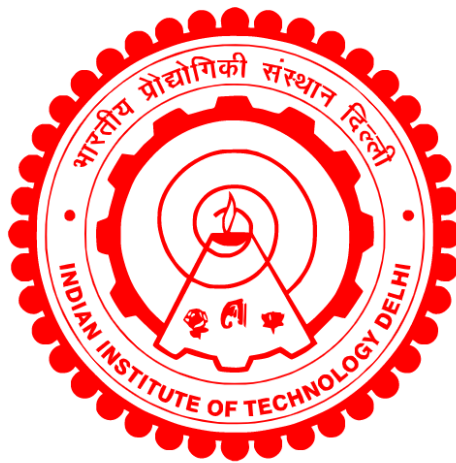


**INVESTIGATING THE ROLE OF MEDIATOR COMPLEX  
SUBUNIT, MED12, IN GLIOBLASTOMA**

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**INDIAN INSTITUTE OF TECHNOLOGY DELHI**

**March 2022**

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SUBUNIT, MED12, IN GLIOBLASTOMA**

by

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**Submitted**

**In fulfillment of the requirements of the degree of Doctor of Philosophy**

**to the**



**INDIAN INSTITUTE OF TECHNOLOGY DELHI**

**March 2022**

*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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## ACKNOWLEDGEMENTS

During the course of my PhD studies, I have received help and support from a number of people and this work would not have been possible without them.

First of all, I would like to thank Prof. Ritu Kulshreshtha, who has been an exceptional mentor over the past five years. I am extremely thankful to her for providing me with this opportunity to work in her lab and her constant guidance and encouragement. She always aspired me to reach my full potential and has been instrumental in teaching me the analytical skills needed to do research. It was really inspiring to work with her, and the journey was remarkable. Most of all she has made me realize the importance of thinking and being perceptive and I would be forever grateful to her for the same.

I express my gratitude towards my student research committee members Prof. D. Sundar, Prof. Preeti Srivastava and Prof. Vivekanandan Perumal for monitoring my research progress and providing their valuable insights and constructive inputs. I would also like to thank them for giving me access to the instrumentation facilities under them that helped me greatly with my experiments.

I sincerely thank Prof. Chitra Sarkar, Dr. Vaishali Suri and Dr. Vikas Sharma at All India Institute of Medical Sciences (AIIMS, New Delhi) for their help and fruitful collaboration. I thank Dr. Hima Makala, Department of Biochemical Engineering and Biotechnology, IIT Delhi, for performing key experiments and data acquisition towards supporting our manuscript. Dr. Hima Makala also needs a special mention here for teaching me certain important experiments that helped me greatly towards completion of my work.

I would also like to express my gratitude towards, Prof. Anuradha Sourirajan, Prof. Kamal Dev, Dr. Garima Bisht and Mr. Rajan Rolta, Shoolini University (Solan, Himachal Pradesh, India) for including me as a collaborator in their studies that widened my research aptitude.

I would like to acknowledge Ministry of Education, Government of India, for financial support during my research work.

Friends and colleagues in the department and institute will always hold a special place in my heart. From teaching me all the small and big experiments to troubleshooting the problems I faced, I thank Omkar, for being a great mentor and friend. A very special thanks to Rabab, Sukanya, Sudipta, Vinay, Anirban, Shivani, Vasu, Anamika, Arun, Pravina, Garima and Indranil for their constant love, support, patience, and for giving me some of my fondest memories of PhD life. Indranil and Garima need a special mention for taking care of all the small things and for providing me with that extra helping hand during my final year. I would also like to mention Sonam, Jananee, Rohit, Aditi and Srimoyee for their constant help throughout my tenure, they were always ready to help with whatever I needed, and I cannot thank them enough for making my journey easy. I would also like to thank all the past and present members of cancer biology lab, Rahul, Aastha, Shravya, Rajat, Anuja, Nidhi, Shikha, Ankita, Deependera, and others for their support and help.

I am thankful to department staff members Anees, Sakshi, Sanjay and Yogesh for their help at all times.

Words are not enough to express my gratitude towards my parents for their unparalleled love, tolerance, patience, and care. I cannot thank my daddy enough for his encouragement at all the times when I was down which helped me overcome all the hardships and complete my thesis. My cousin Kriti deserves a special thanks for her love and care. I consider myself lucky for being blessed with such wonderful parents and family.

Finally, my husband, Prof. Awadhesh Narayan, has been a huge source of inspiration for me. I feel truly fortunate to have him in my life. He has been the guiding light that helped me reach the finish line of my PhD. He stands by me untiringly and I thank him for everything. This work is dedicated to him and to my parents.

## 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। ओवरएक्सप्रेशन 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 एपोटोसिस 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 वीडिआर प्रोटीन के साथ MED12 की सीधी बातचीत का अध्ययन किया। हम बी सेल लिंफोमा 6 (BCL6) की पहचान करते हैं, जो वीडिआर 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 एपोटोसिस 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

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

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

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

<b>CARM1</b>	Coactivator Associated Arginine Methyltransferase 1
<b>GSK3<math>\beta</math></b>	Glycogen Synthase Kinase 3 Beta
<b>MYC</b>	Master Regulator of Cell Cycle Entry and Proliferative Metabolism
<b>BRD4</b>	Bromodomain Containing 4
<b>RAS</b>	Rat sarcoma virus
<b>MEK</b>	Mitogen-activated protein kinase kinase
<b>ERK</b>	Extracellular signal-regulated kinase
<b>EMT</b>	Epithelial to mesenchymal transition
<b>cDNA</b>	Complementary DNA
<b>PBS</b>	Phosphate buffer saline
<b>7 AAD</b>	7-Aminoactinomycin D
<b>PI</b>	Propidium iodide
<b>PE</b>	Phycoerythrin
<b>FITC</b>	Fluorescein isothiocyanate
<b>RIPA</b>	Radioimmunoprecipitation assay buffer