In Silico Study of MIR-124-1 Transcription Factors in Glioblastoma

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Author’s contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

MIR-124-1 is a brain-abundant miRNA, whose expression is important for neuronal tissue division, growth and actions. However, expression of miR-124-1 regulatory mechanisms controlling its actions in neuronal cells in health and diseases still poorly addressed. To understand mechanism for transcriptional and functional regulation of miR-124 in neuronal and glioblastoma cells, this study combined gene expression profiling data and computational transcription factor and microRNA target predictions. The present research focuses on transcription factors and DNA methylation, which are central to miR-124-1 expression regulation. A core promoter sequence of miR-124-1 was predicted to be 500 bp and 100 bp, upstream and downstream of its transcription start sites. Seventy three binding sites of fifty transcription factors in promoter region were found, using MatInspector software. Among these transcription factors, MEIS1, POU3F2, SALL2, ETV1, and MAZ, are known to be brain-enriched transcriptional activators. By using omics data analysis, expression of MAZ, PLAG1 KLF2 as well as a transcriptional repressor ZNF239 showed significant correlation with decline in miR-124-1 expression in glioblastoma cells. Furthermore, a potential CpG island was reported in the promoter, providing another mechanism for transcriptional inhibition of miR-124. As miR-124-1 regulates a number of neuronal physiological and pathological processes, we made an attempt to define its potential targets. A computational prediction of miR-124-1 targets suggested 265 targets with two or more conserved seed sites. Pathway-based analysis of these target genes revealed a significant enrichment for axonal guidance and cancer signaling pathways. At least ten of these targets, SRGAP1, GNAI3, PLXNA3, SEMA5A, SEMA6A, CEBPA, CBL, RASSF5, MITF, and RPS6KB1, showed expected inverse correlation between their

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expression values and miR-124-1 suppression in glioblastoma cells. Taken together, our data form foundation of subsequent future validation researches for miR-124-1 expression regulation including transcription factors and CpG Island within its promoter as well as functional regulation comprising biological pathways controlled by its target genes.

Keywords: Bioinformatics; CpG islands; DNA methylation; glioblastoma; miR-124; neurons; promoter; transcriptional factor.

1. INTRODUCTION

Transcription is regulated by protein factors and small RNA molecules, microRNAs (miRNAs). These micromolecules are a group of evolutionarily conserved small non-coding RNAs (ncRNA) with a length of about ~19-23 nucleotides. A single miRNA can regulate hundreds of gene expression at the post-translational level leading to translational inhibition of destruction of target mRNAs. The simultaneous regulation of several genes by a single miRNA changes biological processes and cells’ physiological activities [1]. microRNA-124 (miR-124) is encoded by three different genes (miR-124-1, miR-124-2, and miR-124-3) in all vertebrates. miR-124-3 resides on chromosome 20, and the other two are on chromosome 8 in the human genome. Though primary transcripts of miR-124 genes (pri-miRNAs) are non-homologous, mature miR-124 sequences are identical and conserved from worm to human. miR-124 is uniquely expressed in nervous tissue and its levels about 100 fold higher than other non-nervous tissues[2,3]. So, the primary role of miR-124 is to participate in neuronal differentiation, development, and biological functions of the brain. Also, miR-124 stimulates neuron outgrowth during neurogenesis and contributes to converting human fibroblasts into neurons [4,5,6,7]. Furthermore, the association between the aberrant expression of miR-124 and neurological disorders, as neurodegenerative and neuroimmune diseases, oxidative stress, stroke, and cancer, was reported previously (Reviewed in [8]). Several studies revealed significant down-regulation of miR-124 in various brain tumors like glioblastoma and medulloblastoma [9,10].

Moreover, its reduction level in neurodegenerative diseases such as Alzheimer’s disease (AD) and Parkinson’s disease (PD) was also reported [11,12]. Therefore, miR-124 had been proposed as a diagnostic and prognostic marker and a therapeutical target for neurological disorders as brain tumors, neurodegenerative disorders, and stroke (Reviewed in [8]). Understanding miRNAs’ expression manners and identifying miRNA profiles can help as biomarkers for the diagnosis and prognosis of target treatments had begun to unfold. Notably, the deletion of only a single miR-124 gene, miR-124-1, in the Mus musculus led to a significant defect in neuronal developmental processes such as smaller brain size and axonal distribution neuronal apoptosis[13]. The molecular mechanism behind the exceptionally high level of miR-124-1 expression in neuronal cells is still unclear. Few studies showed that hypermethylation is associated with transcriptional silencing of miR-124-1 in human cancers, including colon and hematopoietic malignancies [14,15,16]. Besides the transcriptional regulation, the functional nature of miR-124-1 as a negative regulator of its target genes has been reported and linked to neurological functions and disorders (Review in [8]). However, it is still challenging to understand its functional regulation, given the existence of many potential target genes for any miRNA. This is the first work to investigate the regulation mechanism of miR-124-1 transcription in neuronal cells to the best of our knowledge. The study addressed two central questions: Whether brain-enriched transcription factors have binding sites in the proximal promoter of miR-124-1, and whether there are more potential locations of methylation may affect miR-124-1 gene transcription.

2. METHODS

2.1 The Sequence of MIR-124

MIR-124-1 and miR-124-2 are intergenic miRNAs located on human chromosome 8 ranging from 9903388 to 9903472 bp and from 64379149 to 64379257 bp, respectively. MIR-124-3 is located on chromosome 20 ranging from 63178500 to 63178586 bp. The precursor sequences were retrieved from miRBase (http://www.mirbase.org) and aligned using Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi).
2.2 The Sequence of MIR-124 Promoters

The promoter sequences of miR-124-1 and miR-124-2 were obtained using the GenBank, Nucleotide database, National Center for Biotechnology Information (NCBI). DNA methylation analysis and potential transcription factor binding sites were predicted using the Meth Primer and MatInspector programs.

2.3 Meth Primer

CpG islands were predicted across miR-124-1 promoter sequence using the default settings of the Meth Primer program [17] (http://www.urogene.org/methprimer/). The default settings are:

1. The promoter comprises a DNA sequence of at least 200bp in length
2. CG content in the promoter is over 50%
3. The ratio of observed to expect the number of CG dinucleotides is over 0.6

2.4 Genomatix MatInspector

Genomatix MatInspector prediction search (http://www.genomatix.de/) for transcription factor binding sites (TFBSs), several parameters include the core and matrix similarities, which are calculated within MatInspector [18]. The core similarity was set to 1 and >0.95 for the matrix, whereas the optimized matrix threshold was left at the default level.

2.5 Tumour Profile Database

Tumour Profile (http://tumour.bjmu.e-du.cn/) is a database on gene expression profile (GEP) across multiple datasets, mainly microarray transcriptional profiling human normal, non-cancerous, cancerous tissues, and cancer cell lines. Rank-based gene expression (RBE) curves provide an illustration for GEPs in queried tumors.

2.6 Target Scan Human

Target Scan Human (www.targetscan.org/) is In-silico miRNA target prediction algorithms was developed to predict miRNA sites based on the rules and pattern of interaction between mRNAs and miRNAs.

2.7 Pathway Analysis

Pathway analysis was carried out with DAVID [19] (https://david.ncifcrf.gov), online pathway annotation software based on scoring and visualization of the metabolic pathways collected KEGG database. Fisher’s exact test was applied to discriminate if those genes are enriched in the KEGG pathway. P-value < 0.05 cut off was chosen to determine the significant level of pathways.

3. RESULTS

3.1 Low Conservation between Human MIR-124-1 Promoter and MIR-124-2 Promoter Regions

The precursor sequences of human miR-124-1, miR-124-2, and miR-124-3 were extracted from the NCBI database (June 2016), alignments reveal high conservation among them (~89%) (Fig.1). However, mature sequences of miR-124-5p and miR-124-3p are 100% conserved between all three precursors. The pri-miRNA containing the pre-miR-124-2 is known as miR-124-2 host gene (miR-124-2HG), whereas the pre-miR-124-1 appears to be derived from annotated long intergenic non-protein coding RNA 599 (LINC00599). As the promoter sequence of miR-124-1 and miR-124-2 are not characterized yet, the genomic sequences were obtained from GenBank in June 2016 (Supp. 1A, B). The host transcript of pre-miR-124-3 has not been annotated yet; therefore, it is excluded from our work. The sequence comparison of human miR-124-1 and miR-124-2 promoters reveals no conservation between them. This indicates possible different regulatory mechanisms for miR-124-1 and miR-124-2 genes.

3.2 Identification of Potential Transcription Factors Related to the Enriched-Expression of MIR-124-1 in Nervous Tissue

In order to predict the transcription factor binding sites within miR-124-1 core promoter, the sequence on chromosome 8 ranges from 500bp upstream and 100bp downstream of transcription start site (TSS) (Ch: 9903829-9903229) was uploaded in MatInspector (June 2016). Core and matrix similarity cut-off values were set at 1 and 0.95, respectively. As shown in Table 1, 37 transcription factors, including 50 transcription factors, can occupy 73 binding sites in the miR-124-1 promoter region. We investigated whether any of these transcription factors can be responsible for the high expression of miR-124-1 in the brain and other nervous tissues. Therefore, transcription factors were filtered based on their specificity and expression levels in these tissues.
using the UniProt Knowledgebase (UniProtKB), or those transcription factors have at least two binding sites. Seven transcription factors were found brain-enriched; however, at least 5 of them are transcriptional activators (MEIS1, POU3F2, SAL12, ETV1, and MAZ) that can be linked with the high expression of miR-124-1 in neuronal cells (Table 2). Furthermore, at least seven transcription activators (ZNF384, ETV4, ERG, KLF2, ZBTB17, PLAG1, ZNF300) have more than two sites the promoter sequence.

3.3 MAZ, KLF2, PLAG1, and ZNF239 may Involve in the Transcriptional Inhibition of miR-124-1 in Brain Glioblastoma

Several studies have shown the dramatic inhibition in the expression of miR-124-1 in some neurological diseases, glioblastoma, and medulloblastoma [9,10]. The transcriptional regulation is a mechanism that may explain this inhibition. To assess the potential transcription factors involved in this inhibition, we performed an analysis based on omics data. TumourProfile database is used to determine the differential expression of the potential transcriptional regulators of miR-124-1 (Table 2) across glioblastoma and medulloblastoma versus the corresponding control tissues. The mean, Bonferroni correction adjusted P-values (Table 3), and rank-based gene expression (RBE) curves (Fig. 2) were calculated and created at the TumourProfile database between the control normal tissues versus glioblastoma. The P-value is calculated using Fischer’s exact test indicates the probability of the involvement of the genes in the dataset in a given pathway. There are significant levels of co-regulation between miR-124 and MAZ (P-value = 6.00 E-10) as well as miR-124 and KLF2 (P-value = 4.777 E-04), for the expression profile of miR-124 and PLAG1 in brain glioblastoma has shown insignificant correlation. Also, the expression profile of miR-124 and ZNF239 in brain glioblastoma has shown insignificant correlation with (P-value = 1.598 E-05). These correlated genes and miR-124 are likely to be functionally linked and contribute to regulate a common biological pathway.

The expression of brain-enriched transcription factors has not shown a correlation with the decreased level of miR-124-1. However, MAZ, KLF2, and PLAG1 were shown to be significantly downregulated in glioblastoma. KLF2 and PLAG1 were also downregulated in medulloblastoma (Data not shown). Additionally, the expression of ZNF239, a transcriptional repressor with two interaction sites, was found upregulated in glioblastoma cells. These transcription factors, KLF2, PLAG1, and ZNF239, may contribute to the altered expression of the miR-124-1 gene.

3.4 Methylation is a Potential Mechanism for MIIR-124-1 Promoter Inactivation

To examine if the methylation can have a role in the regulation of miR-124-1 expression, we analyzed the existence of CpG islands in human miR-124-1 promoter ranging from 500 bp upstream and 100 bp downstream of TSS (supp.1.C). Based on the MethPrimer program’s algorithm [17] for the prediction of enriched locations of CpG island in genomic DNA, two potential CpG islands (102bp and 167bp) were found in the sequence of the miR-124-1 promoter (Fig. 3A). Our results are in agreement with previous studies that experimentally validated the 102bp CpG island [14]. This prediction’s statistical parameters were the islands of less than 200 bp with CG content greater than 50.0% and observed CpG/expected CpG of more than 0.60 at the 5’ regions of the miR-124-1 promoter.

3.5 Several Potential Targets of MIIR-124-1 are Involved in Neuron Axonal Biogenesis and Cancer Signaling Pathways

Given that miR-124-1 is highly expressed in the neuron, it is expected that the target genes that contribute to its function would be downregulated but upregulated when miR-124-1 is inhibited in neuronal and metastatic cancer cells. To determine target genes of miR-124-1, a computational target prediction program (TargetScanHuman Release 7.1) was applied to suggest thousands of potential targets. Two hundred sixty-five genes were predicted to have at least two conserved seed sites for miR-124-1 (June 2007). We then assessed whether the 265 genes in our target gene list are components of specific signaling pathways. The gene list was uploaded in the DAVID software resource to calculate the enrichment and statistical significance of modified pathways. This analysis shows that eighteen target genes are involved in axonal guidance and cancer signaling pathways (Table 4). To further examine the potential pathophysiologically relevance of endogenous
miR-124-3p candidate targets in regulating glioblastoma, we looked at the expression profile of the target genes in glioblastoma. It is expected the decreased level of miR-124-1 in glioblastoma contributes to the expression levels of these targets. We observed an increase in the expression of several candidate targets such as SRGAP1, GNAI3, PLXNA3, SEMA5A, SEMA6A, CEBPA, CBL, RASSF5, MITF, and RPS6KB1 (Table 5).

4. DISCUSSION

The expression and functional significance of miR-124 have been studied in fundamental neurobiological processes [4,5,6,7], and its inhibition was associated with different neurological diseases as Parkinson’s and Alzheimer’s diseases [11,12]. However, the expression and functional regulation of miR-124-1 remains poorly understood in neuronal cells. A combination of omics-scale data and bioinformatics analyses can provide valuable insights into its neurobiological functions and lead to an understanding of unknown links between transcription regulators and its expression level. Because transcription factors and methylation level are associated with transcriptional control of gene expression, this study hypothesized that an exceptionally high level of miR-124-1 expression in neuron cells could be affected by neuron-enriched transcription factors and unmethylated CpG islands linked to its promoter. The decline in those neuron-enriched proteins and hypermethylation of CpG islands in the miR-124-1 promoter region may significantly decline in neuroblastoma and glioblastoma brain cells. Using the MatInspector bioinformatics tool for binding site prediction, this study showed three brain-enriched transcription factors, MEIS1, Pou3F2, SALL2, ETV1, and MAZ, that potentially combined to ten DNA binding sites to induce miR-124-1 transcription. Inconsistent with miR-124-1 expression and role during neurogenesis [6,7], MEIS1, Pou3F2, SALL2, ETV1, and MAZ are known as neurodevelopmental transcription factors, also having a critical role in vertebral neurogenesis as well as neuronal development, differentiation, and function [20-25]. The available omics data reveal a positive correlation between MAZ and miR-124 expression in glioblastoma tumor cells, but this correlation was not found between miRNA and the other brain-enriched transcription factors. This suggests that MEIS1, Pou3F2, SALL2, and ETV1 are not responsible for perturbation of miR-124-1 regulation in pathological glioblastoma through these transcription factors may interpret the high transcription of miR-124-1 gene in neurons rather non-neuron cells. Interestingly, the transcriptional activators PLAG1 and KLF2 harboring six and four binding sites in miR-124-1 promoter are decreased in glioblastoma cells versus control cells, and repressor ZNF239 expression increased in these cells. Changes in PLAG1, KLF2, and ZNF239 expression may negatively affect miR-124-1 transcription in glioblastoma and medulloblastoma cells.

Methylation is also another mode of miR-124-1 regulation at the transcriptional level. Here, the results identified a CpG dinucleotide-rich region in miR-124-1 promoter located downstream of a previous experimentally validated CpG island [14]. Future investigation is recommended to define MEIS1, Pou3F2, SALL2, ETV1, MAZ, PLAG1, KLF2, and ZNF239 in driving transcription of miR-124-1 as well as to correlate methylation status of CpG islands with the miR-124-1 level in neuron cells. This would explain the link between these transcription factors, methylation status, and miR-124-1 level in the neural tissue pathological conditions.

This work also focused on elucidating the target genes controlled by miR-124-1, which can rationalize how its perturbation can contribute to neuronal pathogenesis. Axon guidance and cancer pathways are aligned with the top pathways of candidate targets for miR-124-1. Consistent with these results, temporal regulation of axon outgrowth and guidance to their target destinations by miR-124 has been reported [26]. Other studies emphasize that axon guidance molecules affected migration, angiogenesis, and cell death in cancer cells. For example, SEMA6A acts as an oncogene because of its distinct angiogenic and apoptotic actions [27], and Slt-Robo GTPase-activating proteins (SRGAPs) adversely regulate cell migration and neurite outgrowth of neuroblastoma cells [28]. This possibility of targeting these genes by miR-124-1 is supported by results, which show an inverse relationship between miR-124-1 expression and target genes in neuroblastoma and normal brain cells. Two of these targets, ITGB1 and LAMC1, are reported to be a direct target for miR-124-1 action [29]. However, the role of miR-124 in suppressing growth in medulloblastoma [30] and glioblastoma cells [30,31,32], both in vitro and in vivo, need further investigations.
miR-124-1  ---------------AGGCCTCT-CTCTCCGTGGTCAGCGGACCTTGATTTAAATGTCCATACAAT
                   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
miR-124-2  ATCAAGATTAGAGG-CTCTGGCTCTCCGTGGTCACAGCGGACCTTGATTAA-TGT-CATACAAT
                   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
miR-124-3  ---------------TGAGGGCCC--CTCTGCGTGGTCACAGCGGACCTTGATTAA-TGTCTATACAAT
                   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |

miR-124-1  TAAGGCAAGCGGTGAATGCCAAGATGGGGCTG
            |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
miR-124-2  TAAGGCAAGCGGTGAATGCCAAGAGGGGAGCCATGGTACGCTGACTTGAA
            |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
miR-124-3  TAAGGCAAGCGGTGAATGCCAAGAGGGGAGCCATGGTACGCTGACTTGAA

Fig 1. Sequence alignment of pre-miR-124-1, pre-miR-124-2, and pre-miR-124-3 showing high conservation among them

The mature sequences within the three pre-miR-214 are completely conserved. Red color indicates the mature sequences of miR-124-5p. Blue color indicate the sequence of miR-124-3p. Multiple sequence alignment was performed using nucleotide BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi)
Table 1. MatInspector prediction of transcriptional families within *Homo sapiens* miR-124-1 promoter

| Matrix family | Matrix | Location | Strand | Matrix similarity |
|---------------|--------|----------|--------|-------------------|
| AHRR          | AHR    | 229-253  | -      | 0.995             |
| BRNF          | POU3F2 | 382-400  | +      | 0.959             |
| BTBF          | ZBTB33 | 70-80    | -      | 0.987             |
| CAAT          | NF-YA  | 149-163  | -      | 0.951             |
| CARE          | CARF   | 359-369  | -      | 0.979             |
| CART          | ALX1   | 396-416  | -      | 0.995             |
| CIZF          | ZNF384 | 76-86    | +      | 0.993             |
|               |        | 261-271  | -      | 0.987             |
| CSEN          | KCNIP3 | 228-238  | +      | 0.953             |
| E2FF          | E2F4   | 165-181  | +      | 0.985             |
|               | E2F1   | 461-477  | -      | 0.952             |
| EBOX          | MYC    | 409-425  | -      | 0.951             |
| EGRF          | EGR2   | 28-46    | -      | 0.984             |
| ETSF          | SPI1   | 26-46    | -      | 0.973             |
|               | ETV1   | 183-203  | -      | 0.968             |
|               |        | 296-316  | -      | 0.954             |
|               |        | 324-344  | -      | 0.968             |
|               |        | 336-356  | -      | 0.961             |
|               |        | 468-488  | +      | 0.95              |
| ETV4          | 316-336 | -     | 0.96  |
|               | 320-340 | -     | 0.96  |
| ERG           | 312-332 | -     | 0.96  |
|               | 332-352 | -     | 0.973 |
| EVI1          | PRDM16 | 171-187  | +      | 0.952             |
| FKHD          | FOXP1  | 101-117  | +      | 0.959             |
|               | FOXX2  | 129-145  | -      | 0.969             |
| GATA          | GATA3  | 132-144  | -      | 0.978             |
| HOMF          | NOBOX  | 400-418  | -      | 0.959             |
| HHEX          | 404-422 | -     | 0.955 |
| HOXC          | MEIS1  | 305-401  | +      | 0.953             |
| KLF2          | 31-47   | -      | 0.977 |
| KLFS          | 51-67   | -      | 0.981 |
|               | 201-217 | -      | 0.991 |
|               | 290-306 | -      | 1      |
| KLF3          | 199-215 | -      | 0.975 |
| KLF4          | 35-51   | -      | 0.981 |
| LEFF          | LEF1   | 103-119  | +      | 0.966             |
| MAZF          | MAZ    | 534-546  | +      | 0.964             |
| MIZ1          | ZBTB17 | 461-471  | -      | 0.959             |
|               | 499-509 | +      | 0.95   |
| MOKF          | ZNF239 | 104-124  | +      | 0.994             |
|               | 373-393 | +      | 0.989 |
|               | 407-427 | -      | 0.984 |
|               | 474-494 | -      | 0.962 |
| MZF1          | MZF1   | 8-18    | +      | 0.974             |
|               | 100-110 | +      | 0.986 |
|               | 157-167 | +      | 0.986 |
|               | 170-180 | +      | 0.992 |
|               | 200-210 | -      | 1      |
|               | 218-228 | -      | 0.992 |
|               | 538-548 | +      | 0.995 |
| NF1F          | NFIC   | 414-434  | +      | 0.963             |
| Matrix family | Matrix | Location | Strand | Matrix similarity |
|--------------|--------|----------|--------|-------------------|
| NKXH | NKX2-5 | 398-416 | - | 1 |
| PLAG | PLAG1 | 7-29 | + | 0.967 |
| | | 30-52 | - | 0.959 |
| | | 44-66 | - | 1 |
| | | 99-121 | + | 0.959 |
| | | 156-178 | + | 0.959 |
| | | 194-216 | - | 0.959 |
| PURA | PURA | 55-67 | - | 0.993 |
| | SALL2 | 204-214 | - | 0.964 |
| | SMAD1 | 293-303 | - | 0.958 |
| | SMAD3 | 143-153 | + | 0.966 |
| | SOX6 | 7 | - | 0.985 |
| | TALE | 104-126 | + | 0.99 |
| | YY1F | 221-237 | + | 1 |
| | | 279-301 | + | 0.961 |
| | ZNFB219 | 55-77 | + | 0.968 |
| | ZKSCAN3 | 87-109 | - | 0.995 |
| | | 42-64 | + | 0.995 |
| | | 206-228 | + | 0.995 |
| | ZFHX | 531-553 | - | 0.976 |
| | ZF07 | ZNF263 | 537-551 | - | 0.954 |
| | ZF11 | ZBTB3 | 438-448 | - | 0.991 |
| | ZF5F | ZBTB14 | 528-542 | + | 0.96 |
| | ZFHX | ZEB1 | 312-324 | + | 0.981 |

Transcriptional families and their members are listed with the position on promoter DNA sequence and strand. The cut-offs were set to ≥0.95 and 1 for matrix and core similarities, respectively.

Table 2. Brain-enriched transcription factors within *Homo sapiens* miR-124-1 promoter

| Transcription factor | ID | Repeat | Function |
|----------------------|----|--------|----------|
| MEIS1 | 4211 | 1 | Transcriptional activator GO:0001077 |
| POU3F2 | 5454 | 1 | Transcriptional activator GO:0001077 |
| KCNIP3 | 30818 | 1 | Transcriptional repressor and corepressor GO:0001078, GO:0003714 |
| SALL2 | 6297 | 2 | Transcriptional activator PMID: 19076363, 25580951, 21362508 |
| ETV1 | 2115 | 5 | Transcriptional activator GO:0001077 |
| MAZ | 4150 | 1 | Transcriptional activator PMID: 14765995, 25013182, 20457603, 18710939 |
| ZNF384 | 171017 | 2 | Positive regulation of transcription from RNA polymerase II promoter GO:0045944 |
| ETV4 | 2118 | 2 | Positive regulation of transcription from RNA polymerase II promoter GO:0045944 |
Transcription factors are listed with their Molecular Functional Annotation using Gene Ontology (GO). Transcription factors with no GO Annotations were given transcriptional functions based on the literature review.

**Table 3. The mean expression intensities of MAZ, KLF2, PLAG1 and ZNF239 in glioblastoma versus normal tissues**

| TFs     | Tissue          | Mean   | Adjusted P value (Bonferroni) |
|---------|-----------------|--------|-------------------------------|
| MAZ     | Brain_normal    | 91     |                               |
|         | Brain_glioblastoma | 84.5  | 6.004E-10                     |
| KLF2    | Brain_normal    | 87     |                               |
|         | Brain_glioblastoma | 81    | 4.777E-04                     |
| PLAG1   | Brain_normal    | 55.8   |                               |
|         | Brain_glioblastoma | 50.1  | 8.745E-05                     |
| ZNF239  | Brain_normal    | 63.2   |                               |
|         | Brain_glioblastoma | 67.1  | 1.598E-05                     |
Fig. 2. Rank-based gene expression (RBE) curves
The inhibition of at the MAZ, KLF2, and PLAG1 mRNA levels in glioblastoma compared to normal tissues. In the line graph of RBE curves, X-axis shows the expression intensity reflected by the rank scores, and Y-axis indicates the sample percentiles at each rank score.

Promoter of miR-124-1 gene, RNA 599 (LINC00599)

Island 1, 102 bp  (184 - 285)
GTGGTCCTCCTCTCCGGCTCCCTCCATCCCCATCTCTCTCCCTCCTTCACTGCACGCACACCGCGCCGC
TTTTAAAATTCCTCTGTGTTTTATCTTC

Island 2, 176 bp  (361 - 536)
CCCTCTCTCTCCTTCCTACAGGACCTTGTATTTAAATGTCCATAACTAATTAGGCAACCGCGTGAATGCA
AGAAATGGGCTGGCTAGACCGCCTCGGAGGGCGCAAGGAGGAGGAGGACCCCGAGCCA
GGCCCTCCGAGAACCTCGGCCCGCAGGCCGC

Fig. 3. A) The potential methylation sites within miR-124-1 promoter
The CpG islands are colored with blue in the genomic DNA whereas the red horizontal lines show the sites of CG dinucleotides. The statistical cutoff was (%GC ≥ 50%, Observed CpG/Expected CpG ≥ 0.60).
B) The sequences of CpG islands are shown and red CGs are indicted in red color
Table 4. The top ranked regulated pathways of candidate miR-124-1 target genes were obtained from the Database for Annotation, Visualization and Integrated Discovery (DAVID)

| Pathways                        | P-value   | Candidate target genes for miR-124-1                                      |
|---------------------------------|-----------|---------------------------------------------------------------------------|
| Axon guidance                   | 6.2E-03   | SRGAP1, SRGAP3, GNAI3, ITGB1, PLXNA3, SEMA5A, SEMA6A                      |
| Pathways in Cancer              | 9.0E-03   | CEBPA, CBL, RASSF5, TRAF3, FZD4, ITGB1, LAMC1, MITF, RXRA, AKT3, ZBTB16  |
| Small cell lung cancer          | 2.3E-02   | TRAF3, ITGB1, LAMC1, RXRA, AKT3                                           |
| Insulin signalling pathway      | 3.0E-02   | CBL, FLOT2, PRKAA2, RHOQ, RPS6KB1, AKT3                                   |
| Acute myeloid leukemia          | 3.9E-02   | CEBPA, , RPS6KB1, AKT3, ZBTB16                                            |

*P-value is calculated using Fischer's exact test indicates the probability of the involvement of the genes in the dataset in a given pathway. Validated target genes are shown in bold.*

Table 5. The mean expression intensities of miR-124-1 candidate target genes in glioblastoma versus normal tissues

| TFs    | Tissue            | Mean | Adjusted P value |
|--------|-------------------|------|------------------|
| SRGAP1 | Brain_normal      | 45.3 |                  |
|        | Brain_glioblastoma| 68.6 | 3.456E-40        |
| GNAI3  | Brain_normal      | 89.5 |                  |
|        | Brain_glioblastoma| 97.5 | 2.996E-53        |
| PLXNA3 | Brain_normal      | 10   |                  |
|        | Brain_glioblastoma| 12  | 2.405E-10        |
| SEMA5A | Brain_normal      | 61   |                  |
|        | Brain_glioblastoma| 82.3 | 6.919E-38        |
| SEMA6A | Brain_normal      | 63   |                  |
|        | Brain_glioblastoma| 78.1 | 8.392E-21        |
| CEBPA  | Brain_normal      | 69.5 |                  |
|        | Brain_glioblastoma| 72.3 | 0.007            |
| CBL    | Brain_normal      | 86.9 |                  |
|        | Brain_glioblastoma| 90.9 | 3.612E-28        |
| RASSF5 | Brain_normal      | 71.8 |                  |
|        | Brain_glioblastoma| 74  | 0.017            |
| MITF   | Brain_normal      | 73   |                  |
|        | Brain_glioblastoma| 76.8 | 2.199E-05        |
| RPS6KB1| Brain_normal      | 61.9 |                  |
|        | Brain_glioblastoma| 76.7 | 2.422E-34        |

5. CONCLUSION

In conclusion, our data provide the basis by which miR-124-1 could regulate signaling pathways relevant to neural growth and cancer and suggest putative brain-enriched transcription factors and methylation mechanisms by which its expression can be controlled during normal and pathological neural states. Our finding suggested that brain-enriched transcription factors have binding sites in the promoter of miR-124-1. Also, there are potential location of methylation that affect miR-124-1 gene transcription. To validate the limitation data, some practice lab technique need to applied and investigate.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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