

**ELUCIDATION OF THE ROLE OF NEURONAL
DIFFERENTIATION FACTORS BELONGING TO THE
NEUROD FAMILY IN GLIOBLASTOMA**

Anirban Jana



**Department of Biochemical Engineering and
Biotechnology**

Indian Institute of Technology Delhi

May 2024

©Indian Institute of Technology Delhi (IITD), New Delhi, 2024

**Elucidation of the Role of Neuronal Differentiation
Factors belonging to the NeuroD family in Glioblastoma**

by

Anirban Jana

Biochemical Engineering and Biotechnology

Submitted

in fulfilment of the requirements of the degree of

Doctor of Philosophy

to the



Indian Institute of Technology Delhi

May 2024

This thesis is dedicated to the most
important women in my life

my mother

Mrs. Sarbani Jana

and

my wife

Mrs. Kumarika Jana

and

my lovely daughter

Anika Jana

Certificate

This is to certify that the thesis entitled “**Elucidation of the Role of Neuronal Differentiation Factors belonging to the NeuroD family in Glioblastoma**” being submitted by **Mr. Anirban Jana** to the Indian Institute of Technology Delhi, for the award of the 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 and the results presented in this thesis have not been submitted in part or full to any other University/ Institute for awarding any degree or diploma.

Prof Ritu Kulshreshtha

Professor

Department of Biochemical Engineering and Biotechnology

Indian Institute of Technology

New Delhi-110016

Date –

Place: New Delhi

Acknowledgments

Throughout my doctoral studies, I had help and support from many people, without whom my work would not have been possible. They have my sincere gratitude and privilege.

First and foremost, I want to express my gratitude to Prof. Ritu Kulshreshtha, who has been a tremendous help throughout the last five years. I am grateful to her for allowing me to work in her lab and pursue my Ph.D. in the Biochemical Engineering and Biotechnology department at IIT Delhi. She always encouraged me to attain my best potential and has helped me develop the analytical abilities needed for research. Working with her was encouraging, and the experience was incredible. Most importantly, my thesis would not have progressed to its current state without her guidance. Her recommendations, criticisms, and remarks aided me in expanding my knowledge of cancer biology.

I am grateful to Prof D. Sundar, Prof Preeti Srivastava, and Prof Vivekanandan Perumal (external expert from Kusuma School of Biological Sciences, IIT Delhi), members of my student research committee for monitoring my study progress and offering valuable insights and constructive recommendations. I also want to thank them for providing me access to their instrumentation facilities.

Prof. Chitra Sarkar, Dr. Vaishali Suri, and Dr. Vikas Sharma of the All India Institute of Medical Sciences (AIIMS, New Delhi) deserve special thanks for their assistance and productive collaboration. Their assistance and helpful advice were quite beneficial for me. Their aid and wise counsel were precious to me.

I would also like to thank Prof. Archana Chugh and Pankhuri Narula (Kusuma School of Biological Sciences, IIT Delhi) for including me as a collaborator that broadened my research aptitude.

I am very thankful to Dr. Kunzang and Dr. Khusboo for their support in performing some experiments and data acquisition that helped me properly shape my study.

I would like to express my gratitude to the Ministry of Education, Government of India, for their financial assistance during my study.

I want to express my gratitude to all who have ever been a part of the CBL lab, IIT Delhi, including Dr. Himesh, Dr. Rahul, Dr. Shivani, Dr. Sonam, Dr. Omkar, Dr. Srishti, Indranil,

Garima, Rahul, Aastha, Anuj, Anubha, Nidhi, Anuja, Rajat, Ravi, Ritanksha, and others for their support and valuable discussion. Sonam, Omkar, Srishti, Indranil, Garima, Anubha, Nidhi, Anuja, Rajat, and Himesh deserve appreciation for their unwavering love, support, and patience, as well as for providing me with some of my fondest Ph.D. memories.

My thanks are dedicated to my friends, especially Dr. Amit Kumar (from CSIR-Institute of Genomics and Integrative Biology, New Delhi), Dr. Surabhi Goyal, Dr. Deepak, Aditi Keshav, Adarsh, Nitika, Pravina, Dr. Sahil, Dr. Biju Jacobs, Swati (IIT Delhi) who helped me in arranging required chemicals, gave me access to the instruments and provided me support in my study throughout.

I appreciate the assistance of department staff members Anees, Sanjay, Ashish, Arjun, Yogesh, Sakshi, and Nitin. Their timely help and support helped me a lot.

Words fail me when it comes to expressing my thanks for my parent's support and love. I owe an immense debt of gratitude to my parents specially, my mother for their unwavering support while I was low, which helped me overcome all my obstacles and finish my thesis. I am also very thankful to my father-in-law, mother-in-law, and brother-in-law for their love and support. Without them, the situation could be more difficult for me. Dr. Ashirbad, my elder brother, deserves special praise for his affection and concern. I feel fortunate to have such a fantastic brother. I also like to thank my sister-in-law Joyashree Jana and my sweet niece Aashree for the unconditional love and support that made my journey easier.

Most importantly, I would like to thank my beautiful wife, Kumarika, and my daughter Anika for their support and motivation during rough times. I consider myself fortunate to have them in my life. They have been a massive inspiration and support for me throughout my journey. I consider myself lucky to be blessed with such a wonderfully supportive family.

Anirban Jana

Abstract:

Neuronal differentiation factors are a class of bHLH transcription factors recently shown to be differentially expressed in various cancers. However, their functions in glioblastoma (GBM) remain little studied so far. Previous studies from our group showed significant downregulation of neuronal differentiation factors, NeuroD2 and NeuroD6, in the TCGA-GBM patient cohort and further demonstrated the tumor-suppressive role of NeuroD2 in GBM under hypoxic conditions. However, the regulation or relevance of NeuroD6 downregulation remained unstudied so far. Here, we show that NeuroD6 is significantly downregulated in Indian GBM patients and GBM cell lines compared to the control brain. NeuroD6 and NeuroD2 both were found to be primarily enriched in the nuclear fraction as compared to the cytoplasmic fraction. Notably, analyses of the GBM patient dataset (TCGA, Firehose Legacy) using c-Bioportal, showed a truncating mutation in only one out of 619 patients, suggesting that NeuroD6 downregulation is not at the genomic level and may be regulated at the epigenetic, transcriptional or post-transcriptional levels. NeuroD6 was also transcriptionally induced by p53 and regulated at the post-transcriptional level by an oncogenic microRNA cluster, miR-421/374b. Our preliminary studies show that NeuroD6 may be regulated by DNA methylation. We further show that NeuroD6 functions as a tumor suppressor in GBM by inhibiting cell proliferation, migration, and tumor formation ability and promoting cell cycle arrest at the G₀G₁ phase in GBM cell lines T98G and A172. We also show that cells overexpressing NeuroD6 display increased apoptosis. Further studies revealed that both NeuroD2 and NeuroD6 regulate GBM cell metabolism by lowering glucose transport and lactate generation, indicating a reduction in glycolysis. Interestingly, overexpression of NeuroD6 and NeuroD2 was shown to lower the levels of oncogenic ID family molecules -ID1, ID2, and ID3 and has a negative feedback loop with ID2. In glucose-depleted conditions, both NeuroD2 and NeuroD6 were shown to accelerate GBM cell death, and when combined with siID2, the inhibition of proliferation was considerably increased. NeuroD2 and NeuroD6 transcript levels were found to be positively correlated in GBM tumors. Co-overexpression of NeuroD6 and NeuroD2 was shown to have a much stronger tumor-suppressive effect on cell proliferation, migration, and spheroid formation ability in GBM. Overall, our work reveals that NeuroD6 and NeuroD2, individually and in combination, may function as diagnostic biomarkers for GBM, and their overexpression may be an attractive option for GBM treatment.

सार:

न्यूरोनल विभेदन कारक बीएचएलएच प्रतिलेखन कारकों का एक वर्ग है जिसे हाल ही में विभिन्न कैंसर मेअलग-अलग रूप से व्यक्त किया गया है। हालाँकि, ग्लियोब्लास्टोमा (जीबीएम) में उनके कार्यों का अब तक बहुत कम अध्ययन किया गया है। हमारे समूह के पिछले अध्ययनों ने टीसीजीए-जीबीएम रोगी समूह में न्यूरोनल विभेदन कारकों, न्यूरोडी2 और न्यूरोडी6 में महत्वपूर्ण गिरावट देखी है और हाइपोक्सिक स्थितियों के तहत जीबीएम में न्यूरोडी2 की ट्यूमर-दमनकारी भूमिका का प्रदर्शन किया है। हालाँकि, न्यूरोडी6 डाउनरेगुलेशन के विनियमन या प्रासंगिकता का अब तक अध्ययन नहीं किया गया है। यहां, हम दिखाते हैं कि नियंत्रण मस्तिष्क की तुलना में भारतीय जीबीएम रोगियों और जीबीएम सेल लाइनों में न्यूरोडी6 को काफी कम विनियमित किया गया है। न्यूरोडी6 और न्यूरोडी2 दोनों को साइटोप्लाज्मिक अंश की तुलना में मुख्य रूप से परमाणु अंश में समृद्ध पाया गया। सी-बायोपोर्टल का उपयोग करके विश्लेषण किए गए पांच चार डेटासेट में से, केवल एक डेटासेट, ग्लियोब्लास्टोमा मल्टीफॉर्म (टीसीजीए, फायरहोज लिगेसी) डेटासेट ने केवल एक रोगी (0.17%) में एक ट्रंकिंग उत्परिवर्तन दिखाया, जो सुझाव देता है कि न्यूरोडी 6 डाउनरेगुलेशन जीनोमिक स्तर पर नहीं है और एपिजेनेटिक, ट्रांसक्रिप्शनल या पोस्ट-ट्रांसक्रिप्शनल स्तरों पर विनियमित किया जा सकता है। न्यूरोडी6 को भी पी53 द्वारा ट्रांसक्रिप्शनल रूप से प्रेरित किया गया था और पोस्ट-ट्रांसक्रिप्शनल स्तर पर एक ऑन्कोजेनिक माइक्रोआरएनए क्लस्टर, एमआईआर-421/374बी द्वारा विनियमित किया गया था। हमारे प्रारंभिक अध्ययन से पता चलता है कि न्यूरोडी6 को डीएनए मिथाइलेशन द्वारा नियंत्रित किया जा सकता है। हम आगे दिखाते हैं कि न्यूरोडी6 जीबीएम में ट्यूमर दमनकर्ता के रूप में कार्य करता है, जो कोशिका प्रसार, प्रवासन और ट्यूमर निर्माण क्षमता को रोकता है और जीबीएम सेल लाइनों टी98जी और ए172 में जी0जी1 चरण में कोशिका चक्र गिरफ्तारी को बढ़ावा देता है। हम यह भी दिखाते हैं कि न्यूरोडी6 को अधिक व्यक्त करने वाली कोशिकाएं एपोटोसिस को बढ़ाती हैं। आगे के अध्ययनों से पता चला कि न्यूरोडी2 और न्यूरोडी6 दोनों ग्लूकोज परिवहन और लैक्टेट उत्पादन को कम करके जीबीएम सेल चयापचय को नियंत्रित करते हैं, जो ग्लाइकोलाइसिस में कमी का संकेत देता है। दिलचस्प बात यह है कि न्यूरोडी6 और न्यूरोडी2 की अधिक अभिव्यक्ति से ऑन्कोजेनिक आईडी परिवार अणुओं -आईडी1, आईडी2 और आईडी3 के स्तर में कमी देखी गई है और आईडी2 के साथ एक नकारात्मक फीडबैक लूप है। ग्लूकोज-क्षीण स्थितियों में, न्यूरोडी2 और न्यूरोडी6 दोनों को जीबीएम कोशिका मृत्यु में तेजी लाने के लिए दिखाया गया था, और जब एसआईडी2 के साथ जोड़ा गया, तो प्रसार का अवरोध काफी बढ़ गया था। जीबीएम ट्यूमर में न्यूरोडी2 और न्यूरोडी6 ट्रांसक्रिप्ट स्तर सकारात्मक रूप से सहसंबद्ध पाए गए। न्यूरोडी6 और न्यूरोडी2 के सह-

ओवरएक्सप्रेशन को जीबीएम में कोशिका प्रसार, प्रवासन और गोलाकार गठन क्षमता पर अधिक मजबूत ट्यूमर-दमनकारी प्रभाव दिखाया गया है। कुल मिलाकर, हमारे काम से पता चलता है कि न्यूरोडी6 और न्यूरोडी2 व्यक्तिगत रूप से और संयोजन में जीबीएम के लिए डायग्नोस्टिक बायोमार्कर के रूप में कार्य कर सकते हैं, और उनकी ओवरएक्सप्रेशन जीबीएम उपचार के लिए एक आकर्षक विकल्प हो सकती है।

Table of Contents

Certificate	i
Acknowledgments	ii
Abstract	iv
Table of contents	vii
List of Figures	xii
List of Tables	xv
Abbreviations	xvi
1. Introduction	1
2. Review of Literature	6
2.1 Glioblastoma	7
2.1.1 WHO grading and classification	8
Classical	9
Proneural	9
Neural	9
Mesenchymal	9
2.1.2 Epidemiology	10
2.1.3 Etiology of GBM	11
2.1.4 Pathogenesis of GBM	12
Site	12
Microscopic and Histological Features of GBM	12
Clinical Presentation	12
2.1.5 Treatment of GBM	13
Surgery	13
Radiation therapy	14
Chemotherapy	14
Advanced in therapy	14
Tumor treating Fields therapy	14
Anti-angiogenic therapy	15
Immunotherapy	15
2.1.6 Metabolic Abnormalities in Glioblastoma	15

2.2 Neuronal Differentiation Factors (NeuroD) belongs to bHLH family	17
2.2.1 Phylogenetic analysis of bHLH molecules and NeuroD	17
2.2.2 NeuroD shares specific conserved motifs	19
2.2.3 Function of different bHLH molecules	20
2.2.4 NeuroD family and functions	20
NeuroD1	21
NeuroD2	22
NeuroD4	22
NeuroD6	22
2.2.5 Role of NeuroD in Cancer	24
2.3 Oncogenic ID genes	25
2.3.1 ID molecules and relation with other bHLH genes	25
2.3.2 IDs promote cell cycle	26
2.3.3 IDs promote stemness	27
2.3.4 ID Proteins Promote Tumor Progression	27
2.3.5 ID Proteins and Glioblastoma	28
2.3.6 ID2 Promotes Cell Survival in glucose Starved Conditions	28
2.4 miRNA	29
2.4.1 miRNA biogenesis and mode of action	30
2.4.2 Cancer and miRNAs	31
2.4.3 Oncogenic miRNAs	32
2.4.4 Tumor suppressor miRNAs	33
3. Objectives	35
4. Material and Methods	37
4. Methodology	38
4.1 Reagent and antibodies	38
4.2 Cell Culture	38
4.3 Patient Data	38
4.4 Plasmids	39
4.5 Cloning of miRNAs cluster miR-421/374b	40
4.6 3'UTR luciferase reporter plasmid construct	40

4.7 In-silico analysis of GBM patient data	40
4.8 Transient Transfection	41
4.9 RNA Isolation and RT-PCR	42
4.10 Stem loop RT PCR	42
4.11 Cell Proliferation MTT assay	43
4.12 Cyquant Cell Proliferation Assay	43
4.13 Colony Formation assay	43
4.14 Soft Agar Assay	43
4.15 Scratch Assay	44
4.16 Boyden Chamber Assay	44
4.17 Caspase 3/7 Glo Assay	44
4.18 PI Annexin V Apoptosis Detection Assay	45
4.19 PI Staining for Cell Cycle Analysis	45
4.20 Generation of Tumor Spheroids	45
4.21 ROS Production Assay	46
4.22 Total ATP and Non-Mitochondrial (Lactic Pathway) ATP Production Measurement	46
4.23 Glucose Deprivation treatment	46
4.24 Quantitative Determination of Lactate	47
4.25 Subcellular Fractionation	47
4.26 3' UTR Luciferase Assay	47
4.27 Promoter luciferase assay	48
4.28 Western Blotting	48
4.29 Chromatin Immunoprecipitation-qPCR (ChIP-qPCR)	48
4.30 Statistical Analysis	49
5. Results	50
5.1 Expression Pattern of Neuronal Differentiation Factors Belonging to NeuroD Family	51
5.1.1 Tissue and Cellular Enrichment of NeuroDs	51
5.1.2 NeuroD2 and NeuroD6 are Downregulated in GBM Patients and Cell Lines	54

5.1.3 NeuroD2 and NeuroD6 both are enriched in leading edge of 3D tumors	61
5.2 Regulation of NeuroD6	63
5.2.1 Genetic mutation profile of NeuroD6 in GBM	63
5.2.2 Demethylation agents promote expression of NeruoD6	63
5.2.3 NeuroD6 is p53 Regulated	65
5.2.4 miR-421/374b Cluster Targets NeuroD6	68
5.3 Functional Characterization of NeuroD6 in GBM	72
5.3.1 Cloning of NeuroD6 CDS region in pcDNA 3.1+ and overexpression	72
5.3.2 NeuroD6 inhibits Cell Proliferation in GBM	73
5.3.3 NeuroD6 inhibits colony formation ability of GBM	74
5.3.4 NeuroD6 Promotes Cell Cycle Arrest at the GoG1 Phase	75
5.3.5 NeuroD6 Inhibits Spheroid Formation Ability of GBM Cells	77
5.3.6 NeuroD6 Inhibits Cellular Migration in GBM Cells	77
5.3.7 NeuroD6 Promotes Apoptosis	78
5.3.8 NeuroD6 Acts as an Antioxidant Molecule in GBM	80
5.4 miR-421/374b Cluster Plays an Oncogenic Role in GBM and negatively regulate NeuroD6	82
5.5 NeuroD2 and NeuroD6 Play a Vital role in GBM Metabolism	86
5.5.1 NeuroD2 and NeuroD6 both decrease glycolysis in GBM	86
5.5.2 NeuroD2 and NeuroD6 both decrease glucose transport in GBM	89
5.6 NeuroD2 and NeuroD6 are negatively correlated with ID genes and promote cell death in glucose starved conditions	90
5.6.1 NeuroD6 and NeuroD2 both inversely correlated with ID genes	90
5.6.2 NeuroD2 and NeuroD6 are negatively correlated with ID2 and promote cell death in glucose starved conditions	92
5.7 NeuroD2/D6 combination therapy shows stronger tumor suppressive impact on different cancer hallmarks	97
5.7.1 NeuroD2 and NeuroD6 are positively correlated	97

5.7.2 The combination of NeuroD2 and NeuroD6 overexpression shows a stronger tumor suppressive impact on GBM cells	99
6. Discussion and Limitations	105
6.1 Discussion	106
6.2 Limitations of the study	112
7. Conclusion Remarks and Future Perspectives	113
7.1 Conclusions	114
7.2 Future Perspectives	116
8. References	117
Appendix 1	144
List of primers	144
Resume of the Author	146

List of Figures

Figure No.	Title	Page No
1	Structural analysis of bHLH molecules	3
2.1	Genomic Alterations and Subtypes of GBM	9
2.2	Epidemiology of brain tumors	11
2.3	Phylogenetic tree of bHLH molecules	19
2.4	NeuroD1 promotes Neuron production	21
2.5	ID genes promote the cell cycle	27
2.6	miRNA biogenesis	31
2.7	Up and downregulated miRNAs in GBM and their function in glioma	34
5.1	Analysis of tissue and cellular enrichment of NeuroDs using ‘The Human Protein Atlas’	53
5.2	Cellular enrichment analysis of NeuroD2 and NeuroD6	54
5.3	TCGA data showing levels of NeuroDs in GBM	56
5.4	TCGA, CCGA, Rembrandt data showing downregulation of NeuroD2 and NeuroD6 is grade dependent in GBM	57
5.5	CGGA data showing downregulation of NeuroD2 and NeuroD6 is downregulated in recurrent Gliomas	59
5.6	CGGA, TCGA, Rembrandt, and Gravendeel data showing relation between NeuroD6 expression and glioma prognosis	60
5.7	NeuroD6 is downregulated in Indian GBM patients and GBM cell lines	61
5.8	Gliovis data (Ivy Gap dataset) showing enrichment of NeuroD6 and NeuroD2 in the leading-edge of tumor microenvironment	62
5.9	Genomic alteration analysis of NeuroD6 using c-Bioportal	63
5.10	CpG island and DNA methylation status of NeuroD6	64
5.11	Diagram showing p53 binding sites (p53REs) at NeuroD6 promoter region	65
5.12	p53 increases NeuroD6 levels	66

5.13	p53 binds to NeuroD6 promoter region and regulate NeuroD6 at the protein levels.	68
5.14	Target scan 8.0 data showing miRNAs that have binding site at 3'UTR region of NeuroD6 and expression level of miR-421 in GBM	70
5.15	The miR-421/374b cluster has binding site at 3'UTR of NeuroD6 and negatively regulates	71
5.16	Confirmation of NeuroD6 expression from NeuroD6 overexpression clone	72
5.17	NeuroD6 downregulates GBM cell proliferation	73
5.18	NeuroD6 decreases colony formation potential in GBM cells	75
5.19	NeuroD6 promotes cell cycle arrest	76
5.20	NeuroD6 downregulates spheroid formation potential	77
5.21	NeuroD6 inhibits GBM cell migration	78
5.22	NeuroD6 promotes apoptosis in GBM	79
5.23	NeuroD6 overexpression brings about increase in the cleaved PARP levels in A172	80
5.24	NeuroD6 is an antioxidant molecule	81
5.25	miR-421/374b plays oncogenic role and inhibits tumor suppressive effect of NeuroD6 in GBM	84
5.26	miR-421/374b plays oncogenic role and inhibits tumor suppressive effect of NeuroD6 in GBM	86
5.27	NeuroD6 and NeuroD2 lowers overall ATP production and glycolysis rate in GBM	88
5.28	NeuroD6 and NeuroD2 glucose transport in GBM	90
5.29	Downregulation of ID1, ID2 and ID3 in NeuroD6 and NeuroD2 upregulated GBM	92
5.30	NeuroD2 and NeuroD6 are downregulated in glucose starved condition and silencing of ID2 increases levels of NeuroD2 and NeuroD6	94
5.31	NeuroD2 and NeuroD6 individually or in combination with si-ID2 promote cell death in glucose starved condition	96
5.32	NeuroD2 and NeuroD6 have positive correlation	98

5.33	NeuroD2 and NeuroD6 combination have better tumor suppressive role in GBM	103
7.1	Schematic diagram representing tumor suppressive role of NeuroD2 and NeruoD6.	114

List of Tables

Table no	Title	Page No.
2.1	Function of different NeuroD molecules	23
3.1	Summary of the clinical data for GBM patients used in this study.	39
5.1	P53 binding sites within 5 kb upstream of NeuroD6 transcription start site	65
5.2	P53 binding sites cloning details	67

Abbreviations

2D	Two Dimensional
3D	Three Dimensional
aa	Amino Acid
7AAD	7-Aminoactinomycin D
ABCG2	Atp Binding Cassette Subfamily G Member 2
Akt	Protein Kinase B
ALDOA	Aldolase A, Fructose-Bisphosphate
ALK	ALK Receptor Tyrosine Kinase
ALL	Acute Lymphocytic Leukemia
AMPK	AMP-Activated Protein Kinase
ANP32A	Acidic Nuclear Phosphoprotein 32 Family Member A
Apaf-1	Apoptotic Peptidase Activating Factor 1
ASCL1	Achaete-Scute Family Bhlh Transcription Factor 1
ATOH1	Atonal Bhlh Transcription Factor 1
ATP	Adenosine 5'-Triphosphate
ATRX	Atrx Chromatin Remodeler
BAX	Bcl2 Associated X, Apoptosis Regulator
BCL2	Bcl2 Apoptosis Regulator
BCL-xL	Bcl2 Like 1
BHF1	Neuronal Differentiation Factor
bHLH	Basic-Helix Loop Helix
Bim	BCL2 Like 11
BNCT	Boron Neutron Capture Therapy
BRAF	B-Raf Proto-Oncogene, Serine/Threonine Kinase
BRN2	Pou Class 3 Homeobox 2
BSA	Bovine Serum Albumin
CA9	Carbonic Anhydrase 9
CCND1	Cyclin D1
CCND2	Cyclin D2
CD133	Cluster Of Differentiation 133

CD24	Cd24 Antigen
CDK4	Cyclin Dependent Kinase 4
CDK6	Cyclin Dependent Kinase 6
cDNA	Complementary DNA
CGGA	Chinese Glioma Genome Atlas
ChIP-qPCR	Chromatin Immunoprecipitation-Quantitative Real Time PCR
CNS	Central Nervous system
CRC	Cyclic Redundancy Check
DCF	Dichlorofluorescein
DCFDA	Dichloro Fluorescein Diacetate
DGCR8	Digeorge Syndrome Critical Region Gene 8
dHAND	Deciduum, Heart, Autonomic Nervous System And Neural Crest Derivatives-Expressed Protein 2
DMEM	Dulbecco's Modified Eagle Medium
DNA	Deoxyribonucleic Acid
E2F	Retinoblastoma-Associated Protein 1
ECL	Enhanced Chemiluminescence
EGFR	Epidermal Growth Factor Receptor
eHAND	Extraembryonic Tissues, Heart, Autonomic Nervous System And Neural Crest Derivatives-Expressed Protein 1
EMT	Epithelial-Mesenchymal Transition
ENO1	Enolase 1
ENO2	Enolase 2
FABP7	Fatty Acid Binding Protein 7
FACS	Fluorescence Activated Cell Sorting
FBS	Fetal Bovine Serum
FCCP	Carbonyl Cyanide-P-Trifluoromethoxyphenylhydrazine
G6PD	Glucose-6-Phosphate Dehydrogenase
GABRA1	Gamma-Aminobutyric Acid Type A Receptor Subunit Alpha1
GAP43	Growth Associated Protein 43

GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
GBM	Glioblastoma Multiforme
GLUT1	Glucose Transporter 1
GLUT2	Glucose Transporter 2
GLUT3	Glucose Transporter 3
GLUT4	Glucose Transporter 4
GSCs	Gbm Stem Cells
GTR	Guided Tissue Regeneration
HES	Hairy And Enhancer Of Split
HEY1	Hes Related Family Bhlh Transcription Factor With Yrpw Motif 1
HIF	Hypoxia Inducible Factor
HIF3A	Hypoxia Inducible Factor 3 Subunit Alpha
HK2	Hexokinase-2
HLH	Helix- Loop-Helix
HOXD10	Homeobox Protein Hox-D10
IC50	Half Maximal Inhibitory Concentration
ID1	Inhibitors Of Differentiation 1
ID2	Inhibitors Of Differentiation 2
ID3	Inhibitors Of Differentiation 3
ID4	Inhibitors Of Differentiation 4
IDH	Isocitrate Dehydrogenase 1
IGF	Insulin-Like Growth Factor
IgG	Immunoglobulin G
IMRT	Intensity Modulated Radiotherapy
Kb	Kilobyte
LDH	Lactate Dehydrogenase
LDLR	Low Density Lipoprotein Receptor
LncRNAs	Long Noncoding Rnas
LRRFIP1	Leucine-Rich Repeat Flightless-Interacting Protein 1

Mash1	Mammalian Achaete Scute Homolog-1
MBD3	Methyl-Cpg-Binding Domain Protein 3
MDM2	Mouse Double Minute 2 Homolog
mETC	Mitochondrial Electron Transport Chain
miRISCs	Mirna-Induced Silencing Complexes
MMP2	Melanoma Mutants, Found Matrix Metalloproteinase-2
MMP9	Melanoma Mutants, Found Matrix Metalloproteinase-9
MRI	Magnetic Resonance Imaging
MSI1	Musashi RNA Binding Protein 1
MTDH	Metadherin
MTT	3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide
MYC	Myc Proto-Oncogene
MYF3	Myogenic Factor 3
MYF5	Myogenic Factor 5
MYOD	Myogenic Differentiation
MYOG	Myogenin
NAC	N-Acetyl Cysteine
NADH	Nicotinamide Adenine Dinucleotide
NEFL	Neurofilament Light Chain
NES	Nestin
NEUROD1	Neuronal Differentiation Factor 1
NEUROD2	Neuronal Differentiation Factor 2
NEUROD4	Neuronal Differentiation Factor 4
NEUROD6	Neuronal Differentiation Factor 6
NEUROG	Neurogenin
NEUROG2	Neurogenin 2
NF1	Neurofibromin 1
NFKB	Nuclear Factor Kappa B Subunit 1
NOS	Nitric Oxide Synthase
OCR	Oxygen Consumption Rate
OLIG2	Oligodendrocyte Transcription Factor 2
P16INK4	Cyclin Dependent Kinase Inhibitor 2a

P21	Cyclin Dependent Kinase Inhibitor 1a
TP53	Tumor Protein P53
PARP	Poly (Adp-Ribose) Polymerase
PBS	Phosphate-Buffered Saline
PDGF	Platelet-Derived Growth Factors
PDGFRA	Platelet-Derived Growth Factor Receptor Alpha
PDH	Pyruvate Dehydrogenase Complex
PDK1	Pyruvate Dehydrogenase Kinase 1
PDP	Pyruvate Dehydrogenase Phosphatase
PEP	Phosphoenolpyruvate
PGAM1	Phosphoglycerate Mutase 1
PI3K	Phosphoinositide 3-Kinase
PK	Pyruvate Kinase
PKLR	Pyruvate Kinase L/R
PKM	Pyruvate Kinase Muscle Isozyme
PKM2	Pyruvate Kinase Muscle Isozyme 2
pRB	Retinoblastoma-Associated Protein
PTEN	Phosphatase And Tensin Homolog
PUMA	P53 Upregulated Modulator Of Apoptosis
qPCR	Quantitative Polymerase Chain Reaction
Raf-1	Raf-1 Proto-Oncogene,
REST	Re1 Silencing Transcription Factor
RhoA	Ras Homolog Family Member A
RIPA	Radioimmunoprecipitation Assay Buffer
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
SDS	Sodium Dodecyl Sulfate
si-ID2	Id2 Silencing
SLC	Solute Carrier
SLC12A5	Solute Carrier Family 12 Member 5
SLIT2	Slit Guidance Ligand 2

SMAD4	Smad Family Member 4
SMARCA4	Swi/Snf Related, Matrix Associated, Actin Dependent Regulator Of Chromatin, Subfamily A, Member 4
SNAI2	Snail Family Transcriptional Repressor 2
SNAIL	Snail Family Transcriptional Repressor 1
SPRY2	Sprouty Rtk Signaling Antagonist 2
SREBP1	Sterol Regulatory Element Binding Transcription Factor 1
SYT1	Synaptotagmin 1
TBX3	T-Box Transcription Factor 3
TCA	Tricarboxylic Acid Cycle
TCGA	The Cancer Genome Atlas
TGF	Transforming Growth Factor
TGF2	Transforming Growth Factor 2
TMZ	Temozolomide
TNF	Tumor Necrosis Factor
TTFA	Thenoyltrifluoroacetone
TWIST	Twist Family Bhlh Transcription Factor 1
VEGF	Vascular Endothelial Growth Factor
WEE1	Wee1 G2 Checkpoint Kinase
WHO	World Health Organization
ZEB	Zinc Finger E-Box Binding Homeobox
ZEB1	Zinc Finger E-Box Binding Homeobox 1
ZEB2	Zinc Finger E-Box Binding Homeobox 2