Abstract

Metastasis is known to be the leading cause of death in aggressive cancers such as triple-negative breast cancer, ovarian cancer, and glioblastoma multiforme. While several molecular markers involved in the regulation of EMT, apoptosis, and oncogenic signaling have been identified, the underlying regulatory framework governing these processes in cancer progression is not well understood. The human KISS1 gene, known to function as a metastasis suppressor gene, lacks extensive investigation in terms of its structural features, regulation, and its role as a whole in the context of multiple types of human cancers. The aim of the present thesis was to explore the role of the KISS1 gene in regulating the processes of transcription, EMT, apoptosis, and signaling in breast, brain, and ovarian cancers.

Initial bioinformatics studies indicated that KISS1 was predicted to have intrinsic disorder, random coil dominance, and nuclear localization propensity. These findings indicated that the protein was most likely a regulatory protein. The three-dimensional structures of the most important transcription factors, namely SP1, MYCN, CDX2, FLI1, GATA2, and HDAC2, were built, followed by molecular docking and long timescale molecular dynamics simulations. These studies indicated differential binding stabilities and adaptability of these transcription factors in the context of the KISS1 promoter. Principal Component Analysis (PCA) and Free Energy Landscape mapping further indicated that these interactions were in stable states. These findings indicated a structural basis of transcriptional regulation. The study was further extended to the MDA-MB-231 and MDA-MB-468 TNBC model cell lines. The study was also extended to the U87MG glioblastoma cell line and SKOV-3 ovarian cancer cell line. The cytotoxicity of Kisspeptin-10 was determined using MTT assays. The study indicated dose-response growth inhibition in all model cell lines. Migration assays indicated that the motility inhibition. Gene and protein expression analyses showed upregulation of epithelial markers such as E-cadherin and pro-apoptotic regulators including BAX and Caspase-3, accompanied by downregulation of mesenchymal markers such as Vimentin and ZEB1 and anti-apoptotic BCL2. Modulation of signalling mediators including Protein kinase A and c-Jun further confirmed pleiotropic regulatory activity. The gene and protein expression studies revealed that epithelial cell markers like E-cadherin, as well as pro-apoptotic regulators like BAX and Caspase-3, were upregulated, while mesenchymal cell markers like Vimentin and ZEB1, as well as anti-apoptotic regulators like BCL2, were downregulated. The regulation of signaling

mediators like Protein kinase A and c-Jun further proved the presence of pleiotropic regulatory activity.

The untargeted metabolomics study identified the reprogramming of metabolism in apoptosis induction and EMT suppression. The differential gene expression study of publicly available datasets also corroborated the findings of the experiments. The study used Kaplan-Meier survival analysis to show that high expression of KISS1 is correlated with better overall survival in breast cancer patient cohorts. Thus, the present thesis combines structural bioinformatics, molecular dynamics, experiments, transcriptomics, and metabolomics to establish the metastasis suppressor role of the KISS1/Kisspeptin. The study provides mechanistic insights into the metastasis suppressor role of the KISS1/Kisspeptin in aggressive types of cancer.