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).

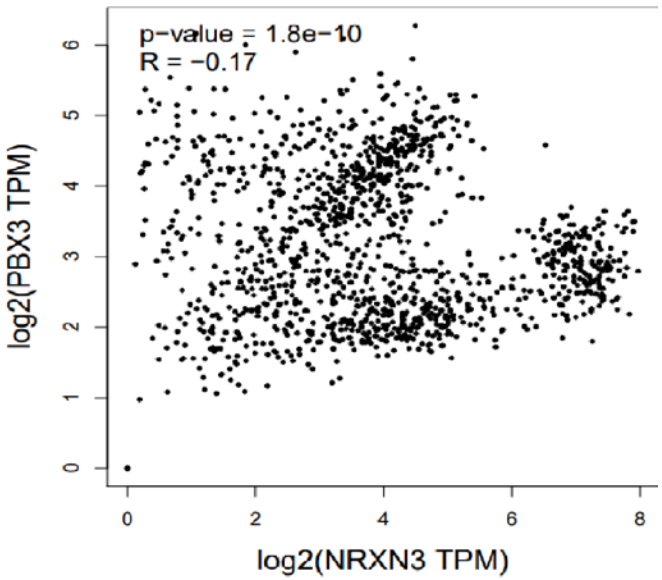

Figure 1: The relationship of NRXN3 and PBX3 genes with glioblastoma.

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.

Financial Disclosure: No financial support was received.

REFERENCES

1. Yool AJ, Ramesh S. Molecular targets for combined therapeutic strategies to limit glioblastoma cell migration and invasion. Front Pharmacol. 2020; 11: 358.

2. Haar CP, Hebbar P, Wallace GC, et al. Drug resistance in glioblastoma: A mini review. Neurochem Res. 2012; 37: 1192-200.

3. Yılmaz A, Altug F, Duz E, et al. Treatment options and effects of survival in glioblastoma multiforme. J Kartal TR. 2012; 23: 25-9.

4. Laffont B, Rayner KJ. MicroRNAs in the pathobiology of atherosclerosis. CJC. 2017; 33: 313-24.

invasion program in glioblastoma. J Exp Clin Cancer Res. 2019; 38.

5. Witwer KW, Halushka MK. Toward the promise of microRNAs- Enhancing reproducibility and rigor in microRNA research. RNA Biol. 2016; 13: 1103-16.

6. Sherin K, Nair AS. Review of computational prediction of competing endogenous RNA. J Proteom Bioinform. 2019; 12.

7. Qi M, Yu B, Yu H, et al. Integrated analysis of a ceRNA network reveals potential prognostic lncRNAs in gastric cancer. Cancer Med. 2020; 9: 1798-817.

8. Fassan M, Dall'Olmo L, Galasso M, et al. Transcribed ultraconserved noncoding RNAs (T-UCRs) are involved in Barret's esophagus carcinogenesis. Oncotarget. 2014; 5: 7162-71.

9. Huang HY, Lin YCD, Li J, et al. miRTarBase 2020: updates to the experimentally validated microRNA-target interaction database. Nucleic Acids Res. 2020; 48: D148-D154.

10. Ergun S. In silico analysis of biomarker potentials of miRNA-mediated ceRNAs in prostate cancer. Dicle Med J. 2018; 45: 415-29.

11. Coronnello C, Benos PV. ComiR: combinatorial microRNA target prediction tool. Nucleic Acids Res. 2013; 41: W159-W164.

12. Us Altay D, Ergun S. In silico analysis of biomarker potentials of miRNA mediated ceRNAs in gastric neoplasms. MBSJHS. 2019; 5: 106-19.

13. Avsar O. Analysis of miRNA-mediated ceRNAs in the pathogenesis of renal cell carcinoma: An in silico approach. HJSE. 2020; 7: 223-38.

14. Bejerano G, Pheasant M, Makunin I, et al. Ultraconserved elements in the human genome. Science. 2004; 304:1321-5.

15. Tang Z, Li C, Kang B, et al. GEPIA: a web server for cancer and normal gene expression profiling and interactive analysis. Nucleic Acids Res. 2017; 45: W98-W102.

16. Zhao Y, Huang W, Kim TM, et al. MicroRNA-29a activates a multi-component growth and

17. Touat M, Idbaih A, Sanson M, et al. Glioblastoma targeted therapy: updated approaches from recent biological insights. Ann Oncol. 2017; 28: 1457-72.

18. Shea A, Harish V, Afzal Z, et al. MicroRNAs in glioblastoma multiforme pathogenesis and therapeutics. Cancer Med. 2016; 5: 1917-46.

19. Morgan R, Pandha HS. PBX3 in cancer. Cancers. 2020; 12.

20. Li YS, Zou Y, Dai DQ. MicroRNA-320a suppresses tumor progression by targeting PBX3 in gastric cancer and is downregulated by DNA methylation. World J Gastrointest Oncol. 2019; 11: 842-56.

21. Pan C, Gao H, Zheng N, et al. Mir-320 inhibits the growth of glioma cells through downregulating PBX3. Biol Res. 2017; 50.

22. Xu X, Bao Z, Liu Y, et al. PBX3/MEK/ERK1/2/LIN28/let-7b positive feedback loop enhances mesenchymal phenotype to promote glioblastoma migration and invasion. J Exp Clin Cancer Res. 2018; 37: 158.

23. Harkin LF, Lindsay SJ, Xu Y, et al. Neurexins 1-3 each have a distinct pattern of expression in the early developing human cerebral cortex. Cereb Cortex. 2017; 27: 216-32.

24. Sun HT, Cheng SX, Tu Y, et al. FoxQ1 promotes glioma cells proliferation and migration by regulating NRXN3 expression. PLoS One. 2013; 8: e55693.

25. Xiang XJ, Deng J, Liu YW, et al. Mir-1271 inhibits cell proliferation, invasion and EMT in gastric cancer by targeting FOXQ1. Cell Physi

ol Biochem. 2015; 36: 1382-94.

26. Kusinska R, Gorniak P, Pastorczak A, et al. Influence of genomic variation in FTO at 16q12.2, MC4R at 18q22 and NRXN3 at 14q31 genes on breast cancer risk. Mol Biol Rep. 2012; 39: 2915-19.

27. Yang Q, Wang R, Wei B, et al. Gene and microRNA signatures are associated with the development and survival of glioblastoma patients. DNA Cell Biol. 2019; 38: 688-99.