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Original Article / Özgün Araştırma

In Silico Analysis of miRNA-mediated ceRNAs as Potential Molecular Biomarkers in Glioblastoma

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Abstract

Objectives: Glioblastoma multiforme (GBM) is defined as the most frequent and lethal form of the primary brain tumors in the central nervous system (CNS) in adults. Recent studies have focused on the identification of the new targets for the diagnosis and treatment of GBM and resulted in great interest for miRNAs due to their regulatory effects in cancer pathogenesis. Thus, we aimed to characterize novel molecular biomarkers for GBM by computational analysis.

Methods: 118 miRNAs that are clinically related with glioblastoma and proven by experimentally were exported through miRTarBase database. 1016 genes projected by these 118 miRNAs were determined via ComiR database. Subsequently, the genes with transcribed ultraconserved regions (T-UCRs) in their exonic regions were designated and the genes which have potential competing endogenous RNA (ceRNA) activities were extracted. Genes with remarkable expression profile differences between glioblastoma and normal brain tissues among ceRNAs that are associated with glioblastoma involving T-UCR were identified.

Results: The statistical analysis of the correlation between PBX3 and NRXN3 genes and glioblastoma was carried out by Spearman correlation test. PBX3 and NRXN3 expression was significantly higher and lower in glioblastoma than in normal brain tissues, respectively. On the other hand, the other genes did not have any remarkable differential expression pattern.

Conclusion: Based on the findings of the current study, it is determined that NRXN3 acts as a tumor suppressor gene and NRXN3 gene is downregulated in GBM. PBX3 gene functions as an oncogene and is upregulated in GBM.

Keywords: Glioblastoma, GBM, miRNA, ceRNA, T-UCR.

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Glioblastomada Potansiyel Moleküler Biyobelirteçler Olarak miRNA Aracılı ceRNA'ların *İn Siliko* Analizi

Öz

Amaç: Glioblastoma multiforme (GBM), yetişkinlerde santral sinir sistemi (SSS)'ndeki primer beyin tümörlerinin en sık görülen ve en öldürücü tipi olarak tanımlanmaktadır. Son yıllardaki çalışmalar, GBM'nin teşhisi ve tedavisi için yeni hedeflerin tanımlanmasına odaklanmış ve kanser patogenezindeki düzenleyici etkileri nedeniyle miRNA'lara büyük ilgi uyandırmıştır. Bu nedenle, bu çalışmada GBM için yeni moleküler biyobelirteçlerin hesaplamalı analizlerle tanımlanması amaçlanmıştır.

Yöntemler: Glioblastoma ile klinik olarak ilişkili olan ve deneysel olarak kanıtlanmış 118 miRNA, miRTarBase veri tabanından elde edildi. Elde edilen 118 miRNA tarafından hedeflenen 1016 gen ComiR veri tabanı aracılığıyla belirlendi. Akabinde, ekzonik bölgelerinde transkribe edilmiş ultra-korunmuş bölgelere (T-UCR) sahip genler belirlendi ve potansiyel olarak endojen rekabetçi RNA (ceRNA) aktivitelerine sahip olan genler ekstrakte edildi. T-UCR içeren glioblastoma ile ilişkili ceRNA'lar arasından glioblastoma ve normal beyin dokuları arasında önemli ekspresyon profili farklılıklarına sahip genler tanımlandı.

Bulgular: PBX3 ve NRXN3 genleri ile glioblastoma arasındaki korelasyonun istatistiksel analizi Spearman koralasyon testi ile gerçekleştirildi. Normal beyin dokularına göre glioblastomada PBX3 gen ekspresyonu daha yüksek iken NRXN3 gen ekspresyonu daha düşüktü. Diğer taraftan, diğer genler anlamlı farklılık gösteren ekspresyon paternine sahip değildi.

Sonuç: Mevcut çalışmanın bulgularına göre, NRXN3 geninin tümör baskılayıcı olarak işlev gördüğü ve GBM'de downregüle edildiği ve PBX3 geninin onkogen olarak görev aldığı ve GBM'de upregüle edildiği belirlendi.

Anahtar kelimeler: Glioblastoma, GBM, miRNA, ceRNA, T-UCR.

INTRODUCTION

Glioblastoma is classified as primary glioblastoma multiforme (GBM) which is seen in 80% of cases with the onset approximately at age 62, and secondary GBM which is derived from oligodendrogliomas or astrocytomas with the onset at age 45 on average[1]. GBM is defined as the most frequent and lethal form of the primary brain tumors in the central nervous system (CNS) in adults and classified as Grade IV by the World Health Organization[2]. Approximately 1/3 of primary brain tumors is glioblastoma multiforme. The diagnosis and treatment of GBM is challenging and treatment options have not altered over many years even its high frequency[3].

MicroRNAs (miRNAs) that are non-coding and short (18-22 nucleotides) RNA molecules are expressed in the cells of many organisms. miRNAs modulate gene and protein expression by degrading target mRNA or blocking translation. Thousands of miRNA genes have been designated in the genomes of many organisms such as plants, animals. Approximately 60% of the human genome and nearly every gene clusters are estimated to be regulated by miRNAs. MicroRNAs are key players of numerous biological functions and disruption of the function of miRNAs cause to many diseases such as cancer and neuropsychiatric diseases. Moreover, in recent years, miRNA regulation of physiology of cells, miRNA therapeutics, xenomiRs, and miRNA biomarkers have been receiving a great deal of attention by researchers[4,5].

Competing endogenous RNAs (ceRNAs) are transcripts that compete for microRNA binding, modulating each other's functions post-transcriptionally. miRNAs bind to microRNA response elements (MREs) in the 3'UTRs of target mRNA. ceRNAs consist of various RNA transcript types such as protein-encoding mRNAs, circRNAs, pseudogenes, and lncRNAs. It has been proposed that many RNAs may interact with each other via MREs. The

repressive action of miRNA is deactivated by "ceRNAs" or "miRNA sponges". The ceRNAs which have many MREs for a miRNA show multiple interactions and it is resulted with a complex regulatory network. Dysregulation of ceRNA network leads to various human diseases such as cancer. ceRNAs are key players of carcinogenesis and molecular pathways are affected by ceRNA interactions. Hence, underlying molecular mechanisms of cancer may be elucidated by the analysis of ceRNAs[6,7].

Ultra-conserved regions (UCRs) are non-coding DNA sequences and conserved among mice, rats, and human beings. UCRs were discovered in mice, rats, and human genomes by bioinformatics tools in 2004. More than 90% of ultra-conserved regions are transcribed (T-UCRs) in normal tissues and are modulated at the level of transcription in carcinogenesis. The expression levels of T-UCRs have tissue-specific pattern. Recent studies conducted with genome-wide expression profiling approach have demonstrated that T-UCRs show divergent profiles in various cancer types and support their roles in tumorigenesis[8].

One of the most aggressive cancers, glioblastoma is challenging for treatment. Recent studies have focused on the determination of the new targets for the diagnosis and treatment of GBM and resulted in great interest for miRNAs due to their regulatory effects in normal conditions and cancer pathogenesis. According to the properties of miRNAs, it is aimed to identify novel molecular biomarkers for GBM by in silico analysis in this study.

METHODS

miRNA selection

First of all, the miRNAs that are implicated in the pathogenesis of glioblastoma were selected. In this regard, one hundred and eighteen miRNAs that are clinically related with glioblastoma and proven by experimentally were exported through miRTarBase database. Extensive information about experimentally verified miRNA-target interactions was obtained from the miRTarBase database. Scientists can apply for the database in order to confirm novel targets of miRNA[9,10].

miRNA-mediated ceRNAs analysis

One thousand sixteen genes projected by these one hundred and eighteen miRNAs were determined using the ComiR database. The genes with ComiR score greater than 0.8685 were taken into account in this study. ComiR is an online web server for combinatorial miRNA target estimation and has a free access for academic users. ComiR estimates the potential of being targeted by a group of microRNAs for a mRNA in fly, mouse, worm or human genomes and each one may have zero, one or more targets on its 3'UTR. In identification the modulator potential of a mRNA from a cluster of miRNAs, ComiR uses the levels of miRNA expression which are provided by the users in a combination of relevant machine learning techniques and thermodynamic modeling in order to make more certain estimations. For each gene, the tool indicates the possibility of being functional target of a group of miRNAs according to the relative miRNA expression levels[10-12]. It is expected that RNA transcripts of the given genes have potential ceRNA activities for the microRNAs and this regulation may occur via miRNA-sponging mechanism[13].

Matching of ceRNAs with the genes containing T-UCR

Ultra-conserved regions (UCRs) in the human genome were determined by Bejerano et al.[14]. The genes involving these regions classified as downstream, upstream, and exonic based on the localization within the gene[14]. In this study, the genes with T-UCR in their exonic regions were designated and the genes which have potential ceRNA activities were extracted in the previous analysis.

Analysis of differential gene expression between glioblastoma and normal brain tissues

The genes with remarkable expression differences between brain tissue and glioblastoma multiforme from glioblastoma-associated ceRNAs involving T-UCR were designated via Gene Expression Profiling Interactive Analysis (GEPIA) database[10,12]. GEPIA that is user-friendly web tool delivers normal and cancer gene expression and interactive analysis data[15].

Analysis of the correlation between PBX3 and NRXN3 genes in glioblastoma

Analysis of differential gene expression ensures to find the tumor-specific genes by comparing normal and tumor groups[10,12]. The statistical analysis of the relationship between PBX3 and NRXN3 genes and glioblastoma was carried out by the use of Spearman correlation test.

RESULTS

In the current study, one hundred and eighteen miRNAs that are clinically associated with glioblastoma and proven by experimentally by the use of miRTarBase database are shown in Table I. One thousand sixteen genes that are simultaneously targeted by these 118 miRNAs were shown in supplementary I. The genes with ComiR score greater than 0.8685 were taken into account in this study. The genes which include T-UCR in exons based on the study of Bejerano et al.[14] was determined and afterwards, the ones which have potential ceRNA activities were extracted and are shown in Table II.

Supplementary I: The genes targeted by these 118 glioblastoma-associated miRNAs simultaneously.

Gene ID	ComiR equal abundance score
SCYL3	0.9157
LASP1	0.914
CFLAR	0.9162
SARM1	0.8693
FKBP4	0.9142
THSD7A	0.8691
KMT2E	0.8685
ZNF263	0.9143
MAP3K9	0.916
TTC22	0.9112
GAS7	0.9159
E2F2	0.9151
CDKL5	0.8693
ST3GAL1	0.8685
REV3L	0.9225
IDS	0.8685
ZNF200	0.9121
LRRC23	0.9112
HFE	0.8686
SLC7A14	0.8689
NUDCD3	0.8692
IGF1	0.8691
PRDM11	0.9222
NRXN3	0.8686
SLC45A4	0.9155
GRAMD1B	0.8689
NDUFS1	0.8695
KPNA6	0.8688
AGPAT4	0.8692
POU2F2	0.9223
SNX1	0.8694
IKZF2	0.9157
UBA6	0.869
GAB2	0.9137
DAPK2	0.869
ADAM28	0.9155
HDAC9	0.9155
SNX29	0.8691
RSF1	0.8691
ADAMTS6	0.9156
H6PD	0.8693
NEDD4L	0.8688
KIAA2022	0.8689
HEBP2	0.8695
MPHOSPH9	0.9133
SIKE1	0.8689
FOXN3	0.869
AP5M1	0.9226
EIF2AK2	0.8695
KMT2C	0.8697
ATP2B4	0.9146
RIOK2	0.9133
BCAT1	0.8693
MON2	0.9224
EPN1	0.9162
ZNF275	0.9153
HIPK2	0.8695
UHRF1BP1	0.8686
GNAI3	0.9162
WDR3	0.9224

PKN2	0.9145
SLK	0.9144
MTHFD2	0.9148
SLC9A7	0.8691
CD84	0.8694
ATXN3	0.9237
DNTTIP2	0.9149
RRP15	0.869
ROCK1	0.8689
PSME4	0.9155
NEDD4	0.8691
GNB5	0.9159
PTPN3	0.9152
EXOC5	0.9159
RAD18	0.8685
MGAT4A	0.8688
ZFYVE26	0.9223
RPS6KA6	0.8693
SMC1A	0.9234
CHFR	0.8694
TRHDE	0.9161
P4HA2	0.9114
IGF2BP2	0.9128
MGLL	0.9145
IPCEF1	0.869
ADD2	0.8693
RASAL2	0.9162
ZNF37A	0.8688
FNDC3B	0.9155
WDR62	0.8688
BCAP29	0.9151
SEC31B	0.9151
RBM7	0.8689
RBMS2	0.8694
PLXNA2	0.869
PAG1	0.8686
MBNL3	0.9162
PPP1R12B	0.8696

DNAJC10	0.9162
DCX	0.9159
ACER3	0.8687
PIK3C3	0.8685
N4BP2	0.8691
RUNX1T1	0.8687
RIF1	0.8694
RAB21	0.8696
CDH7	0.9161
MEF2C	0.9134
BZW1	0.9119
PGR	0.8695
FAM135A	0.9158
ERC1	0.8687
XPO1	0.869
LYRM2	0.9157
ZNF264	0.9162
SSH1	0.8696
MAP3K4	0.9134
PTPN4	0.8687
C20orf194	0.9145
MAVS	0.8696
NOS1	0.8689
ZBTB25	0.9161
ARHGAP4	0.9143
GPATCH2L	0.9162
PCBP4	0.9199
ZNF268	0.9224
PDPR	0.8686
TNRC6A	0.8695
DTX2	0.9096
RGS17	0.8685
SNAP23	0.9116
AGO1	0.9162
GPATCH2	0.9156
ECHDC1	0.9118
CBX5	0.9226
FKBP5	0.8692

CDC5L	0.9142
CDC34	0.9106
MTAP	0.9157
CECR2	0.915
DDTL	0.9106
MAPK1	0.8695
ADRBK2	0.8692
TFIP11	0.91
RBFOX2	0.8688
MTMR3	0.8687
MIEF1	0.9142
KIAA0930	0.9151
DDHD1	0.9162
VTI1B	0.8685
SPTLC2	0.8688
GALNT16	0.9152
DICER1	0.9155
ZC3H14	0.9162
RPS6KA5	0.9237
KIAA0391	0.9219
SLC52A3	0.9124
ST8SIA5	0.8695
CEP192	0.8693
RNMT	0.9154
LIPG	0.8695
ANKRD12	0.915
MIB1	0.915
PGRMC1	0.9103
ALG13	0.9151
PORCN	0.9126
KLF8	0.9149
FGF14	0.9161
FNDC3A	0.915
STK24	0.9235
KATNAL1	0.9152
INTS6	0.8696
NFAT5	0.8696
LONP2	0.8687

CCDC113	0.9147
SLC7A6	0.9157
ESRP2	0.9134
MLYCD	0.8694
GSPT1	0.8689
GGA2	0.9152
XYLT1	0.8694
HOMER2	0.916
EHD4	0.8687
ATP8B4	0.8687
DTWD1	0.8696
SLC30A4	0.8686
MYEF2	0.8689
FZD3	0.9226
UBE2W	0.9161
TUBB4A	0.9126
AKAP8	0.9139
AVL9	0.8687
CDK6	0.8694
ITGB8	0.869
TTC26	0.914
TFEC	0.8685
HOXA1	0.9116
PLEKHA8	0.9158
AP1S1	0.9112
C1GALT1	0.8688
TMEM106B	0.8696
FKTN	0.9224
TGFBR1	0.915
AKNA	0.9146
KCNT1	0.9222
RGP1	0.8686
ABCA2	0.8685
CCNJ	0.9133
PLEKHA1	0.8696
BMPR1A	0.916
CPEB3	0.9158
FBXL20	0.8695
INTS2	0.9114
LUC7L3	0.8688
GABRA4	0.8695
CLNK	0.9142
CTSC	0.869
DTX4	0.9148
CCND1	0.9122
CBL	0.9161
CARS	0.913
SOX6	0.8691
CAPRIN2	0.9159
DUSP16	0.9143
C12orf49	0.8694
TBC1D30	0.9156
CNOT2	0.9129
KRR1	0.869
ST8SIA1	0.869
FRK	0.8696
SOD2	0.8692
RNF8	0.8687
ZNF451	0.8694
ASCC3	0.9139
KIAA1244	0.8694
SLC16A10	0.9225
IMPG1	0.869
GHR	0.9153
COL4A3BP	0.9149
PRLR	0.9225
SKP1	0.9161
CPEB4	0.9146
KPNA1	0.9156
UBE3A	0.9221
XRN1	0.9154
BBX	0.9159
KIAA1257	0.8685
HEMK1	0.9241
ACVR2B	0.9161
ABCC5	0.9144
KLHL24	0.9152
INO80D	0.8696
TTL	0.9161
TFCP2L1	0.8693
DNAJC27	0.8685
APC2	0.9142
TTC31	0.9139
PAPOLG	0.8685
ELMOD3	0.9131
GGCX	0.8692
ZNF142	0.8691
HDLBP	0.9124
PLCL1	0.8692
KYNU	0.8696
AAK1	0.9162
ARID3A	0.9209
PLEKHA3	0.9237
TNR	0.8694
GPX7	0.9113
KCNC4	0.8697
MEF2D	0.9156
C1orf21	0.9162
TROVE2	0.9155
MTR	0.8691
RIMS3	0.869
AKT3	0.8688
CTBS	0.8688
TMED5	0.8686
DR1	0.8695
PTBP2	0.8696
DIEXF	0.8693
SLC5A9	0.9113
SGIP1	0.8688
ADGB	0.916
MED28	0.9162
SLC16A7	0.8696
DCLRE1B	0.9138
CCND2	0.9222

CYP20A1	0.8695
TRPM6	0.9135
TRIM67	0.9157
FBXW2	0.9161
RBM18	0.8686
ONECUT2	0.9241
YLPM1	0.9152
NEK9	0.9154
DNAL1	0.8693
NRDE2	0.8695
ZNF410	0.9126
YIPF4	0.8695
FAM178A	0.9146
HELLS	0.9155
MOB3B	0.869
B4GALT4	0.8686
ACVR2A	0.9152
ODF2L	0.8685
ZNF644	0.9106
SEPT7	0.8689
CHST3	0.915
SLC25A16	0.8686
SPRYD7	0.9117
NLN	0.9219
ATPAF1	0.9145
ACVR1C	0.8687
LPGAT1	0.9224
PARD6B	0.9151
RAB22A	0.8691
BCAS4	0.8689
STAMBP	0.8687
HIF3A	0.9155
NQO2	0.916
ATXN1	0.8694
SH3TC1	0.9219
ATP5S	0.9225
GGA3	0.9154
GTF3C4	0.8688
POLR1B	0.9153
THOC2	0.9141
MED1	0.8686
GPCPD1	0.915
TMX4	0.9152
AP5S1	0.9155
MKKS	0.9155
RALY	0.9223
CEP250	0.916
AMOT	0.9155
AGO3	0.9226
THRA	0.9143
PCNXL4	0.9162
MASP1	0.915
HELB	0.9161
RAP1B	0.8696
RAB3IP	0.8691
PTPRB	0.869
DYRK2	0.9158
ZNF835	0.9126
HIP1	0.8688
FOXP2	0.9224
MKLN1	0.9161
TMOD2	0.8693
ICE2	0.9158
ARPP19	0.869
CALML4	0.9137
KCNC1	0.8689
PRRG3	0.8685
ATP8B3	0.9158
DDA1	0.9155
TULP4	0.8689
PXDN	0.9132
PGPEP1	0.9156
ZNF557	0.8687
ZNF341	0.9215
NFATC1	0.9121
RAB11FIP4	0.9154
LRRC41	0.9107
ENOSF1	0.9147
GRSF1	0.8688
PCBD2	0.869
SCO1	0.8693
STARD13	0.9137
LARGE	0.8687
MYO18B	0.8687
FAM83F	0.8697
MBD2	0.9218
WNT2B	0.916
MYCN	0.9119
CRB1	0.9223
KLRD1	0.9237
AGO4	0.9153
BTF3L4	0.8685
DAGLA	0.9156
FADS2	0.9131
CLOCK	0.9159
DZIP1	0.8685
MTO1	0.9161
ZC3H10	0.8693
CD164	0.914
REPS1	0.8687
USP15	0.8696
CPM	0.8689
KIAA0513	0.8693
SLC9A5	0.9149
RC3H1	0.8692
TTLL4	0.9121
ALDH1L2	0.9158
USP44	0.9192
SLC41A2	0.9146
ALPK3	0.8687
LIMD2	0.9224
KAT7	0.8695
SKIL	0.922
UGGT1	0.9157

ARHGEF39	0.9131
YIPF3	0.9113
SLC22A23	0.8688
TAF8	0.9159
CPEB2	0.9205
RAB30	0.8694
SLCO5A1	0.9155
TMPRSS4	0.8686
UNC13C	0.9159
CTDSPL2	0.9151
THBS1	0.8687
ITGA11	0.9223
ADAM10	0.8695
TTLL7	0.869
IFI44L	0.9152
ADAMTS14	0.9123
SSFA2	0.9157
ABI2	0.8697
PARP9	0.9151
CNOT6L	0.8689
KIAA1644	0.922
NDUFA9	0.8686
TARBP2	0.9126
ACVRL1	0.913
ANKRD52	0.9234
ZNF740	0.9158
WDFY2	0.8691
NOVA1	0.9159
SYT16	0.8696
SLC38A6	0.9145
NAA30	0.9234
RAB15	0.9215
TSPAN3	0.9159
IGF1R	0.9224
ABHD2	0.8693
NTRK3	0.9226
DET1	0.9138
ZNF710	0.9147

FTO	0.9161
NKD1	0.8691
GFOD2	0.9156
PCTP	0.9129
GNAL	0.8685
C18orf21	0.9084
GALNT1	0.9129
GAREM	0.8685
TP53	0.913
TBCD	0.9108
TRIM65	0.9114
RNF165	0.9161
WTIP	0.8695
POU2F1	0.9237
ABL2	0.916
RGS16	0.9116
LHX9	0.9155
SNX27	0.8693
GABPB2	0.916
SYT14	0.8691
ACP1	0.9116
PLEKHA6	0.9156
PTPN7	0.9114
SYT2	0.8691
TEX261	0.9145
ZC3H8	0.9158
KIAA1715	0.8692
GULP1	0.869
SPAG16	0.922
LIMD1	0.9161
ZNF660	0.9158
MUC4	0.9146
TBCK	0.869
SPATA5	0.8689
METTL14	0.9157
USP53	0.9148
UGT3A1	0.9154
SSBP2	0.869

PPIP5K2	0.8696
BDP1	0.9145
TNFAIP8	0.9159
ATG12	0.9151
ARHGAP26	0.8693
PCYOX1L	0.9138
G3BP1	0.869
GFOD1	0.8691
IRAK1BP1	0.8687
MMS22L	0.9155
FAXC	0.8694
CLVS2	0.8695
RNF217	0.9225
SHPRH	0.8695
PURB	0.8691
CASK	0.8691
KDM6A	0.9143
DIAPH2	0.916
FAM135B	0.9154
VLDLR	0.916
UGCG	0.9136
SNX30	0.9221
NR6A1	0.9237
A1CF	0.9225
EIF4EBP2	0.8688
CNNM2	0.8696
INTS4	0.9135
SOGA1	0.8695
PCDH15	0.8689
CDH8	0.9154
LPHN3	0.916
PDCD4	0.913
CD226	0.8696
FREM2	0.8691
DCP1B	0.9121
THRB	0.8689
GXYLT1	0.9158
AKAP6	0.9161

THRSP	0.9104
ADAMTS12	0.9151
C4orf33	0.8689
WWC2	0.8686
GABRA2	0.8688
GFRA1	0.8695
CACUL1	0.8694
RABGAP1L	0.8687
PTPN14	0.8694
EPG5	0.8689
ATP5A1	0.8686
GUCY1A2	0.9226
ZNF773	0.8692
FARP1	0.9157
ZNF117	0.8685
SREK1IP1	0.9223
SMARCA5	0.8685
RANBP2	0.9117
ASAP1	0.9222
PTPRD	0.9149
CNKSR3	0.9237
SREK1	0.8686
HS2ST1	0.869
MSI2	0.8689
CHST9	0.9162
OTULIN	0.8685
LRRK1	0.9162
ENAH	0.9161
GPR26	0.8691
ADAMTS5	0.8688
PIEZO2	0.8687
APOOL	0.869
ATP6V1C1	0.9151
PDZD9	0.9115
PPARGC1B	0.9224
LSM11	0.8689
AFF2	0.8693
PSD3	0.8694

MMP16	0.8693
BACH1	0.9127
ANKRD9	0.8686
UQCRB	0.8688
AIFM1	0.9129
FBXO32	0.8689
B3GNT7	0.9134
ATP2B2	0.8685
STEAP2	0.8689
HYDIN	0.9151
MYO1E	0.8687
KCNJ6	0.8696
TSPAN18	0.9152
DGKI	0.8696
UBN2	0.8695
BRAF	0.8693
AP3S2	0.8688
WIPI2	0.9142
PAFAH2	0.914
XKR8	0.9115
EYA3	0.8687
CLSTN2	0.8696
PPP1R15B	0.9142
AGPAT6	0.9151
ELK4	0.9161
TNNI1	0.869
IGF2BP1	0.9235
SCUBE1	0.8692
STARD9	0.9204
ACE	0.9124
BSDC1	0.9125
ZBTB8A	0.869
ZNF362	0.9125
TRAPPC10	0.8685
ICOSLG	0.9152
TAOK1	0.8691
MFSD12	0.9099
PLXDC1	0.8686

IKZF3	0.9161
ACOX1	0.8688
TMEM143	0.9099
FMNL3	0.9162
TREML1	0.9102
CCNF	0.9226
PRKAA2	0.8693
CTRC	0.9147
RBBP4	0.916
UBXN10	0.8686
NFIA	0.8693
ZNF326	0.8693
SLC30A7	0.8689
VANGL2	0.914
ACP6	0.9234
WDR26	0.8686
REL	0.9162
DISC1	0.8688
FAM84A	0.869
DUSP19	0.8685
SMARCAD1	0.9141
EOGT	0.9132
EIF4E3	0.8694
LRRC58	0.8692
CCDC141	0.8686
ICA1L	0.8692
RYBP	0.9155
RPP14	0.8692
RBM47	0.9146
APBB2	0.9158
TTC14	0.9158
SENP2	0.9151
IFT122	0.9153
SFMBT1	0.9111
CDC25A	0.9184
INTU	0.8696
RNF123	0.9139
MFSD8	0.9144

WDR41	0.9142
GPX8	0.9147
RICTOR	0.8692
DCBLD1	0.9118
KIF6	0.8685
USP49	0.8692
DLC1	0.913
ADCY1	0.8694
TP53INP1	0.8685
KIAA1958	0.9225
STRBP	0.9147
HDX	0.9151
BRWD3	0.8685
SLITRK5	0.9226
CFL2	0.8691
SUGT1	0.9162
PGM2L1	0.9154
SLC16A9	0.9141
AMER2	0.8695
PDZD8	0.8692
FAM204A	0.8696
CLEC1B	0.9142
FUNDC2	0.8689
AGBL2	0.919
CPSF2	0.9162
ARL5B	0.8689
ADAMTS15	0.9135
HIF1AN	0.9226
SPINT1	0.9096
ARIH1	0.9237
SYNPO2L	0.9135
TRIM44	0.8696
TPP1	0.9134
TRIM66	0.8692
PRTG	0.9225
PKD1L2	0.9138
NA	0.9224
TMED3	0.8696

GALR1	0.9161
TVP23A	0.9143
SLFN5	0.8689
GREM1	0.8696
SGSM1	0.9145
PBX3	0.9124
FBXO22	0.9162
IRGQ	0.916
ZNF226	0.9155
ANKRD11	0.8691
ZNF641	0.9223
TTYH1	0.9213
MAPK1IP1L	0.9159
POLR3D	0.9216
FAM84B	0.9151
TET2	0.9152
ANKRD49	0.914
IRS1	0.8688
MECP2	0.8692
RAB3B	0.8696
SH3TC2	0.8694
SHE	0.8688
PTAFR	0.9212
HIC2	0.923
TOR1AIP2	0.8691
MAP3K2	0.869
TMEM154	0.8695
GPR37L1	0.8689
TMEM192	0.9225
NIPA1	0.9152
RNF150	0.9161
USP38	0.9233
CRTAP	0.8687
KRT78	0.9121
LONRF2	0.8692
SERPINB9	0.9147
NUDCD2	0.9224
SGCD	0.8692

ATF7	0.9155
TMEM126B	0.911
NETO2	0.8688
CLCN5	0.9239
KCND3	0.8687
ZNF562	0.8695
GATM	0.9106
SYNPO	0.9148
ZNF556	0.9156
NEGR1	0.8696
DPAGT1	0.9135
ALG14	0.9161
ARNT2	0.9155
FUT9	0.8695
ZNF24	0.869
PDP2	0.8689
FAM222B	0.9144
BNC2	0.8694
PARP14	0.9151
TNKS	0.8688
STOX2	0.8686
SMARCC1	0.9135
ZNF417	0.9126
PEAK1	0.9162
NABP1	0.916
XCR1	0.9155
RNF213	0.8689
PHC3	0.9161
CBX2	0.9148
SWSAP1	0.9097
CD34	0.8687
CYB561D1	0.8686
MGA	0.9156
ATP2A2	0.9155
CNTNAP2	0.8685
IGDCC3	0.9216
MYO1H	0.9103
SLCO2A1	0.9126

TMEM167A	0.8687
SH3PXD2B	0.8687
C4orf32	0.8692
FZD4	0.8685
PDE12	0.916
CA5A	0.916
VCPIP1	0.8691
YPEL2	0.915
CADM2	0.869
SMAD2	0.9162
EIF3F	0.9155
ALG10B	0.8695
RPS6KB2	0.9185
MLXIP	0.9157
SLC35E3	0.8696
ZDHHC21	0.8694
JAKMIP2	0.8689
SPRYD4	0.9162
RNF152	0.916
ZNF843	0.9137
MTX3	0.9153
SLC38A9	0.9127
POLE	0.8693
SCN4B	0.9146
RIMKLA	0.8691
RPS6KA3	0.9153
HIC1	0.915
PAWR	0.9159
MIEF2	0.9133
SAMD12	0.916
IL17RA	0.8687
ARL6IP6	0.9131
AMER3	0.9146
NT5DC1	0.8689
CSRNP3	0.8693
PXT1	0.9124
CLK3	0.8696
ARID3B	0.9221

C14orf28	0.9215
ZNF154	0.8686
SOCS4	0.9221
FGD6	0.869
PLD5	0.9159
ZNF609	0.869
TSPYL5	0.9146
YOD1	0.9146
GPR157	0.9144
LRRC57	0.916
AEN	0.9132
NME9	0.9126
ZNF678	0.922
RFX7	0.869
RNF41	0.8688
RTKN2	0.8685
MGAT4C	0.8697
CREB3L2	0.9159
RGMA	0.8695
HHIPL1	0.9156
FIGN	0.9237
PLCXD1	0.9155
MXRA7	0.9158
PAPPA	0.9223
C16orf72	0.9224
PLCXD3	0.9156
CEP63	0.9151
GJC1	0.9157
CALN1	0.8694
POTEC	0.9218
ZNF623	0.8688
MACC1	0.8686
KREMEN1	0.9154
KCTD16	0.8695
B3GALT5	0.9162
TMPRSS2	0.9123
FAM120C	0.8688
GOLGA6L4	0.9143

PCDH9	0.9162
SDR42E1	0.9225
FLRT2	0.9162
FAM43A	0.9107
PURA	0.8695
ZBTB37	0.9237
TNFAIP8L1	0.9139
RAD51D	0.8695
IFNLR1	0.9145
BRCC3	0.9122
LSAMP	0.8693
LMLN	0.9158
PBX1	0.9158
C16orf52	0.9146
YTHDF3	0.8686
PIGP	0.8693
IKZF1	0.8687
PTCH1	0.9161
CYP2R1	0.9128
MARC1	0.8688
ZNF555	0.8686
KPNA4	0.9225
FSD2	0.8686
PPARA	0.9161
NAP1L1	0.9226
SESTD1	0.8691
TET3	0.9221
LIN28B	0.9235
TMEM256-PLSCR3	0.9117
FAM122A	0.9151
SHISA7	0.8688
ZC3H6	0.9161
NCR3LG1	0.869
ZNF793	0.8685
ZNF383	0.8689
CENPP	0.8687
RALGAPA2	0.9144

ASAH2	0.9151
PTAR1	0.9224
PARVB	0.8688
VWC2	0.8694
SNTN	0.9217
BEND4	0.916
NA	0.9137
PTPLAD2	0.9225
KCTD21	0.9122
NDUFA4	0.9197
FAM179A	0.9159
PTPRT	0.9158
PLEKHG4	0.8685
RYR1	0.8686
SRGAP3	0.9157
LCOR	0.8691
FUT4	0.8685
ZNF774	0.8692
ZNF765	0.9225
TSC22D2	0.869
ZNF605	0.8693
IPO4	0.9148
GDAP2	0.9224
TPK1	0.9138
MAN2A2	0.9145
HDAC2	0.869
SLC22A25	0.9138
WNK3	0.8687
ZKSCAN5	0.8685
TECPR2	0.9152
ZNF512B	0.9213
ZNF431	0.9225
NF1	0.8687
COL27A1	0.9144
POTEI	0.9142
NHLRC2	0.9162
FLNA	0.8692
SRGAP1	0.9226

LRRC8B	0.8688
NOL4L	0.8686
C6orf141	0.9121
DDI2	0.8689
TRIM33	0.8685
LRP10	0.9151
CDC42SE1	0.9144
EME2	0.915
ZNF81	0.8687
ERO1L	0.9154
PLCG2	0.9154
FCHSD1	0.9198
ZNF121	0.869
MBP	0.8694
MRPL42	0.8696
ZNF248	0.9149
CACNA1E	0.8695
HELZ	0.9161
ZKSCAN8	0.8691
ASPH	0.8691
ZNF26	0.8696
NRARP	0.9106
ZNF587	0.9158
MDM4	0.9162
IPO9	0.9158
SLC5A3	0.8693
CNOT7	0.8691
LRIG2	0.8695
MAP3K3	0.9135
ATG9A	0.9146
EFCAB2	0.8689
CHIC1	0.9155
PHACTR4	0.9148
PBX2	0.9108
FAM155A	0.8692
PSORS1C2	0.9099
FBXO48	0.8689
PCDHA4	0.9224

TRIM13	0.9158
SLC35B4	0.915
ZBTB10	0.9158
TMEM170B	0.8689
GPR56	0.9148
C15orf59	0.9153
C5orf51	0.9156
ONECUT3	0.9158
NYNRIN	0.9194
ATP10A	0.8686
PBX2	0.9108
PSORS1C2	0.9105
VGLL3	0.8695
TRIM71	0.9239
METTL6	0.9151
XKR4	0.9162
PRR22	0.9135
C17orf51	0.869
FGFR1OP	0.8696
GIMAP1	0.9148
NRAS	0.915
SYNJ2BP	0.916
LEPROT	0.8687
RPS29	0.8691
ZNF891	0.9226
VSTM5	0.9139
PEX26	0.9237
SIAH3	0.9158
CCDC7	0.9141
PLXNA4	0.916
APOL6	0.8693
PBX2	0.9108
PBX2	0.9108
PSORS1C2	0.9105
PBX2	0.9108
PSORS1C2	0.9105
KIAA0040	0.8685
PBX2	0.9108

ARHGEF38	0.9146
TMEM189	0.8693
ARHGAP8	0.9207
AMACR	0.913
PEG10	0.9158
NA	0.9113
MARS2	0.9106
PRR5-ARHGAP8	0.9092
FMN1	0.8694
DNAH10OS	0.8686
PCDHA10	0.8693
ATXN7L3B	0.9224
NA	0.8685
SOGA3 KIAA0408	0.9224
NOX5	0.8689

ZNF432	0.9142
CUX1	0.8695
P2RX5-TAX1BP3	0.8686
ITGB3	0.9139
NA	0.9149
RBM15B	0.8685
XKR7	0.8688
TMEM178B	0.9225
GAN	0.924
NA	0.8687
NA	0.9139
C19orf84	0.9112
RNF115	0.8695
ZNF850	0.9156
NA	0.9201

TRABD2B	0.8689
SLC25A53	0.8687
NUDT3	0.8694
GRIN2B	0.9226
ZBTB8B	0.9225
SOCS7	0.8689
GOLGA6L9	0.9147
ZNF280B	0.9147
DDTL	0.9107
TTYH1	0.9135
TTYH1	0.9135
NA	0.9141
RBFOX2	0.8688
ZNF8	0.9161

Table I: miRNAs implicated in the pathogenesis of glioblastoma.

hsa-let-7a-1	hsa-mir-137	hsa-mir-181b-2	hsa-mir-21	hsa-mir-30c-1	
hsa-let-7a-2	hsa-mir-139	hsa-mir-181c	hsa-mir-210	hsa-mir-30c-2	hsa-mir-455
hsa-let-7a-3	hsa-mir-142	hsa-mir-181d	hsa-mir-218-1	hsa-mir-31	hsa-mir-486
hsa-let-7d	hsa-mir-143	hsa-mir-183	hsa-mir-218-2	hsa-mir-3163	hsa-mir-491
hsa-mir-101-1	hsa-mir-145	hsa-mir-184	hsa-mir-22	hsa-mir-32	hsa-mir-504
hsa-mir-101-2	hsa-mir-146a	hsa-mir-18a	hsa-mir-221	hsa-mir-323a	hsa-mir-539
hsa-mir-106a	hsa-mir-146b	hsa-mir-193a	hsa-mir-222	hsa-mir-323b	hsa-mir-7-1
hsa-mir-10a	hsa-mir-148a	hsa-mir-195	hsa-mir-224	hsa-mir-326	hsa-mir-7-2
hsa-mir-10b	hsa-mir-149	hsa-mir-196b	hsa-mir-23b	hsa-mir-328	hsa-mir-7-3
hsa-mir-124-1	hsa-mir-151a	hsa-mir-19a	hsa-mir-25	hsa-mir-329-1	hsa-mir-708
hsa-mir-124-2	hsa-mir-153-1	hsa-mir-19b-1	hsa-mir-26a-1	hsa-mir-329-2	hsa-mir-873
hsa-mir-124-3	hsa-mir-153-2	hsa-mir-19b-2	hsa-mir-26a-2	hsa-mir-342	hsa-mir-885
hsa-mir-125b-1	hsa-mir-155	hsa-mir-200	hsa-mir-27b	hsa-mir-34a	hsa-mir-9-1
hsa-mir-125b-2	hsa-mir-15a	hsa-mir-200b	hsa-mir-29a	hsa-mir-367	hsa-mir-9-2
hsa-mir-1260a	hsa-mir-16-1	hsa-mir-205	hsa-mir-29c	hsa-mir-376a-1	hsa-mir-9-3
hsa-mir-128-1	hsa-mir-16-2	hsa-mir-206	hsa-mir-302a	hsa-mir-376a-2	hsa-mir-92a-1
hsa-mir-128-2	hsa-mir-17	hsa-mir-208a	hsa-mir-302b	hsa-mir-381	hsa-mir-92a-2
hsa-mir-1305	hsa-mir-181a-1	hsa-mir-208b	hsa-mir-302c	hsa-mir-425	hsa-mir-95
hsa-mir-130a	hsa-mir-181a-2	hsa-mir-20a	hsa-mir-302d	hsa-mir-451a	hsa-mir-99a
hsa-mir-134	hsa-mir-181b-1	hsa-mir-20b	hsa-mir-30a	hsa-mir-452	

The genes with remarkable expression profile differences between glioblastoma and normal brain tissues among glioblastoma-associated ceRNAs involving T-UCR were defined. Expression of PBX3 gene was significantly higher and NRXN3 gene expression was remarkably lower in glioblastoma than in normal brain tissues according to the current analysis. On the other hand, the other genes did not show any remarkable expression differences (Table III).

Table II: The glioblastoma-associated ceRNAs that match the genes containing T-UCR in the exonic regions.

uc.378	251	***NRXN3***
uc.184	230	***CPEB4***
uc.33	312	***PTBP2***
uc.414	246	***THRA***
uc.280	220	***PBX3***
uc.393	275	***CLK3***

Table III: Expression values of ceRNAs with T-UCR that are associated with glioblastoma in normal brain tissues and glioblastoma.

*NRXN3**	**1.84**	**18.41**
CPEB4	14.04	11.54
PTBP2	12.1	11.96
THRA	99.62	144.88
*PBX3**	**19.54**	**3.29**
CLK3	32.92	29.86

**shows remarkably differential expression profile between normal brain tissues and glioblastoma*

The statistical analysis of the relationship between PBX3 and NRXN3 genes and glioblastoma multiforme was carried out via GEPIA database. It was determined that PBX3 and NRXN3 genes were significantly correlated with glioblastoma based on the Spearman correlation analysis (Figure 1) (p=0.0014; R=-0.17).

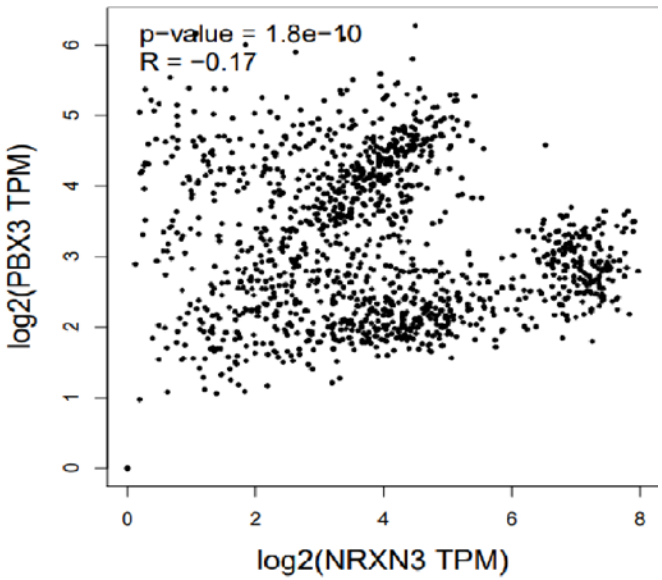

Figure 1: The relationship of NRXN3 and PBX3 genes with glioblastoma.

DISCUSSION

Glioblastoma which is the most frequent and aggressive form of primary malignancies in adult human brains is characterized by tumor heterogeneity, diffuse invasion, drug resistance, and rapid growth. It has been clarified that miRNAs are implicated in tumorigenesis. Moreover, it has been observed that expression levels of miRNAs are differed between pathological and normal tissues. Recent studies have subclassified glioblastoma into five clinically and genetically distinct subtypes according to miRNA expression profiles and it has been supposed that miRNAs are important for the phenotypic characteristics of the subclasses[16,17]. The median survival time of patients with GBM is approximately 14 to 16 months despite standard treatment options and there is no cure at present. In recent years,

studies in this field have been focused on the identification of new targets for diagnostics and therapeutics for GBM. It is supposed that detection and quantifying miRNAs in serum and tissues will become a standard tool for diagnosis and prognosis of GBM and have a great potential for personalized treatment strategies[18]. In this regard, based on the idea that miRNAs are implicated in the pathogenesis of glioblastoma, we aimed to determine novel molecular biomarkers for GBM through *in silico* analysis that uses glioblastoma-specific microRNAs, identifies their combinatorial target genes which have potential ceRNA activities. In this study, 118 microRNAs correlated with glioblastoma were obtained from miRTarBase database (Table I). The genes with ComiR score greater than 0.8685 were listed through 1016 genes that are simultaneously targeted by these 118 miRNAs. The genes with T-UCR in their exonic regions were selected based on the study of Bejerano et al.[14]. Subsequently, the genes which show potential ceRNA activities were extracted (Table II). Then, the genes with remarkable expression diffrences between GBM and normal brain tissues were extracted from glioblastoma-associated ceRNAs that include T-UCR. While PBX3 gene was highly expressed in GBM than in normal brain tissues, NRXN3 gene was significantly less expressed in GBM than in normal brain tissues according to the analysis in this study. On the other hand, other genes did not show any significant differences in expression pattern. According to the findings of the Spearman correlation analysis, PBX3 and NRXN3 genes were shown to have remarkable relationship with GBM.

PBX3 is a member of Pre-B-cell leukemia homeobox family and implicated in early development and several biological processes in adulthood. The location of PBX3 gene is on chromosome 9q33.3. PBX3 as a transcription factor shows a stable interaction with DNA and binds to DNA with a consensus sequence (TGATTGATTTGAT). It has been demonstrated that PBX3 is commonly associated with cancer and overexpressed in several types of cancers such as hematological malignancies and colorectal cancers. Moreover, PBX3 activates numerous signaling pathways such as MAPK/ERK signaling pathway. PBX3 functions as an oncogene and is implicated in the regulation of biological functions such as stimulating proliferation, colony formation, cell survival, and invasion[19,20]. It has been demonstrated that PBX3 is upregulated in gastric cancer cells and apoptosis is induced by targeting PBX3 gene in gastric cancer[20]. In a study conducted with glioma cell lines, it has been shown that PBX3 was overexpressed[21]. Xu et al. reported that PBX3 was significantly associated with invasion of GBM cells and mesenchymal transition[22].

Neurexins (NRXNs) are a family of neuronal-specific cell surface proteins and they are implicated in cell recognition and adhesion. Moreover, the presynaptic terminal proteins are involved in synaptogenesis, neurotransmitter release and synaptic transmission and are also essential for the development and function of synapses. NRXN genes are differentially spliced into numerous isoforms[23,24]. It is known that FoxQ1 as a potential oncogene may induce tumor cell proliferation and migration by targeting NRXN3 gene in a direct way[25]. It has been reported that FoxQ1 stimulated cell proliferation and migration of glioma by suppressing NRXN3 gene and suggested that NRXN3 gene might be a tumor suppressor[24]. In the study conducted with breast cancer patients, G allele carriers in rs10146997 of NRXN3 gene was statistically related to the development of breast cancer[26]. It has been reported that NRXN3 gene expression was downregulated in the samples of GBM[27].

NRXN3 and PBX3 genes were associated with GBM in this present study and they were

suggested to have potential roles in carcinogenesis. It has been supposed that NRXN3 acts as a tumor suppressor gene and its expression is decreased in GBM according to the analysis in this study. On the other hand, PBX3 gene is suggested to function as an oncogene and is upregulated in GBM according to the in silico analysis.

CONCLUSION

The present study investigated the correlation of NRXN3 and PBX3 genes with GBM and this study supports the potential roles for the genes in the pathogenesis of glioblastoma. Additionally, further *in vivo* and *in vitro* studies are needed in order to elucidate tumor suppressor role of NRXN3 and oncogenic activity of PBX3 in GBM.

Ethics Committee Approval: This study did not require any ethical approval.

Declaration of Conflicting Interests: The authors declare that they have no conflict of interest.

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