MIRNA THERAPY FOR GLIOBLASTOMA TREATMENT

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DEPARTMENT OF BIOCHEMICAL ENGINEERING AND BIOTECHNOLOGY

INDIAN INSTITUTE OF TECHNOLOGY DELHI

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by

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Submitted

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Dedicated to my loved ones

CERTIFICATE

This is to certify that the thesis entitled “miRNA therapy for Glioblastoma treatment”, being submitted by Mr. Indranil Mondal 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.

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Indranil Mondal

Date

ABSTRACT

Glioblastoma (GBM), the most aggressive central nervous system (CNS) tumor, remains incurable despite medical progress. Understanding GBM's molecular processes is vital for therapy development. In recent years, microRNAs (miRNAs) have been found to exhibit altered expression in GBM and other cancers, influencing key hallmarks. This study focuses on miR-210, a highly oncogenic miRNA in GBM, and aims to unravel its role in reprogramming energy metabolism – an emerging hallmark of cancer. To uncover its role in tumor metabolism, aldehyde dehydrogenase 5 family member A1 (ALDH5A1) was identified and validated as a direct target of miR-210 in GBM. Here, we report the role of miR-210/ALDH5A1 axis in GBM metabolism for the first time. We show that ALDH5A1 is downregulated in GBM patients and play an important role in cellular metabolism. By measuring various parameters like glucose uptake, extracellular lactate, ATP, and extracellular acidification rate (ECAR), we demonstrate that miR-210 promotes the glycolysis of GBM while ALDH5A1 inhibits it. We also demonstrate that ALDH5A1 overexpression significantly increases the oxygen consumption rate (OCR) GBM cells, while miR-210 inhibits mitochondrial respiration. Finally, we established a tumor suppressor role of ALDH5A1 in GBM where it inhibited cellular proliferation, 3D-spheroid formation, and reactive oxygen species (ROS) accumulation. Additionally, the study explores miR-210 as a potential therapeutic target and introduces a novel nanocarrier designed for the *in vitro* delivery of anti-miR-210 to GBM cells. Considering the challenges of delivering small RNA-based therapeutics into cells, a unique transglutaminase (TGase)-based nanocarrier was developed, designed in the shape of a blooming flower. These TGase nanoflowers (TGNFs), functionalized with PEI and with a mean diameter of 61nm, effectively complexed with anti-miR-210, enhancing its cellular uptake and facilitating its downregulation. The TGNFs exhibited >90% encapsulation efficiency for anti-miR-210, demonstrated non-cytotoxicity, did not adversely affect the liver and kidney health of CD1 mice, protected the cargo from serum nucleases, and prevented replacement by other polyanion moieties. Delivering anti-miR-210 to GBM cell lines U-87 MG and LN229 using the TGNFs led to a substantial reduction in cellular proliferation and migration, along with the induction of apoptosis. Overall, this research validates a novel target of miR-210, sheds light on its role in GBM metabolism, and presents a unique nanocarrier for delivering its antagonist to GBM cells.

सार

ग्लियोब्लास्टोमा (जीबीएम), सबसे आक्रामक सीएनएस ट्यूमर, चिकित्सा प्रगति के बावजूद लाइलाज बना हुआ है। जीबीएम की आणविक प्रक्रियाओं को समझना चिकित्सा विकास के लिए महत्वपूर्ण है। हाल के वर्षों में, माइक्रोआरएनए (एमआईआरएनए) को जीबीएम और अन्य कैंसर में परिवर्तित अभिव्यक्ति प्रदर्शित करते हुए पाया गया है, जो प्रमुख लक्षणों को प्रभावित करते हैं। यह अध्ययन एमआईआर-210 पर केंद्रित है, जो जीबीएम में एक अत्यधिक ऑन्कोजेनिक एमआईआरएनए है, और इसका उद्देश्य ऊर्जा चयापचय को पुन: प्रोग्राम करने में इसकी भूमिका को उजागर करना है - जो कैंसर की एक उभरती हुई पहचान है। ट्यूमर चयापचय में इसकी भूमिका को उजागर करने के लिए, एल्डिहाइड डिहाइड्रोजनेज 5 परिवार के सदस्य A1 (ALDH5A1) की पहचान की गई और GBM में miR-210 के प्रत्यक्ष लक्ष्य के रूप में मान्य किया गया। यहां, हम पहली बार GBM चयापचय में miR-210/ALDH5A1 अक्ष की भूमिका की रिपोर्ट करते हैं। हम दिखाते हैं कि ALDH5A1 GBM रोगियों में डाउनरेगुलेट होता है और सेलुलर चयापचय में महत्वपूर्ण भूमिका निभाता है। ग्लूकोज ग्रहण, बाह्यकोशिकीय लैक्टेट, एटीपी, और बाह्यकोशिकीय अम्लीकरण दर (ECAR) जैसे विभिन्न मापदंडों को मापकर, हम प्रदर्शित करते हैं कि miR-210 GBM के ग्लाइकोलाइसिस को बढ़ावा देता है जबकि ALDH5A1 इसे रोकता है। हम यह भी प्रदर्शित करते हैं कि ALDH5A1 ओवरएक्प्रेशन ऑक्सीजन खपत दर (OCR) GBM कोशिकाओं को काफी बढ़ा देता है, जबकि miR-210 माइटोकॉन्ड्रियल श्वसन को रोकता है। अंत में, हमने जीबीएम में ALDH5A1 की एक ट्यूमर दमनकारी भूमिका स्थापित की, जहां इसने सेलुलर प्रसार, 3डी-गोलाकार गठन और प्रतिक्रियाशील ऑक्सीजन प्रजातियों (आरओएस) संचय को रोक दिया। इसके अतिरिक्त, अध्ययन एक संभावित चिकित्सीय लक्ष्य के रूप में miR-210 की खोज करता है और GBM कोशिकाओं में एंटी-miR-210 की इन विट्रो डिलीवरी के लिए डिज़ाइन किया गया एक नया नैनोकैरियर पेश करता है। कोशिकाओं में छोटे आरएनए-आधारित चिकित्सीय पहुंचाने की चुनौतियों को ध्यान में रखते हुए, एक अद्वितीय ट्रांसग्लूटामिनेज़ (टीजीज़)-आधारित नैनोकैरियर विकसित किया गया था, जिसे एक खिलते हुए फूल के आकार में डिज़ाइन किया गया था। ये TGase नैनोफ्लॉवर (TGNFs), PEI के साथ क्रियाशील और 61nm के औसत व्यास के साथ, एंटी-miR-210 के साथ प्रभावी ढंग से जटिल होते हैं, इसके सेलुलर अवशोषण को बढ़ाते हैं और इसके डाउनरेगुलेशन को सुविधाजनक बनाते हैं। टीजीएनएफ ने एंटी-एमआईआर-210 के लिए 90% एनकैप्सुलेशन दक्षता का प्रदर्शन किया, गैर-साइटोटॉक्सिसिटी का प्रदर्शन किया, सीडी1 चूहों के लीवर और किडनी के स्वास्थ्य पर प्रतिकूल प्रभाव नहीं डाला, सीरम न्यूक्लिअस से कार्गो की रक्षा की, और अन्य पॉलीअनियन मोएटीज़ द्वारा प्रतिस्थापन को रोका। टीजीएनएफ का उपयोग करके जीबीएम सेल लाइनों यू-87 एमजी और एलएन229 में एंटी-एमआईआर-210 पहुंचाने से एपोप्टोसिस के प्रेरण के साथ-साथ सेलुलर प्रसार और प्रवासन में काफी कमी आई। कुल मिलाकर, यह शोध एमआईआर-210 के एक नए लक्ष्य को मान्य करता है, जीबीएम चयापचय में इसकी भूमिका पर प्रकाश डालता है, और जीबीएम कोशिकाओं तक इसके प्रतिपक्षी पहुंचाने के लिए एक अद्वितीय नैनोकैरियर प्रस्तुत करता है।

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ABBREVIATIONS

[2-N-(7-nitrobenz-2-oxa-1,2-diaxol-4-yl) amino]-2-deoxyglucose	2-NBDG
1,2-dioleyl-3-dimethylammonium propane	DODAP
1,2-dioleyloxy-N,N-dimethyl-3-aminopropane	DODMA
2'-7'-Dichlorodihydrofluorescein diacetate	DCFDA
3' untranslated region	3' UTR
3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide	MTT
3-phosphoinositide-dependent protein kinase 1 (PDPK1)-mediated phosphoglycerate kinase 1	PGK1
Adeno-associated viruses	AAVs
Adenosine diphosphate	ADP
Adenosine triphosphate	ATP
Aldehyde dehydrogenase 5 family member A1	ALDH5A1
Alpha-ketoglutarate	α-KG
Angiopoietin 1	ANG1
Angiopoietin 24	ANG24
Antigen-presenting cells	APCs
Antisense oligonucleotides	ASOs
Apolipoprotein E	ApoE
ATP binding cassettee	ABC
Blood brain barrier	BBB
Blood-tumor barrier	BTB
Bovine serum albumin	BSA
Brain-derived neurotrophic factor	BDNF
carbonic anhydrase 9	CA9
Carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone	FCCP
Cell-penetrating peptides	CPPs
Central Brain Tumor Registry of the United States	CBTRUS
Central nervous system	CNS
Chimeric Antigen Receptor T-cell	CAR-T

Chinese glioma genome atlas	CGGA
Cholangiocarcinoma	CCA
CLear cell renal cell carcinoma	CCRCC
Clustered regulatory interspaced short palindromic repeats	CRISPR
Colorectal cancer	CRC
Cytochrome c oxidase assembly protein	COX10
D-2-hydroxyglutarate	D-2HG
Damage-associated molecular patterns	DAMPs
DiGeorge syndrome critical region 8	DGCR8
Dimethyl sulfoxide	DMSO
Disease specific survival	DSS
Dulbecco's Modified Eagle Medium	DMEM
Dynamic light scattering	DLS
Electron transport chain	ETC
Encapsulation efficiency	EE
Epidermal growth factor receptor	EGFR
Erythropoietin	EPO
Extracellular acidification rate	ECAR
Field emission scanning electron microscopy	FESEM
Fluorescein amidite	FAM
Food and drug administration	FDA
Fourier transform infrared spectroscopy	FTIR
Gamma-aminobutyric acid	GABA
Gamma-aminobutyric acid type A receptor alpha1	GABRA1
Glioblastoma	GBM
Glioma stem cells	GSCs
Glucose-6-phosphate	G6P
Glyceraldehyde 3-phosphate dehydrogenase	GAPDH
Hepatocellular carcinoma	HCC
Hexokinase 1	HK1

Hypoxia response elements	HREs
Hypoxia-inducible factor A	HIF1A
Interleukin-6	IL-6
Iron-sulfur cluster scaffold homolog	ISCU
Isoccitrate dehydrogenase	IDH
Locked nucleic acid	LNA
Magnetic resonance	MR
miRNA	MicroRNA
Monoclonal antibody	mAb
Nanographene oxide	NGO
Neurofilament light polypeptide	NEFL
Nicotinamide Adenine Dinucleotide Phosphate Hydrogen	NADPH
Noncoding RNA	ncRNA
Nonhomologus end joining	NHEJ
Non-small cell lung cancer	NSCLC
Nuclear factor kappa B	NF-kB
O6-methylguanine DNA methyltransferase	MGMT
Oncogenic miRNAs	OncomiRs
Oncogenic virus	OV
Oral squamous cell carcinoma	OSCC
Overall survival	OS
Oxidative phosphorylation	OXYPHOS
Oxygen consumption rate	OCR
Pathogen-associated molecular patterns	PAMPs
Phenylmethylsulfonyl fluoride	PMSF
Phosphatase and tensin homolog	PTEN
Phosphate-buffered saline	PBS
Phosphoenol pyruvate	PEP
Platelet Derived Growth Factor Receptor Alpha	PDGFRA
Poly (ADP-ribose) polymerase	PARP

Poly (lactic-co-glycolic acid)	PLGA
Polyacrylamide gel electrophoresis	PAGE
Poly-dimethylaminoethyl methacrylate	PDMAEMA
Polyethylene glycol	PEG
Polyethylenimine	PEI
Polyglycerol	PG
Poly-β-amino esters	PBAE
Post transcriptional gene scilencing	PTGS
Programmed cell death ligand 1	PD-L1
Progression free survival	PFS
Proliferating cell nuclear antigen	PCNA
Prolyl 4-hydroxylase, beta polypeptide	P4HB
Pyruvate dehydrogenase kinase 1	PDK1
Pyruvate kinase M	PKM
Quantitative real time polymerase chain reaction	qRT-PCR
Rabies virus glycoprotein	RVG
Reactive oxygen species	ROS
Receptor tyrosine kinases	RTKs
Regulator of differentiation 1	ROD1
Retinoblastoma-associated protein	RB
Ribose-5-phosphate	R5P
RNA-induced silencing complex	RISC
Severe adverse events	SAEs
Sex determining region Y-box 2	SOX2
Site directed mutagenesis	SDM
Small molecule inhibitors of miRNAs	SMIRs
Sodium dodecyl sulfate	SDS
Solid lipid nanoparticles	SLNs
Solute carrier family 12 members 5	SLC12A5
Spherical nucleic acids	SNAs

Stable nucleic acid-lipid particles	SNALPs
Subunit D of succinate dehydrogenase complex	SDHD
Succinic semialdehyde dehydrogenase	SSADH
Synaptotagmin 1	SYT1
TAR RNA-binding protein	TRBP
Telomerase Reverse Transcriptase	TERT
Temozolomide	TMZ
The cancer genome atlas	TCGA
Toll like receptors	TLRs
Transglutaminase	TGase
Transglutaminase nanoflowers	TGNFs
Transmission electron microscopy	TEM
Tris-buffered saline with 0.1% Tween 20	TBST
Tumor Treating Fields	TTFs
Vascular endothelial growth factor A	VEGFA
Wharton's jelly mesenchymal stem cells	WJMSCs
World health organization	WHO