

UNRAVELLING THE ROLE OF MIR-196A IN GLIOBLASTOMA

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UNRAVELLING THE ROLE OF MIR-196A IN GLIOBLASTOMA

by

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CERTIFICATE

This is to certify that the thesis entitled “**Unravelling the role of miR-196a in glioblastoma**”, being submitted by **Ms. Sonam Takkar** 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 my 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

Glioblastoma (GBM) is the most common and aggressive malignant primary brain tumor in humans. Despite of advances in medical management of solid tumors, the mortality rates of GBM patients remain high, which urge for a better understanding of GBM pathogenesis and improvement in its therapeutic strategy. Hypoxia has been correlated with the aggressive form of glial tumors, their poor prognosis and resistance to various therapies. MicroRNAs (miRNAs) have emerged as key players in cellular transformation and tumorigenesis and have shown great potential for cancer diagnostics and therapeutics. The present study is based on previous studies from our lab showing alteration in the miRNA profile in GBM cell line U87MG in response to severe hypoxia. Here, we take forward our previous studies to identify hypoxia regulated miRNAs that play critical role in hypoxia signalling and studying its clinical implications. A total of sixteen miRNAs were further validated by qRT-PCR and nine were found to be hypoxia regulated in GBM cells. Among these, miR-196a was found to be highly induced in response to hypoxia in a HIF dependent manner. miR-196a was also found to be significantly up-regulated in TCGA-GBM and Indian GBM patient cohorts. The high expression of miR-196a was shown to be associated with poor prognosis in GBM patients. We did functional characterization of miR-196a in GBM cell lines, U87MG and A172 using both inhibition and overexpression approach in normoxia as well as hypoxia. miR-196a overexpression was found to induce cellular proliferation, migration and colony forming potential and inhibit apoptosis in U87MG and A172 cell lines while miR-196a inhibition using anti-miR-196a showed opposite results suggesting oncogenic role of miR-196a in GBM. We further identified targets of miR-196a using a combination of bioinformatic and biochemical approaches. Notably, we found that miR-196a not only downregulates tumor suppressor genes but may also be involved in up-regulating the levels of specific oncogenes. We further unveiled that NRAS, AJAP1, TAOK1 and COL24A1 are direct targets of miR-196a. Our study also reported complex competitive regulation of oncogenic NRAS by miR-196a, miR-146a and let-7g in GBM. Analysis of microarray gene expression data obtained by miR-196a inhibition under hypoxia elucidated the role of miR-196a in HIF, Calcium Adhesion, Wnt and Cell Adhesion pathways. Interestingly, miR-196a was found to positively regulate the expression of various genes involved in induction or stabilization of HIFs and maintenance of hypoxic conditions, thereby suggesting the existence of an indirect miR-196a/HIF positive loop under hypoxia. Overall,

our work identifies novel axis of HIF-1/miR-196a/NRAS in GBM and suggests its prognostic and therapeutic significance.

सार

ग्लियोब्लास्टोमा (जीबीएम) मनुष्यों में सबसे आम और आक्रामक घातक प्राथमिक ब्रेन ट्यूमर है। ठोस ट्यूमर के चिकित्सा प्रबंधन में प्रगति के बावजूद, जीबीएम रोगियों की मृत्यु दर उच्च बनी हुई है, जो जीबीएम रोगजनन की बेहतर समझ और इसकी चिकित्सीय रणनीति में सुधार के लिए आग्रह करते हैं। हाइपोक्सिया को ग्लायल ट्यूमर के आक्रामक रूप, उनके खराब रोग का निदान और विभिन्न उपचारों के प्रतिरोध के साथ सहसंबद्ध किया गया है। सूक्ष्म आरएनए (miRNAs) सेलुलर ट्रांसफॉर्मेशन और ट्यूमरजेनेसिस में प्रमुख खिलाड़ी के रूप में उभरे हैं और कैंसर निदान और उपचार के लिए काफी संभावनाएं दिखाई हैं। वर्तमान अध्ययन गंभीर हाइपोक्सिया के जवाब में जीबीएम सेल लाइन U87MG में miRNA प्रोफ़ाइल में परिवर्तन दिखाने वाली हमारी लैब से पिछले अध्ययनों पर आधारित है। यहाँ, हम हाइपोक्सिया विनियमित miRNAs की पहचान करने के लिए अपने पिछले अध्ययनों को आगे बढ़ाते हैं जो हाइपोक्सिया सिग्नलिंग में महत्वपूर्ण भूमिका निभाते हैं और इसके नैदानिक प्रभाव का अध्ययन करते हैं। कुल सोलह miRNAs को आगे क्यूआरटी-पीसीआर द्वारा मान्य किया गया था और नौ जीबीएम कोशिकाओं में नियंत्रित हाइपोक्सिया पाए गए थे। इनमें, एमआईआर-196ए (miR-196a) को एचआईएफ निर्भर तरीके से हाइपोक्सिया के जवाब में अत्यधिक प्रेरित पाया गया। miR-196a को टीसीजीए-जीबीएम और भारतीय जीबीएम रोगी सहकर्मियों में भी काफी हद तक नियंत्रित किया गया। miR-196a की उच्च अभिव्यक्ति को जीबीएम रोगियों में खराब रोगनिरोध से जुड़ा हुआ दिखाया गया था। हमने दोनों जीबीएम सेल लाइनों, U87MG और A172 में miR-196a के कार्यात्मक लक्षण वर्णन और नॉरमोक्सिया के साथ-साथ हाइपोक्सिया में निषेध और ओवरएक्प्रेशन दृष्टिकोण का उपयोग किया। miR-196a अभिव्यक्ति में वृद्धि A172 और U87MG सेल लाइनों में सेलुलर प्रसार, माइग्रेशन और कॉलोनी बनाने के लिए संभावित पाया गया था और एपोप्टोसिस को कम करने के लिए पाया गया था, जबकि miR-196a ने एंटी-miR-196a का उपयोग करते हुए विपरीत परिणाम दिखाए थे, जीबीएम में miR-196a की ऑन्कोजेनिक भूमिका का सुझाव दिया था। हमने आगे जैव-रासायनिक और जैव-रासायनिक दृष्टिकोणों के संयोजन का उपयोग करके miR-196a के लक्ष्यों की पहचान की। विशेष रूप से, हमने पाया कि miR-196a न केवल ट्यूमर शमन जीन को डाउनग्रेड करता है, बल्कि विशिष्ट ऑन्कोजेन्स के स्तर को विनियमित करने में भी शामिल हो सकता है। हमने आगे खुलासा किया कि NRAS, AJAP1, TAOK1 और COL24A1 miR-196a के प्रत्यक्ष लक्ष्य हैं। हमारे अध्ययन ने जीबीएम में miR-196a, miR-146a और let-7g द्वारा ऑन्कोजेनिक NRAS के जटिल प्रतिस्पर्धी विनियमन की भी सूचना दी। हाइपोक्सिया के तहत miR-196a निषेध द्वारा प्राप्त माइक्रोएरे जीन अभिव्यक्ति डेटा का विश्लेषण HIF, कैल्शियम आसंजन, डब्ल्यूएनटी और सेल आसंजन मार्गों में miR-196a की भूमिका को स्पष्ट करता है। दिलचस्प बात यह है कि miR-196a को HIF की प्रेरण या स्थिरीकरण और हाइपोक्सिक स्थितियों के रखरखाव में शामिल विभिन्न जीनों की अभिव्यक्ति को सकारात्मक रूप से विनियमित करने के लिए पाया गया था, जिससे हाइपोक्सिया के तहत एक अप्रत्यक्ष miR-196a / HIF पॉजिटिव लूप के अस्तित्व का सुझाव दिया गया था। कुल मिलाकर, हमारा काम जीबीएम में HIF-1 / miR-196a / NRAS के उपन्यास अक्ष की पहचान करता है और इसके पूर्वानुमान और चिकित्सीय महत्व का सुझाव देता है।

CONTENTS

Title	Page No.
CERTIFICATE	i
ACKNOWLEDGEMENTS	ii-iii
ABSTRACT	iv-vi
CONTENTS	vii-viii
LIST OF FIGURES	ix-x
LIST OF TABLES	xi
ABBREVIATIONS	xii-xiv
CHAPTER 1: INTRODUCTION	1-5
CHAPTER 2: REVIEW OF LITERATURE	6-28
2.1 Glioblastoma.....	7
2.1.1 Classification.....	8
2.1.2 Diagnosis and prognosis.....	10
2.1.3 Current treatment modalities.....	12
2.2 Hypoxia.....	13
2.2.1 Hypoxia Regulated Transcription factors.....	14
2.2.2 Hypoxia regulated pathways.....	16
2.2.3 Role of Hypoxia in Glioblastoma.....	17
2.3 MicroRNA.....	18
2.3.1 Biogenesis.....	18
2.3.2 Mode of action.....	20
2.3.3 MicroRNAs and Cancer.....	21
2.3.4 Clinical utility of miRNAs.....	22
2.3.5 Hypoxic regulation of miRNAs.....	24
2.4 GBM and miRNAs.....	26
CHAPTER 3: OBJECTIVES	29-30
CHAPTER 4: MATERIALS AND METHODS	31-41
4.1 Cell culture.....	32
4.2 Hypoxia Treatment.....	32
4.3 Oligos and plasmids.....	32
4.4 Transient transfections.....	33
4.5 RNA isolation and cDNA synthesis.....	33
4.6 Stem loop RT PCR.....	33
4.7 Real-Time qRT-PCR.....	33
4.8 Selection of candidate hypoxia regulated miRNAs.....	34
4.9 HIF inducibility of candidate-miRNA.....	34
4.10 Prediction of HREs in the promoter of miRNAs.....	34
4.11 Construction of promoter luciferase reporter constructs.....	34
4.12 Chromatin Immunoprecipitation Assay.....	35
4.13 Cloning of candidate-miRNAs.....	35
4.14 Cell Viability assay.....	36
4.15 Clonogenic Assay.....	36
4.16 Caspase 3/7 activity Assay.....	36
4.17 PE Annexin V apoptosis detection assay.....	37
4.18 Wound Healing Assay.....	37
4.19 Transwell chamber migration assay.....	37

4.20 3D Spheroid formation Assay.....	37
4.21 miRNA Target Prediction.....	38
4.22 Construction of 3' UTR luciferase constructs.....	38
4.23 Dual luciferase Assay.....	39
4.23.1 Promoter luciferase assay.....	39
4.23.2 3'UTR luciferase assay.....	39
4.23.3 TGFβ reporter luciferase assay.....	39
4.24 Western Blot.....	39
4.25 Glioblastoma Patient Samples.....	40
4.26 Microarray Analysis.....	40
4.27 Statistical Analysis.....	41
CHAPTER 5: RESULTS.....	42-76
5.1 Identification of various hypoxia regulated miRNAs in GBM.....	43
5.1.1 <i>In silico</i> pathway analysis of candidate miRNAs.....	43
5.1.2 Validation of miRNA levels in response to hypoxia.....	45
5.1.3 Cloning of selected candidate miRNAs.....	46
5.1.4 Effect of Hypoxia regulated miRNA on cellular viability.....	47
5.2 To study the mechanism of regulation of candidate miRNA under hypoxia.....	48
5.2.1 miR-196a expression increases with decreasing oxygen concentration.....	48
5.2.2 miR-196a is hypoxia inducible in a HIF-1 dependent manner.....	49
5.3 To study the clinicopathological association of candidate miRNAs in GBM.....	51
5.3.1 Diagnostic and prognostic significance of miR-196a in GBM.....	51
5.3.2 miR-196a is up-regulated in Indian GBM patients.....	53
5.4 To study the biological functions of candidate miRNA in GBM.....	53
5.4.1 miR-196a promotes cell proliferation under hypoxia and normoxia....	53
5.4.2 miR-196a inhibits cellular apoptosis under hypoxia and normoxia.....	56
5.4.3 miR-196a promotes 3D tumor spheroid forming capacity of GBM cells.....	59
5.4.4 miR-196a promotes cellular migration under hypoxia and normoxia.....	60
5.4.5 miR-196a does not induce TGFβ-signaling under hypoxia.....	63
5.5 Identification biologically relevant target transcripts of candidate miRNA.....	64
5.5.1 Analysis of miR-196a target genes.....	64
5.5.2 miR-196a targets NRAS, AJAP1, TAOK1 and COL24A1 in GBM.....	65
5.5.3 NRAS is the direct target of miR-196a.....	67
5.5.4 NRAS functions as on oncogene in GBM.....	69
5.5.5 Complex regulation of NRAS by miR-196a, miR-146a and let-7g.....	72
5.5.6 miR-196a gene signature under hypoxia in GBM.....	74
5.5.7 Complex interaction of miR-196a and HIF pathway in GBM.....	76
CHAPTER 6: DISCUSSION.....	77-82
CHAPTER 7: CONCLUDING REMARKS AND FUTURE DIRECTIONS.....	83-85
7.1 Concluding Remarks.....	84
7.2 Future Directions.....	85
BIBLIOGRAPHY.....	86-109
APPENDIX I.....	110-114
APPENDIX II.....	115-119
RESUME OF THE AUTHOR.....	120-123

LIST OF FIGURES

Figure No.	Title	Page No.
2.1	Genetic alterations in primary and secondary GBM.	8
2.2	WHO 2016 grading of CNS tumors.	9
2.3	GBM subtypes based on genetic alterations.	10
2.4	An overview of effect of decreasing oxygen concentration on cell metabolism.	13
2.5	Pictorial representation of hypoxia induced activation of HIF pathway.	15
2.6	Hypoxia Responsive Transcription factors.	16
2.7	Regulation of HIF pathway under hypoxic and normoxic conditions.	17
2.8	Overview of miRNA biogenesis.	20
2.9	Regulation of crucial biological processes by miRNAs in cancer.	22
2.10	Various approaches used for miRNA based therapy.	23
2.11	An overview of various strategies used for miRNA inhibition.	24
2.12	Effect of miRNA on various biological functions in glioma.	27
5.1	<i>In silico</i> pathway analysis of selected miRNAs.	45
5.2	qPCR analysis of cloned miRNA precursors.	47
5.3	Selection of candidate miRNA on basis of its effect on cell proliferation.	48
5.4	miR-196a expression at varying oxygen concentration.	49
5.5	miR-196a is regulated by HIF-1 under hypoxia.	51
5.6	miR-196a is up-regulated in GBM patients and cell lines and high its levels are associated with poor survival in GBM patients.	52

5.7	miR-196a is up-regulated in Indian GBM patients.	53
5.8	miR-196a increases cell viability under normoxic and hypoxic conditions in U87MG and A172 cells.	55
5.9	miR-196a promotes clonogenic potential in both U87MG and A172 cells.	56
5.10	miR-196a inhibits cellular apoptosis.	58
5.11	miR-196a promotes 3D tumor spheroid formation in U87MG cells.	60
5.12	miR-196a promotes cellular migration under hypoxia and normoxia.	63
5.13	miR-196a doesn't induce TGF β -signaling.	63
5.14	Analysis of miR-196a target genes.	64
5.15	Validation of predicted miR-196a target genes.	65
5.16	Expression pattern of AJAP1, COL24A1, TAOK1 and NRAS in different subtypes of GBM.	66
5.17	AJAP1, COL24A1, TAOK1 and NRAS are targets of miR-196a in GBM.	67
5.18	NRAS is the direct target of miR-196a.	68
5.19	NRAS functions as an oncogene in GBM.	71
5.20	Complex regulation of NRAS by miR-196a, miR-146a and let-7.	74
5.21	Validation of microarray data by qPCR in U87MG and A172 cells.	75
5.22	miR-196a promotes HIF induced hypoxia.	76
7.1	A schematic view deciphering the role of miR-196a in GBM in context of hypoxia biology.	84

LIST OF TABLES

Table No.	Title	Page No.
5.1	List of selected candidate hypoxia regulated miRNAs	43
5.2	<i>In silico</i> pathway analysis of selected miRNAs	44
5.3	Validation of selected miRNAs by qPCR analysis.	46
5.4	Pathway analysis of miR-196a gene signature under hypoxia.	75

ABBREVIATIONS

AGO2	Argonaute
AJAP1	Adherens Junctions Associated Protein 1
AKT	Protein kinase B
ARID4B	AT-Rich Interaction Domain 4B
ARNT	Aryl Hydrocarbon Receptor Nuclear Translocator
ATP	Adenosine Triphosphate
ATRX	ATP-dependent helicase
Bcl-2	B-cell lymphoma 2)
bHLH	basic Helix-Loop-Helix
BIRC6	Baculoviral IAP repeat-containing protein 6
BSA	Bovine Serum Albumin
CA9	Carbonic Anhydrase 9
CDK6	Cyclin-dependent kinase 6
cDNA	Complementary DNA
CO₂	Carbon dioxide
COL3A1	Collagen Type III Alpha 1 Chain
COL24A1	Collagen type XXIV alpha 1 chain
CSC	Cancer Stem Cells
CT	Computed Tomography
COX2	Cyclooxygenase-2
CREB	cAMP response element-binding protein
CYBB	NADPH oxidase 2
DGCR8	DiGeorge syndrome chromosomal [or critical] region 8
DNA	Deoxyribonucleic acid
ECL	Enhanced chemiluminescence
ECM	Extracellular matrix
EGFR	Epidermal Growth Factor Receptor
EMT	Epithelial to Mesenchymal Transition
EP300	E1A Binding Protein P300
EPO	Erythropoietin
ERBB2	Erythroblastic oncogene B
FACS	Fluorescence-activated Cell Sorting
FBS	Fetal Bovine Serum
FIH	Factor-inhibiting HIF
FOXO1	Forkhead Box O1
GABRA1	Gamma-aminobutyric acid type A receptor alpha 1 subunit
GAPDH	Glyceraldehyde 3-Phosphate Dehydrogenase
GATA1	GATA Binding Protein 1
GBM	Glioblastoma Multiforme
GGT1	Gamma-Glutamyltransferase 1
GLUT1	Glucose transporter
HDAC1	Human histone deacetylase 4
H & E	Haematoxylin- and Eosin
HGI2	Hypoxia-inducible protein 2
HIF	Hypoxia Inducible Factor
HIF-1α	Hypoxia Inducible Factor1A

HOX	Homeobox
HRE	Hypoxia Response Element
HRM	Hypoxia Regulated microRNAs
HRP	Horseradish peroxidase
IDH1	Isocitrate Dehydrogenase 1
iNOS	Inducible nitric oxide synthase
KPS	Karnofsky Performance Scale
lincRNA	Large intergenic noncoding RNAs
MAPK	Mitogen-Activate Protein Kinases
MGMT	O-6-Methyl Guanine DNA Methyl Transferase
miRISCs	miRNA-induced silencing complexes
mRNA	Messenger RNA
miRNA/miR	MicroRNA
MMP	Matrix Metalloproteases
MREs	miRNA response elements
MRI	Magnetic Resonance Imaging
mTOR	Mammalian target of rapamycin
MTT	3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide
NCCS	National Centre for Cell Sciences
NEFL	Neurofilament
NF1	Neurofibromin 1
NFκB	Nuclear Factor kappa B
NFκBIA	NF-kappa-B-Inhibitor Alpha
NRAS	NRAS Proto-Oncogene
ncRNAs	non-coding RNAs
ODD	Oxygen-Dependent Degradation domain
OPN	Osteopontin
PARP	poly-ADP ribose polymerase
PAS	Per-Arnt-Sim
PBS	Phosphate Buffer Saline
PBST	Phosphate Buffer Saline with Tween-20
PCR	Polymerase Chain Reaction
PDGFRA	Platelet Derived Growth Factor Receptor
PE	Phycocerythrin
PET	Positron emission tomography
PET membrane	Polyethylene Terephthalate membrane
PHDs	prolyl-hydroxylases
PI3K	phosphoinositide 3-kinase
PIPES	piperazine-N,N'-bis(2-ethanesulfonic acid)
PMSF	Phenylmethylsulfonyl fluoride
PPARγ	Peroxisome proliferator-activated receptor gamma
pri-miRs	primary-miRNAs
PTEN	Phosphatase and tensin homolog
PUMA	p53 up-regulated modulator of apoptosis
qRT-PCR	Quantitative Reverse Transcription-PCR
qPCR	Quantitative real-time PCR
RECK	Reversion-inducing-cysteine-rich protein with kazal motifs
RhoA	Ras homolog gene family, member A

RNA	Ribonucleic acid
ROCK	Rho-associated coiled-coil kinases
ROS	Reactive oxygen species
RT	Room Temperature
RNA	Ribonucleic Acid
RUFY3	RUN And FYVE Domain Containing 3
SDS-PAGE	Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis
shRNA	short hairpin RNA
SLC12A5	Solute carrier family 12 member 5
sRNA-Seq	Small RNA Sequencing(deep sequencing)
STAT3	Signal transducer and activator of transcription 3
STY1	Synaptotgamin 1
TAOK1	Thousand and One Amino Acid Protein Kinase 1
TARPB2	TAR RNA-BINDING PROTEIN 2
TCEAL7	Transcription Elongation Factor A Like 7
TCGA	The Cancer Genome Atlas
TEAD3	TEA domain transcription factor 3
TGF-β	Transforming growth factor beta
TIMP3	TIMP Metallopeptidase Inhibitor 3
TMZ	Temozolomide
TRANSFAC	TRANScription FACtor database
TWIST1	Twist-related protein 1
TWIST2	Twist Family BHLH Transcription Factor 2)
UBE2C	Ubiquitin-conjugating enzyme E2C
UTR	Untranslated Region
VEGF	Vascular Endothelial Growth Factor
VHL	Von Hippel- Lindau
WHO	World Health Organization
XPO5	Exportin-5
ZEB 1	Zinc finger E-box-binding homeobox 1
ZEB 2	Zinc finger E-box-binding homeobox 2
ZMYND11	Zinc Finger MYND-Type Containing 11
3D	Three Dimensional
7-AAD	7-Aminoactinomycin D