INVESTIGATING THE ROLE OF MEDIATOR COMPLEX SUBUNIT, MED12, IN GLIOBLASTOMA

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by

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Dedicated to
my loved ones

CERTIFICATE

This is to certify that the thesis entitled "**Investigating the role of mediator complex subunit, MED12, in glioblastoma**", being submitted by **Mrs. Srishti Srivastava** 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

GBM is the most life-threatening tumor of the central nervous system. In spite of recent therapeutic advancements, maximum survival of GBM patients remains a dismal of 12-19 months. Understanding the molecular landscape of the disease is the way forward in precision medicine. The mediator complex is a set of proteins, essential for eukaryotic gene expression. Because of its vast array of regulatory functions in transcription, mediator complex is an imperative tool for regulation of gene expression. It communicates the signal from stimuli induced transcription factors to transcription machinery and drive the expression of downstream genes. Thus, any alteration in its structure or function may bring about miscommunication of the external signal and aberrant gene transcription leading to various diseases. Abnormal expression/mutations of specific mediator genes have been associated with progression of various cancers however, its role and status in GBM remains largely unknown. Our work focuses on one subunit of the kinase module of the mediator complex, MED12. We show that MED12 is overexpressed in various GBM patient cohorts including Indian GBM patients and cell lines. We further show that the expression of MED12 decreases with the grade of tumor, being higher in the low-grade tumors as compared to GBM indicating its possible involvement in the early events of gliomagenesis. The expression of MED12 correlates positively with IDH mutations, which is again found majorly in low-grade gliomas. Correlation of MED12 expression with patient prognosis gave varied results. In case of GBM, we found that its high expression is associated with worse prognosis in certain patient cohorts while in some cohorts the expression is not significantly correlated with prognosis. Further, in case of low-grade glioma, in certain cases, its high expression correlates with better prognosis. Functional characterization of MED12 using both overexpression and knockdown approach revealed that it promotes cell proliferation, migration and inhibits apoptosis in GBM cell lines. Transcriptome analysis post MED12 knockdown revealed Vitamin D receptor (VDR) pathway to be one of the key pathways affected by MED12 in GBM. We studied direct interaction of MED12 with VDR protein using docking studies and co-immunoprecipitation assay. We identify B cell lymphoma 6 (BCL6), a secondary regulator of VDR signaling, to be directly regulated by MED12 through a combination of chromatin immunoprecipitation, qRT-PCR and western analyses. We further show that MED12 brings about the inhibition of tumor protein 53 (p53) levels and

apoptosis partly through induction of BCL6 in GBM. Overall, this stands as the first report of MED12 overexpression and involvement in GBM pathogenesis and identifies MED12 as an important mediator of VDR signaling and an attractive molecule for development of new therapeutic interventions.

सार

ग्लियोब्लास्टोमा केंद्रीय तंत्रिका तंत्र का सबसे जानलेवा ट्यूमर है। हाल की चिकित्सीय प्रगति के बावजूद, ग्लियोब्लास्टोमा के रोगियों की अधिकतम उत्तरजीविता 12-19 महीनों की निराशाजनक है। रोग के आणविक परिदृश्य को समझना सटीक चिकित्सा में आगे का रास्ता है। मिडिएटर कॉम्प्लेक्स प्रोटीन का एक सेट है, जो यूकेरियोटिक जीन अभिव्यक्ति के लिए आवश्यक है। प्रतिलेखन में नियामक कार्यों के अपने विशाल सरणी के कारण, जीन अभिव्यक्ति के नियमन के लिए मिडिएटर कॉम्प्लेक्स एक अनिवार्य उपकरण है। यह सिग्नल प्रेरित ट्रांसक्रिप्शन कारकों से ट्रांसक्रिप्शन मशीनरी तक सिग्नल का संचार करता है और डाउनस्ट्रीम जीन की अभिव्यक्ति को संचालित करता है। इस प्रकार, इसकी संरचना या कार्य में किसी भी परिवर्तन से बाहरी संकेत और असामान्य जीन प्रतिलेखन का गलत संचार हो सकता है, जिससे विभिन्न रोग हो सकते हैं। विशिष्ट मिडिएटर जीन की असामान्य अभिव्यक्ति / उत्परिवर्तन विभिन्न कैंसर की प्रगति से जुड़े रहे हैं, हालांकि, ग्लियोब्लास्टोमा में इसकी भूमिका और स्थिति काफी हद तक अज्ञात है। हमारा काम मिडिएटर कॉम्प्लेक्स,के काइनेज मॉड्यूल के एक सबयूनिट, MED12 पर केंद्रित है। हम दिखाते हैं कि MED12 भारतीय ग्लियोब्लास्टोमा रोगियों और सेल लाइनों सहित विभिन्न ग्लियोब्लास्टोमा रोगी समूहों में अतिप्रवाहित है। हम आगे दिखाते हैं कि MED12 की अभिव्यक्ति ट्यूमर के ग्रेड के साथ घट जाती है, ग्लियोब्लास्टोमा की तुलना में निम्न-श्रेणी के ट्यूमर में अधिक होने से ग्लियोमेजेनेसिस की शुरुआती घटनाओं में इसकी संभावित भागीदारी का संकेत मिलता है। MED12 की अभिव्यक्ति सकारात्मक रूप से IDH उत्परिवर्तन के साथ संबंध रखती है, जो फिर से निम्न-श्रेणी के ग्लियोमा में प्रमुख रूप से पाई जाती है। रोगी रोग निदान के साथ MED12 अभिव्यक्ति के सहसंबंध ने विविध परिणाम दिए। ग्लियोब्लास्टोमा के मामले में, हमने पाया कि इसकी उच्च अभिव्यक्ति कुछ रोगी समूहों में बदतर रोग का निदान से जुड़ी है, जबकि कुछ समूहों में अभिव्यक्ति रोगी के अस्तित्व के साथ महत्वपूर्ण रूप से सहसंबद्ध नहीं है। इसके अलावा, निम्न-श्रेणी के ग्लियोमा के मामले में, कुछ मामलों में, इसकी उच्च अभिव्यक्ति बेहतर पूर्वानुमान के साथ सहसंबद्ध होती है। ओवरएक्प्रेशन और नॉकडाउन दृष्टिकोण दोनों का उपयोग करते हुए MED12 के कार्यात्मक लक्षण वर्णन से पता चला है कि यह सेल प्रसार, प्रवास को बढ़ावा देता है और ग्लियोब्लास्टोमा सेल लाइनों में एपोप्टोसिस को रोकता है। ट्रांसक्रिप्टोम विश्लेषण पोस्ट MED12 नॉकडाउन ने विटामिन डी रिसेप्टर (VDR) मार्ग को ग्लियोब्लास्टोमा में MED12 से प्रभावित प्रमुख मार्गों में से एक होने का खुलासा किया। हमने डॉकिंग अध्ययन और सह-प्रतिरक्षाअवक्षेपण परख का उपयोग करते हुए वीडीआर प्रोटीन के साथ MED12 की सीधी बातचीत का अध्ययन किया। हम बी सेल लिंफोमा ६ (BCL6) की पहचान करते हैं, जो वीडीआर सिग्नलिंग का एक द्वितीयक नियामक है, जिसे क्रोमेटिन प्रतिरक्षा अवक्षेपण, क्यूआरटी-पीसीआर और पश्चिमी विश्लेषणों के संयोजन के माध्यम से MED12 द्वारा सीधे विनियमित किया जाना है। हम आगे बताते हैं कि MED12 ट्यूमर प्रोटीन 53 (p53) के स्तर और एपोप्टोसिस को आंशिक रूप से ग्लियोब्लास्टोमा में BCL6 के शामिल होने के माध्यम से रोकता है। कुल मिलाकर, यह MED12 की अति-अभिव्यक्ति और ग्लियोब्लास्टोमा रोगजनन में भागीदारी की पहली रिपोर्ट के रूप में खड़ा है और MED12 को VDR सिग्नलिंग के एक महत्वपूर्ण मध्यस्थ और नए चिकित्सीय हस्तक्षेपों के विकास के लिए एक आकर्षक अणु के रूप में पहचानता है।

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ABBREVIATIONS

GBM	GBM
VDR	Vitamin D receptor
BCL6	B cell lymphoma 6
PCR	Polymerase chain reaction
qRT-PCR	Quantitative real time pcr
IDH	Isocitrate dehtdrogenase
RNA pol II	RNA polymerase II
PIC	Preinitiation complex
CTD	Carboxyl terminal domain
SWI/SNF	SWItch/Sucrose Non-Fermentable
CHD	chromodomain helicase DNA-binding
CDS	Coding sequence
VEGFA	Vascular endothelial growth factor A
VEGFR2	Vascular endothelial growth factor receptor 2
RIG 1	Retinoic acid-inducible gene I
WHO	World Health Organization
GAPDH	Glyceraldehyde phosphate dehydrogenase
FBS	Fetal bovine serum
FACS	Fluorescence associated cell sorting
NOS	Not otherwise specified
ATRX	ATP-dependent helicase ATRX
Ep-GBM	Epithelial-like GBM
CDKN2A	Cyclin-dependent kinase inhibitor 2A

PTEN	Phosphate and tensin homolog
BRAF	V-raf murine sarcoma viral oncogene homolog B1
TERT	Telomerase reverse transcriptase
CIC	Capicua transcriptional repressor
FUBP1	Far upstream element binding protein 1
NOTCH1	Notch receptor 1
MYB	MYB Proto-Oncogene
FGFR1	Fibroblast growth factor receptor 1
MAPK	Mitogen activated protein kinase
NF1	Neurofibromin 1
PI3K	Phosphatidylinositol 3-kinase
mTOR	mammalian target of rapamycin
WNT	Wingless and Int-1
EGFR	Epidermal growth factor receptor
TGF	Transforming growth factor
TGFBR2	Transforming growth factor beta receptor 2
RB	Retinoblastoma protein
PDGFRA	Platelet derived growth factor receptor alpha
SYT1	Synaptotagmin 1
SLC12A5	Solute carrier family 12 members 5
GABRA1	Gamma-aminobutyric acid type A receptor alpha1
NEFL	Neurofilament light polypeptide
MGMT	O6-methylguanine-DNA methyltransferase
TTF	Tumor treating fields

CAR	chimeric antigen receptors
DMSO	Dimethyl sulfoxide
DMEM	Dulbecco's modified Eagle's medium
CD133	Cluster of differentiation 133
MTT	3- (4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide
ACTB	Beta actin
ACT	Adoptive cell therapy
SRB	suppressor of RNA Polymerase B
TRAP	thyroid hormone receptor proteins
TFIIH	Transcription factor II Human
TFIID	The transcription Factor II D
CCNC	Cyclin C
CDK8	Cyclin dependent kinase 8
NFkB	Nuclear factor kappa-light-chain-enhancer of activated B cells
SOX	SRY-related HMG-box genes
SEC	Super elongation complex
OPA	Odd-paired motif
RTK	Receptor tyrosine kinase
MBD	Mediator binding domain
REST	RE1-Silencing Transcription factor
Gli3a	GLI Family Zinc Finger 3
CBP	CREB binding protein
NICD	Notch intracellular domain
JMJD6	Jumonji Domain Containing 6

CARM1	Coactivator Associated Arginine Methyltransferase 1
GSK3β	Glycogen Synthase Kinase 3 Beta
MYC	Master Regulator of Cell Cycle Entry and Proliferative Metabolism
BRD4	Bromodomain Containing 4
RAS	Rat sarcoma virus
MEK	Mitogen-activated protein kinase kinase
ERK	Extracellular signal-regulated kinase
EMT	Epithelial to mesenchymal transition
cDNA	Complementary DNA
PBS	Phosphate buffer saline
7 AAD	7-Aminoactinomycin D
PI	Propidium iodide
PE	Phycoerythrin
FITC	Fluorescein isothiocyanate
RIPA	Radioimmunoprecipitation assay buffer