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## ***Introduction***

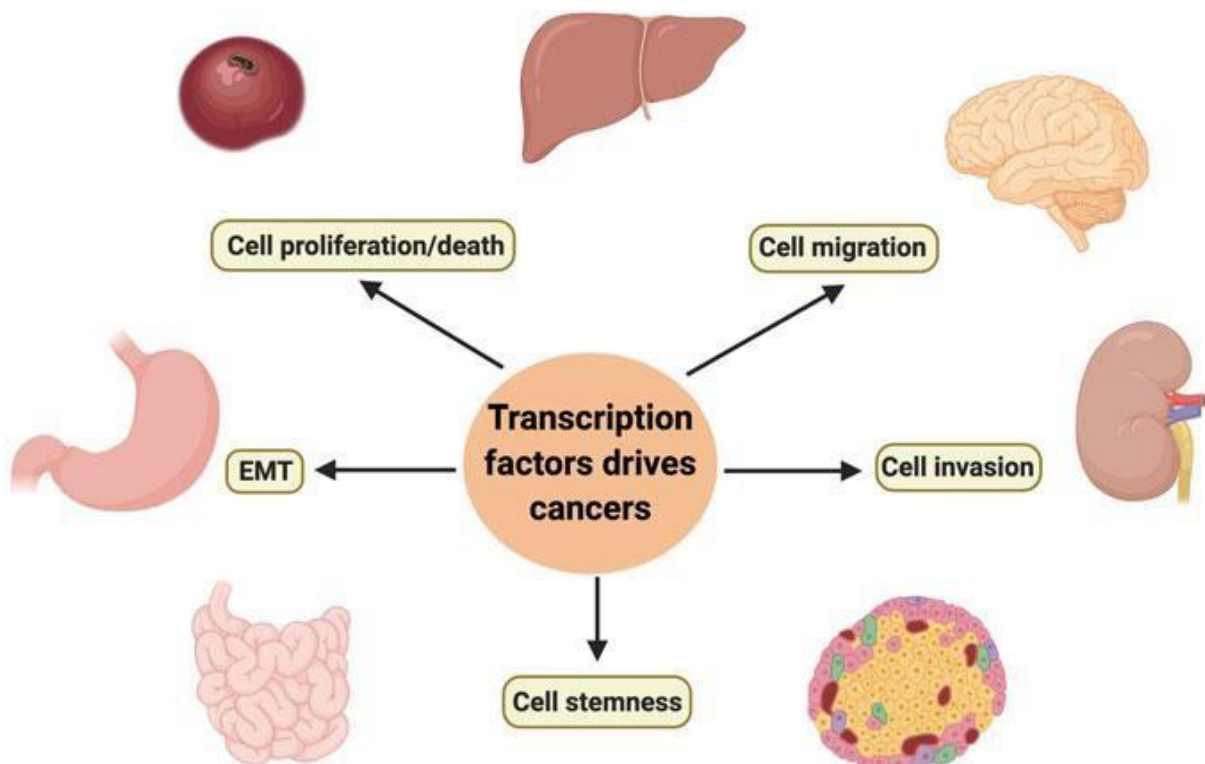
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Cancer remains one of the leading causes of morbidity and mortality worldwide. According to recent global cancer statistics, more than 19 million new cancer cases are diagnosed annually, with over 10 million cancer-related deaths reported each year, underscoring the substantial public health burden imposed by malignancies (Sung et al., 2021). Current statistics also suggest that breast cancer is the most frequently diagnosed cancer-type across the world, forming nearly 2.3 million new cancer patients annually and accounting for 11–12% of all newly diagnosed cancer patients. In addition, breast cancer also ranks among the most frequently occurring cancer-related deaths among females, resulting in over 680,000 cancer-related fatalities annually (Sedeta et al., 2023). Among various types of breast cancer, triple-negative breast cancer (TNBC) constitutes over 15–20% of all breast cancer patients and is associated with aggressive characteristics, early metastasis, and a high recurrence rate with a lack of targeted cancer therapy. Although ranked lower with regard to cancer incidence compared to breast cancer, ovarian cancer also ranks as one of the deadliest cancer types. Worldwide, there are more than 310,000 new ovarian cancer patients who are diagnosed every year, and there are more than 200,000 deaths recorded worldwide. However, the high death rate has been attributed to the fact that, for ovarian cancer patients, about 70% are already suffering from advanced stages of metastatic disease. The most common type of ovarian cancer is high-grade serous ovarian carcinoma, which is linked with early peritoneal spread, drug resistance, and recurrence (Łukasiewicz et al., 2021).

Glioblastoma, the most aggressive primary brain tumour, accounts for approximately 45–50% of all malignant primary brain tumours (Sipos et al., 2025). Although the global incidence is relatively lower compared to breast and ovarian cancers, approximately 3–5 cases per 100,000 individuals annually, glioblastoma has a disproportionately high mortality rate. The median survival remains approximately 12–15 months despite maximal surgical resection, radiotherapy, and chemotherapy. A five-year survival rate of less than 7% evidence presses the urgent need for deeper mechanistic understanding and novel therapeutic strategies (Davis, 2016).

The conceptual model by Douglas Hanahan and Robert A. Weinberg, popularly known as the Hallmarks of Cancer, describes the biological capabilities acquired during tumour development. Among these hallmarks, the activation of invasion and metastasis is considered the most lethal attribute, since metastatic dissemination accounts for the majority of worldwide cancer-related deaths (Guan, 2015). Metastasis is a highly coordinated multistep process involving epithelial–mesenchymal transition, extracellular matrix degradation, intravasation, survival in circulation, extravasation, and colonisation of distant organs (van Zijl et al., 2011). These events are tightly regulated through complex transcriptional, signalling, and epigenetic networks, including:

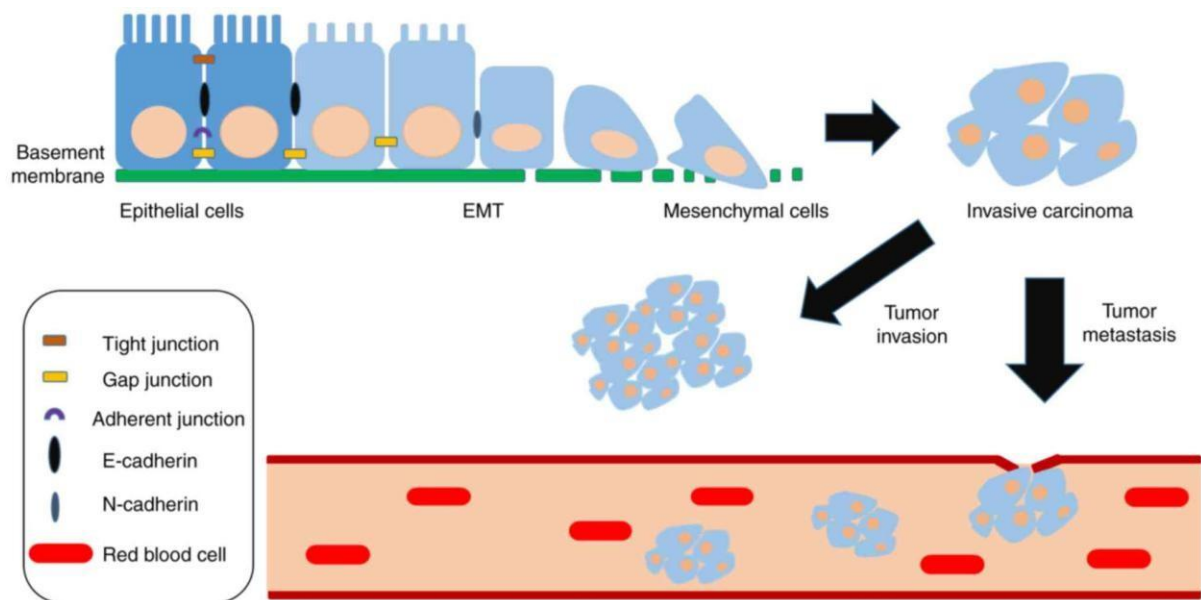
1. **Transcription factors** are considered the master regulators of oncogenic reprogramming. Proteins like SP1, MYCN, CDX2, FLI1, GATA2, and HDAC2 directly regulate gene expression programs responsible for proliferation, differentiation, stemness, invasion, and chromatin remodelling(Vishnoi et al., 2020). Since transcription factors often act at the top of regulatory hierarchies, even slight changes in their activities might provide significant changes in downstream gene networks(Baumgart et al., 2013). The oncogenic TFs, such as MYCN, amplify proliferative and metabolic pathways, while epigenetic regulators like HDAC2 alter chromatin accessibility and impact global transcriptional landscapes(Lodrini et al., 2013). Thus, the study of transcription factors and their activities become imperative to understand how cancer cells reprogram their gene expression profiles toward gaining aggressive phenotypes and how metastasis suppressor genes may become transcriptionally silenced in the absence of direct mutation(R. Liu et al., 2021).



*Figure 1: Elucidated role of transcription factors in cancer ((A. Zhang et al., 2023)*

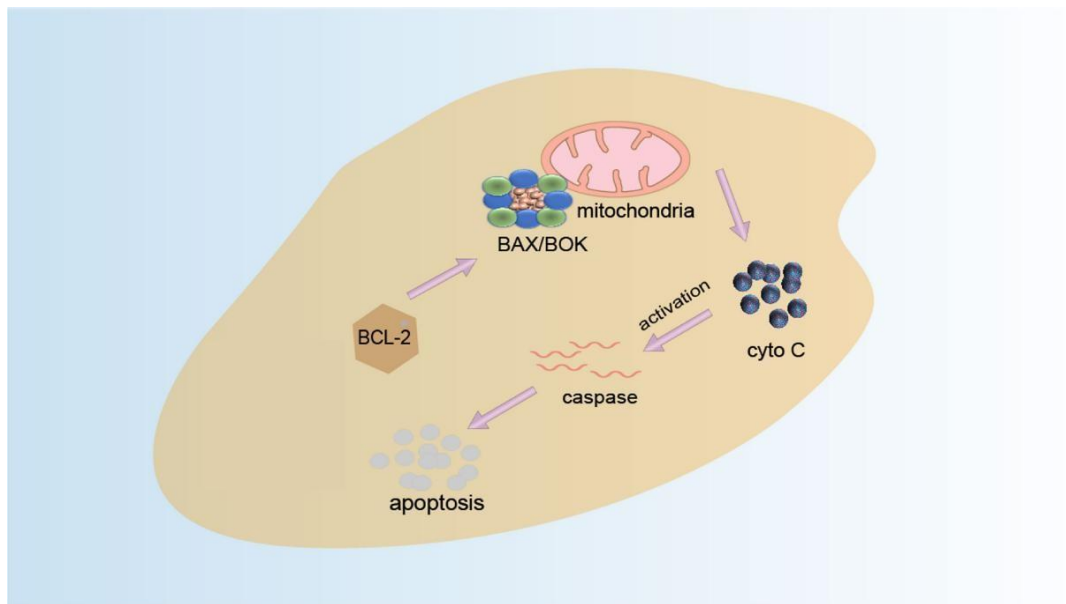
2. **Adhesion markers** are crucial for evaluating metastatic potential. Markers like E-cadherin, N-cadherin, Vimentin, CD44, ZEB1, and Beta-catenin are important for epithelial-mesenchymal transition, an essential phenomenon for cancer cells to become motile and invade other tissues(Kallergi et al., 2011). Usually, the absence of E-cadherin disorganises epithelial cell-cell interactions. Conversely, overexpression of N-cadherin and Vimentin increases cell mobility(Cao et al., 2019). CD44 facilitates the growth of cancer stem cells and treatment resistance, and ZEB1 feeds back by repressing epithelial-specific genes while activating those

related to mesenchymal cells. By exploring adhesion markers, it is important to note that this phenomenon harbours quantifiable molecular events for evaluating metastatic potential and how static epithelial cells transform into motile cells with a mesenchymal phenotype(Primeaux et al., 2022).



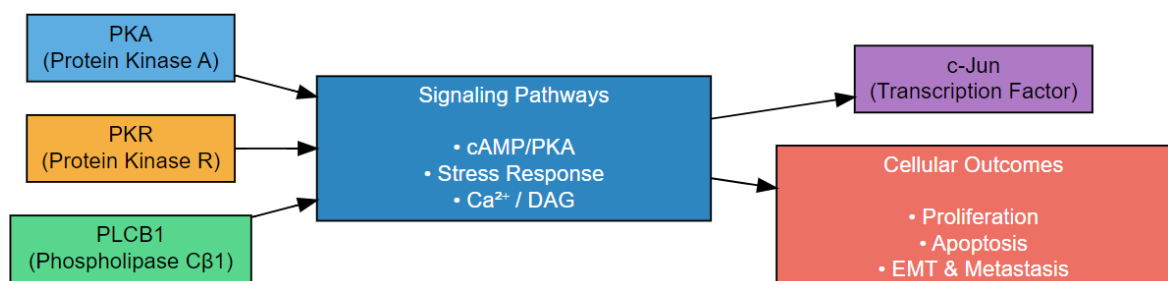
*Figure 2: Mechanism followed by adhesion markers in progression and metastasis of cancer. (M. Li et al., 2018)*

3. **Apoptosis regulators** serve as crucial guardians against cell death. A balance between pro-apoptotic proteins (e.g., BAX) and anti-apoptotic proteins (e.g., BCL2) controls mitochondrial membrane integrity and activation of the intrinsic apoptotic pathway(Brunelle & Letai, 2009). Activated effector molecules, such as Caspase-3, Caspase-8, and Caspase-9, carry out programmed cell death. Resistance to apoptosis is a common hallmark of cancers and plays a major role in chemoresistance and tumour recurrence(Singh et al., 2019). The study of apoptosis regulators is important because it helps to detail whether molecular interventions result in true cytotoxicity or rather mere suppression of proliferation. Moreover, understanding the apoptotic machinery opens up possibilities for identifying therapeutic vulnerabilities aimed at restoring programmed cell death to malignant cells(Pistritto et al., 2016).



*Figure 3: Mechanism followed by major apoptosis regulators in control of cancer. (Qian et al., 2022)*

4. In the case of **intracellular signalling molecules**, the overall effect of the signalling molecules would be to ensure integration of the extracellular signals and transcriptional responses. Proteins like Protein kinase A, EIF2AK2, Phospholipase C beta 1, and c-Jun are only a few of the signaling molecules of interest (Filteau et al., 2015). For instance, the signaling pathways regulated by these molecules, like the phosphorylation cascade, the stress response, the inflammatory response, and the activation of transcription factors, would be the ones whose consistent activation promotes oncogenic growth and, in the long run, metastatic ability. This is evidenced by the fact that since signaling molecules are often the nodal point of cell regulation, alterations in signaling molecules would affect various pathways like proliferation, apoptosis, and EMT (Ardito et al., 2017).



*Figure 4: Role of signalling molecules in cancer (Image by Bio render)*

5. Another layer of post-transcriptional control is the addition of **microRNAs**, which help fine-tune the expression of genes. They can bind to the complementary mRNA strand, modifying transcript stability and translation efficiency. Altered expression of microRNAs has the effect of inactivating tumour suppressor genes and strengthening oncogene expression, contributing to the metastasis and drug resistance of tumours(Ankasha et al., 2018). One of the most vital aspects of considering the role of microRNAs in relation to metastasis and tumour formation is the fact that, unlike the discrepancies between mRNA expression and the production of protein, microRNAs offer a wider scope of understanding related to the regulation of genes. Their presence in biofluids makes them ideal biomarkers, and understanding them is critical since they are dynamic regulatory molecules stabilising or amplifying oncogenic and metastatic pathways(Hussen et al., 2021).

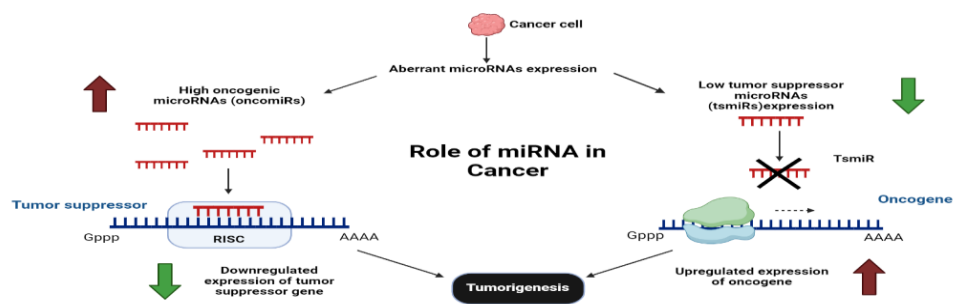


Figure 5: Role of miRNA in cancer (Image by Bio render)

Within such a complicated regulatory network, the human *KISS1* gene was found to be a key mediator of metastasis suppression involving other key molecules. *KISS1* was first discovered for its capacity to prevent the metastasis of melanoma. Kisspeptins are recognised to activate signal transduction in a G-protein-coupled receptor named *KISS1R*. (Beck & Welch, 2010). Besides its traditional function as a peptide precursor, current studies show that *KISS1* also has characteristics of an intrinsically disordered, nuclear-prone, regulatory protein. Structural analysis suggests *KISS1* is composed mainly of random coil, is basic, and has the potential for nuclear localization. Importantly, *KISS1* expression is down-regulated in aggressive breast and ovarian cancer, where it is linked to invasion signatures in glioblastoma. The significance of *KISS1* in metastasis biology is highlighted by its position at the core of regulatory cascades. It is transcription factors that regulate its expression, signaling cascades that activate it, adhesions that mark its downstream role in

metastasis suppression, apoptosis-related factors that carry out its role in survival, and miRNAs that may post-transcriptionally stabilize its expression. KISS1 is therefore not only an individual metastasis suppressor, but an organizer in metastasis regulatory cascades(Beck & Welch, 2010). Bioinformatic and structural studies have revealed KISS1 to carry characteristics of an intrinsically disordered protein comprised primarily of flexible random coils and to have nuclear localisation potential. Intrinsically disordered proteins have been identified as regulatory hubs displaying considerable versatility in their capacity to interact with various partners and accommodate different binding interfaces. KISS1 structural versatility might facilitate transcription-related, signalling, and adhesive events within metastasis suppression pathways (Harihar & Welch, 2023).

In spite of its known biological importance, there are many gaps in the knowledge of this protein. Most of the research on the subject has been concerned with expression profiling and clinical association. For instance, the structural details of the interactions of KISS1 with transcription factors, the dynamic stability of these interactions, and the integration of KISS1 with apoptosis and signalling have not been investigated by using molecular docking and long-timescale molecular dynamics simulation techniques. In addition, there has been limited research on the effects of disorder on the regulatory plasticity of the KISS1 molecule(Shah, Pillai, et al., 2025).

Considering that metastatic breast cancer, recurrent ovarian cancer, and glioblastoma present a high mortality rate, understanding upstream regulatory targets that have the potential to maintain metastasis suppressor gene expression is a matter of critical urgency(Steege et al., 2003). In this regard, metastasis suppressor pathways would provide a therapeutic tool that, unlike conventional therapeutic modalities, would reprogram cancer cells to less aggressive cancer types instead of eliminating proliferative cancer cells(Vaidya & Welch, 2007).

### ***Research Gap***

Despite extensive research into cancer, metastasis remains the leading cause of death in breast cancer, ovarian cancer, and glioblastoma. Many molecular regulators, including transcription factors, adhesion markers, mediators of apoptosis, signalling molecules, and microRNAs, have been investigated independently. However, several fundamental gaps remain. First, though the human *KISS1* gene is widely recognized as a metastasis suppressor, the majority of studies have focused on expression profiling and clinical correlation rather than structural and mechanistic investigation(Maqsood et al., 2025). The three-dimensional architecture, intrinsic disorder characteristics, and dynamic regulatory interactions of KISS1 remain poorly characterised. Secondly, the concerted regulation of KISS1 by transcription factors like SP1, MYCN, CDX2, FLI1, GATA2, and HDAC2 has not been modelled or validated systematically by structure-based and thermodynamics approaches. Static gene regulation models do not explain the conformational plasticity and stability of these interactions under physiological conditions(Duan et al., 2022). Third, even though adhesion markers E-cadherin, N-

cadherin, vimentin, and ZEB1, apoptosis regulators BAX and BCL2, and the tumor-signaling mechanism through c-Jun and Protein Kinase A are established tumor progression markers, their integration with a KISS1-mediated metastasis suppressor molecule concept has not been studied. Fourth, the KISS1/Kisspeptin tumor-signaling pathways in various aggressive tumor types, namely triple-negative breast cancer, glioblastoma, and ovarian cancer, have not been comparatively analysed by a unified mechanism. Lastly, while the literature has primarily focused on either *in silico* prediction or *in vitro* experimentation individually, a holistic model for explaining the KISS1/Kisspeptin-mediated mechanisms of triple-negative breast cancer by encompassing structural bioinformatics, dynamic simulation, transcriptional regulation, EMT regulation, and apoptosis modulation has not been established (Song & Zhao, 2016a).

### ***Hypothesis and Objectives:***

#### **Objective 1: Unravelling the transcriptional regulation of Kisspeptin-1 in breast, ovarian and glioblastoma cancers.**

- a) Identification of potential transcription factor binding sites via *In silico* approach.
- b) Unraveling the identified transcriptional factors regulating the expression of Kisspeptin-1 via *In vitro* approach.
- c) *In vitro* analysis of Kisspeptin-1 regulation through miRNAs and telomerase.

#### **Objective 2: Investigating the effect of Kisspeptin -1 on identified adhesion and signalling molecules in breast, ovarian and glioblastoma cancers.**

- a) Studying the Kisspeptin-1 mediated expression of adhesion molecules.
- b) Evaluating the expression of downstream signalling molecules of Kisspeptin-1.

### ***Rationale of the study***

- In view of these gaps, there is a compelling need to systematically investigate the molecular mechanisms underlying kisspeptin-mediated metastasis suppression in aggressive cancers. The present study is rationally designed to integrate *in silico* transcription factor analysis, protein–DNA docking, and molecular dynamics simulations with *in vitro* functional validation to elucidate the transcriptional regulatory landscape associated with the KISS1 gene.
- By focusing on TNBC as the primary model and extending the investigation to ovarian cancer and GB, this study aims to identify conserved and context-specific transcriptional regulatory mechanisms governed by kisspeptin signaling. Emphasis on key transcription factors implicated in EMT, invasion, and cellular plasticity will provide mechanistic insight into how kisspeptin influences gene expression programs driving tumor aggressiveness.

- Understanding the transcriptional and signaling networks regulated by exogenous kisspeptin may not only advance fundamental knowledge of metastasis suppression but also identify potential molecular targets for therapeutic intervention. Given the limited treatment options and poor prognosis associated with TNBC, ovarian cancer, and GB, elucidating kisspeptin-mediated regulatory mechanisms holds significant translational relevance.

### ***How the Present Thesis Addresses These Gaps***

The present thesis follows a multi-layered and integrative strategy to overcome these limitations systematically:

- Advanced methods of **bioinformatics and molecular dynamics** are developed and employed in **Chapter 1** to predict the structural, physicochemical, and dynamic characteristics of KISS1, followed using protein-DNA docking and simulations enabling the interaction of transcription factors and KISS1 at the molecular level.
- Further, in **Chapter 2**, the functional validation of the role of the pleiotropic nature of KISS1 in **triple-negative breast cancer** is demonstrated through a variety of transcription factors, EMT, apoptosis, and signaling molecules, thus indicating the ability of KISS1 to simultaneously control various cancer hallmark pathways.
- The anti-invasive and EMT-suppressive activities of Kisspeptin-10 are explored in **glioblastoma** in **Chapter 3**, and the chapter expands the field of metastasis suppressor biology understanding of control of EMT transitions in highly invasive brain along with the functional validation and miRNA regulatory network.
- In **Chapter 4**, the role of Kisspeptin-10 was studied in **ovarian cancer**, where similar anti-invasive and apoptosis-modulating activities were observed to those in other epithelial cancer along with the functional assay described by peritoneal dissemination.