5 mg/mL in PBS) was added to each well and incubated for 4 h at 37 °C. The resulting formazan crystals were dissolved in 150 µL of DMSO, and absorbance was measured at 570 nm using a microplate reader. Cell viability (%) was calculated relative to untreated control wells. IC_{10} and IC_{50} values for Kisspeptin-10 and Doxorubicin were derived using nonlinear regression (four-parameter logistic model) fitted to MTT dose-response curves (GraphPad Prism 10).

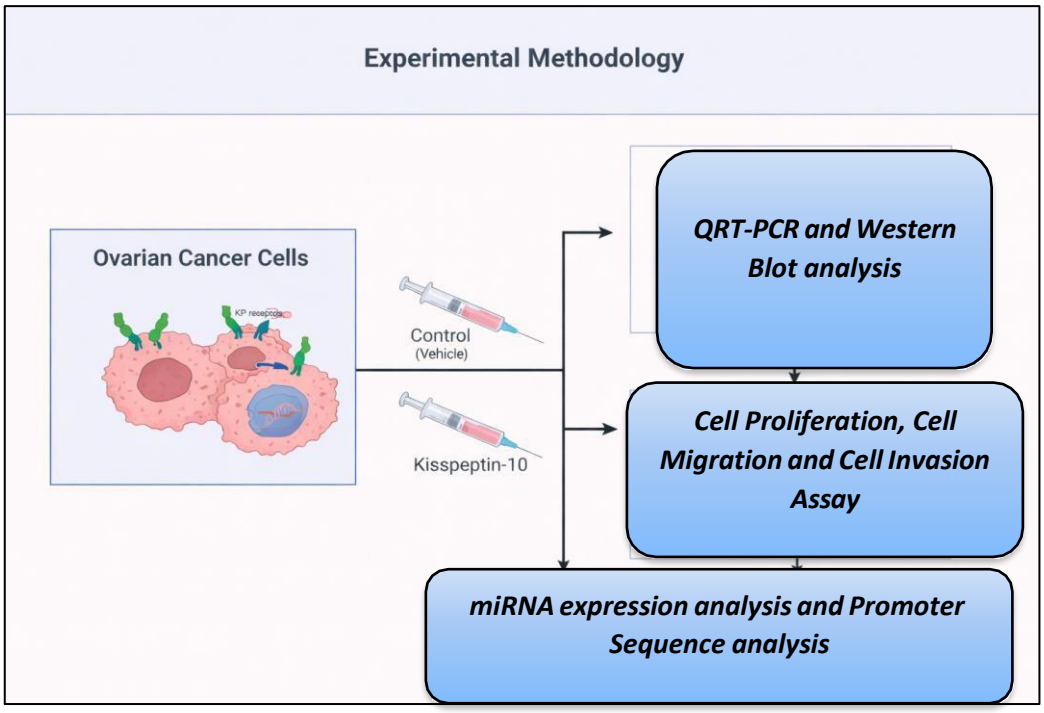


Figure 4.3: *Experimental regime followed for in vitro experiments for SKOV3 cells*

Treatments were divided into five groups:

1. Control (untreated)
2. Kisspeptin-10 at IC_{10} and IC_{50} concentrations: 10.34 nM (K10- IC_{10}) and 72.28 nM (K50- IC_{50})
3. Doxorubicin at IC_{10} and IC_{50} concentrations: (positive control) at nM 22.48nM (D10-IC10) and 117.nM (D50- IC_{50})

4.3.2 Cell Migration Assay

The protocol for SKOV3 cells was followed as mentioned in Chapter 2- 2.3.3.

4.3.3 Cell Invasion Assay

The protocol for SKOV3 cells was followed as mentioned in Chapter 2- 2.3.4.

4.3.4 miRNA Isolation and Quantitative Real-Time PCR (qPCR)

The protocol for SKOV3 cells was followed as mentioned in Chapter 2- 2.3.6 for miRNA 200c, miRNA 345 and miRNA 577.

4.3.5 Total RNA Isolation and cDNA Preparation Quantitative Real-Time PCR (qPCR)

The protocol for SKOV3 cells was followed as mentioned in Chapter 2- 2.3.5- SP1, GATA2, CDX2, HDAC2, NMYC, FLI1, ZEB1, ECAD, NCAD, CD44, VIMENTIN, BCATENIN, PKA, PKR, PLCB1 and CJUN.

4.3.6 Western Blot Analysis

The protocol for SKOV3 cells was followed as mentioned in Chapter 2- 2.3.7.

Statistical Analysis

The protocol for SKOV3 cells was followed as mentioned in Chapter 2- 2.3.7.

4.3.7 Bioinformatics and Promoter Analysis

Promoter sequences (−1500 bp upstream to +200 bp downstream of the transcription start site) of KISS1 and hTERT genes were retrieved from the Ensembl database. Transcription factor binding site (TFBS) prediction was performed using the HOCOCO v11 database, focusing on binding motifs for SP1, CDX2, GATA2, FLI1, and ZEB1. Promoter occupancy and motif enrichment were visualised using JASPAR and FIMO (MEME Suite).

4.4.1 Promoter analysis reveals shared transcription factor binding between KISS1

To explore the potential transcriptional interconnectivity between *KISS1 and hTERT* regulation, promoter sequences (−2000 to +100 bp relative to TSS) were analysed using the HOCOMOCO v11 motif database. The analysis revealed multiple SP1, ZEB1, and GATA2 motifs within both *KISS1* and *hTERT* promoter regions, suggesting the possibility of shared transcriptional control or reciprocal feedback regulation.

SP1 motifs were predominantly clustered within the proximal 500 bp region of hTERT, consistent with previous experimental evidence of its role in telomerase activation. ZEB1 motifs appeared distributed within both distal and proximal promoter regions, implying potential repression of hTERT under Kisspeptin-induced ZEB1 downregulation. CDX2 and FLI1 motifs showed moderate enrichment, indicating context-dependent promoter occupancy.

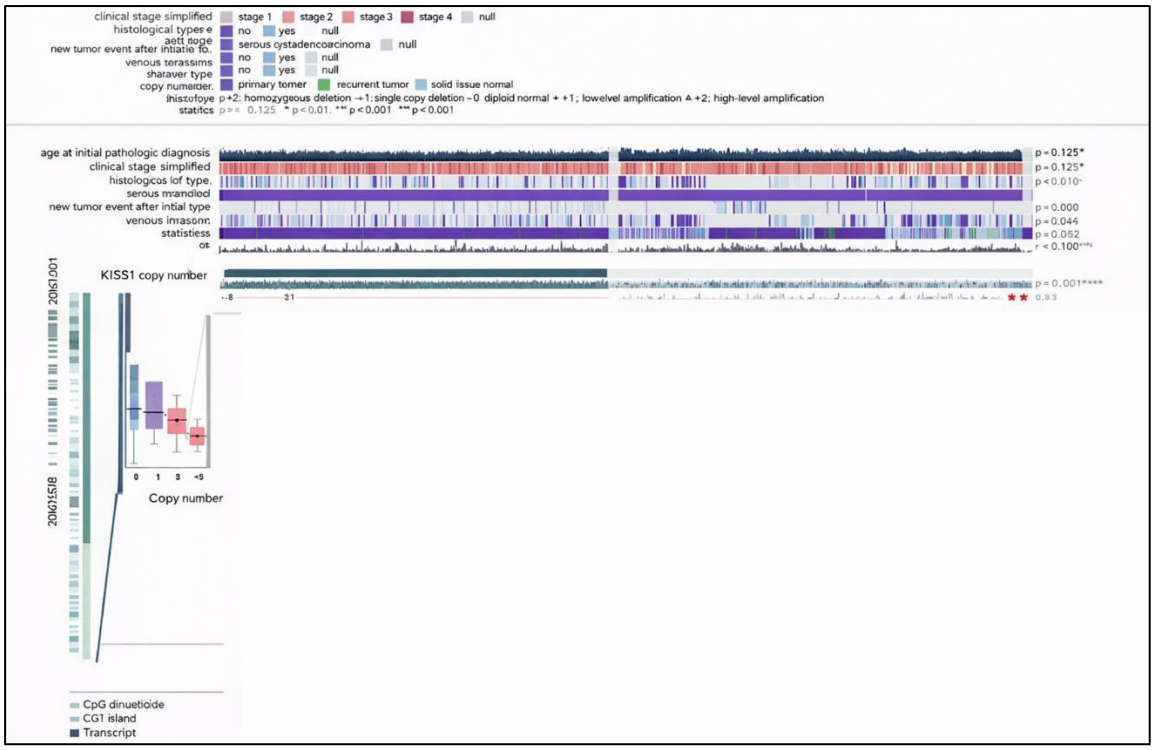


***Figure 4.4**: KISS1 Promoter Region using MoLoTool.*

S. No.	Chr	Seq name	Sequence (5′ → 3′)	Logo	P-value ($-\log_{10}$)	Start	End	Strand	Uniprot ID	Human Gene	Mouse Gene	Shield Region
1	1	chr1: GRCh38:1	TATTATATGCATG		8.59	596	609	+	CDX2_HUMAN	CDX2	Cdx2	1–6
2	1	chr1: GRCh38:1	GTTTATACCCGGC		4.138	296	307	−	CDX2_HUMAN	CDX2	Cdx2	1–7
3	1	chr1: GRCh38:1	CTTTTATTCGCTC		5.657	2209	6721	+	CDX2_HUMAN	CDX2	Cdx2	1–15
4	1	GRCh38:1	ACGGAAGACCGG		4.251	5610	2123	+	FIJI_HUMAN	FLI1	Fli1	1–0
5	1	GRCh38:1	ACGGAAATATCG		4.813	1538	2123	+	FIJI_HUMAN	FLI1	Fli1	1–3
6	1	GRCh38:1	CCAGAACTGTG		4.160	4937	4948	+	FLI1_HUMAN	FLI1	Fli1	1–6
7	1	GRCh38:1	ACTGATACAC		4.317	4816	4773	+	GATA2_HUMAN	GATA2	Gata2	1–5
8	1	GRCh38:1	GAGGCGAGGCCGC		4.220	519	532	+	SPI1_HUMAN	Three-finger Kruppel-related	SPI1	1–1
9	1	GRCh38:1	ATTGGGCCCGGGA		4.411	3882	5719	+	SPI1_HUMAN	Three-finger Kruppel-related	Spi1	1–8
10	1	GRCh38:1	GCGGCCGCGCAGC		5.655	2728	6402	+	SPI1_HUMAN	Three-finger Kruppel-related	Spi1	1–10
11	1	GRCh38:1	CTGCGCGCGCCGT		5.171	6745	6693	+	SPI1_HUMAN	Three-finger Kruppel-related	Spi1	1–11
12	1	GRCh38:1	GCGGCGCGCGCCG		4.264	5446	6681	+	SPI1_HUMAN	Three-finger Kruppel-related	Spi1	1–13

Figure 4.5: *Sequence Motif Location for each motif in MoLoTool.*

4.4.2 Effect of Kisspeptin-10 and Doxorubicin on SKOV-3 cell viability

The cytotoxic effects of Kisspeptin-10 and Doxorubicin on SKOV-3 ovarian cancer cells were evaluated using the MTT assay. Cell viability was expressed as a percentage relative to untreated control cells, which were normalised to 100%. Both Kisspeptin-10 and Doxorubicin induced a concentration-dependent reduction in cell viability. Increasing concentrations of Kisspeptin-10 resulted in a progressive decline in cell viability, with marked reductions observed at higher nanomolar concentrations. A similar dose-dependent decrease in viability was observed for Doxorubicin, although higher concentrations were required to achieve comparable cytotoxic effects. Nonlinear regression analysis of normalised percentage cell viability data was performed to determine the half-maximal inhibitory concentration (IC_{50}). Kisspeptin-10 exhibited a lower IC_{50} value (72.28 nM) compared to Doxorubicin (117.23 nM), indicating that Kisspeptin-10 possesses greater cytotoxic potency in SKOV-3 cells.

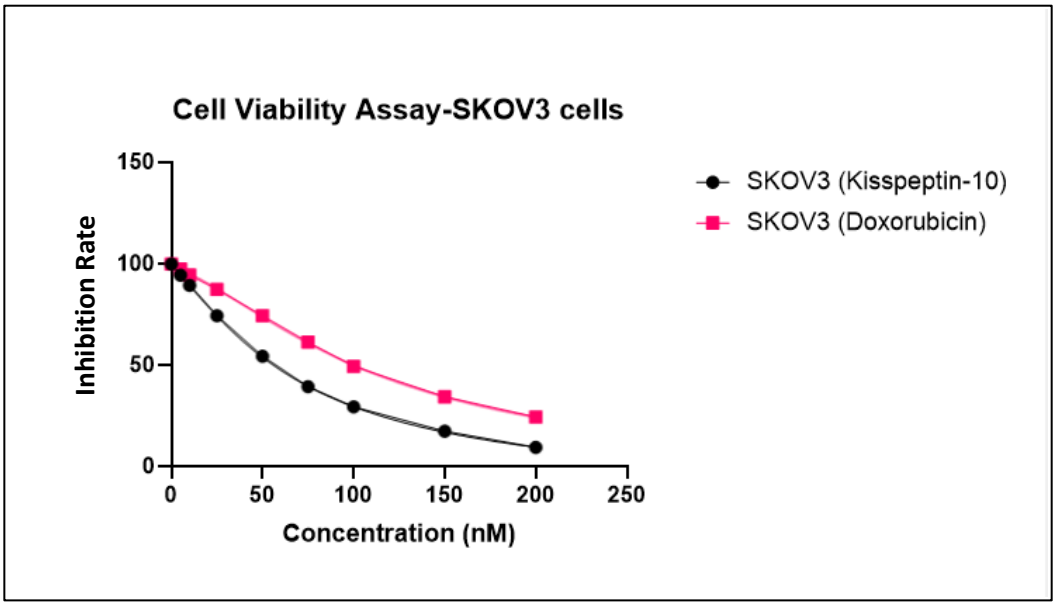


Figure 4.6: *Cell Cytotoxicity Assay for SKOV3 cells*

4.4.3 Kisspeptin-10 Inhibits Migration of SKOV3 cells

To investigate the effect of Kisspeptin-10 on ovarian cancer cell migration, a wound-healing assay was performed at 0-, 12-, and 24-hours following treatment. Untreated control cells exhibited progressive wound closure over time, reflecting the intrinsic migratory capacity of SKOV3 cells. In contrast, Kisspeptin-10 treatment significantly impaired wound closure in a dose-dependent manner. At both 12 and 24 hours, cells treated with K10 showed a marked reduction in migratory activity compared to control cells. Notably, the K50-treated group exhibited the lowest extent of wound closure, indicating a more pronounced inhibition of migration at higher Kisspeptin-10 concentrations. Quantitative analysis confirmed that wound closure percentages in both K10 and K50 groups were significantly lower than those observed in untreated controls at all assessed time points. These findings demonstrate that Kisspeptin-10 effectively suppresses glioblastoma cell migration, with greater inhibitory effects observed at higher concentrations.

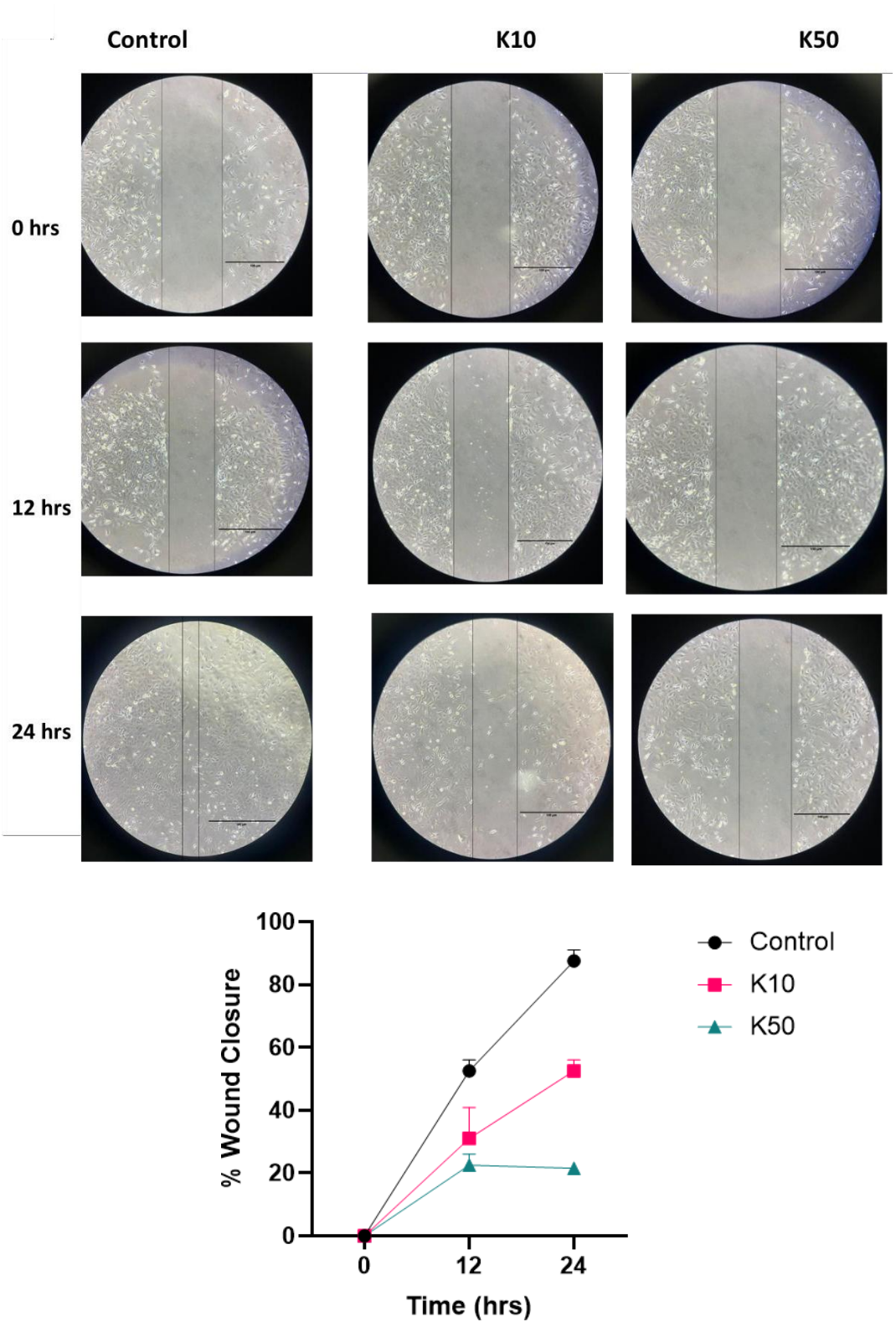


Figure 4.7: *Cell Migration Assay for SKOV3 cells*

4.4.4. Kisspeptin-10 inhibits invasive Potential of SKOV3 cells

Treatment with Kisspeptin-10 resulted in a significant reduction in the number of invading cells. Both K10- and K50-treated groups exhibited markedly decreased invasion compared to the control group, with the most substantial inhibition observed in the K50 group. Quantitative analysis confirmed that the reduction in invasion was statistically significant for both treatment conditions relative to the control.

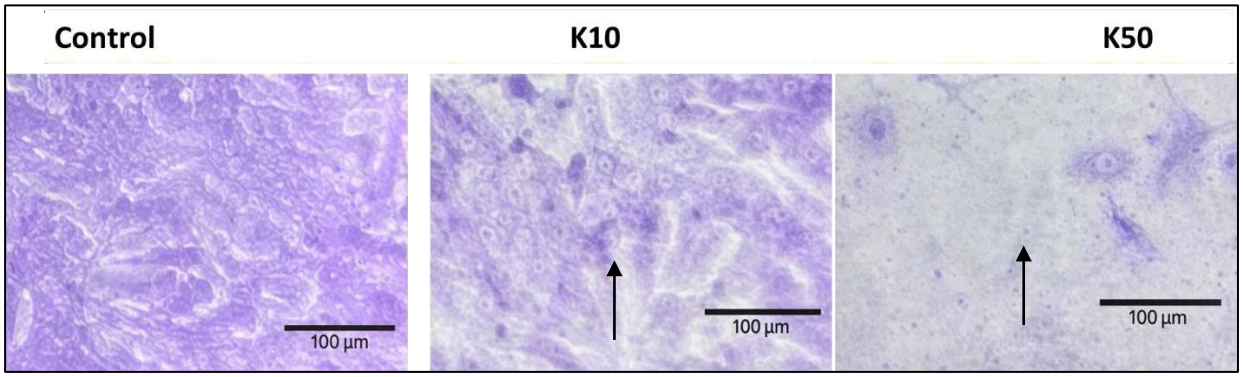


Figure 4.8: *Cell Invasion Assay for SKOV3 cells (Arrows indicates decreased invasion)*

4.4.4 Effect of Kisspeptin-10 on gene expression in SKOV-3 cells

The effects of Kisspeptin-10 and Doxorubicin on the expression of transcription factors, signalling molecules, and EMT-associated markers were evaluated in SKOV-3 ovarian cancer cells using quantitative real-time PCR (qRT-PCR). Gene expression levels were normalised to the housekeeping gene, and relative expression was calculated using the $2^{-}\Delta\Delta Ct$ method, with untreated control cells set to unity.

Regulation of transcription factors

Kisspeptin-10 treatment resulted in a dose-dependent modulation of transcription factor expression. At the IC_{10} concentration, SP1 expression increased modestly to 1.20 ± 0.04-fold, while a stronger induction was observed at the K50 (1.35 ± 0.05-fold). Similar dose-dependent upregulation was noted for GATA2 (K10: 1.20 ± 0.04, K50: 1.55 ± 0.06), CDX2 (K10: 1.10 ± 0.04, K50: 2.58 ± 0.06), and NMYC (K10: 1.09 ± 0.04, K50: 1.13± 0.06).

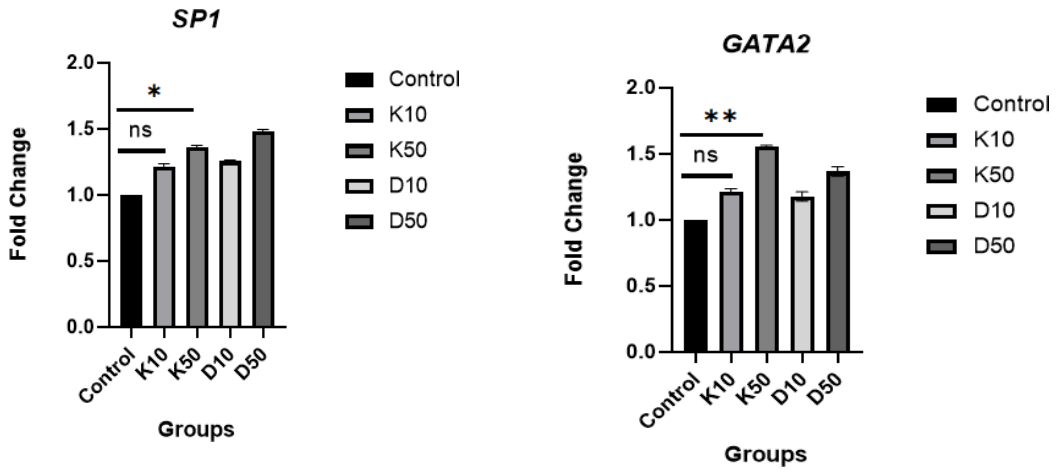
In contrast, transcription factors associated with chromatin remodelling and mesenchymal transition were downregulated following Kisspeptin-10 treatment. ZEB1 expression was reduced to 0.95 ± 0.03-fold at K10 and further suppressed at K50 (0.65 ± 0.04-fold). Similarly, HDAC2 and FLI1 showed moderate but consistent downregulation, with HDAC2 decreasing to 0.93 ± 0.03-fold and FLI1 to 0.76 ± 0.04-fold at K50.

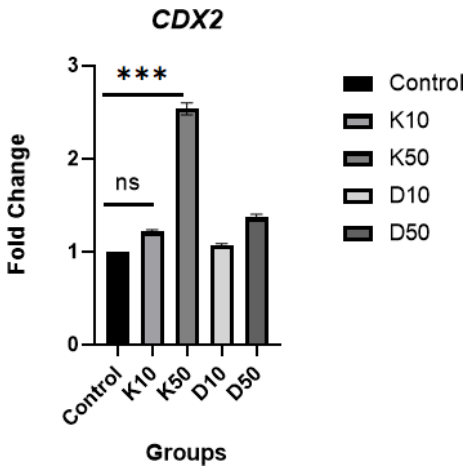
Modulation of signalling molecules

Analysis of signalling regulators revealed significant Kisspeptin-10–mediated activation of intracellular signalling pathways. Expression of PLCB1, PKA, and PKR (EIF2AK2) increased substantially following Kisspeptin-10 treatment. At IC_{10}, PLCB1 expression increased to 1.45 ± 0.05-fold, while at K50 it reached 1.75 ± 0.06-fold. Comparable induction patterns were observed for PKA (K10: 1.45 ± 0.05, K50 1.75 ± 0.06) and PKR (K10: 1.45 ± 0.05, K50: 1.75 ± 0.06). Conversely, CJUN expression was significantly suppressed by Kisspeptin-10, decreasing to 0.95 ± 0.03-fold at K10 and 0.65 ± 0.04-fold at K50, indicating inhibition of pro-proliferative and stress-responsive signalling pathways.

Regulation of EMT and adhesion-associated markers

Kisspeptin-10 significantly altered the expression of EMT and adhesion markers in SKOV-3 cells. Expression of epithelial markers was markedly enhanced, with E-cadherin (ECAD) increasing to 2.15 ± 0.05-fold at K10 and 3.65 ± 0.06-fold at K50. In contrast, mesenchymal markers were significantly downregulated following Kisspeptin-10 treatment. N-cadherin (NCAD) expression decreased to 0.85 ± 0.03-fold at K50, while Vimentin and CD44 were reduced to 0.65 ± 0.03-fold and 0.59 ± 0.04-fold, respectively. These changes indicate a coordinated suppression of EMT-associated gene expression.

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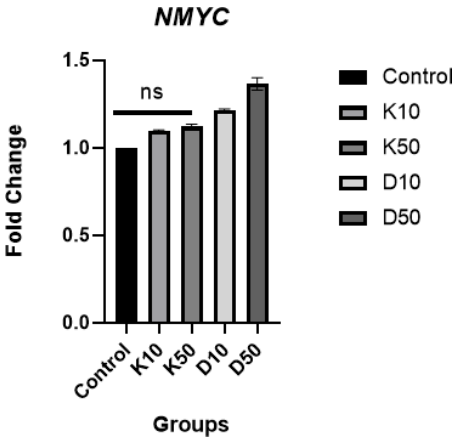
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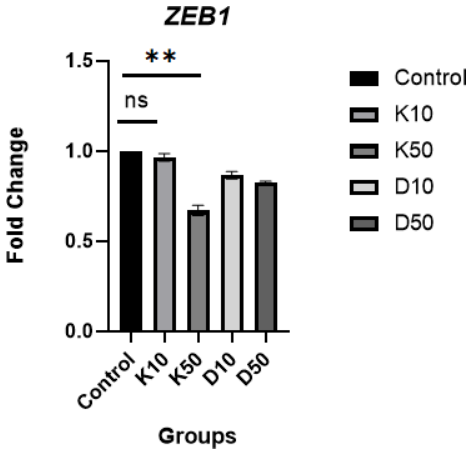
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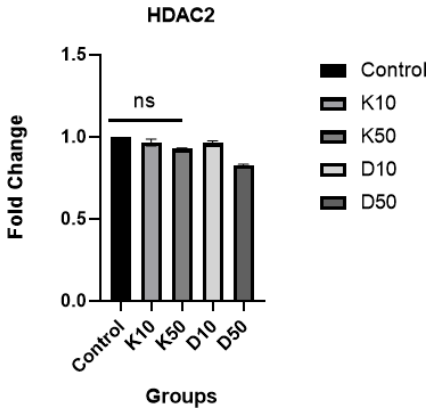
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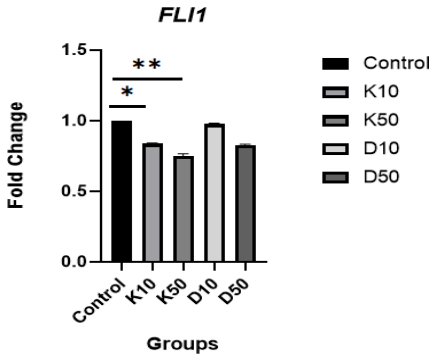
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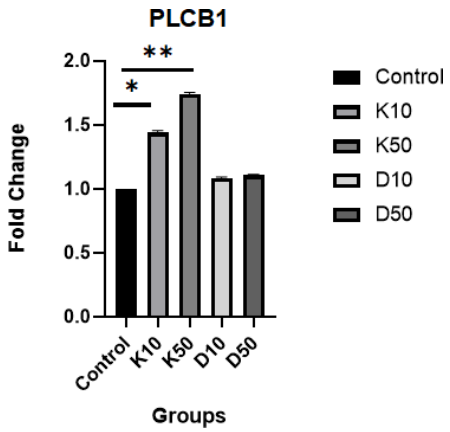
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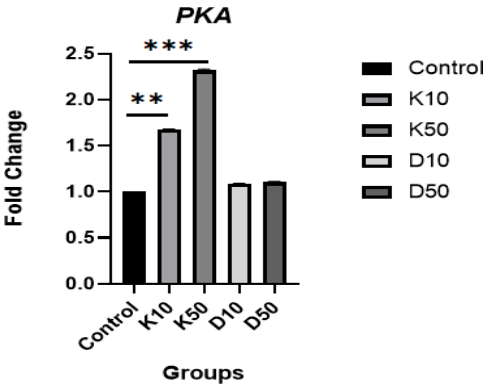
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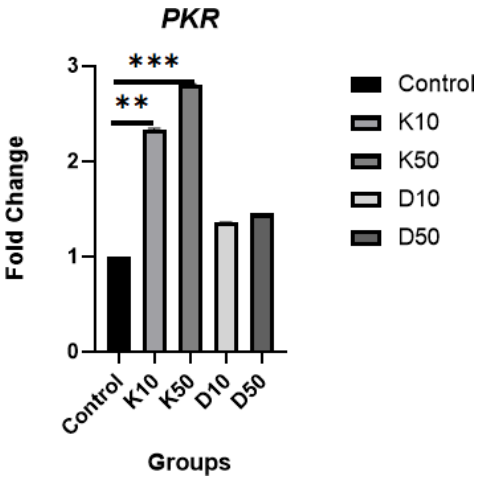
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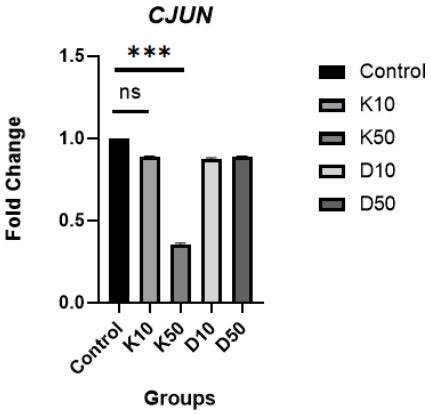
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ns
Control
K10
K50
D10
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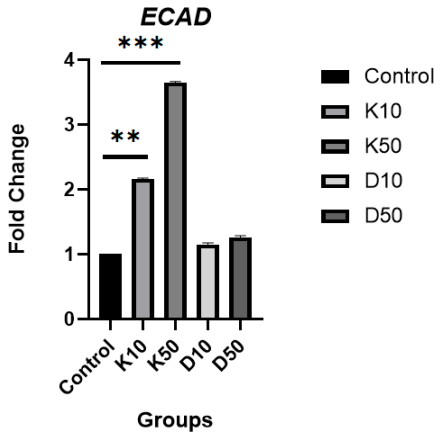
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Fold Change
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Control
K10
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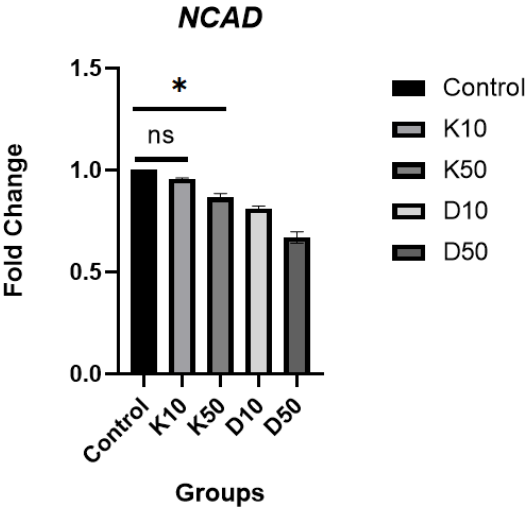
Groups


NCAD
Fold Change
1.5
1.0
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ns
Control
K10
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D10
D50
Groups

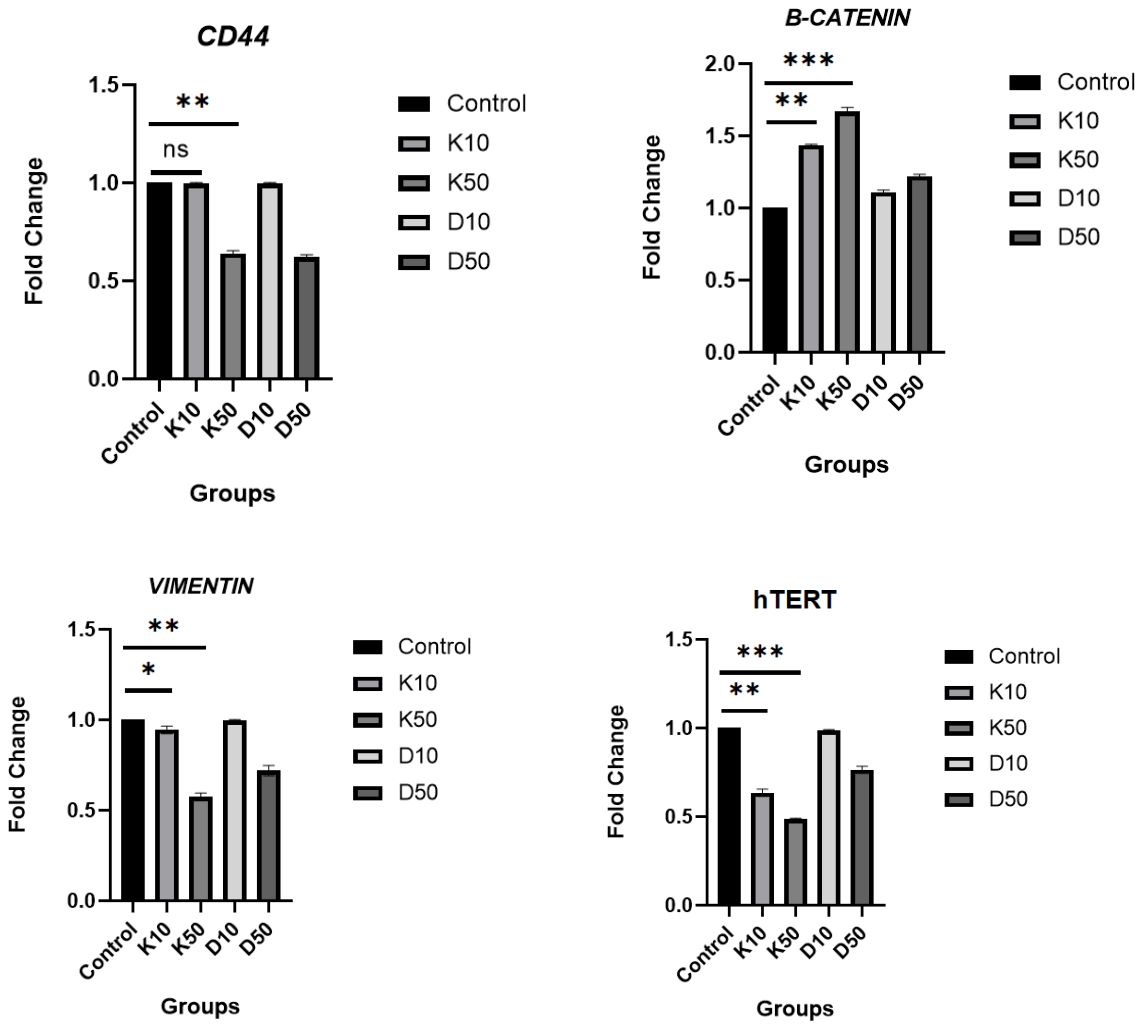

Figure 4.9: Gene expression analysis for SKOV3 cells

Table 4.1: Consolidated Gene Expression profiles of Transcription factors, Adhesion markers, and Signalling molecules for SKOV3 cells

Gene	Control	K10	K50	D10	D50
SP1	1.00	1.21 ± 0.04	1.35 ± 0.05	1.25 ± 0.04	1.40 ± 0.05
GATA2	1.00	1.20 ± 0.04	1.55 ± 0.06	1.15 ± 0.04	1.34 ± 0.05
CDX2	1.00	1.10 ± 0.04	2.58 ± 0.06	1.05 ± 0.03	1.36 ± 0.04
NMYC	1.00	1.09 ± 0.04	1.13± 0.06	1.15 ± 0.04	1.32± 0.03
ZEB1	1.00	0.95 ± 0.03	0.63 ± 0.04	0.85 ± 0.03	0.83 ± 0.04
HDAC2	1.00	0.95 ± 0.03	0.93 ± 0.03	0.95 ± 0.03	0.80 ± 0.04
FLI1	1.00	0.83± 0.03	0.76 ± 0.04	0.98± 0.03	0.80 ± 0.01
PLCB1	1.00	1.45 ± 0.05	1.75 ± 0.06	1.05 ± 0.03	1.10 ± 0.03

PKA	1.00	1.67 ± 0.05	2.75 ± 0.06	1.03 ± 0.03	1.09 ± 0.03
PKR (EIF2AK2)	1.00	2.31 ± 0.05	2.79 ± 0.06	1.35 ± 0.05	1.45 ± 0.05
CJUN	1.00	0.89 ± 0.03	0.35 ± 0.04	0.85 ± 0.03	0.89 ± 0.04
ECAD	1.00	2.15 ± 0.05	3.64 ± 0.06	1.13 ± 0.03	1.24 ± 0.04
NCAD	1.00	0.95 ± 0.03	0.85 ± 0.03	0.80 ± 0.03	0.65 ± 0.04
CD44	1.00	0.95 ± 0.03	0.65 ± 0.04	1.00 ± 0.03	0.63 ± 0.01
β-CATENIN	1.00	1.43 ± 0.05	1.65 ± 0.06	1.10 ± 0.03	1.21 ± 0.04
VIMENTIN	1.00	0.93 ± 0.03	0.59 ± 0.03	1.00 ± 0.03	0.70 ± 0.04
HTERT	1.00	0.62±0.02	0.49±0.01	0.98±0.01	0.78±0.03

4.4.5 In Vitro Validation of miRNA Expression in SKOV-3 Cells

Quantitative real-time PCR (qRT-PCR) was performed to evaluate the expression of miR-200, miR-345, and miR-577 in SKOV-3 ovarian cancer cells following treatment with Kisspeptin-10 and Doxorubicin. Cells were treated with Kisspeptin-10 at IC_{10} (K10) and IC_{50} (K50) concentrations, while Doxorubicin at IC_{10} (D10) and IC_{50} (D50) served as a positive control. miRNA expression levels were normalised to endogenous small RNA controls and expressed as relative fold change compared to untreated control cells. Kisspeptin-10 treatment resulted in a significant, dose-dependent increase in the expression of miR-200. A moderate elevation in miR-200 levels was observed at the K10, while a higher fold increase was detected at the K50 groups compared to untreated controls.

Similarly, miR-345 expression was significantly upregulated following Kisspeptin-10 treatment, with greater induction observed K50 to K10. The increase in miR-345 expression displayed a clear dose-dependent pattern across the Kisspeptin-10 treatment groups. In addition, miR-577 expression was non-significantly increased in Kisspeptin-10–treated SKOV-3 cells, exhibiting a concentration-dependent rise from K10 to K50 when compared to control cells.

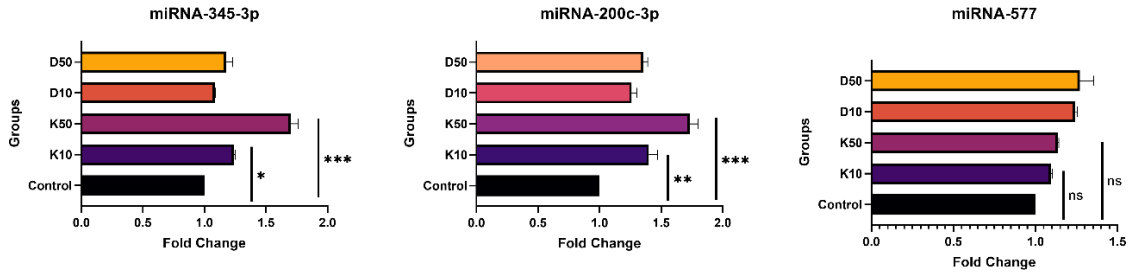

Figure 4.10: *miRNA expression analysis for SKOV3 cells*

4.4.6 Western Blot analysis

Western blot analysis was conducted to assess the effect of Kisspeptin-10 on the protein level of ZEB1 and SP1 in SKOV-3 ovarian cancer cells. The analysis was done using β-actin as a control. The analysis showed that the protein level of ZEB1 did not change significantly in all treatment groups in comparison to the control group (ns). Kisspeptin-10 (K10 and K50) and Doxorubicin (D10 and D50) treatment groups showed a constant level of protein expression close to the control group, indicating that the level of the ZEB1 protein in the ovarian cancer cell line was not significantly affected by the treatment. On the other hand, the level of SP1 showed a significant effect following treatment with Kisspeptin-10. Although the K10 group showed a moderate increase in the level of SP1 in comparison to the control group (*$p < 0.05$), the K50 group showed a higher level of SP1 in comparison to the control group (**$p < 0.01$). On the other hand, the level of SP1 in the Doxorubicin treatment groups (D10 and D50) was found to be down-regulated in comparison to the control group.

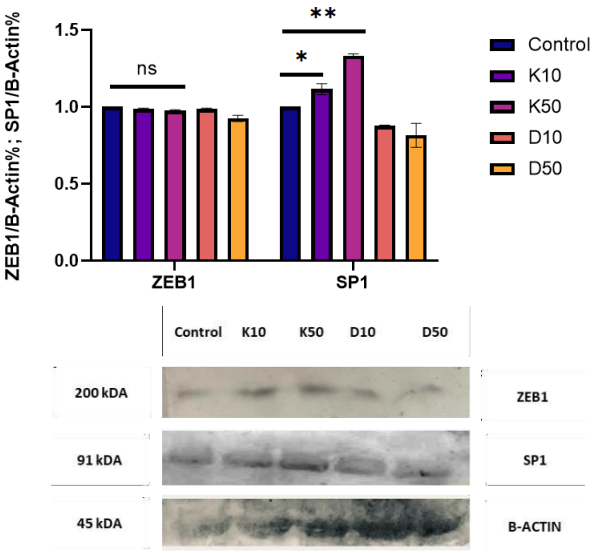

Figure 4.11: *Protein expression analysis for SKOV3 cells.*

DISCUSSION

Ovarian cancer exhibits a great degree of cellular plasticity, can spread, and is commonly resistant to traditional chemotherapeutic regimens, leading to poor clinical outcomes(X. Guo et al., 2026). Traditional drugs such as Doxorubicin have mainly cytotoxic activity based on DNA damage and oxidative stress, but recently it has been suggested that the effective therapeutic management should also target the molecular pathways regulating tumour aggressiveness, EMT and metastatic capacity. Here, the effects of external Kisspeptin-10 in SKOV-3 human ovarian cancer cells were studied in a novel manner, combining the cell survival outcome with transcriptional, signalling and post-transcriptional regulatory modulations to gain insights into its overall cellular effect(Kciuk et al., 2023).

The result of the MTT assay for cell viability indicated that kisspeptin-10 induced a significant decrease in cell viability of SKOV-3 cells in a dose-dependent manner and that it had a lower IC50 value than doxorubicin. As a normalised pharmacological measurement, IC50 was considered a robust indicator for drug potency and was not influenced by the assay-specific variation. Higher potency of Kisspeptin-10(He et al., 2016). The IC_{50} value is lower, which means that inhibition of cell viability to the same extent requires a much lower concentration of Kisspeptin-10 than Doxorubicin. Notably, this decrease in viability is associated with profound transcriptional reprogramming, indicating that Kisspeptin-10 is not acting as a general cytotoxic stress but rather as a regulated cellular mechanism(Song & Zhao, 2016b).

At the transcriptional level, Kisspeptin-10 produced a concerted, dose-dependent regulation of a set of transcription factors. Upregulation of SP1, GATA2, CDX2 and NMYC was accompanied by downregulation of ZEB1, HDAC2 and FLI1. This pattern is more reflective of a coordinated transcriptional reprogramming than of a lattice of randomly perturbed genes. While a subset of these upregulated transcription factors has been reported to have context-dependent protumour roles, their upregulation in conjunction with suppression of EMT and viability indicates their potential role in orchestrated transcriptional and cellular state transitions. By contrast, ZEB1 downregulation is a major result since ZEB1 is a key EMT and cellular plasticity regulator. Reduction of ZEB1 in concert with HDAC2 depletion suggests a relief from chromatin-based repression of epithelial genes and the neutralisation of EMT programs(Patel et al., 2026).

Kisspeptin-10 had a profound effect on the expression of intracellular signalling molecules, such as PLCB1, PKA, PKR, and CJUN; expression was inhibited. These are indicative of the recruitment of receptor-mediated signalling cascades downstream of KISS1R activation(Mead et al., 2007b). PLCB1 and PKA are major components of the second messenger and kinase signalling cascades, respectively,

and PKR is a stress-responsive kinase that modulates translational homeostasis and apoptotic vulnerability. CJUN, a member of the AP-1 transcription complex implicated in survival and stress adaptation, was significantly downregulated. Taken together, these signalling alterations can indicate a potential transition from pro-survival and proliferative signalling to controlled stress and growth regulatory pathways(Xia et al., 2007). Although doxorubicin displayed a very strong effect on cell viability and, to a lesser extent, precise expression control of RBM39, it produced a somewhat weaker and less coherent regulation of these signalling proteins, highlighting differences in the modes of action of cytotoxic chemotherapy and peptide-based signalling modulation(Xu et al., 2022).

Among the most remarkable results of Kisspeptin-10 treatment (Kp-10) was on EMT and its related genes, such as adhesion. Kisspeptin-10 triggered the upregulation of epithelial markers E-cadherin and β-catenin and the downregulation of mesenchymal/stemness-related markers N-cadherin, Vimentin, and CD44. Uncontrolled EMT is a key promoter of ovarian cancer progression, invasion, and therapy resistance; suppression of EMT is tightly associated with decreased metastatic potential. Re-expression of epithelial markers and loss of mesenchymal features were indicative of a more stable epithelial phenotype. While the KISS1-derived peptide KP-10 was not directly tested in any migration or invasion assays, the transcriptional signature is highly indicative of decreased motility and invasive potential and in agreement with the known function of the KISS1/KISS1R system as a metastasis suppressor(Ciaramella et al., 2018).

The transcriptional coherence reported here is also supported by the analysis of promoter sequences. *In silico* screening against the HOCOMOCO transcription factor binding motif database identified overrepresentation of the binding sites of Kisspeptin-controlled transcription factors such as SP1, GATA2, CDX2, etc., in the promoter sequences of the regulated transcripts. This indicates that the changes in the expression of these transcription factors could be responsible for the changes in gene expression observed. Enrichment of AP-1–related motifs in mesenchymal gene promoters is also consistent with the observed repression of CJUN and could underlie decreased expression of at least some EMT-associated targets. While the presence of a promoter motif does not prove direct binding, it does provide mechanistic validity for the coordinated transcriptional regulation observed in response to Kisspeptin-10(Vicente et al., 2012).

In addition to transcriptional regulation, posttranscriptional regulation via microRNAs (miRNAs) is yet another layer of cellular regulation that is modulated by Kisspeptin-10. *In vitro* confirmation revealed a pronounced upregulation of miR-200, miR-345 and miR-577 in a dose-dependent manner in SKOV-3 cells after the treatment with Kisspeptin-10. miR-200 is well established in its regulation of epithelial and mesenchymal states via bidirectional interactions with EMT-related transcription factors. miR-345 and miR-577 have been shown to regulate apoptosis, survival signalling and EMT-related gene

expression. These miRNAs appear to be modulated in a concerted manner, parallel to the transcriptional changes and indicative of post-transcriptional stabilisation of the cellular state induced by Kisspeptin-10. Although miRNA targets weren't experimentally validated in this work, the observed changes at the mRNA level, concordant with miRNA expression, do support their participation in the regulatory network induced by Kisspeptin-10(Pu et al., 2019).

Combining these results, we can propose a multi-level mechanistic model in which Kisspeptin-10 activates KISS1R-dependent signalling cascades at the transcriptional and post-transcriptional levels of regulatory networks. These networks collectively influence transcription factors, signalling molecules, chromatin modulators and miRNAs to suppress EMT, attenuate cellular plasticity, and impair cell viability. Conventional chemotherapy drugs induce cytotoxic damage to cells, but Kisspeptin-10 appears to have a regulatory effect on the pathways of tumour growth and aggressiveness(Hu et al., 2019).

Although these are exciting insights, there are obvious limitations that should be considered. This study was limited to one ovarian cancer cell line, and therefore, extension to other models is needed to evaluate the broader applicability. In addition, although transcriptional and miRNA changes strongly suggest functional implications in terms of migration, invasion and apoptosis, these need to be confirmed by direct functional assays and by validation at the protein level. Future studies, including chromatin immunoprecipitation, miRNA target validation, migration and invasion assays and *in vivo* models, will be required to further dissect the therapeutic potential of Kisspeptin-10(S. Li et al., 2010).

In Summary, this work provides evidence for the powerful action of Kisspeptin-10 on SKOV-3 ovarian cancer cells, leading to decreased cell viability and synchronised transcriptional and posttranscriptional reprogramming. Kisspeptin-10 induces a less aggressive cellular manifestation through its effects on signalling pathways, transcription factors, EMT-related genes and miRNAs. These data lay a solid molecular groundwork to explore further approaches based on Kisspeptin as modulatory routes in ovarian cancer therapy.

CONCLUSION

In the present study, we provide novel evidence that exogenous Kisspeptin-10 potently inhibits cell survival of SKOV-3 serous epithelial ovarian cancer cells and is capable of orchestrating a molecular response at the transcriptional signalling and at post transcriptional regulation of gene expression. The treatment with kisspeptin-10 also affected key transcription factors and intracellular signalling pathways, along with dose-dependent changes in expression of EMT- and adhesion-related genes. These molecular changes were accompanied by dramatic changes in miRNA expression, including the upregulation of miR-200, miR-345 and miR-577, suggesting a defined regulatory response rather than a general cytotoxic reaction.

Together, the combination of cell survival, gene expression, promoter assay and miRNA profiling data underpin a model of Kisspeptin-10 as a reprogramming agent that alters ovarian cancer cells to a less malignant phenotype. Inhibition of EMT-related molecular programs, decreased migration and invasion, increased apoptotic sensitivity - Kisspeptin-10 hits several hallmarks of ovarian cancer. These observations provide a molecular basis for the preclinical evaluation of Kisspeptin-based approaches as regulatory therapeutics in serous epithelial ovarian cancer.

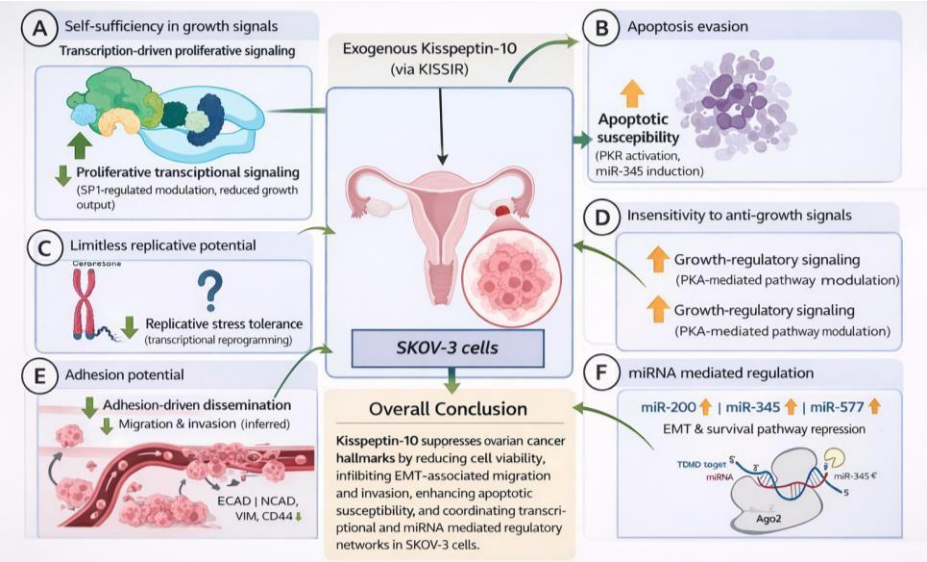

Figure 4.12: *Conclusion Image for the role of Kisspeptin-10 in Ovarian Cancer*