# REGULATORY AND FUNCTIONAL DISSECTION OF MIR-191 IN BREAST CANCER.

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# REGULATORY AND FUNCTIONAL DISSECTION OF MIR-191 IN BREAST CANCER.

**by**


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# CERTIFICATE

This is to certify that the thesis entitled "**Regulatory and Functional Dissection of miR-191 in Breast Cancer.**", being submitted by **Miss Neha Nagpal** to the Indian Institute of Technology Delhi, for the award of degree of **Doctor of Philosophy**, is a record of bonafide research work carried out by her, which has been prepared under our supervision and guidance of conformity with the rules and regulations of "Indian Institute of Technology Delhi". The research reports and the results presented in this thesis have not been submitted in part or full to any other University/ Institute for the award of any degree or diploma.

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# ABSTRACT

MicroRNAs are post transcriptional gene regulators that play a critical role in normal as well as disease associated cellular processes. Research in the past decade has shown extensive association between deregulation of specific miRNA levels and cancer. miR-191 is one such microRNA that was found to be abnormally expressed in more than twenty different cancers and various other diseases. However, the functional relevance of altered miR-191 levels remains little studied.

In this thesis, we aimed to understand the basics behind aberrant regulation of miR-191 and to elucidate its functional domain in breast cancer. This study reveals miR-191 as an estrogen-inducible onco-miR in breast cancer, which promotes several hallmarks of cancer including enhanced cell proliferation, migration, chemoresistance and survival in tumor microenvironment. miR-191 is a direct estrogen receptor (ER) target and our results suggest existence of a positive regulatory feedback loop. Further, we show that the ER coactivator MED1 plays an important role in the transcriptional regulation of miR-191. We show miR-191 as a critical mediator of estrogen-mediated cell proliferation. Investigations of mechanistic details of miR-191 functions identify several cancer-related genes like BDNF, CDK6 and SATB1 as miR-191 targets. miR-191 and SATB1 show inverse correlation of expression. miR-191 mediated enhanced cell proliferation and migration are partly dependent on targeted downregulation of SATB1. Further, functional validation of estrogen:miR-191:SATB1 link suggests a cascade initiated by estrogen that induces miR-191 in ER-dependent manner to target SATB1, a global chromatin remodeler, thereby contributing to estrogen-specific gene signature to regulate genes like ANXA1, PIWIL2, CASP4, ESR1/ESR2, PLAC1 and SOCS2 involved in breast cancer progression and migration. Overall, the identification of estrogen/ER/miR-191/SATB1 cascade seems to be a significant pathway in estrogen signaling in breast cancer with miR-191 as oncogenic player.

We also explored the influence of tumor microenvironment on the function and regulation of miR-191. We found that miR-191 is a hypoxia inducible microRNA in a HIF (hypoxia inducible factor)-dependent manner and suggest existence of miR-191/HIF positive feedback loop. miR-191 overexpressing cells showed better proliferation, migration and survival under hypoxia as compared to the control cells while anti-miR-191 treated cells showed the opposite. We further established that miR-191 is a critical regulator of TGFβ-signaling and promotes cell migration by regulating TGFβ2 expression under hypoxia. The levels of TGFβ2 mRNA were found to be significantly higher in miR-191 overexpressing cells as compared to the control in 2D and 3D breast tumor models while *vice versa* was seen on miR-191 inhibition. The existence of both miR-191:TGFβ2 and miR-191:HuR interactions have been shown to control TGFβ2 levels under hypoxia. Notably, the levels of several HIF and TGFβ pathway genes (like VEGFA, CA9, SMAD3, EGFR, ERBB4, CXCR4, CTGF, & BMP4) were found to be higher in miR-191 overexpressing cells. Lastly, anti-miR-191 treatment given to breast tumor spheroids leads to a drastic reduction in spheroid tumor volume.

Altogether, our results suggest a critical role of miR-191 in both estrogen and hypoxia-induced cancer progression and suggest that miR-191 inhibition may offer a novel therapy for breast tumors.

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