Functional Characterization of Hypoxia associated long non-coding RNAs and anti-cancer drug development for Glioblastoma treatment

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by

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Dedicated to my loved ones

CERTIFICATE

This is to certify that the thesis entitled **— Functional Characterization of Hypoxia associated long non-coding RNAs and anti-cancer drug development for Glioblastoma treatment**, being submitted by **Ms. Garima Yadav** 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), a highly aggressive brain tumor is marked by the presence of pseudopalisading region and necrotic areas (PAN), which are closely linked to the tumor's increased malignancy, invasiveness, and poor prognosis. This study investigates the involvement of long non-coding RNAs (lncRNAs) in these hallmark regions of GBM, providing insights into their potential role in driving tumor progression. Using RNA-seq data from the IVY Glioblastoma Atlas Project, we identified 40 differentially expressed lncRNAs in PAN compared to Peripheral Tumor (PT) regions, implicating pathways such as mTOR, MAPK, and NF-kB signaling. Co-expression network analysis revealed MIR210HG as a key lncRNA, that is positively correlated with hypoxia-inducible genes like VEGFA and CA9. MIR210HG is a hypoxia-inducible lncRNA, and its induction was found to be mediated by hypoxia-inducible factor 1 and 2 (HIF-1/HIF-2). The expression of MIR210HG is significantly upregulated in GBM tissues compared to normal brain tissues across various datasets, with the highest levels observed in GBM Grade IV, correlating with poor prognosis. Functional analysis demonstrated that MIR210HG promotes cell proliferation and regulates the cell cycle, enhancing G1/S progression in GBM cells. Additionally, MIR210HG was found to promote cell migration and regulate epithelial-mesenchymal transition (EMT) markers, potentially contributing to GBM invasiveness. The downstream target prediction indicated a potential role of MIR210HG in the collagen synthesis, as silencing MIR210HG resulted in reduced COL5A1 expression. The miRNA-lncRNA interaction analysis revealed miR-503 as a potential regulator of MIR210HG and COL5A1 in GBM. Additionally, The Pan cancer expression analysis of MIR210HG through various tumor datasets demonstrated that MIR210HG is significantly upregulated in most cancers and increased with the tumor stage in a subset of them. The prognostic analysis revealed high MIR210HG expression is associated with poor overall and disease-free survival in specific cancer types. Genetic alteration analysis showed minimal alterations in the MIR210HG locus, indicating that overexpression in cancers is not due to gene amplification. The correlation of MIR210HG with promoter methylation was found to be significantly negative in nature in majority of cancers depicting the possible

epigenetic regulation of expression of MIR210HG. Notably, MIR210HG showed negative correlations with immune cells and thus may have strong impact on the tumor microenvironment. Functional analysis indicates its association with hypoxia, angiogenesis, metastasis, and DNA damage repair processes. MIR210HG was found to interact with several proteins and potentially regulate chromatin modifications and transcriptional regulation. These findings highlight the oncogenic role of MIR210HG and suggest its potential as a diagnostic and therapeutic target in many cancers. In addition, coumarin derived triazole compounds were screened for their anti-cancer properties. The candidate compounds showed anti-proliferative and migration inhibitory properties along with an increase in cell apoptosis. The candidate compounds were further shown to downregulate the levels of MIR210HG and miR-210-3p in GBM. Overall, this research identifies lncRNA signature in the GBM tumor microenvironment and its association with cancer pathways and disease prognosis. We further demonstrate pro-oncogenic role of MIR210HG in GBM and provide a mechanistic insight. Additionally, we identify new drug compounds with a potential to target MIR210HG in GBM.

सारांश

ग्लियोब्लास्टोमा (GBM) एक अत्यधिक आक्रामक मस्तिष्क ट्यूमर है, जो हाइपोक्सिक क्षेत्रों और नेक्रोसिस की उपस्थिति से विशेष रूप से छद्मपलिसेडिंग क्षेत्र और नेक्रोटिक क्षेत्रों (PAN) में चिह्नित होता है। ये क्षेत्र ट्यूमर की बढ़ती घातकता, आक्रामकता, और खराब पूर्वानुमान से निकटता से जुड़े होते हैं। इस अध्ययन में GBM के इन महत्वपूर्ण क्षेत्रों में लंबी गैर-कोडिंग RNA (lncRNA) की भागीदारी की जांच की गई है, जो ट्यूमर की प्रगति में इनकी संभावित भूमिका पर अंतर्दृष्टि प्रदान करता है। IVY Glioblastoma Atlas Project से प्राप्त RNA-seq डेटा का उपयोग करके, हमने PAN की तुलना में पेरिफेरल ट्यूमर (PT) क्षेत्रों में 40 भिन्न रूप से व्यक्त lncRNAs की पहचान की, जो mTOR, MAPK, और NF-kB सिग्नलिंग जैसी पथों से जुड़े हैं। सह-अभिव्यक्ति नेटवर्क विश्लेषण में MIR210HG को एक प्रमुख lncRNA के रूप में पहचाना गया, जो हाइपोक्सिया-प्रेरित जीन जैसे VEGFA और CA9 के साथ सकारात्मक रूप से सहसंबद्ध है। MIR210HG एक हाइपोक्सिया-प्रेरित lncRNA है, और इसका प्रेरण हाइपोक्सिया-प्रेरित फैक्टर 1 और 2 (HIF-1/HIF-2) द्वारा मध्यस्थित पाया गया। विभिन्न डेटा सेटों में सामान्य मस्तिष्क ऊतकों की तुलना में GBM ऊतकों में MIR210HG का अभिव्यक्ति स्तर काफी अधिक पाया गया, विशेष रूप से ग्रेड IV GBM में, जो खराब पूर्वानुमान से सहसंबद्ध था। कार्यात्मक विश्लेषण ने दिखाया कि MIR210HG कोशिका प्रसार को बढ़ावा देता है और GBM कोशिकाओं में G1/S प्रगति को बढ़ाते हुए कोशिका चक्र को नियंत्रित करता है। इसके अलावा, MIR210HG ने कोशिका प्रवासन को बढ़ावा दिया और एपिथेलियल-मेसेंकाइमल ट्रांज़िशन (EMT) मार्करों को नियंत्रित किया, जिससे GBM की आक्रामकता में संभावित योगदान हुआ। डाउनस्ट्रीम टारगेट प्रेडिक्शन ने कोलेजन संश्लेषण में MIR210HG की संभावित भूमिका का संकेत दिया, क्योंकि MIR210HG को साइलेंस करने पर COL5A1 अभिव्यक्ति में कमी देखी गई। miRNA-lncRNA इंटरैक्शन विश्लेषण ने miR-503 को GBM में MIR210HG और COL5A1 का संभावित नियामक बताया। इसके अतिरिक्त, विभिन्न ट्यूमर डेटा सेटों के माध्यम से किए गए पैन-कैंसर अभिव्यक्ति विश्लेषण ने दिखाया कि MIR210HG अधिकांश कैंसरों में काफी अधिक अभिव्यक्त होता है और कुछ कैंसरों के एक उपसमूह में ट्यूमर के चरण के साथ बढ़ता है। पूर्वानुमान विश्लेषण से पता चला कि MIR210HG की उच्च अभिव्यक्ति कुछ कैंसर प्रकारों में खराब समग्र और रोग-मुक्त जीवन प्रत्याशा से जुड़ी है। आनुवांशिक परिवर्तन विश्लेषण ने MIR210HG लोकेस में न्यूनतम परिवर्तन दिखाए, जो इंगित करता है कि कैंसर में इसकी ओवरएक्सप्रेशन जीन एम्पलीफिकेशन के कारण नहीं है। MIR210HG का प्रमोटर मिथाइलेशन के

साथ सहसंबंध अधिकांश कैंसरों में नकारात्मक पाया गया, जो MIR210HG की अभिव्यक्ति के संभावित एपिजेनेटिक नियमन को दर्शाता है। विशेष रूप से, MIR210HG ने प्रतिरक्षा कोशिकाओं के साथ नकारात्मक सहसंबंध दिखाया और इस प्रकार इसका ट्यूमर माइक्रोएनवायरनमेंट पर मजबूत प्रभाव हो सकता है। कार्यात्मक विश्लेषण इसके हाइपोक्सिया, एंजियोजेनेसिस, मेटास्टेसिस और डीएनए क्षति मरम्मत प्रक्रियाओं के साथ जुड़ाव का संकेत देता है। MIR210HG को कई प्रोटीनों के साथ इंटरैक्ट करते हुए पाया गया और यह संभावित रूप से क्रोमैटिन संशोधनों और ट्रांसक्रिप्शनल नियमन को नियंत्रित कर सकता है। ये निष्कर्ष MIR210HG की ओंकोजेनिक भूमिका को उजागर करते हैं और इसे कई कैंसरों में नैदानिक और चिकित्सीय लक्ष्य के रूप में सुझाते हैं। इसके अतिरिक्त, क्यूमारिन से व्युत्पन्न ट्रायाज़ोल यौगिकों को उनके एंटी-कैंसर गुणों के लिए स्क्रीन किया गया। उम्मीदवार यौगिकों ने GBM में MIR210HG और miR-210-3p के स्तर को कम करने के साथ-साथ कोशिका अपोप्टोसिस में वृद्धि, कोशिका प्रसार और प्रवासन को रोकने वाले गुण दिखाए। कुल मिलाकर, यह शोध GBM ट्यूमर माइक्रोएनवायरनमेंट में lncRNA हस्ताक्षर की पहचान करता है और इसके कैंसर पथों और रोग के पूर्वानुमान से संबंध को दर्शाता है। हम GBM में MIR210HG की प्रो-ओंकोजेनिक भूमिका को प्रदर्शित करते हैं और यांत्रिक अंतर्दृष्टि प्रदान करते हैं। इसके अलावा, हम GBM में MIR210HG को लक्षित करने की क्षमता वाले नए औषध यौगिकों की पहचान करते हैं।

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ABBREVIATIONS

Adrenocortical carcinoma	ACC
Bladder urothelial carcinoma	BLCA
Breast invasive carcinoma	BRCA
Cervical squamous cell carcinoma and endocervical adenocarcinoma	CESC
Cholangiocarcinoma	CHOL
3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide	MTT
Colon adenocarcinoma	COAD
Diffuse large B-cell lymphoma	DLBC
Esophageal carcinoma	ESCA
Glioblastoma	GBM
Head and Neck squamous cell carcinoma	HNSC
Kidney Chromophobe	KICH
Kidney renal clear cell carcinoma	KIRC
Kidney renal papillary cell carcinoma	KIRP
Acute Myeloid Leukemia	LAML
Brain Lower Grade Glioma	LGG
Liver hepatocellular carcinoma	LIHC
Lung adenocarcinoma	LUAD
Lung squamous cell carcinoma	LUSC
Mesothelioma	MESO
Non-small-cell lung cancer	NSCLC
Ovarian serous cystadenocarcinoma	OV
Pancreatic adenocarcinoma	PAAD
Pheochromocytoma and Paraganglioma	PCPG
Prostate adenocarcinoma	PRAD
Rectum adenocarcinoma	READ

Sarcoma	SARC
Skin Cutaneous Melanoma	SKCM
Stomach adenocarcinoma	STAD
Testicular Germ Cell Tumors	TGCT
Thyroid carcinoma	THCA
Thymoma	THYM
Uterine Corpus Endometrial Carcinoma	UCEC
Uterine Carcinosarcoma	UCS
Blood brain barrier	BBB
Cellular tumor regions	CT
Infiltration into the brain tissue	IT
Microvascular proliferation	MVP
Pseudopalisades around necrosis	PAN
Bovine serum albumin	BSA
Central Brain Tumor Registry of United State	CBTRUS
Dimethyloxaloylglycine	DMOG
Central nervous system	CNS