3 Epigenetic Mechanisms of Glioblastoma

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Abstract: Aberrant DNA methylation is a common event in the genesis and progression of tumors. The application of next-generation sequencing enables the identification and mapping of DNA methylation and its derivatives, 5fC and 5hmC, to base-pair resolution. This chapter describes nine novel hypermethylation genes and six hypomethylation genes, identified by constructing a DNA methylation profile, in glioblastoma. Abnormal promoter methylation and histone modifications were associated with differential expression of miRNAs in glioblastoma: miR-185 reversed global DNA methylation and the methylation level of the hypermethylation genes by targeting DNMT; and miR-101 regulated histone methylation of hypomethylation genes by targeting EED, EZH2, and DNMT3A. The long noncoding RNA CASC2c directly bound to miR-101 via microRNA response elements, and there was a reciprocal repression between CASC2c and miR-101. Despite being competitors they both led to the overexpression of their target hypomethylation genes CPEB1, PRDM16, and LMO3.

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Taken together, glioblastoma is a complicated pathological process with deregulated methylation and histone modifications. Focal differentially methylated region and differentially methylated site studies will be helpful for the identification of regulatory elements of transcription. Studies of intragenic and distant intergenic alterations in DNA methylation will help elucidate the nature of epigenetic deregulation in glioblastoma.

Key words: Glioblastoma; Histone modification; lncRNA; Methylation; miRNA

Introduction

Aberrant DNA methylation patterns have been shown to be common events in the genesis and progression of tumors (1). In cancer cells, a general decline in the level of methylated cytosine (genomic hypomethylation) is accompanied by local locus-specific hypermethylation (2, 3). Genomic hypomethylation contributes to genetic instability and proto-oncogene hypomethylation, which is responsible for their stronger expression (4). In addition, functional silencing of tumor-associated genes is usually associated with local promoter hypermethylation (5). Thus, alterations in tumor cell DNA methylation patterns contribute to abnormal gene expression and malignant phenotypes. Glioblastoma multiforme is the most common and aggressive primary central nervous system tumor in adults. Abnormal DNA methylation is responsible for glioblastoma genesis, development, and malignancy progression (6). Promoter hypermethylation and epigenetic silencing of the MGMT gene have been widely described in glioma (7–9). Several genes that are involved in key cellular functions such as the cell cycle (10), tumor suppression (11–15), DNA repair (16, 17), tumor invasion (18), and apoptosis (19) have been shown to be silenced in association with promoter hypermethylation in malignant glioblastoma. Despite these important findings, aberrant DNA methylation on genome-wide scale is still not fully understood in glioblastoma. This chapter describes nine novel hypermethylation genes and six hypomethylation genes, identified by constructing a genome-wide DNA methylation profile, in glioblastoma.

Methylomes of Glioblastoma

DNA METHYLATION PROFILE IN GLIOBLASTOMA

MeDIP-chip was used to investigate the whole-genome differential methylation patterns between glioblastoma and nontumor brain samples (20). A total of 104 hypomethylated and 524 hypermethylated regions were identified in glioblastoma. Of these, 70 hypomethylated and 361 hypermethylated regions were CpG islands (Figure 1A). Thirty hypomethylated and 199 hypermethylated regions were mapped to the unannotated gene regions (Figure 1B). Meanwhile, 74 hypomethylation and 325 hypermethylation regions were mapped to

annotated gene regions comprising the promoter region, intragenic region, and the regions downstream. Furthermore, 81.1% (60 of 74) of hypomethylated and 66.5% (216 of 325) of hypermethylated regions mapped to the promoter regions of annotated genes (Figure 1C). Twenty-seven hypomethylated and 53 hypermethylated regions mapped to CpG islands as well as the promoters of known genes (Figure 1D). Thus, a number of new differential methylation regions (DMRs) were shown to exist in unannotated genomic regions as well as the promoter regions, intragenic regions, and regions downstream of known genes in glioblastoma. The promoter hypermethylated genes exist predominately on chromosomes 1, 2, 3, 17, and x, while the promoter hypomethylated genes were mainly distributed on chromosomes 1, 11, 16, 19, 20, and 22 (Figure 1D). The functional and pathway analyses of these differential promoter methylated genes were performed by the DAVID bioinformatics tools. Most of the differential promoter methylated genes belonged to signaling networks that played critical roles in regulation of transcription, neurological process, ion transport, cell adhesion, apoptosis, and regulation of tumor development (Tables 1 and 2). Promoter hypermethylated genes that were correlated to

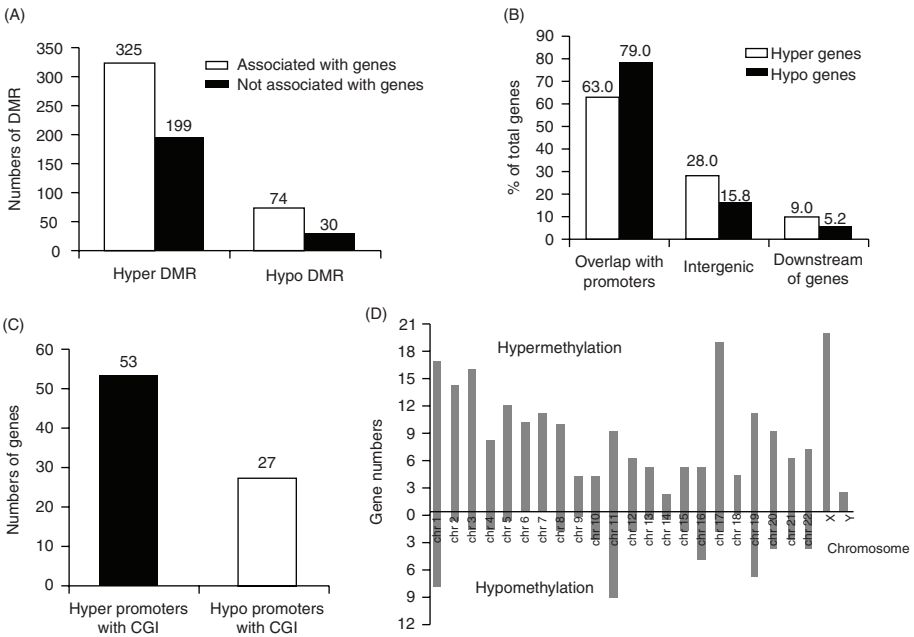


Figure 1 Genome-wide analysis of DMRs in primary glioblastoma. (A) DMRs (differentially methylated regions) are correlated with or without genes. (B) Distribution of DMRs is correlated with genes. Most identified DMRs are mapped to gene promoters. (C) Numbers of DMRs are mapped to both gene promoters and CpG islands. (D) Chromosomal distribution of 60 promoter hypomethylated genes and 216 promoter hypermethylated genes (Zuping Zhang, Guiyuan Li. Study on epigenetic mechanisms of glioma. Doctoral thesis, Central South University, 2009; 12).

TABLE 1 **Gene Ontology and KEGG Pathway Enrichment Analysis of Promoter Hypermethylated Genes Identified by MeDIP-Chip (Zuping Zhang. Doctoral thesis. Central South University, 2009)**

Gene Ontology Analysis	Promoter Hypermethylation Genes
Cell communication and intracellular signal transduction	22 Genes: ANKDD1A, OPN1MW, PKP1, PCDHB13, PI4KA, HSH2D, KNDC1, KCNMB3SST, DIRAS3, PTAFR, KCNN3, OR10Q1, CD81, ABRA, CASP9, FYN, MBP, OR10H5, RCVRN, GPR31, KCNMB2, TDGF1,
Neurological system process, and synaptic and nerve impulse transmission	20 Genes: SIX3, PI4KA, RCVRN, PCDHB13, OR10H5, MBP, KCNMB2, KCNMB3, CLN3, MPZ, S100P, TRPV1, DLGAP2, PROM1, FYN, SYPL1, SST, GAD1KCNN3, OR10Q1, HTR1D
Negative regulation of biological process, metabolic process, cellular process, and transcription activity	16 Genes: PAIP2, TDGF1, RASSF1, DEDD2, GRLF1, SALL4, RASSF2, TMSB4Y, CFTR, CLN3, GDNF, SIX3, DKK4, SST, ST18, B4GALNT2
Chemical homeostasis, homeostatic process, ion homeostasis, regulation of pH, and biological quality	13 Genes: KCNMB3, CCKAR, CLN3, MPZ, TRPV1, CYP11B2, RPH3AL, DNAJC16, DEDD2, MB KCNMB2, MBP, EDNRB
Brain development, neurons generation, and migration	9 Genes: FYN, SIX3, CFTR, NNAT, CCKAR, ROBO2, GRLF1, GDNF, LRRC4
Cell and biological adhesion, homophilic cell adhesion, and Cadherin	13 Genes: SDK1, FBLN7, PCDHB13, CLDN18, PKP1, CD300A, ROBO2, PCDHA12, PARVB, FGF6, FLRT1FERMT3, PCDHA8, PCDHA13
Ion transport, calcium channel, cation channel activity, gated channel activity, and ion transmembrane transporter activity	10 Genes: KCNMB3, TRPV1, FYN, CACNG7, KCNN3, TRPV3, CACNG1, SLC5A11, KCNK10, KCNMB2
Migration and motility of cell, localization of cell, cellular morphogenesis during differentiation, and cellular structure morphogenesis	9 Genes: S100P, TDGF1, EDNRB, SST, FYN, CCKAR, GDNF, ROBO2, CFTR
Actin binding and cytoskeletal protein binding	8 Genes: TMSB4Y, PDE4DIP, FYN, PARVB, ABRA, C14 or f49, RPH3AL, PHACTR3
Apoptosis induction by extracellular signals	2 Genes: SST, DEDD2
Neuroactive ligand–receptor interaction	6 Genes: EDNRB, PTAFR, HTR1D GH2, SST, CCKAR, TRPV1
KEGG Pathway Analysis	**Promoter Hypermethylation Genes**
MAPK signaling pathway	4 Genes: MAP2K3, CACNG7, FGF6, CACNG1
WNT signaling pathway	1 Gene: DKK4
JAK-STAT signaling pathway	1 Gene: GH2

TABLE 2 **Gene Ontology and KEGG Pathway Enrichment Analysis of Promoter Hypomethylation Genes Identified by MeDIP-Chip (Zuping Zhang. Doctoral thesis. Central South University, 2009)**

Gene Ontology Analysis	Promoter Hypomethylation genes
Cell communication and intracellular signal transduction	14 Genes: ABR, OR1L6, FKBP8, DRD4, SORBS1, GP1BB, OR10G4, BAD, C9, OR51S1, CCRL2, OR8A1, SFN, MLNR
Protein metabolic, cellular metabolic, and biopolymer metabolic process	11 Genes: CPEB1, C9, FKBP8, TUBB4Q, PRSS33, FUT5, NRBP2, PSMF1, OR51S1, KLHL21, TUBB8
Transport including metal ion transport and cation transport, ion channel activity	10 Genes: ACCN1, ABCC12, SLC5A9, SLC2A9, KCNK4, TUBB4Q, TUBB8, MFSD3, SLC28A1, SORBS1
Hydrolase activity and serine hydrolase activity	5 Genes: ABCC12, OR51S1, TUBB4Q, TUBB8, PRSS33
Regulation of gene expression, transcription, DNA binding, and transcription factor activity	5 Genes: CPEB1, LMO3, PHF13, TOX2, NAT14
Nervous system and organ development, and system development	3 Genes: ACCN1, ABR, IGSF8
Cell death, apoptotic program, and induction of apoptosis	3 Genes: BAD, C9, SFN
KEGG Pathway Analysis	**Promoter Hypomethylation Genes**
Neuroactive ligand–receptor interaction	3 Genes: SCT, DRD4, MLNR
Insulin signaling pathway	2 Genes: BAD, SORBS1

human tumor development included EDNRB, ARHI, FYN, GIPC2, GDNF, RASSF1, RASSF2, and ARHI (21–25).

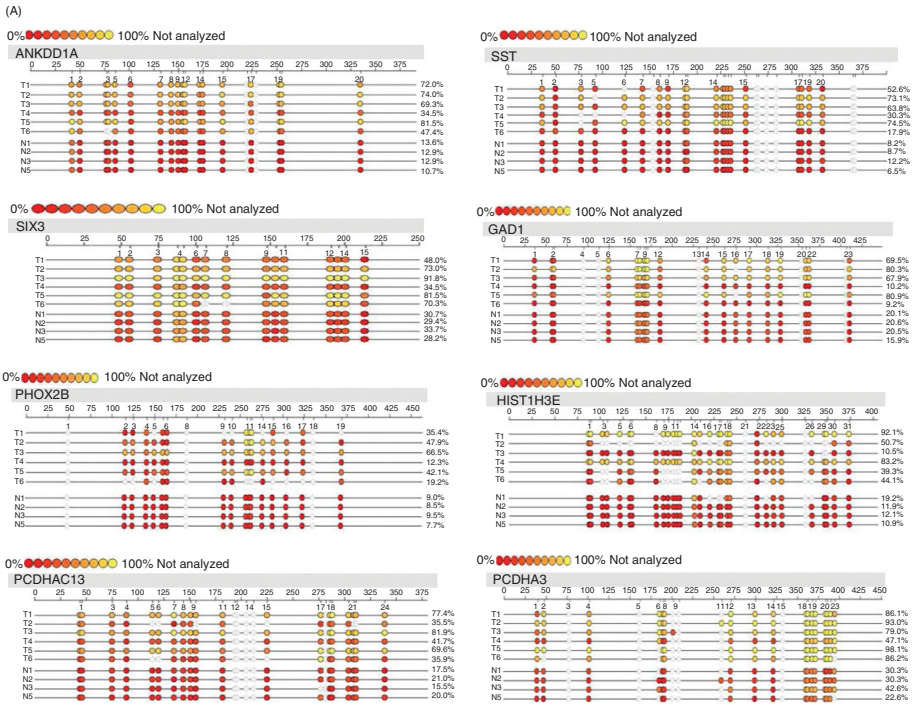
NINE NOVEL HYPERMETHYLATION GENES IN GLIOBLASTOMA

Sequenom MassARRAY platform quantitative analysis confirmed that LRRC4, ANKDD1A, GAD1, SIX3, SST, PHOX2B, PCDHA8, HIST1H3E, and PCDHA13 were the nine novel promoter hypermethylation genes in glioblastoma (Figure 2B). LRRC4 (GeneBank: AF196976) is not only a brain-specific gene but also a novel candidate for tumor suppression. Methylation-mediated inactivation of LRRC4, SIX3, and ANKDD1A has been verified as a frequent and glioblastoma-specific

event (15). SIX3 is a novel negative transcriptional regulator and acts as a tumor suppressor that directly represses the transcription of AURKA and AURKB in glioblastoma (26). ANKDD1A inhibits the transcriptional activity of HIF1α to alter hypoxia microenvironment by directly interacting with FIH1. The tumor-specific methylation of ANKDD1A indicates that it could be used as a potential epigenetic biomarker and also as a possible therapeutic target for glioblastoma.

SIX NEW HYPOMETHYLATION GENES IN GLIOBLASTOMA

Signalmap software was used to select the following 12 genes from the 74 hypomethylated regions screened with the methylation chip: F10, POTEH, CPEB1, LMO3, ELFN2, PRDM16, CD207, BAD, NRBP2, SLITRK5, SLC44A2, and PGP These genes were tested in a large scale of samples by BSP, which revealed that there is no difference between the methylation levels of CD207, BAD, NRBP2, SLITRK5, SLC44A2, and PGP in glioblastoma tissues and in normal brain tissues. F10 (27), POTEH (28), CPEB1 (29), LMO3 (30), ELFN2, and PRDM16 (31) were hypomethylated in glioblastoma tissues. They were confirmed to be novel hypomethylated genes in glioblastoma. Because of their higher expression, and poor outcomes in patients harboring these genes, these hypomethylated genes could be



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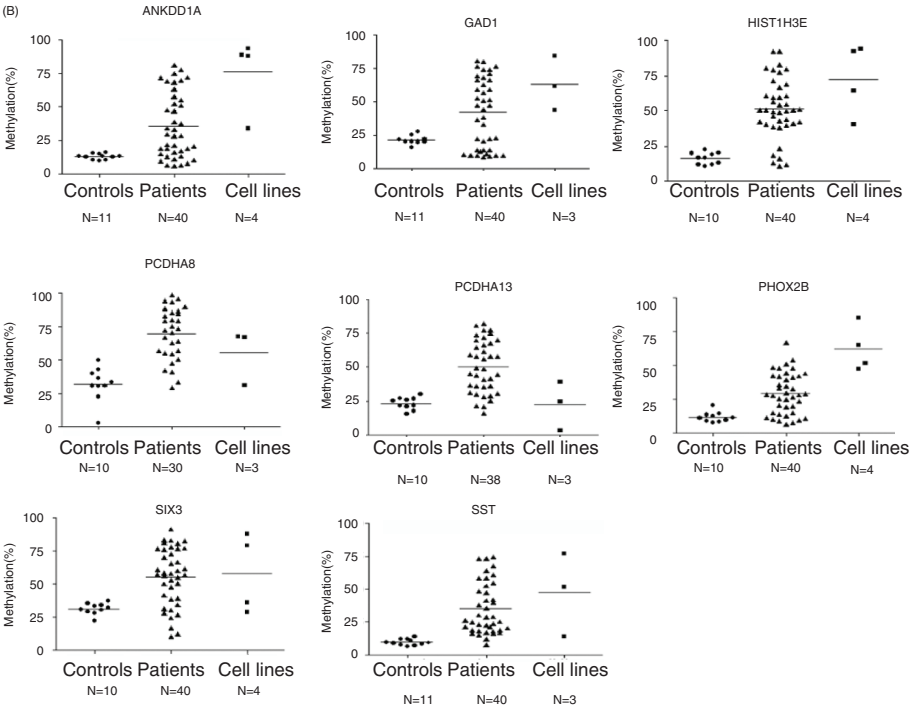

Figure 2 MassARRAY methylation analysis for glioblastoma. (A) MeDIP-chip assay detected hypermethylation genes' promoter methylation levels. Mass ARRAY assay were carried out using patients' samples gDNA screened by microarray. The position of CpG dinucleotides were marked by circles within the sequence (straight line), and the methylation levels were marked by different circle colors. Gray circles represent CpG sites that could not be analyzed. The base-pair position in the gene sequence used the ruler on top and CpG sites number at the bottom. In all tested genes, glioma samples have significant hypermethylation in promoter regions compared with normal controls. N1, N2, N3, and N5 represent normal brain, while T1, T2, T3, T4, T5, and T6 represent glioma samples. Glioma samples and normal samples were matched for age and sex. (B) Methylation levels of eight selected genes were identified by MeDIP-chip in glioma patients, normal controls, and glioma cell lines. DNA methylation was analyzed using MassARRAY assay. The gene names were marked in the top of each graph. N represents the number of cases for this research. In the top of each graph, the results of an individual gene are represented (20).

regarded as important prognostic markers of glioblastoma. While hypomethylation of F10 was correlated to patients' age, high expression of POTEH and hypomethylation of PRDM16 were related to astrocytoma pathology grade (Table 3). Thus, high expression of POTEH and hypomethylation of PRDM16 could be considered as important markers in the progression of glioblastoma. The glioblastoma tissues with a high POTEH expression level or PRDM16 hypomethylation would be more malignant than those with a low POTEH expression level or PRDM16 hypermethylation.

TABLE 3 Correlation between Methylation Status and Protein Expression of Hypomethylation Genes and Clinical Parameters of Astrocytoma Patients

	hypomethylation		Sex		Age(years)[a]		Grade	
Variables	+	−	Male	Female	<median	≥median	Low grade (I+II)	High grade (III+IV)
FX(score)								
<8	12	9	9	12	12	9	10	11
≥8	67	8	45	30	32	43	46	29
P		0.001		0.162		0.239		0.126
POTEH(score)								
<8	27	12	23	16	15	24	29	10
≥8	51	6	31	26	29	28	27	30
P		0.006		0.656		0.230		0.008
CPEBl(score)								
<8	18	7	17	8	14	11	14	11
≥8	25	0	17	8	9	16	12	13
P		0.010		1.000		0.781		0.571
LMO3(score)								
<8	9	4	10	3	8	5	8	5
≥8	34	3	24	13	19	18	18	19
P		0.043		0.508		0.526		0.424
ELFN2(score)								
<8	12	6	11	7	9	9	10	8
≥8	29	3	23	9	18	14	16	16
P		0.034		0.434		0.670		0.706
PRDM16(score)								
<8	7	4	8	3	7	4	7	4
≥8	36	3	26	13	20	19	19	20
P		0.016		0.704		0.468		0.382

Note: Immunohistochemistry score ≥8 represents the high expression; <8 represents the low expression. The high expression of FX, POTEH, CPEB1, ELFN2, LMO3, and PRDM16 were companied by their hypomethylation in glioblastoma. And the hypomethylation of POTEH, CPEB1, LMO3, and ELFN2 did not have statistically significant correlation with sex, age, or histological grades. The hypomethylation of F10 was correlated to patients' ages, and high expression of POTEH and hypomethylation of PRDM16 were related to the pathology grade of astrocytoma.

miRNA and Methylation Genes in Glioblastoma

Epigenetic modifications encompass DNA methylation, chromatin remodeling, noncoding RNA expression, and histone tail modifications. Methylation modification is important for a proper genome function by maintaining chromatin structure, chromosome stability, and transcription. Histones are the protein moiety around chromatin, which is packaged by DNA, and their N-terminal tails can suffer a variety of post-translational modifications, such as methylation, acetylation, sumoylation, ubiquitination, phosphorylation, and ADP ribosylation (32, 33). MicroRNAs (miRNAs) are 20–22 nucleotide (nt) noncoding RNAs that bind to the 3' untranslated region of the target mRNA to form RNA-induced silencing complexes, which lead to the down regulation of genes by causing mRNA destabilization and/or translational inhibition (34, 35). miRNAs, as both targets and effectors, play a critical role in regulation of DNA methylation (36–39). Moreover, miRNAs can regulate DNA methylation by targeting the DNA methylation machinery.

THE LRRC4-AP-2-miR-182 LOOP

As miR-381 and miR-182 could facilitate glioblastoma cell growth *in vitro* and *in vivo*, they are regarded as potential therapeutic biomarkers in glioblastoma (40). The downregulation of miR-381 or miR-182 arrested cell cycle of glioblastoma cells in the G0/G1 phase and inhibited their proliferation by suppressing E2F3 and upregulating phosphorylated Rb. LRRC4 was the co-target gene of miR-381 and miR-182, and its expression was inversely correlated with miR-381, miR-182, and BRD7 in glioblastoma. Knockdown of miR-182 and miR-381 inhibited LRRC4-mediated binding of AP-2/SP1/E2F6/c-Myc to BRD7 by ERK/MAPK and PI-3K/AKT (40). Transcription of miR-182 was induced by the transcription factor AP-2, as predicted by online software and confirmed by ChIP. miR-182 inhibited the expression of LRRC4, and LRRC4 inhibited the expression and transcription of AP-2 by negatively regulating the ERK/MAPK and PI-3K/AKT signaling pathways. This indicated that the LRRC4-AP-2-miR-182-LRRC4 loop is involved in glioblastoma development (40).

THE LRRC4-miR-185/SP1-DNMT1 LOOP

LRRC4 upregulation induced the expression of miR-185, and the LRRC4-miR-185/SP1-DNMT1-LRRC4 loop played a key role in glioblastoma: miR-185 inhibited cell motility, invasion, and proliferation; and DNMT1, one of the most important DNA methyltransferases, maintained methylation. miR-185 upregulation inhibited DNMT1 and decreased global methylation through HPLC-DAD; it also downregulated the expression of nine novel hypermethylated genes (SIX3, SST, LRRC4, GAD1, PCDHA8, PHOX2B, PCDHA13, ANKDD1A, and HIST1H3E) (20). Thus, miR-185 acts as a tumor suppressor by targeting DNMT1 to decrease global methylation and recover hypermethylation of these genes. The GO methodology is a success-oriented probabilistic system performance analysis technique. Based on GO methodology, miR-185 was also considered to be involved in Rho GTPase activity. RhoA and CDC42 were the direct targets of miR-185, and

the expression of these two molecules was negatively correlated with miR-185 in glioblastoma. Overexpression of miR-185 reduced the growth and migration of glioblastoma cells by inhibiting RhoA, CDC42 directly, and VEGFA indirectly (20). In summary, LRRC4 could regulate miRNAs as a tumor suppressor gene. These processes constitute multiple circuits, including LRRC4-miR-185-DNMT1-LRRC4, LRRC4-SP1-DNMT1-LRRC4, and LRR C4-AP-2-miR-182-LRRC4. These circuits take part in the development of glioblastoma with multiple feedback mechanisms (Figure 3).

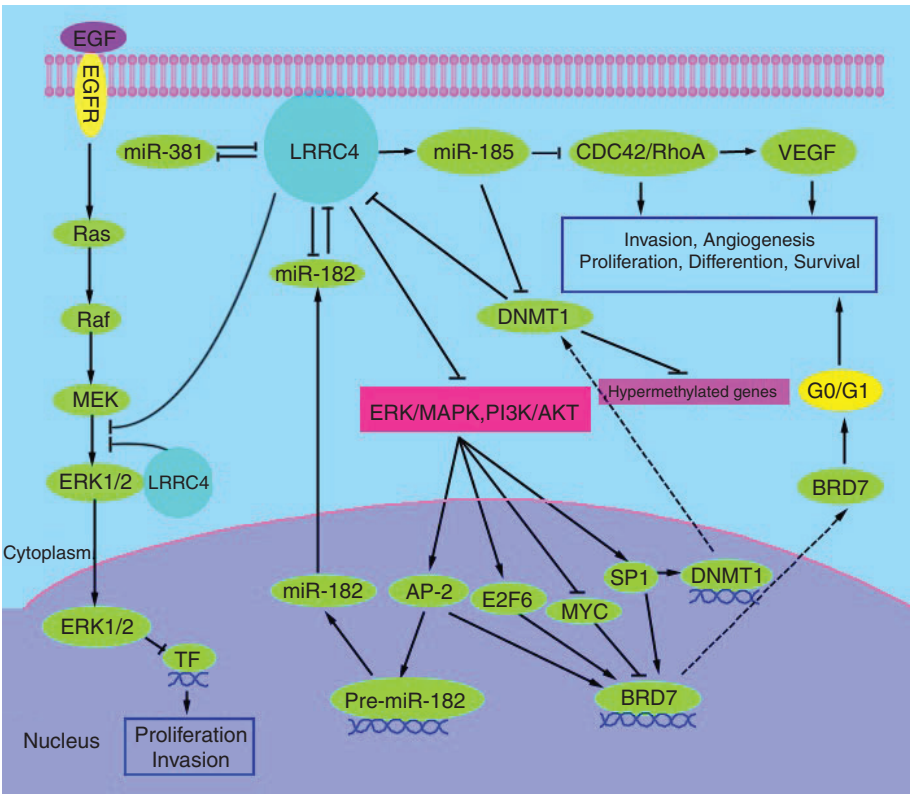

Figure 3 The regulation networks of hypermethylated genes, miRNA, DNMT, transcript factors, and target genes in glioblastoma. LRRC4-AP-2-miR-182-LRRC4loop: LRRC4 is a common target of miR-182 and miR-381, and miR-182 and miR-381 inhibit LRRC4 expression; meanwhile, the re-expression of LRRC4 also decreases miR-182 and miR-381 expression. The transcription of miR-182 is induced byAP-2; however, LRRC4 also inhibits the expression of AP-2 through negatively regulating the ERK/MAPK and PI-3K/AKT signaling pathways. The LRRC4-AP-2-miR-182-LRRC4 loop was formed among LRRC4, miR-182, and AP-2. LRRC4-miR-185-DNMT1-LRRC4 loop: The re-expression of LRRC4 increases miR-185 expression, while miR-185 decreases global methylation by targeting DNMT1 and increases the expression of LRRC4. LRRC4-SP1-DNMT1-LRRC4loop: DNMT1 is positively regulated by SP1, and it increases the expression ofLRRC4, while LRRC4 also inhibits SP1 by negatively regulating the ERK/MAPK and PI-3K/AKT signal pathway (Adapted from Mol Cancer 2011;10:124.)

miR-101 AND HYPOMETHYLATION GENES

It has been shown that miR-101 is downregulated in multiple tumors, including glioblastoma, and acts as a tumor suppressor. Interestingly, the novel hypomethylation genes CPEB1, PRDM16, ELFN2, and LMO3 were predicted to be targeted by miR-101. CPEB1, PRDM16, and ELFN2 were verified to be the direct target genes of miR-101; however, LMO3 was not the direct target (Figure 4). miR-101 suppressed CPEB1 expression by reversing the CPEB1 promoter methylation status. Furthermore, miR-101 reversed CPEB1 promoter methylation status by regulating the methylation-related histones H3K27me3, H3K4me2, H4K20me3, and H3K9me3. In addition, the decreased expression of CPEB1 triggered senescence in a p53-dependent manner (Figure 4). miR-101 downregulated LMO3 expression by reversing the LMO3 promoter methylation status, inhibiting the presence of the methylation-related histones H3K27me3 and H3K4me2, and increasing the presence of H4K20me3 and H3K9me3 on the promoter. miR-101 reduced the occupancy of H3K27me3 through suppressing EED, DNMT3A, and EZH2, and reduced the H3K9me3 occupancy on the LMO3 promoter by PHF8, G9a, SUV39H1, and SUV39H2. Moreover, miR-101 inhibited LMO3 expression by reducing MZF1 and USF (Figure 4). miR-101 also reduced PRDM16 expression by affecting the PRDM16 promoter methylation status. miR-101 was related to an increase in H4K20me3 and H3K9me3 and a decrease in the methylation-related histones H3K27me3 and H3K4me2 on the PRDM16 promoter. In addition, miR-101 suppressed PRDM16 expression by targeting DNMT3A, which decreased histones H3K27me3 and H3K4me2 at the PRDM16 core promoter (Figure 4). In addition, miR-101 also reduced H3K27me3 occupancy at the core promoter of the hypermethylation gene LRRC4 and reversed the LRRC4 methylation level through targeting EED, EZH2, and DNMT3A (Figure 4).

LncRNA, miRNA, and Methylation in Glioblastoma

INTERACTION OF LNCRNA AND miRNA

lncRNAs regulate gene transcription (41), chromatin remodeling (42), post-transcriptional processing of mRNA (43), and competing endogenous RNA (ceRNA) (44, 45). Emerging evidence suggests that lncRNAs communicate with ncRNAs, mRNAs, proteins, and genomic DNA, and act as tethers, guides, decoys, and scaffolds (46, 47). lncRNAs can participate in the ceRNA regulatory network and act as endogenous miRNA sponges to compete for binding of miRNA through MRE, which is “the letters” of the RNAcode (48). ceRNAs and miRNAs repress reciprocally and form a double-negative feedback loop (49, 50). Online promoterInspector and promoterScan softwares predicted that the CpG island status on the promoter of pre-miR-101-1 and pre-miR-101-2 is not the reason for the lower expression of miR-101. Furthermore, the LOH in chromosomes 1p31(pre-miR-101-1) and 9p24 (pre-miR-101-2) was insufficient to lower the expression of miR-101 in glioblastoma. The software programs DIANA LAB and miRanda were used to search for lncRNAs and there were 18 lncRNAs predicted to form putative binding sites with miR-101. However, only six lncRNAs have been confirmed to

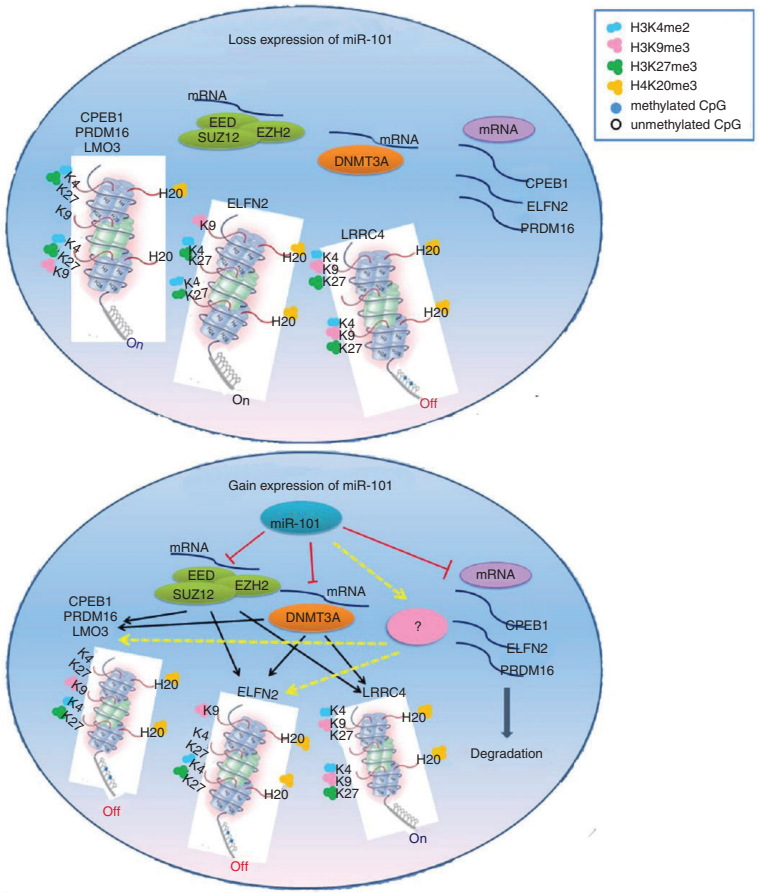

Figure 4 The networks of miRNA, gene methylation, and histone modification in glioblastoma. The hypomethylated genes CPEB1, PRDM16, and ELFN2 are target genes of miR-101, but LMO3 is not, and the expression of CPEB1, PRDM16, and ELFN2 are inhibited directly by miR-101. Moreover, miR-101 also indirectly suppresses the expression of CPEB1, ELFN2, PRDM16, and LMO3 and affects their methylation levels by targeting EZH2, EED, and DNMT3A and regulating histone methylation; miR-101 decreases the occupancy of H3K4me2 and H3K27me3 at CPEB1, ELFN2, PRDM16, and LMO3 core promoter and increases the H3K9me3 and H4K20me3 occupancy at CPEB1, PRDM16, and LMO3 core promoter by targeting EZH2, EED, and DNMT3A; then, it recovers the methylation levels of CPEB1, ELFN2, PRDM16, and LMO3 gene promoter, and indirectly downregulates the expression of these hypomethylation genes. miR-101 does not bind to 3,UTR of hypermethylated gene LRRC4, but it remains to upregulate the expression of LRRC4. miR-101 decreases the occupancy of H3K27me3 at LRRC4 core promoter and induces hypomethylation of LRRC4 by targeting EZH2, EED, and DNMT3A. In short, miRNAs can not only directly regulate expression of hyper-/hypo- methylation genes by binding to 3′-UTR of genes but also regulate the methylation level and gene expression through histone and DNA methylation modification by targeting histone and DNA methyltransferases (Xiaoping Liu. Docotor's thesis. Central South University, 2012).

show significantly different expression between glioblastoma and normal brain tissues. CASC2c was bound to miR-101 directly by MRE of miR-101, and there was a reciprocal repression between CASC2c and miR-101. The higher expression of CASC2c is one of the reasons for the low expression of miR-101. CASC2c is also the target of miR-101 and commonly exists in the RISC complex with miR-101. A high level of CASC2c positively regulated the expression of pre-miR-101; however, in the processing from pre-miR-101 to mature miR-101, CASC2c negatively regulated the expression of Dicer and inhibited the expression of mature miR-101 in glioblastoma (51). CASC2c is a long noncoding RNA and provided evidence that high expression of CASC2c occurred in glioblastoma. Knockdown CASC2c suppressed the proliferation, migration, and invasion *in vitro* and glioblastoma tumorigenesis *in vivo*.

EFFECT OF LNCRNA ON METHYLATION GENES IN GLIOBLASTOMA

CASC2c functions as a suppressor of miR-101 or as a competitor of its target genes such as CPEB1, PRDM16, and LMO3. The expression of hypomethylation genes CPEB1, PRDM16, and LMO3 was increased in glioblastoma, and the depletion of CASC2c led to their repression. Thus, in normal tissue, CASC2c, miR-101, and target genes keep a balance by competitive restriction. In glioblastoma, because of the high expression of CASC2c; low expression of miR-101; or the overexpression of CPEB1, PRDM16, and LMO3 as a result of hypomethylation status of its promoter, the balance of this regulatory feedback is lost. In terms of complexity of molecular mechanisms in tumors, a cause-and-effect relationship among CASC2c, miR-101, CPEB1, PRDM16, and LMO3 could not be established. Despite this, miR-101might be a core unit, and MRE of miR-101 is important for the crosstalk among CASC2c, miR-101, and their target genes in glioblastoma.

Conclusion

The application of next-generation sequencing enabled DNA methylation and its derivatives, 5fC and 5hmC, to be mapped at base-pair resolution. These studies have offered novel viewpoints into the distribution, dynamics, and function of DNA methylation in vertebrate genomes. Focal DMRs and DM site studies will be helpful for the discovery of regulatory elements of transcription factors, which may participate in specific gene regulation *in vivo*. Model systems should be used to test the functionality of individual DM sites or DMRs identified in epigenomic profiles. In the near future, studies of intragenic and distant intergenic alterations in DNA methylation will help elucidate the nature of epigenetic deregulation in diseases, especially for glioblastoma.

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