# Molecular mechanisms and potential prognostic effects of REST and REST4 in glioma (Review)

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**Abstract.** Glioma refers to a tumor of the brain and central nervous system, which is characterized by high incidence, high mortality and high recurrence rate. Although the association between glioma and the repressor element silencing transcription factor (REST) has been reported by numerous studies, the complicated regulatory mechanisms underlying REST remain unknown. REST is a transcriptional repressor that undergoes alternative splicing to produce splicing variants when transcribed. Previous studies have demonstrated that alternative splicing may serve a role in the outcome of glioma. The present review discussed the mutual relationship among REST, REST4 and glioma. It was concluded that increased REST expression in glioma may be associated with poor prognosis; and REST4, an AS variant of REST, also functions to regulate glioma by suppressing REST. In addition, the present review discussed the regulation of REST and its target genes in glioma, and identified factors that induce REST alternative splicing, particularly in glioma. These findings suggest that REST may be considered a prognostic factor, which can be predictive of patient outcome.

**Contents**



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## 1. Introduction

Glioma, which refers to a tumor of the brain and central nervous system, accounts for 27% of all brain and CNS tumors, and 80% of malignant brain and CNS tumors diagnosed in the United States (1). The World Health Organization (WHO) classifies glioma into numerous histological subtypes; anaplastic astrocytoma (WHO grade Ⅲ) and glioblastoma (WHO grade IV) are the most common. Due to the high mortality, high recurrence and low survival rates associated with this malignancy, the median overall survival time is <1 year (2). At present, radiotherapy, plus concomitant and adjuvant chemotherapy (temozolomide), following surgical resection represents the recommended therapeutic strategy for the treatment of newly diagnosed glioma (3). However, these cytotoxic therapies exhibit little improvement on overall survival, and are associated with severe toxic side effects. Therefore, further understanding the pathogenesis of glioma, and identifying novel strategies for the treatment of glioma, is required.

## 2. Repressor element silencing transcription factor

Molecular markers associated with the proliferation, apoptosis, invasion and metastasis of glioma have previously been reported. The present review aimed to evaluate the role of the transcriptional repressor, repressor element silencing transcription factor (REST). REST, which is also known as neuron-restrictive silencer factor (NRSF), is located at the 4q12 human chromosomal region, and is comprised of 4 exons and 3 introns. The full length of the REST gene is 24 kb. REST is widely expressed in various types of cancer, including glioma, lung cancer (4) and breast cancer (5). Furthermore, the expression levels of REST were demonstrated to increase with age, and elderly people possess higher REST expression compared with younger individuals (6,7). Regardless of age, the expression of REST may also be upregulated when neural cells become malignant (8,9). In addition, a previous study identified a positive correlation between the expression of REST and the malignant degree of glioma (10).

The protein size of REST is 1,097 amino acids. As a transcriptional repressor, REST may inhibit its target

genes by binding to the neuron-restrictive silencer element (NRSE) (11,12). By connecting with two distinct corepressors, mSin3 and CoREST, REST is able to regulate the expression of neuronal genes, via the recruitment of histone deacetylase complex (HDAC) to the promoters of REST-regulated genes in neuronal and non-neuronal cells (13,14). In addition, REST has been demonstrated to act as a negative regulator of genes associated with numerous aspects of neuronal function, including neurogenesis, neural differentiation and preserving the specific neural phenotype (15-17). Furthermore, REST has been implicated to serve various roles in numerous cellular environments in the nervous system (18).

## 3. REST in glioma: Poor prognosis

In glioma, REST acts as an oncogene, which promotes proliferation and invasion of glioma cells. A previous study of 21 medulloblastoma tumor specimens demonstrated that all tumor specimens expressed higher levels of REST compared with the adjacent normal cerebellum tissue sections (6 strongly and 11 weakly) (19). Furthermore, when treated with REST-VP16, which is a competitor of endogenous REST/NRSF for DNA binding, the potential of REST intracranial tumorigenicity was suppressed and the growth of established tumors in nude mice was inhibited (19). These results indicated that overexpression of REST may contribute to medulloblastoma tumorigenesis and accelerate the proliferation of tumor cells. Furthermore, Blom *et al* (20) further hypothesized that REST may not directly influence glioma tumorigenesis but contribute to tumor development beyond the DNA level. The mRNA expression levels of REST were increased 2-5-fold in glioma tissue compared with in normal cerebral cortex tissues. Notably, when REST expression was knocked out in mice, the apoptotic and neuronal differentiation programs of malignant glioblastoma multiforme cell-derived xenograft tumors were activated (9). Taken together, these data suggested that REST may promote glioma development and is elevated in glioma compared with adjacent normal tissues. Furthermore, the mRNA expression levels of REST were significantly increased in grade III-IV glioma compared with in grade I-II glioma (10), thus suggesting that the expression of REST is positively correlated with the degree of glioma malignancy. However, low expression of REST was also detected in mouse neuroblastoma cell lines, such as NS20Y (6,21,22). Due to this discrepancy, further research is required to validate the expression levels of REST in various species.

Due to the aforementioned expression profiles of REST in glioma, the present review hypothesized that there may be an association between REST expression levels and the prognosis of patients with glioma. Numerous studies have demonstrated that high REST expression is associated with poor prognosis in glioma. Wagoner and Roopra reported that patients with 'REST enhanced malignancies' (REM) tumors exhibited a significantly more aggressive disease course compared with patients with non-REM tumors (23), thus suggesting that enhanced REST may induce a more aggressive disease, and patients who express lower REST may have a better outcome. To verify these findings, a mouse xenograft experiment was conducted, which demonstrated that the injection of 'high REST' glioma cells into mice resulted in reduced survival compared with mice injected with 'low REST' glioma cells (8). In addition, elevated REST levels were associated with poor overall and event free survival in human patients with medulloblastoma (24). A further study in Chinese patients with neuroblastoma demonstrated that patients with late stage (grade IV) glioma who had higher REST activity were associated with poor survival compared with those in early stages (grades I and III combined) (25). Based on these experimental results, it is indicated that patients with glioma and high REST expression may have a poor prognosis.

## 4. Regulation and function of REST4

*Alternative splicing of REST.* It has been reported that >90% of human genes, including phosphatase and tensin homolog, p53 (26), neurofibromatosis type I (27), and ATP binding cassette subfamily C member 1 (28), undergo alternative splicing, which contributes to the diversity of the transcriptome and proteome (29-31); there is no exception for REST. Several splicing variants of REST, including REST, REST1, REST4 and REST5 (Fig. 1), have been identified. However, among these variants, only REST4 and REST5 have been detected in neuronal tissues (32). REST4, with the insertion of exon N between exon III and IV of the REST gene, results in the early termination of translation, and has been reported to include the N-terminal repression domain and number 1-5 zinc finger motifs, whereas REST5 lacks the number 5 zinc finger motif (32,33). In addition, it has been demonstrated that the number 5 zinc finger motif is of crucial importance for the nuclear targeting of REST (34). Accordingly, it may be hypothesized that only REST4 maintains the ability of nuclear targeting in neuronal tissues.

*Inducing factors of REST4.* Recently, factors that regulate alternative splicing of REST and induce REST4 have been identified. Neural-specific Ser/Arg repeat-related protein of 100 kDa (nSR100/SRRM4) is a transcriptional repressor of genes required for neurogenesis. Raj *et al* (35) demonstrated that nSR100 can directly induce alternative splicing of REST transcripts, and thus produce the REST isoform, REST4. However, in non-neural cells, REST inhibits the expression of nSR100. In addition, pioglitazone, a highly selective peroxisome proliferator-activated receptor γ (PPARγ) agonist that is used to treat diabetes, has been reported to increase the expression of REST4 in HepG2 cells (36). In addition, protein kinase A, which inhibits repression of the cholinergic gene locus by REST, has been demonstrated to promote the production of REST4, whereas its inhibitor, H89, was able to suppress the expression of REST4 in PC12 cells (37).

*REST4 weakens REST function.* Since it lacks the C-terminal repression domain of REST and the CoREST binding domain, REST4 loses the transcriptional silencing ability of REST; however, it can suppress the silencing function of REST by inhibiting the binding of NRSF/REST to repressor element-1 (RE-1)/NRSE. This process is known as the antisilencer mechanism of gene regulation (37,38). In addition, REST4 has been determined to be localized to the nucleus, and this nuclear-targeting signal exists in the zinc-finger domains (21). However, the complicated mechanism by which REST4

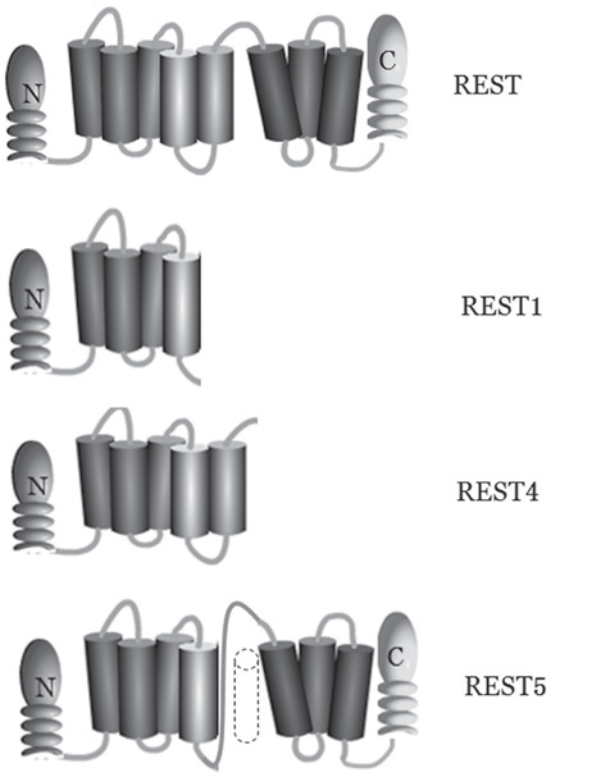


Figure 1. Protein structure of REST, REST1, REST4 and REST5. REST contains the N-terminal repression domain, C-terminal repression domain and number 1-8 zinc fingers. However, REST1 contains the N-terminal repression domain and number 1-4 zinc fingers, REST4 contains the N-terminal repression domain and number 1-5 zinc fingers, and REST5 does not contain the number 5 zinc finger. REST, repressor element silencing transcription factor.

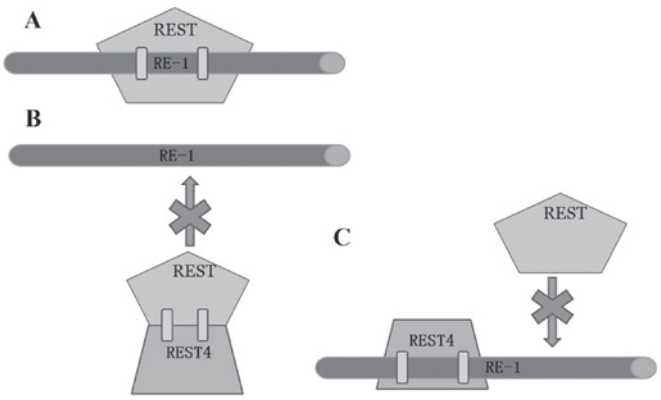


Figure 2. Relationship between RE-1, REST and REST4. (A) In normal conditions, REST can target RE-1/NRSE to serve a role in transcriptional repression. (B) REST4 may form a heterodimer with REST blocking the ability of REST to bind to RE-1/NRSE. (C) REST4 may combine with RE-1/NRSE to inhibit REST binding to RE-1/NRSE. NRSE, neuron-restrictive silencer element; RE-1, repressor element-1; REST, repressor element silencing transcription factor.

interferes with REST remains controversial (Fig. 2). Previous studies have indicated that REST4 forms a heterodimer with REST, blocking the ability of REST to bind to its DNA recognition sequence, RE-1/NRSE (37,39). It has also been suggested that REST4 combines with RE-1/NRSE; however, the protein-DNA interaction is considerably weaker than that of REST, thus resulting in weakened transcriptional repression (40). Furthermore, a recent study indicated that human glioma tissues that expressed REST4 exhibited reduced REST mRNA expression compared with tissues that did not express REST4 (10). Based on these findings, the present review hypothesized that REST4 may combine with REST; therefore, REST4 may be considered a therapeutic marker that can inhibit the expression of REST, so as to prevent the proliferation of glioma cells and tumor growth. Further studies are required to validate the comprehensive cell biological processes by which REST4 affects REST.

## 5. Regulation of REST expression in glioma

Recently, HDAC inhibitors (HDACIs) have been suggested to possess therapeutic potential for patients with REST-positive medulloblastoma. In REST-positive medulloblastoma, HDACIs, such as benzamides (MS-275) and suberoylanilide hydroxamic acid, were reported to decrease REST protein expression via a post-transcriptional mechanism. With the decline in REST expression, one of its target genes, synapsin 1, was increased, thus resulting in inhibition of glioma cell growth (24).

A previous study indicated that REST alone cannot induce tumorigenesis in neural cells (41). In addition, some human medulloblastomas coexpress abnormally high levels of Myc and REST (42). Majumder demonstrated that in the Myc plus REST-expressing group, but not in the control group, tumors arose in the mouse cerebellum (43). In addition, the Wnt pathway has been reported to activate REST gene transcription by stabilizing the β-catenin protein (44); this pathway may be associated with Myc-REST-mediated medulloblastoma tumorigenesis (43).

β-transducin repeats-containing protein (β-TrCP) is an E3 ubiquitin ligase, which has been reported to ubiquitinate REST, and derepress REST target genes (23,45). Therefore, loss of β-TrCP expression can worsen glioma progression by reducing REST degradation and enhancing REST function. The target genes of REST in glioma have recently been verified; there were only 17 target genes extracted in cell line data and only 14 target genes in tumor samples (25), but not 24 genes, which were previously detected in three non-neuronal cells (46). In addition, the target genes were slightly different in the cell line and tumor samples (25).

In addition to the aforementioned factors, telomere repeat-binding factor 2 (TRF2) has been reported to influence the growth of glioma via the regulation of REST expression. A recent study indicated that TRF2 depletion may inhibit the proliferation and reduce the survival of glioma by activating DNA checkpoints, and rendering cells vulnerable to apoptosis. In addition, the potential underlying mechanism may be that depletion of TRF2 derepresses REST target genes resulting in cell cycle arrest and the acquisition of differentiated neuron properties, including the expression of neuronal proteins (47). Another potential mechanism suggests that depletion of TRF2 may reduce REST levels in glioma, so as to increase the levels of β-III tubulin and L1 cell adhesion molecule (L1CAM), two neuronal proteins that are REST target genes (48). However, how β-III tubulin and L1CAM influence glioma remains unknown (Fig. 3).

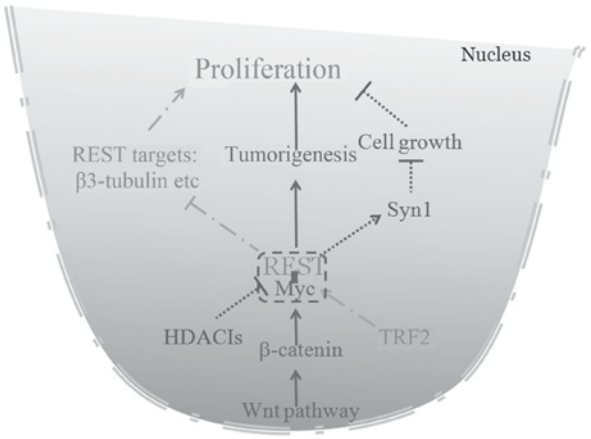


Figure 3. Regulation of REST expression, which affects glioma cell proliferation. Arrows indicate promotion or induction, whereas blunt arrows indicate inhibition or decrease. The three types of line, dotted, solid and dashed lines, represent three regulating pathways. HDACIs, histone deacetylase inhibitors; REST, repressor element silencing transcription factor; Syn1, synapsin 1; TRF2, telomere repeat-binding factor 2.

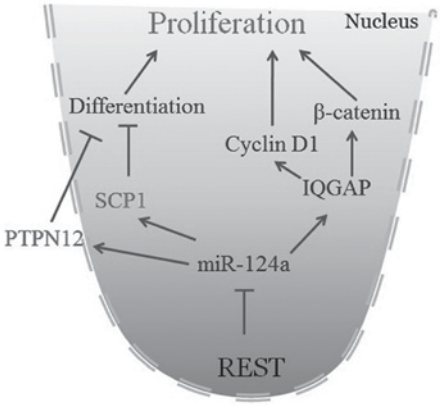


Figure 4. REST inhibits miR-124a so as to promote glioma cell proliferation in various manners. Arrows indicate promotion or induction, whereas blunt arrows indicate inhibition or decrease. IQGAP1, IQ motif containing GTPase activating protein 1; PTPN12, protein tyrosine phosphatase, non-receptor type 12; REST, repressor element silencing transcription factor; SCP1, synaptonemal complex protein 1.

In addition to these aforementioned factors, pioglitazone, an antidiabetic drug, has also been reported to affect REST expression in glioma. When U87 cells were treated with 50 and 100 $\mu$M pioglitazone, the relative expression levels of REST were significantly decreased and cell growth was inhibited (10). As a result, pioglitazone may be a potential treatment for glioma with high REST expression. When treating glioma combined with diabetes, doctors should keep in mind that pioglitazone may influence glioma progression via REST. However, this hypothesis requires further experiments to verify it in humans.

## 6. Gene regulation by REST in glioma

MicroRNAs (miRNAs) are essential regulators of tissue specificity (49), and REST has been reported to be associated with numerous miRNAs (50). Among them, miRNA (miR)-124a is the most representative miRNA in glioma (Fig. 4), whose overexpression is associated with improved prognosis (51). Notably, REST has been demonstrated to repress miR-124a gene expression in glioma through binding to RE-1 (52). Therefore, miR-124a levels may be decreased in high REST glioma. In addition, an increase in miR-124a expression has been reported to reduce the expression of synaptonemal complex protein 1 (SCP1) and protein tyrosine phosphatase, non-receptor type 12 (PTPN12) (9), two small phosphatases that inhibit differentiation and increase proliferation, respectively (53). Therefore, it may be concluded that REST maintains the self-renewal and tumorigenic potential of glioma cells through suppression of miR-124a and dysregulation of SCP1 and PTPN12. Furthermore, Lu *et al* demonstrated that the IQ motif containing GTPase activating protein 1 (IQGAP1) is a direct target of miR-124a in glioma cells. miR-124a restoration can suppress the expression of IQGAP1 and β-catenin. Furthermore, IQGAP1 suppression was able to inhibit cell proliferation and invasion by suppressing β-catenin and downstream cyclin D1 (54). Therefore, REST may inhibit glioma cell differentiation, so as to promote proliferation and invasion, via the miR-124a and IQGAP1 pathway.

## 7. Conclusion

REST is a negative regulator of genes, which exerts important functions in glioma. High REST expression has been detected in glioma, particularly in high-grade glioma. The involvement of some biological factors on the regulation of REST may explain the expression variation in glioma. The majority of studies have demonstrated that REST has a vital effect on glioma proliferation and progression. Furthermore, REST expression has been reported to be associated with glioma outcome; increased REST expression may result in shorter overall survival. Notably, REST may be considered a prognostic factor, and reducing REST expression may be a potential therapeutic strategy for the treatment of glioma.

REST4 is a splicing variant of REST that can inhibit its expression. Although the underlying mechanism remains to be elucidated, inducing REST4 does appear to decrease REST expression. Therefore, it may be hypothesized that the inducing factors of REST4, such as nSR100 and PPARγ, may decrease the expression of REST so as to ease glioma progression and improve the prognosis of patients. However, this hypothesis requires further laboratory and clinical exploration.

However, it remains to be determined how REST impacts glioma progression and prognosis. In addition, it is unknown as to whether the REST signaling pathway may exert the same effects in glioma as is it does in non-neural cancer. The function of REST4 in glioma also remains to be elucidated, and it remains to be determined whether the expression levels of REST4, similar to REST, will influence glioma outcome. Therefore, further studies should focus on promoting clinical developments of REST and its splicing variants in glioma. In addition, the interplay between REST and REST4 in glioma remains controversial. Further *in vitro* and *in vivo* studies of REST and REST4 may identify the underlying mechanisms. In addition, further exploration is required

to determine the complete signaling pathway of REST expression in glioma.

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