

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

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INDIAN INSTITUTE OF TECHNOLOGY DELHI
FEBRUARY 2015**

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

by

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Submitted

in fulfilment of the requirements of the degree of

Doctor of Philosophy

to the



Department of Biochemical Engineering & Biotechnology

Indian Institute of Technology Delhi

February 2015

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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ACKNOWLEDGEMENTS

First of all, I would like to thank God for providing me this opportunity and giving me patience to endure all the obstacles to help me to accomplish my dreams. I strongly believe that it is only because of the God's grace that I have completed it successfully.

I have no words to express my deep sense of gratitude to Dr. Ritu Kulshreshtha for her guidance, discussions, encouragement and constructive criticism throughout the entire course of study. It was her inspiration and moral support that helped me to complete my work successfully.

I am extremely thankful to Prof. P. K. Roychoudhury for his constant support and encouragement. I am grateful to him for allowing me to use his lab facilities. His enthusiasm guided me all the way during my Ph. D. tenure.

I am highly indebted to my SRC members, Prof. Saroj Mishra, Prof. Prashant Mishra and Dr. Biswajit Kundu (external expert, Kusuma School of Biological Sciences, IIT Delhi) for monitoring my work progress and giving valuable suggestions.

I would like to express my deep sense of gratitude to Late Prof. J. K. Deb for providing me an opportunity to work under his supervision. I am thankful to him for his moral support, enthusiasm, doubts clarifications and valuable suggestions.

I am grateful to Prof. Cathrin Briskin, EPFL; Switzerland, for providing me an opportunity to learn in vivo experimentation under her guidance during IITD-EPFL PhD Exchange Program. I really admire her support and encouragement.

Heartfelt thanks to Prof. Alok Bhattacharya, JNU, for his help and support. His permission to use his lab facilities was crucial for the successful completion of my work. I am grateful to the students at his lab for their help and cooperation.

I am extremely thankful to Dr. Jitendra K Thakur, NIPGR, for his constant help, valuable suggestions and encouragement.

I would like to thank Dr. Vivekanandan Perumal, KSBS, IITD, for allowing me to use his lab facilities. He has been a great support during the initial phase of my journey.

I am thankful to Hafiz, Ph. D. student, JNU, for his valuable time and suggestions to help me in my experiments. He has been a constant support throughout the journey.

I am thankful to Kannu, Penny, Ana and Georgios, students, EPFL; Switzerland, for being there for me. Their practical training, doubt clarifying and valuable suggestions helped me a lot. Ana and Penny you both are wonderful friends, thanks a lot for being there with me.

I would like to thank Divya di, Vinay & Richa, students at NIPGR, for their constant help and support for western blotting experiments.

I have no words to express my heartfelt thanks to some angels in my life, my friends, Sayoni and Kalpana for their valuable friendship, love and care. The journey would have been much tougher or even impossible without them.

I am indebted to Shwetha and Shabana for teaching me the basics of biochemical engineering and helping me through the course work. I am thankful to both of them for being very good friends and helping me with their valuable time.

My friends Shazia (di), Aditi, Rahul, Lexy, Anisha, Gaurav, and Mamta (di) deserve special mention for their encouragement during critical times. It was their love, care, attention, and support that helped me through the difficulties.

I am thankful to my labmates and other colleagues, Shivani, Sonam, Rahul, Rakhi, Pooja, Shilpi, Jyoti, Gautam, Ritesh, Alex, Anamika, Sharadha, Surbhi, Dhara, Swati, Tenzin, Pavan, Pawan, Stefan (Sir), Suneer, Ruchika, Hanindar, Vivek, Bhuvan, Bhavna, Tanvi, Abhishek and Hemant for their help and cooperation.

I am extremely thankful to Rana Sir, Yogesh Bhaiya and Sunil for their constant help and support. I am thankful to Kirti, Gauri, Neera Mam, Bhagwan Das ji, Ratan Sir, SitaRam ji and Mukesh Sir for their timely help and support.

Finally, I take this opportunity to express my deepest gratitude to my parents for their love, care, & concern. I am able to complete it only because of their moral support, enthusiasm and encouragement. I consider myself lucky for being blessed with such wonderful parents, who helped me realize my dreams. I would like to thank my siblings Payal and Darpan for their love, care and support.

I acknowledge Council of Scientific and Industrial Research (CSIR), New Delhi for providing the financial support during the thesis work.

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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 TGF β -signaling and promotes cell migration by regulating TGF β 2 expression under hypoxia. The levels of TGF β 2 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:TGF β 2 and miR-191:HuR interactions have been shown to control TGF β 2 levels under hypoxia. Notably, the levels of several HIF and TGF β 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.

CONTENTS

Title	Page No.
CERTIFICATE.....	i
ACKNOWLEDGEMENTS.....	iii-v
ABSTRACT.....	vii-viii
CONTENTS.....	xi-xiii
LIST OF FIGURES.....	xv-xix
LIST OF TABLES.....	xxi
ABBREVIATIONS.....	xxiii-xxvi
Chapter 1 INTRODUCTION.....	1-5
Chapter 2 OBJECTIVES.....	7
Chapter 3 REVIEW OF LITERATURE.....	9-59
3.1 Breast Cancer.....	9
3.1.1 Pathology of breast cancer.....	9
3.1.1.1 Familial breast cancer.....	9
3.1.1.2 Sporadic breast cancer.....	10
3.1.1.3 Non-invasive breast cancer.....	10
3.1.1.3a Ductal Carcinoma <i>in situ</i>	11
3.1.1.3b Lobular Carcinoma <i>in situ</i>	11
3.1.1.4 Invasive breast cancer.....	11
3.1.1.5 Metastatic breast cancer.....	12
3.1.2 Breast tumor classification.....	13
3.1.3 Breast tumor markers.....	14
3.1.4 Breast cancer classification based on marker receptors.....	14
3.1.4.1 Estrogen receptor.....	15
3.1.4.2 Progesterone receptor.....	19
3.1.4.3 Her2/neu receptor.....	19
3.2 microRNAs.....	19
3.2.1 Discovery of microRNAs.....	21
3.2.2 Biogenesis of microRNAs.....	22
3.2.2.1 Nuclear miRNA cleavage.....	22
3.2.2.2 Cytoplasmic processing & RISC assembly.....	23
3.2.3 Mechanism of action.....	25

3.2.3.1 Target recognition.....	25
3.2.3.2 mRNA cleavage.....	25
3.2.3.3 Translational repression.....	26
3.2.4 Regulation of microRNA Expression.....	29
3.2.5 Functional dissection of microRNAs.....	31
3.2.6 microRNAs and cancer connection.....	32
3.2.7 microRNAs in disease therapeutics.....	35
3.3 microRNAs and Breast Cancer.....	38
3.4 miR-191.....	38
3.4.1 miR-191/425 cluster.....	38
3.4.2 Regulation of miR-191.....	41
3.4.3 miR-191 and Cancer connection.....	41
3.4.3.1 Acute Myeloid Leukemia and miR-191.....	42
3.4.3.2 Acute Lymphoblastic Leukemia and miR-191.....	42
3.4.3.3 Childhood Acute Lymphoblastic Leukemia and miR-191.....	43
3.4.3.4 Mixed Lineage Leukemia and miR-191.....	43
3.4.3.5 Lymphoma and miR-191.....	43
3.4.3.6 Breast Cancer and miR-191.....	43
3.4.3.7 Colon Cancer and miR-191.....	44
3.4.3.8 Gastric Cancer and miR-191.....	44
3.4.3.9 Skin Cancer and miR-191.....	44
3.4.3.10 Hepatic Cancer and miR-191.....	45
3.4.3.11 Pancreatic cancer and miR-191.....	45
3.4.3.12 Thyroid and follicular carcinoma and miR-191.....	45
3.4.3.13 Prostate/ Ovarian Cancer and miR-191.....	46
3.4.3.14 Osteosarcoma and miR-191.....	46
3.4.3.15 Lung Cancer and miR-191.....	46
3.4.3.16 Retinoblastoma and miR-191.....	46
3.4.3.17 Oral Squamous Cell Carcinoma and miR-191.....	46
3.4.4 miR-191 in diseases other than cancer.....	47
3.4.5 miR-191 as normaliser.....	53
3.4.6 miR-191 in development and differentiation.....	54
3.4.7 Targets of miR-191.....	55
3.4.7.1 SOX4.....	56

3.4.7.2 SATB1.....	56
3.4.7.3 NDST1.....	56
3.4.7.4 CDK6.....	56
3.4.7.5 RIOK3.....	57
3.4.7.6 MXI1.....	57
3.4.7.7 MDM4.....	57
3.4.7.8 EGR1.....	58
3.4.7.9 TIMP3.....	58
3.4.8 Prospects of miR-191 as potential prognostic marker and novel therapeutic target.....	58
Chapter 4 MATERIALS AND METHODS.....	61-80
4.1 Cell culture.....	61
4.2 Hypoxia/Stress/Radiation/Drug Treatment.....	61
4.3 Hormonal Treatment.....	62
4.4 Oligos & Plasmids.....	62
4.5 Transient & Stable transfections.....	63
4.6 Construction of stable polyclonal cell lines.....	63
4.7 Cell proliferation assay.....	64
4.8 Trypan Blue Assay.....	65
4.9 Colony formation assay.....	65
4.10 Soft Agar assay.....	66
4.11 Cell Cycle analysis.....	66
4.12 Scratch Assay.....	67
4.13 Boyden Chamber Assay.....	67
4.14 Mitochondrial Transmembrane Potential ($\Delta\Psi_m$).....	68
4.15 3-D spheroid generation.....	68
4.16 Total RNA isolation & cDNA synthesis.....	69
4.17 Microarray expression profiling and analysis.....	70
4.18 Stem-loop RT-PCR.....	70
4.19 Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR).....	71
4.20 Target Prediction.....	74
4.21 Construction of reporter luciferase constructs.....	74
4.22 Dual Luciferase assay.....	76
4.23 Immunofluorescence.....	77

4.24 Western Blotting.....	78
4.25 Chromatin Immunoprecipitation.....	79
Chapter 5 MIR-191 IS ESTROGEN REGULATED AND FUNCTIONS AS AN ONCOGENIC REGULATOR IN HUMAN BREAST CANCER.....	81-138
5.1 miR-191 is overexpressed in breast cancer cell lines.....	81
5.2 Biological effects of miR-191.....	82
5.2.1 miR-191 promotes breast cancer cell proliferation.....	82
5.2.2 miR-191 promotes G1/S and G2/M transitions in cell cycle.....	88
5.2.3 miR-191 promotes breast cancer cell migration.....	90
5.2.4 miR-191 promotes stress- and chemo-resistance.....	91
5.3 Regulation of miR-191: Role of estrogen & its receptors.....	93
5.3.1 miR-191 is an estrogen regulated miRNA.....	93
5.3.2 miR-191 levels are estrogen receptor dependent.....	94
5.3.3 Transcriptional regulation of miR-425 in breast cancer.....	100
5.3.4 Role of mediator complex in the transcriptional regulation of miR-191/425 cluster in breast cancer.....	101
5.3.4.1 Transcriptional regulation of miR-191/425 cluster: Role of MED1.....	101
5.3.4.2 ER α dependent overexpression of miR-191/425 is abolished by MED1 silencing.....	103
5.3.4.3 Transcriptional regulation of miR-191/425 cluster occurs through recruitment of ERs and MED1 on its promoter.....	105
5.3.4.3a ERs bind to the EREs upstream of miR-191/425 to regulate its expression.....	105
5.3.4.3b ER α mediated recruitment of MED1 on the EREs in the promoter of miR- 191/425.....	108
5.4 Identification of biologically relevant targets of miR-191.....	112
5.4.1 Effect of miR-191 on whole transcriptome.....	112
5.4.2 <i>In silico</i> analysis to search for targets of miR-191.....	115
5.4.3 Effect of miR-191 on target genes.....	117
5.5 SATB1: a bonafide target of miR-191.....	120
5.5.1 Conservation of miR-191 binding site across various species.....	120
5.5.2 Confirmation of SATB1 as miR-191 target.....	121
5.5.3 Inverse correlation of levels of miR-191 & SATB1 in context of ER.....	123

5.5.4 Functional repercussions of miR-191 mediated downregulation of SATB1.....	125
5.6 Estrogen mediated cellular effects are miR-191 dependent.....	129
5.6.1 Estrogen mediated cellular proliferation is miR-191 dependent.....	129
5.6.2 Commonality of gene expression signature between estrogen & miR-191.....	132
5.7 Discussion.....	135
Chapter 6 HIF-INDUCIBLE MIR-191 PROMOTES MIGRATION IN BREAST CANCER THROUGH COMPLEX REGULATION OF TGFβ-SIGNALING IN HYPOXIC MICROENVIRONMENT.....	139-186
6.1 miR-191 is stress and DNA damage inducible.....	139
6.2 miR-191 is hypoxia inducible in a HIF dependent manner.....	140
6.3 Existence of a positive feedback loop between HIF and miR-191.....	147
6.4 miR-191 promotes hypoxia mediated breast tumor aggressiveness.....	152
6.5 miR-191 induces TGF β -signaling under hypoxia.....	159
6.6 TGF β 2: A direct miR-191 target in hypoxic microenvironment.....	162
6.7 miR-191 mediated downregulation of HuR in hypoxia leads to induction of TGF β 2.....	165
6.8 TGF β 2 is a critical mediator of miR-191 induced breast cancer migration.....	177
6.9 Anti-miR-191 therapy brings about reduction in MCF7 3D tumor spheroids volume.....	181
6.10 Discussion.....	186
Chapter 7 CONCLUDING REMARKS & FUTURE DIRECTIONS.....	193-196
7.1 Concluding Remarks.....	193
7.2 Future Directions.....	195
LIST OF PUBLICATIONS (OUT OF THE PRESENT WORK).....	197
BIBLIOGRAPHY.....	199-245
APPENDIX.....	247-264
RESUME OF THE AUTHOR.....	265-268