REGULATORY AND FUNCTIONAL CHARACTERIZATION OF MIR-490 IN GLIOBLASTOMA

OMKAR SUHAS VINCHURE

DEPARTMENT OF BIOCHEMICAL ENGINEERING AND BIOTECHNOLOGY

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by

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Submitted

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to the

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To my parents and family,

And to all the small things in life that have a big and seemingly unreal impact

CERTIFICATE

This is to certify that the thesis entitled "**Regulatory and functional characterization of miR-490 in Glioblastoma**", being submitted by **Mr. Omkar Suhas Vinchure** 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 him, 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.

Dr. Ritu Kulshreshtha

Associate Professor,

Department of Biochemical Engineering & Biotechnology,

Indian Institute of Technology Delhi,

New Delhi- 110016.

Date:

Place:

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Omkar Suhas Vinchure

Date

ABSTRACT

Glioblastoma (GBM) continues to be the most lethal cancer type of the central nervous system with patients exhibiting a median survival rate of a meagre 14 months post diagnosis. Currently practiced therapeutic regimen (surgical resection with chemo-radio therapy) has proven inadequate in extending the lifespan and/or quality of life of GBM patients. A detailed understanding of molecular alterations governing GBM is thus essential for development of novel therapeutic options. Recent evidence has highlighted the importance of deregulated activity of chromatin remodeler EZH2 and telomere maintenance mechanisms in GBM with both implicated in gliomagenesis. However, a detailed understanding of their regulators and mediators remain poorly explored in GBM. In this report, through analyses of the ChIP-seq data performed using H3K27me3 (EZH2) antibody in Indian glioma patient cohort we identified a novel microRNA target of EZH2 namely miR-490-3p in GBM. We showed that miR-490 was significantly downregulated in low and high grade Indian glioma patient samples, GBM cell lines, as well as in The Cancer Genome Atlas (TCGA) patient cohort. Its downregulation is mediated *via* EZH2-mediated histone methylation and upstream DNA methylation as confirmed from its upregulation post EZH2 siRNA and 5-Azacytidine treatment, respectively. Functional characterization revealed that miR-490 inhibited cell viability, tumorigenicity, migration and Epithelial-to-Mesenchymal-Transition (EMT) in GBM cells with downregulation of multiple EMT transcription factors and pro-migratory genes. We showed that miR-490 directly targeted TGFBR1 and TGIF2 of the TGF-β signaling. TGIF2, a novel target, was shown to promote migration and EMT that could partially be rescued by miR-490-3p overexpression. Besides, we also showed that miR-490 directly targeted TRF2, TNKS2 and SMG1, belonging to the telomere maintenance mechanism and increased telomere dysfunction induced foci formation suggesting that miR-490 regulates telomere maintenance in GBM. Overexpression of miR-490 also resulted in induction of global DNA damage as seen from 53BP1 foci formation and increase in p-γH2AX levels. Also, miR-490 was shown to inhibit TRF2-mediated telomere maintenance hallmarks as seen by reduced stemness (SOX2 and SOX4 levels) and increased senescence (downregulation of SIRT1 and accumulation of H3K9me3 marks). It also initiated the downstream DNA Damage Response (DDR) leading to p53 pathway activation and induction of REST target genes TUBB3 and L1CAM. This response was dependent on p53 status of cells. Interestingly, positive correlation of TRF2 with these

hallmarks highlights the importance of miR-490 mediated targeting of telomere maintenance which could be of therapeutic importance in GBM.

Taken together, suppression of miR-490 is associated with events promoting glioma progression namely upregulation of EZH2 and telomere maintenance. The inhibition of TGF-β signaling and telomere maintenance by miR-490 in GBM assigns significance to miR-490 as a novel therapeutic agent in GBM especially since these pathways are cooperatively known to enhance GBM aggressiveness.

सार

ग्लियोब्लास्टोमा (GBM) केंद्रीय तंत्रिका तंत्र का सबसे घातक कैंसर प्रकार है जिसके मरीज निदान के बाद 14 महीने की औसत उत्तरजीविता दर प्रदर्शित करते हैं। वर्तमान में चिकित्सीय पद्धति (कीमो-रेडियो थेरेपी के साथ शल्य चिकित्सा स्नेह) GBM रोगियों के जीवनकाल और / या गुणवत्ता को बढ़ाने में अपर्याप्त साबित हुआ है। इस प्रकार GBM को नियंत्रित करने वाले आणविक परिवर्तनों की एक विस्तृत समझ उपन्यास चिकित्सीय विकल्पों के विकास के लिए आवश्यक है। हाल के साक्ष्यों ने GBM में क्रोमेटिन रिमोडेलर EZH2 और टेलोमेयर रखरखाव तंत्र की अविनियमित गतिविधि के महत्व को रेखांकित किया है और दोनों ग्लिओमाजन्यता में लक्षित हैं। हालाँकि, GBM में उनके नियामकों और मध्यस्थों की विस्तृत समझ की जाँच विस्तार से नहिं की गइ है। इस रिपोर्ट में, Chip-seq डेटा के विश्लेषण के माध्यम से भारतीय ग्लियोमा रोगी समूह में H3K27me3 एंटीबॉडी का उपयोग करते हुए हमने GBM में EZH2 के microRNA लक्ष्य अर्थात् miR-490-3p की पहचान की है। हमने दिखाया कि miR-490 निम्न और उच्च श्रेणी के भारतीय ग्लियोमा रोगी के नमूनों, GBM सेल लाइनों और साथ ही द कैंसर जीनोम एटलस के रोगी समूह में काफी दमन पाया गया था। इसका दमन EZH2 की मध्यस्थता वाले हिस्टोन मेथिलेशन और डीएनए मेथिलेशन के माध्यम से होता है जिसकी EZH2 siRNA और 5-Azacytidine उपचार से पुष्टि की गई है। कार्यात्मक लक्षण वर्णन में पता चला है कि कई EMT प्रतिलेखन कारकों और समर्थक-प्रवासी जीनों के दमन के साथ GBM कोशिकाओं में miR-490 ने सेल व्यवहार्यता, ट्यूमरजन्यता, माइग्रेशन और एपिथेलियल से मेसेंकायमल ट्रांजीशन (इ एम् टी) को बाधित किया। हमने दिखाया कि miR-490 ने TGF-β सिग्नलिंग के TGFBR1 और TGIF2 को सीधे टारगेट किया। TGIF2 माइग्रेशन और इ एम् टी को बढ़ावा देने के लिए दिखाया गया था जिसे आंशिक रूप से miR-490-3p ओवरएक्प्रेशन द्वारा कम किया जा सकता है। इसके अलावा, हमने यह भी दिखाया कि miR-490 ने टेलोमेयर रखरखाव तंत्र से संबंधित TRF2, TNKS2 और SMG1 को सीधे टारगेट किया और telomere dysfunction induced foci के गठन को प्रेरित किया जिसने सुझाव दिया कि miR-490 GBM में टेलोमेयर रखरखाव को नियंत्रित करता है। MiR-490 के ओवरएक्प्रेशन के कारण डीएनए की क्षति के परिणामस्वरूप 53BP1 foci गठन और p-γH2AX स्तरों में वृद्धि देखी गई। इसके अलावा, miR-490 को TRF2 की मध्यस्थता वाले टेलोमेयर रखरखाव हॉलमार्क को बाधित करने के लिए दिखाया गया था जिसमें कम स्टेमनेस (SOX2 और SOX4 स्तर) और ज्यादा सेनेसेन्स (SIRT1 की गिरावट और H3K9me3 अंक का संचय) दिखे। इसने डीएनए डैमेज रेस्पोंस की शुरुआत की, जिसके कारण p53 पाथवे और REST लक्ष्य जीन TUBB3 और L1CAM को सक्रिय किया। यह प्रतिक्रिया कोशिकाओं के p53 स्थिति पर निर्भर थी। दिलचस्प बात यह है कि इन हॉलमार्क के साथ TRF2 का सकारात्मक सहसंबंध miR-490 के महत्व को रेखांकित करता है जो कि टेलोमेयर रखरखाव को लक्षित करता है जो जीबीएम में चिकित्सीय महत्व का हो सकता है।

miR-490 का दमन ग्लिओमा प्रगति को बढ़ावा देने वाली घटनाओं के साथ जुड़ा हुआ है जो कि EZH2 के अपगमन और टेलोमेयर रखरखाव हैं। जीबीएम में miR-490 द्वारा TGF-β सिग्नलिंग और टेलोमेयर रखरखाव का निषेध GBM में एक उपन्यास चिकित्सीय तत्त्व के रूप में miR-490 को विशेष रूप से महत्व देता है क्योंकि ये GBM की आक्रामकता को सहकारी रूप से बढ़ाने के लिए जाने जाते हैं।

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ABBREVIATIONS

3’ UTR	3’ Untranslated Region
53BP1	TP53 Binding Protein 1
ADAM17	ADAM Metallopeptidase Domain 17
AGO	Argonaute
ALT	Alternative lengthening of telomeres
ATM	Ataxia telangiectasia mutated
ATR	Ataxia telangiectasia and Rad3-related
BRCA1	Breast Cancer Type 1 Susceptibility Protein
CAR	Chimeric antigen receptor
CCL5	Chemokine (C-C motif) ligand 5
CD133	Cluster of differentiation 133
ChIPseq	Chromatin Immunoprecipitation sequencing
CHK2	Checkpoint kinase 2
CHRM2	Cholinergic Receptor Muscarinic 2
circRNAs	Circular RNAs
CNS	Central Nervous System
CSC	Cancer Stem Cell
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
DAPI	4′,6-diamidino-2-phenylindole
DGCR8	DiGeorge syndrome critical region 8
DMEM	Dulbecco’s modified Eagle’s medium
DMSO	Dimethyl sulphoxide
DNMTs	DNA methyltransferases
ECM	Extracellular matrix
EGFR	Epidermal growth factor receptor
EMT	Epithelial to mesenchymal transition
EMT-ATFs	EMT-activating transcription factors
EZH2	Enhancer of Zeste Homolog 2

FACS	Fluorescence activated cell sorting
FBS	Fetal Bovine Serum
GAPDH	Glyceraldehyde phosphate dehydrogenase
GBM	Glioblastoma
HDR	homology directed repair
ICAM1	Intercellular Adhesion Molecule 1
IDH1	Isocitrate dehydrogenase
L1CAM	L1 Cell Adhesion Molecule
lncRNAs	Long non-coding RNAs
MGMT	O6-methylguanine DNA methyltransferase
miRISC	miRNA
miRNAs	microRNAs
MMP	Matrix metalloproteinase
MTT	3- (4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide
NF1	Neurofibromatosis type 1
NHEJ	Non-homologous end joining
NOTCH1	Notch receptor 1
NOXA	Phorbol-12-Myristate-13-Acetate-Induced Protein 1
NSC	Neural Stem Cell
P53β	p53 β
PAI-1	Plasminogen Activator Inhibitor 1
PARP1	poly(ADP-ribose) polymerase 1
PASHA	Partner of DROSHA
PCR	Polymerase chain reaction
PD-1	Programmed cell death protein 1
PDGFR	Platelet-derived growth factor
PD-L1	Programmed death-ligand 1
PIK3R1	Phosphoinositide-3-Kinase Regulatory Subunit 1
PML	Promyelocytic Leukemia
POT1	Protection Of Telomeres 1

PRC2	Polycomb Repressor Complex 2
Pre-miRNA	Precursor miRNA
Pri-miRNA	Primary miRNA
PTEN	Phosphatase and tensin homolog
qRT-PCR	Quantitative reverse transcriptase PCR
Rap1	Repressor / Activator Protein 1
RBPs	RNA binding proteins
REST	RE1
RHOA	Ras Homolog Family Member A
RNAi	RNA interference
siRNA	small interfering RNA
SIRT1	Sirtuin 1
SMG1	Nonsense Mediated MRNA Decay Associated PI3K Related Kinase
SMURF1	SMAD Specific E3 Ubiquitin Protein Ligase 1
SNAI	Snail family transcriptional repressor
SOX2	SRY (sex determining region Y)-box2
SOX4	SRY-Box Transcription Factor 4
SVZ	Subventricular zone
TCGA	The Cancer Genome Atlas
TERRA	Telomeric repeat-containing RNA
TGF- β	Transforming growth factor β
TGIF2	TGF β Induced Factor Homeobox 2
TIFs	Telomere dysfunction Induced Foci
TIN2	TRF1- and TRF2-Interacting Nuclear Protein 2
TMM	Telomere maintenance mechanism
TMZ	Temozolomide
TNKS2	Tankyrase 2
TP53	Tumour protein p53
TPM	TERT promoter mutation
TPP1	ACD Adrenocortical dysplasia protein homolog gene

TR4	Testicular nuclear receptor 4
TRF1	Telomeric repeat-binding factor 1
TRF2	Telomeric repeat-binding factor 2
TTF	Tumour-treating field
TUBB3	Tubulin Beta 3 Class III
TWIST	Twist-related protein
VIM	Vimentin
WHO	World Health Organization
ZEB	Zinc-finger enhancer binding
ZO-1	Zonula occludens- 1