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ORIGINAL ARTICLE

Expression profiling of RE1-silencing transcription factor (*REST*), REST corepressor 1 (*RCOR1*), and Synapsin 1 (*SYN1*) genes in human gliomas

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Summary

Purpose: *The repressor element 1 (RE-1) silencing transcription factor (REST) is a transcription factor which represses the expression of neuronal differentiation-related genes including SYN1 gene. CoREST, encoded by RCOR1 gene, binds to the REST protein for remodeling of chromatin structure. Although there is a relation among REST, RCOR1, and SYN1 genes, the role of these genes in glioma tumors is still unclear. In this study, expressions of REST, RCOR1, and SYN1 genes were detected in primary cultures derived from tumor samples of diffuse astrocytoma (DA), anaplastic oligodendroglioma (AO), and glioblastoma multiforme (GBM) cases.*

Methods*: Expression profiles were analysed by RT-qPCR and the copy number variations were examined with qPCR in primary cultures. ChIP assay was performed to show binding characteristics of REST and CoREST proteins on promoter region of SYN1 gene.*

Results: *Means of relative expression for REST were as follows: 0.7898, 0.7606, and 0.7318 in DA, AO, and GBM groups, respectively. For RCOR1, expression means in DA, AO, and GBM groups were 0.7203, 0.7334, and 0.7230, respectively. SYN1 expression means were as follows: 0.3936, 0.3192, and 0.3197 in DA, AO, and GBM groups, respectively. Neither gain nor loss of copy numbers were detected for REST and RCOR1 genes in all groups. Copy loss for SYN1 was detected in primary culture of a DA case. REST and CoREST presented positive precipitation pattern on promoter region of SYN1 gene.*

Conclusions: *Expressions of REST and RCOR1 genes may downregulate SYN1 expression in gliomas. Low expression pattern of SYN1 may maintain cancer stem-like phenotype which contributes to development of gliomas.*

Key words: *gene expression, gliomas, RCOR1, REST, SYN1*

Introduction

Gliomas are central nervous system tumors that originate from glial cells such as astrocytes or oligodendrocytes which maintain and support the physiology of neuron cells. According to World Health Organization, gliomas are classified as astrocytoma (grade II), oligoastrcytoma (grade II), oligodendroglioma (grade II), anaplastic astrocytoma/oligodendroglioma (grade III) and glioblastoma (grade IV) [1]. Classification system of gliomas is based on the presence of necrosis in tumor tissue, mitotic activity, and vascular or micro-vascular proliferation [1]. Histopathological features of gliomas are associated with tumor recurrence, therapy response, and median survival of patients. Glioblastoma tumors exhibit resistance to therapy and tumor recurrence after therapy. Besides, medi-

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an survival for glioblastoma patients ranges from 14 to 16 months [2,3]. Studies indicate that gene mutations, co-deletions, and extra-chromosomes are related with WHO grading and the prognosis of gliomas [4].

The repressor element 1 (RE-1) silencing transcription factor (REST) is a transcription factor consisting of DNA binding and repressor domains [5,6]. DNA binding domain of REST binds to RE-1 sequence on promoter region of target gene, resulting in repression of expression [6]. It is known that REST protein suppresses several neural differentiation-related genes in the non-neuronal cells, while *REST* expression is down-regulated in neuronal precursor cells [5,7-11]. In carcinogenesis, *REST* can act as a tumor suppressor or an oncogene. A splice variant of *REST* gene in small cell lung cancer cells (SCLC) causes production of truncated mature mRNA and this mRNA variant down-regulates function of wild type REST protein [12]. Contrary to its tumor suppressor function in SCLC, *REST* can act as an oncogene in neuroblastoma and medulloblastoma tumors and over-expression of *REST* has been identified for these tumors [13,14]. Single nucleotide polymorphisms of *REST* leading to amino acid substitutions are observed in primary and secondary glioblastomas [15]. Furthermore, these polymorphisms in *REST* are detected in other gliomas such as astrocytoma, oligoastrocytoma, oligodendrogliomas [15]. In addition to mutations, amplification of *REST* is detected in glioblastomas [15]. *REST* expression is found in GBM specimens and maintains self-renewal and tumor-initiating capacity of GBM stem-like cells. High *REST* expression also induces aggressive properties of GBM tumors in *in vivo* studies [16,17].

Repressor domain of REST protein (RD2) interacts with REST corepressor 1 which is encoded by the *RCOR1* gene. REST and CoREST are assembled with the histone demethylase LSD1/KDM1A and the histone deacetylases 1 and 2 (HDAC1/2) proteins to modify chromatin structure [6]. Chromatin repression by this complex leads to down-regulation of gene expression. For histone modification, a complex consisting of both REST and CoREST may interact with different proteins such as SUMO and human positive coactivator 4 (PC4) [18,19]. All three members of the CoREST family proteins are expressed in brain tissue and this expression profile is observed at common territories of brain. However, interactions of CoREST with HDAC proteins in histone remodeling complex may show specific affinity depending on CoREST family members [20]. REST and CoREST can also regulate the expression of the *SYN1* gene. *SYN1* gene expression is up-regulated during brain development and this gene is expressed in adult neural tissue. Repression of the *REST* gene with second-generation antisense oligonucleotides increases *SYN1* expression in liver cells [21]. *SYN1* promoter has REST binding motifs and its expression is directly downregulated by REST protein [22]. Although there is a link among *REST, RCOR1*, and *SYN1* genes, interplay of these genes in glioma tumors is still unclear.

In this study, expressions of *REST, RCOR1*, and *SYN1* genes were analyzed in primary cell cultures derived from tumor samples of DA, AO, and GBM cases. In order to demonstrate the correlation between copy number status and expression values, RT-qPCR was used. To show the binding features of REST and CoREST transcription factor on promoter region of *SYN1* gene, chromatin immunoprecipitation assay was performed for glioma derived primary cultures.

Methods

Patients

Forty-nine patients with brain tumor (18 female, 31 male) were recruited into the study. Informed consent was obtained from each patient and the study was approved by the Local Ethics Committee of Ege University, School of Medicine. The distribution of tumors according to histopathology was as follows: 11 DA (22.5%), 8 AO (16.3%), and 30 GBM (61.2%). The mean age was 53.00 ± 13.92 years. Demographic and clinical features of cases are presented in Table 1. Primary cell cultures of the tumor tissues were cultured in BIOAMF-1 basal medium (Biological Industries, Israel) containing to 10,000U/ml penicillin, 10mg/ml streptomycin, 2 mM L-glutamine at 37°C in a humidified 95% air and 5% CO_2 atmosphere. Expanded cell cultures of the third passage were used.

Isolation of total RNA and genomic DNA

In order to detect mRNA expression of *REST, RCOR1*, and *SYN1* genes, total RNA were isolated from primary tumor cell cultures. High Pure RNA Isolation Kit (Roche Applied Science, Mannheim, Germany) was used to isolate total RNA from primary tumor cell cultures according to manufacturer's instructions. Total RNA was converted to cDNA with using Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics, Mannheim, Germany). cDNA samples were used for gene expression analysis. For detection of copy number variations of *REST, RCOR1*, and *SYN1* genes, genomic DNA samples were used. High Pure PCR Template Preparation Kit for Genomic DNA (Roche Diagnostics,

Table 1. Demographic and clinical features of glioma cases

Gender	*Case number*	*Age, years, mean*	*Standard deviation*	*Min age (years)*	*Max age (years)*
F	18	49.78	15.40	15	73
M	31	54.87	12.87	30	79
Total	49	53.00	13.92	15	79
Diagnosis			*Grade*	*Case number*	*%*
Diffuse astrocytoma			II	11	22.5
Anaplastic oligodendroglioma			III	8	16.3
Glioblastoma			IV	30	61.2
Total				49	100

F: female, M: male

Mannheim, Germany) was used to obtain DNA samples according to manufacturer's instructions.

Detection of gene expression and copy number variation

For the detection of gene expression, 2.6 µl cDNA were mixed with 7.4 µl Light Cycler PCR Master Mix and sterile bidistilled water was added to PCR mix. The expression studies of *REST, RCOR1*, and *SYN1* genes were performed with Real Time Online qRT-PCR according to the Taqman protocol. Relative Quantification software (Roche Diagnostics GmbH, Mannheim, Germany) was used for data analysis. Absolute quantification method was used for the expression study. For absolute quantification, ten-fold serial dilutions were used to create standard curve for endogeneous control (*GAPDH*) (LightCycler h-G6PDH Housekeeping Gene Set, Roche, cat no: 03261883001). Cycle threshold (CT) values were obtained for both target genes and the endogeneous control in all cases. These CT values for each sample were transformed to copy numbers for every gene according to standard curve of *GAPDH* (efficiency:2.137, slope:-3.032, intercept:38.29). The expression data was normalized to that of the *GAPDH* gene to calculate relative ratio. UPL probe system was also used to detect copy number variation (CNV) and the PCR reaction was performed with Real Time Online qRT-PCR. Probes and primers for detection of CNV were designed with Probe Finder Software. Contrary to gene expression assay, genomic DNA was used for CNV study. Relative ratios of *REST, RCOR1*, and *SYN1* genes were obtained with log2 presented copy numbers compared to diploid DNA material from WI-38 cell line. All probe and primer sequences for gene expression and CNV studies are given in Table 2.

Chromatin immunoprecipitation

Chromatin immunoprecipitation assay was performed to show the binding features of REST and CoREST proteins on promoter region of *SYN1* gene using REST (Millipore, 17-641) and CoREST ChIP antibodies (Millipore, 07-455). Human RNA Polymerase II ChampionChIP Antibody Kit (Sabiosciences, Germany-QIAGEN GmbH) was used as positive and negative control. Assays were performed for each antibody for 24 cases. Champion ChIP-One Day Kit (Sabiosciences, Germany-QIAGEN GmbH) was used for ChIP assay. Experimental steps were performed according to the manufacturer's instructions. Primary tumor cell cultures obtained from tumor tissues were cultured until their numbers reached to 4-6x10^6. Chromatin structures in cell lysates were sheared with Sonicator S-4000 (Misonix Ultrasonic Liquid Processors, QSonica, LLC, Newtown, CT). Cell lysates were sheared under the following settings: amplitude: 30; power: 0.5 W; time: 2 sec ON and 15 sec OFF; total time: 16 sec. Size of chromatin samples were between 300 bp and 1000bp. For precipitation, non-immune serum for negative control, target specific antibody, and anti-RNA polymerase II antibody for positive control were used. After obtaining the DNA promoter fragments, qPCR was performed. DNA samples precipitated with REST and Co-REST antibodies were examined by qPCR with SYBR Green I for promoter of *SYN1* gene. The DNA was subjected to 30 cycles of PCR. REST and ChampionChIP Antibody Kit includes specific control qPCR primers (SYN I and GAPDH, Human IGX respectively) used for chromatin immunoprecipitation. CT values were not observed for IG samples according to 30 cycles. For data analysis, positive control was achieved from precipitated DNA by RNA polymerase II antibody with using *GAPDH* proximal promoter primers. *SYN1* promoter primers were used for samples. Copy numbers of precipitated DNA with REST antibody, CoREST antibody, and RNA polymerase II antibody were calculated for samples according to standard curve of *GAPDH*. Copy numbers of REST and CoREST were normalized to obtain fold chance with respect to copy number of RNA polymerase II for a sample. Primers for ChIP assay were given in Table 2.

Statistics

Means, standard deviations, maximum-minimum values, and 95% confidence interval (CI) were exam-

Table 2. Primer and probe sequences of *REST*, *RCOR1* and *SYN1* genes for gene expression, copy number variation and ChIP analysis

Primer and probe sequences used for gene expression analysis			
Gene	*Forward primer*	*Reverse primer*	*Probe*
GAPDH	gaaggtgaaggtcggagtc	gaagatggtgatgggatttc	caagcttcccgttctcagcc
REST	tggaaaatgcaactatttttcaga	tctgagaacttgagtaaggacaaagt	caggagaa
RCOR1	cgaggactaaaactagtgtgatgg	tgcctcttccagttcatcct	ggaggag
SYN1	gacggaagggatcacatcat	ctggtggtcaccaatgagc	ggtggtgg
Primer and probe sequences used for detection of gene copy number			
Gene	*Forward primer*	*Reverse primer*	*Probe*
REST	ggctatttcgtgacatttaaacac	tctgagaacttgagtaaggacaaagt	caggagaa
RCOR1	cgaggactaaaactagtgtgatgg	tcccctaagtggttagacaacac	ggaggag
SYN1	tgaaagcacggtgttactgc	ctggtggtcaccaatgagc	ggtggtgg
Primer sequences used for ChIP			
Gene	*Forward primer*	*Reverse primer*	*Probe*
SYN1	tgggttttaggaccaggatg	ggtgctgaagctggcagt	-

Table 3. Expression profiles of *REST, RCOR1*, and *SYN1* genes in glioma derived primary cultures. RR defines means of relative ratios in cases of specific group. Mean describes means of relative ratios and copy numbers of target and endogenous control genes for all groups

REST gene expression			
Grade	*REST*	*GAPDH*	*RR*
Diffuse astrocytoma	1.07E+08±1.32E+08	4.86E+09±5.36E+09	0.7898±0.0708
Anaplastic oligodendroglioma	9.55E+08±1.73E+09	9.58E+10±1.85E+11	0.7606±0.0598
Glioblastoma	2.12E+08±8.64E+08	1.16E+10±5.29E+10	0.7318±0.1975
Mean	3.1E+08±9.88E+08	2.38E+10±8.79E+10	0.7495±0.1603
RCOR1 gene expression			
Grade	*RCOR1*	*GAPDH*	*RR*
Diffuse astrocytoma	1.93E+07±2.19E+07	4.86E+09±5.36E+09	0.7203±0.0621
Anaplastic oligodendroglioma	2.61E+08±5.19E+08	9.58E+10±1.85E+11	0.7334±0.0807
Glioblastoma	6.52E+06±1.40E+07	1.16E+10±5.29E+10	0.7239±0.3469
Mean	5.09E+07±2.20E+08	2.38E+10±8.79E+10	0.7246±0.2729
SYN1 gene expression			
Grade	*SYN1*	*GAPDH*	*RR*
Diffuse astrocytoma	7.34E+05±1.31E+06	1.54E+10±1.98E+10	0.3936±0.2228
Anaplastic oligodendroglioma	1.25E+07±2.32E+07	9.58E+10±1.85E+11	0.3192±0.3081
Glioblastoma	3.41E+06±1.24E+07	1.76E+10±5.47E+10	0.3197±0.2900
Mean	4.30E+06±1.37E+07	2.99E+10±8.80E+10	0.3362±0.2757

ined with descriptive statistics for gene expression, copy number variation, and ChIP results of *REST, RCOR1,* and *SYN1* genes in gliomas. One-way analysis of variance (ANOVA) was performed to examine data for groups. Relationship of genes was presented with correlation analysis. Relations between gene copy numbers and gene expression were analyzed with regression analysis. In analysis of all parameters for all groups, discriminate analysis was performed. SPSS v15.0 software was used for statistical analysis and a p value < 0.05 was accepted as statistically significant.

Results

Expression profiles of REST, RCOR1, and SYN1 genes

REST expression in primary cultures derived from tumor samples of 49 cases was examined

with RT-PCR. *GAPDH* gene was used as the housekeeping gene to normalize data for obtaining relative ratios. Means of relative expression for *REST* were as follows: 0.7898 in DA group, 0.7606 in AO group, and 0.7318 in GBM group (Table 3). There was no significant difference for *REST* expression according to groups (p>0.05). Mean value of *REST* expression in the total group was 0.774. Means of relative expression ratio for *RCOR1* were 0.7203, 0.7334, and 0.7230 for DA, AO, and GBM groups, respectively (Table 3). *RCOR1* gene expression was similar among all groups. Relative expression means of *SYN1* were 0.3936, 0.3192, and 0.3197 for DA, AO, and GBM groups, respectively (Table 3). There was no significant difference for mean values of *SYN1* expression among groups (p>0.05). Means of *SYN1* expression were lower than means of *REST* and *RCOR1* among groups. In primary tumor cell cultures of 2 of AO cases and 7 of GBM cases, no *SYN1* expression was found. Contrary to DA group, there was no *SYN1* expression in 25% of all AO and GBM groups.

Copy number variation of REST, RCOR1, and SYN1 genes

Neither gain nor loss of copy numbers was detected for the *REST* gene in all groups. In addition, there was no significant difference in terms of copy numbers of *REST* among groups. There was not copy number gain or loss for *RCOR1* in groups. Copy number of *SYN1* could not be detected in the primary tumor cell cultures of 2 DA cases, 3 AO cases, and 7 GBM cases. Copy loss was only detected in a primary tumor cell culture derived from a DA case. There was no significant difference in terms of copy numbers for *SYN1* among groups. Copy number variation results are presented in Figure 1. Correlation analysis of the copy numbers and expression results are displayed in Table 4.

Chromatin immunoprecipitation for promoter region of SYN1 gene

DNA samples obtained from primary tumor cell cultures were precipitated with antibodies against REST, CoREST, and RNA polymerase II proteins. ChIP assay was performed to show the binding of REST and CoREST proteins on promoter region of *SYN1*. RNA polymerase antibody was used as the positive control. Copy numbers obtained from REST and CoREST antibodies were normalized to that of the copy

Table 4. Correlations among expressions and copy number variations of *REST, RCOR1*, and *SYN1* genes in glioma derived primary cultures

		REST	*CoREST*	*SYN1*	*CNV-REST*	*CNV-CoREST*	*CNV-Syn1*
REST	Pearson correlation	1					
	p (2-tailed)						
	N	53					
CoREST	Pearson correlation	-0.372 **	1				
	p (2-tailed)	0.006					
	N	53	53				
SYN1	Pearson correlation	0.296 *	-0.056	1			
	p (2-tailed)	0.031	0.689				
	N	53	53	53			
CNV-REST	Pearson correlation	-0.050	-0.181	-0.005	1		
	p (2-tailed)	0.729	0.204	0.972			
	N	51	51	51	51		
CNV-CoREST	Pearson correlation	-0.137	-0.124	0.009	0.640 **	1	
	p (2-tailed)	0.339	0.385	0.952	0.000		
	N	51	51	51	51	51	
CNV-Syn1	Pearson correlation	-0.106	-0.081	-0.193	-0.183	-0.111	1
	p (2-tailed)	0.514	0.621	0.232	0.265	0.500	
	N	40	40	40	39	39	40

* p< 0.05
** p< 0.01

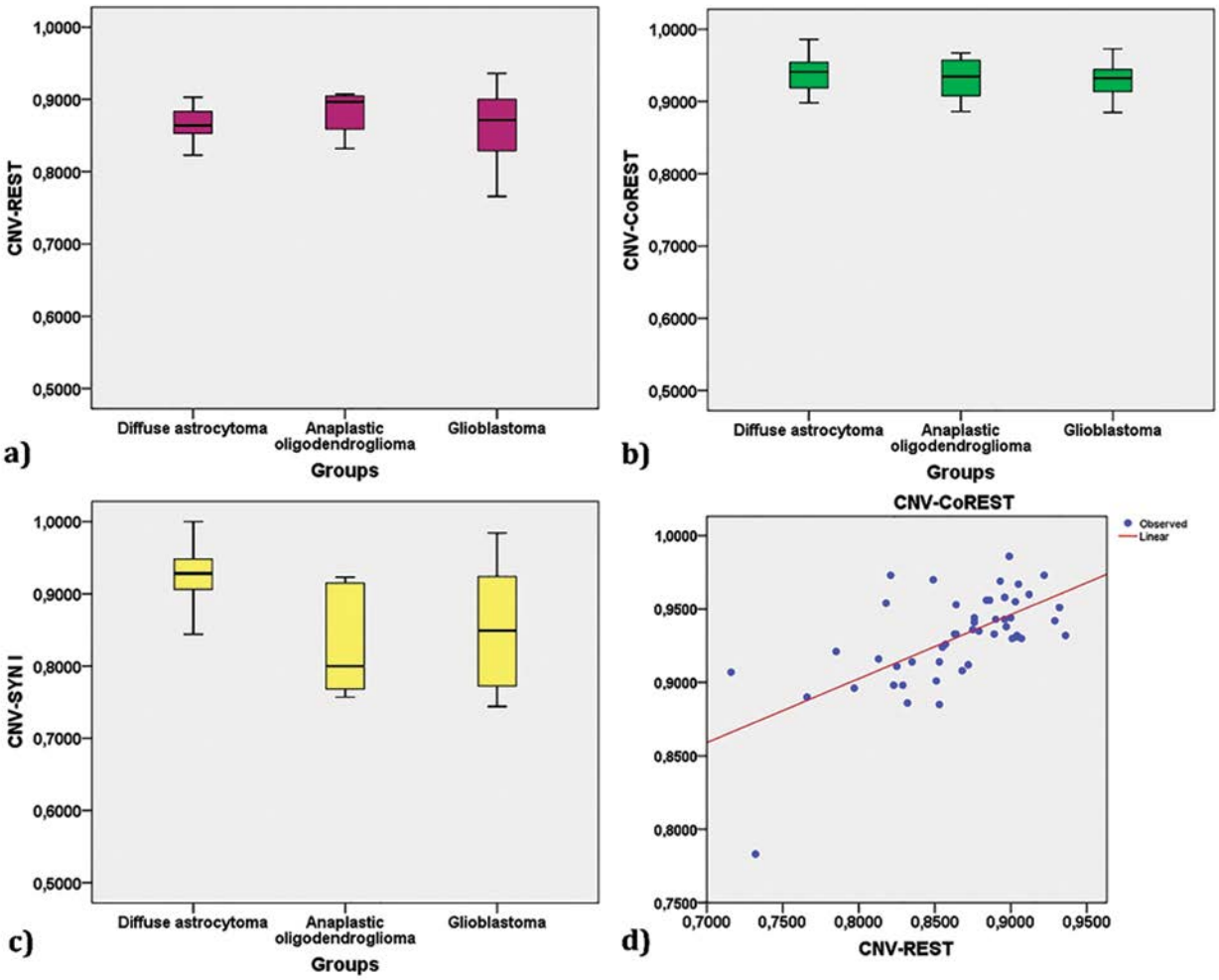

Figure 1. A: Copy number variations of *REST* were 0.8655±0.0266 in DA, 0.8699±0.0608 in AO and 0.8617±0.0521 in GBM (p>0.05). **B:** CNV of *RCOR1* were 0.9389±0.0265 in DA, 0.9186±0.0602 in AO and 0.9305±0.0243 in GBM (p>0.05). **C:** CNV of *SYN1* were 0.4021±1.5914 in DA, 0.8326±0.0805 in AO and 0.8539±0.0839 in GBM (p>0.05) genes in glioma derived primary cultures. **D:** There was a positive correlation between *REST* and *RCOR1* copy numbers (p=0.000).
CNV: Copy number variations, DA:diffuse astrocytoma, AO:anaplastic oligodendroglioma, GBM:glioblastoma multiforme

number data obtained from RNA polymerase II antibody for samples. Both REST and CoREST presented positive precipitation patterns on the promoter region of the *SYN1*. Relative immunoprecipitation ratios of REST protein were 1.0558, 1.0670, and 1.0365 for DA, AO, and GMF groups, respectively (Table 5). Means of relative ratio for CoREST were detected as 1.0765, 1.0834, and 1.0651 for DA, AO, and GBM groups, respectively (Table 5). For both proteins, significant differences for relative ratios were not observed among tumor groups. In AO group, *SYN1* expression exhibited 30% correlation within relative ratios of REST and CoREST immunoprecipitation. According to discriminate analysis with all parameters including expression, copy number and ChIP results of all genes in all groups, 89.5% of all cases were compatible with their clinical data.

Discussion

REST protein regulates expressions of several genes including neuronal differentiation-related genes. This transcriptional repressor is composed of a DNA binding domain and a repressor domain [6]. DNA binding domain of REST protein binds to specific DNA sequence, called as the RE-1 binding site/neuron-restrictive silencer element, on promoter region of target genes [6]. In response to REST protein binding on RE-1 binding site, neuronal differentiation-related genes in non-neuronal cells are repressed to maintain differentiated phenotype [23]. *REST* is normally expressed in both embryonic and neuronal stem cells. However, this expression pattern is diminished during differentiation process and *REST* expression is rarely observed in differentiated neurons [6,24-28].

It has been shown that *REST* is expressed

Table 5. Chromatin immunoprecipitation analysis of REST and CoREST proteins for promoter region of *SYN1* gene. ChIP experiments were performed in 24 cases. Mean describes means of fold changes in groups according to RNA polymerase II antibody. For each group, 95% confidence interval limits are shown. Minimum and maximum fold changes in groups are given as min and max

	Grade	*N*	*Mean ± SD*	*95% confidence interval*	*Min*	*Max*
CHIP-REST	2	4	1.0588 ± 0.0688	(0.9492-1.1683)	0.9872	1.1513
	3	6	1.0670 ± 0.0471	(1.0176-1.1164)	1.0096	1.1138
	4	14	1.0365 ± 0.0688	(0.9968-1.0763)	0.9131	1.1677
	Total	24	1.0479 ± 0.0630	(1.0213-1.0745)	0.9131	1.1677
CHIP-CoREST	2	4	1.0765 ± 0.0374	(1.0170-1.1360)	1.0404	1.1290
	3	6	1.0834 ± 0.0514	(1.0295-1.1374)	1.0123	1.1322
	4	14	1.0651 ± 0.0429	(1.0403-1.0898)	0.9816	1.1594
	Total	24	1.0716 ± 0.0432	(1.0533-1.0898)	0.9816	1.1594

in both GBM cell lines and GBM tumor samples [16,29]. Conti et al. found *REST* expression in GBM tumors whose mRNA level ranged from 2- to 5-fold compared to normal cerebral cortex tissues [16]. Wagoner et al. examined *REST* function between gliomas and non-neoplastic brain tissue by a bioinformatic approach which is based on the use of available microarray datasets [30]. In bioinformatic analysis, expressions of *REST* target genes in glioma tumors are down-regulated when compared to non-neoplastic brain tissue. With respect to our data, *REST* expression at mRNA level was detected in all groups and means of *REST* expression among groups were similar. Means of relative expression for *REST* were 0.7898 in DA group, 0.7606 in AO group, and 0.7318 in GBM group. Conti et al. identified low or high copy number amplification at 4q12 in GBM tumor specimens [16]. On the other hand, Wagoner et al. observed that amplification at 4q12 is often occurring without amplification of *REST* in gliomas [30]. We sought to determine if mRNA expression of *REST* would be in line with copy number status. Thus, expression studies were accompanied with CNV studies. Neither gains nor losses of copy numbers were detected for *REST*. Our data suggest that *REST* is expressed in glioma tumors. *REST* expression may not be frequently accompanied with copy number status in gliomas.

In addition to *REST*, *RCOR1* may also be associated with *REST* to induce tumorigenesis. Repressor domain 2 of REST protein binds to CoREST protein to form functional protein complex for chromatin remodeling in the nucleus [6]. CoREST protein shows coexistence with different proteins such as HDAC and LSD1 to form chromatin remodeling complex [18,19]. Binding of CoREST with LSD1 on nucleosomal structure can lead to posttranscriptional modification of histone tails, leading to silencing of neuronal differentiation-related genes [6,18,19,31]. Although REST protein is implicated in chromatin remodeling with CoREST protein to regulate expression of target genes, *RCOR1* has not been well examined in gliomas. In the current study, all primary tumor cell cultures showed expression patterns for *RCOR1*. There was no significant difference in terms of expressions of *RCOR1* among tumor groups. In addition, neither copy losses nor copy gains for *RCOR1* were detected. There was a positive correlation between *REST* and *RCOR1* copy numbers (p=0.000). Malignant transformation processes in brain tissue may be provided due to aberrant *REST* expression in neurogenesis. In addition, *RCOR1* may be in partnership with *REST* to contribute tumorigenesis in brain tissue.

SYN1 is one of the neuronal genes which is associated with formation and maintenance of synaptic contacts [32]. It is known that REST protein binds to promoter of *SYN1*, resulting in down-regulation [22,33]. Second-generation antisense oligonucleotide mediated suppression of *REST* increases *SYN1* expression in differentiated liver cells [21]. In order to demonstrate binding patterns of REST and CoREST proteins on *SYN1* promoter, ChIP assay was performed and relative ratios were detected for each patient. However, significant differences for relative ratios were not observed among groups. In the AO group, *SYN1* expression exhibited 30% correlation within relative ratios of REST and CoREST immunoprecipitation.

Copy number variation and expression profiles for *SYN1* were also examined for all primary cultures. Means of *SYN1* expression were lower than means of *REST* and *RCOR1* expressions among groups. *SYN1* expression was not detected in primary tumor cell cultures of 2 AO and 7 GBM cases. This result may indicate that *SYN1* expression was repressed in 25% of all AO and

GBM group. In addition, there was a positive correlation between expression values of *SYN1* and *REST* (p=0.031). Copy number of *SYN1* could not be detected in 12 of all cases. Copy loss for *SYN1* was only detected in a primary tumor cell culture derived from a DA case. There was no significant difference in terms of copy numbers for *SYN1* among groups. *SYN1* expression may not be related to copy number variation due to rare occurrence. However, *SYN1* expression may be negatively regulated by REST and CoREST proteins in gliomas. In the current study, primary cell cultures derived from tumor samples were used. Culture media with growth factors were applied to induce differentiation for obtaining tumor cells in specific phenotype [34,35]. In order to prevent cells from phenotypic drift in culture condition, low passage number and culture medium without growth factors were used. However, compared to tumor tissue, gene expression profile may exhibit difference due to adaptation of cells in any culture condition. Not identifying an effect of cell culture conditions on gene expression pattern may be a limitation for this study.

Inhibition of *REST* leads to differentiation and maturation of the stem cell-derived dopamine neuronal progenitors [36]. *REST* also provides self-renewal features of glioblastoma stem-like cells (GSC). Besides, knockdown of *REST* in GSCs generates tumors with higher apoptotic and lower invasive properties [17]. Knockdown of *REST* in GBM cells, not stem-like cells, also shows similar results regarding self-renewal and tumor-initiating capacity [16]. It is known that high grade brain tumors have high percentages of cancer stem-like cells. In addition, cancer stem like-cells are directly related with resistance to chemo-/radiotherapy and increased recurrence rates after surgery [37-40]. Maintenance of GSCs properties may be supported by low level expression of *SYN1*, which involves terminal differentiation of neurons and is regulated by *REST*.

In conclusion, our findings revealed that expressions of *REST* and *RCOR1* may cause down-regulation of *SYN1* in gliomas. Deregulated expression of *SYN1* may maintain cancer stem-like phenotype which contributes to development of gliomas. This phenotype may be associated with the grades of gliomas leading to poor prognosis and resistance to chemotherapy.

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Conflict of interests

The authors declare no confict of interests.

References

1. Louis DN, Ohgaki H, Wiestler OD et al. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol 2007;114:97-109.
2. Choucair AK, Levin VA, Gutin PH et al. Development of multiple lesions during radiation therapy and chemotherapy in patients with gliomas. J Neurosurg 1986;65:654-658.
3. Thakkar JP, Dolecek TA, Horbinski C et al. Epidemiologic and molecular prognostic review of glioblastoma. Cancer Epidemiol Biomarkers Prev 2014;23:1985-1996.
4. Vigneswaran K, Neill S, Hadjipanayis CG. Beyond the World Health Organization grading of infiltrating gliomas: advances in the molecular genetics of glioma classification. Ann Transl Med 2015;3:95.
5. Coulson JM. Transcriptional regulation: cancer, neurons and the REST. Curr Biol 2005;15:R665-668.
6. Majumder S. REST in good times and bad: roles in tumor suppressor and oncogenic activities. Cell Cycle 2006;5:1929-1935.
7. Ballas N, Battaglioli E, Atouf F et al. Regulation of neuronal traits by a novel transcriptional complex. Neuron 2001;31:353-365.
8. Hakimi MA, Bochar DA, Chenoweth J, Lane WS, Mandel G, Shiekhattar R. A core-BRAF35 complex containing histone deacetylase mediates repression of neuronal-specific genes. Proc Natl Acad Sci USA 2002;99:7420-7425.
9. Ballas N, Grunseich C, Lu DD, Speh JC, Mandel G. REST and its corepressors mediate plasticity of neuronal gene chromatin throughout neurogenesis. Cell 2005;121:645-657.
10. Ballas N, Mandel G. The many faces of REST oversee epigenetic programming of neuronal genes. Curr Opin Neurobiol 2005;15:500-506.
11. Mandel G, Fiondella CG, Covey MV, Lu DD, Loturco JJ, Ballas N. Repressor element 1 silencing transcription factor (REST) controls radial migration and temporal neuronal specification during neocortical development. Proc Natl Acad Sci USA 2011;108:16789-16794.

12. Coulson JM, Edgson JL, Woll PJ, Quinn JP. A splice variant of the neuron-restrictive silencer factor repressor is expressed in small cell lung cancer: a potential role in derepression of neuroendocrine genes and a useful clinical marker. Cancer Res 2000;60:1840-1844.
13. Fuller GN, Su X, Price RE et al. Many human medulloblastoma tumors overexpress repressor element-1 silencing transcription (REST)/neuron-restrictive silencer factor, which can be functionally countered by REST-VP16. Mol Cancer Ther 2005;4:343-349.
14. Negrini S, Prada I, D'Alessandro R, Meldolesi J. REST: an oncogene or a tumor suppressor? Trends Cell Biol 2013;23:289-295.
15. Blom T, Tynninen O, Puputti M et al. Molecular genetic analysis of the REST/NRSF gene in nervous system tumors. Acta Neuropathol 2006;112:483-490.
16. Conti L, Crisafulli L, Caldera V et al. REST controls self-renewal and tumorigenic competence of human glioblastoma cells. PloS One 2012;7(6):e38486.
17. Kamal MM, Sathyan P, Singh SK et al. REST regulates oncogenic properties of glioblastoma stem cells. Stem Cells 2012;30:405-414.
18. Ouyang J, Shi Y, Valin A, Xuan Y, Gill G. Direct binding of CoREST1 to SUMO-2/3 contributes to gene-specific repression by the LSD1/CoREST1/HDAC complex. Mol Cell 2009;34:145-154.
19. Das C, Gadad SS, Kundu TK. Human positive coactivator 4 controls heterochromatinization and silencing of neural gene expression by interacting with REST/NRSF and CoREST. J Mol Biol 2010;397:1-12.
20. Barrios AP, Gomez AV, Saez JE et al. Differential properties of transcriptional complexes formed by the CoREST family. Mol Cell Biol 2014;34:2760-2770.
21. Sedaghat Y, Bui HH, Mazur C, Monia BP. Identification of REST-regulated genes and pathways using a REST-targeted antisense approach. Nucl Acid Ther 2013;23:389-400.
22. Paonessa F, Latifi S, Scarongella H, Cesca F, Benfenati F. Specificity protein 1 (Sp1)-dependent activation of the synapsin I gene (SYN1) is modulated by RE1-silencing transcription factor (REST) and 5'-cytosine-phosphoguanine (CpG) methylation. J Biol Chem 2013;288:3227-3239.
23. Schoenherr CJ, Paquette AJ, Anderson DJ. Identification of potential target genes for the neuron-restrictive silencer factor. Proc Natl Acad Sci USA 1996;93:9881-9886.
24. Sun YM, Greenway DJ, Johnson R et al. Distinct profiles of REST interactions with its target genes at different stages of neuronal development. Mol Biol Cell 2005;16:5630-5638.
25. Sun YM, Cooper M, Finch S et al. Rest-mediated regulation of extracellular matrix is crucial for neural development. PloS One 2008;3(11):e3656.
26. Gao Z, Ure K, Ding P et al. The master negative regulator REST/NRSF controls adult neurogenesis by restraining the neurogenic program in quiescent stem cells. J Neuroscience 2011;31:9772-9786.
27. Singh SK, Veo BL, Kagalwala MN, Shi W, Liang S, Majumder S. Dynamic status of REST in the mouse ESC pluripotency network. PloS One 2012;7(8):e43659.
28. Lee M, Ji H, Furuta Y, Park JI, McCrea PD. p120-catenin regulates REST and CoREST, and modulates mouse embryonic stem cell differentiation. J Cell Sci 2014;127:4037-4051.
29. Zhang D, Li Y, Wang R et al. Inhibition of REST Suppresses Proliferation and Migration in Glioblastoma Cells. Int J Mol Sci 2016;3;17: E664.
30. Wagoner MP, Roopra A. A REST derived gene signature stratifies glioblastomas into chemotherapy resistant and responsive disease. BMC Genomics 2012;13:686.
31. Baron R, Vellore NA. LSD1/CoREST is an allosteric nanoscale clamp regulated by H3-histone-tail molecular recognition. Proc Natl Acad Sci USA 2012;109:12509-12514.
32. Cesca F, Baldelli P, Valtorta F, Benfenati F. The synapsins: key actors of synapse function and plasticity. Prog Neurobiol 2010;91:313-348.
33. Taylor P, Fangusaro J, Rajaram V et al. REST is a novel prognostic factor and therapeutic target for medulloblastoma. Mol Cancer Ther 2012;11:1713-1723.
34. Engstrom PG, Tommei D, Stricker SH, Ender C, Pollard SM, Bertone P. Digital transcriptome profiling of normal and glioblastoma-derived neural stem cells identifies genes associated with patient survival. Genome Med 2012;4:76.
35. Galli R, Binda E, Orfanelli U et al. Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. Cancer Res 2004;64:7011-7021.
36. Trzaska KA, Reddy BY, Munoz JL, Li KY, Ye JH, Rameshwar P. Loss of RE-1 silencing factor in mesenchymal stem cell-derived dopamine progenitors induces functional maturity. Mol Cell Neurosci 2008;39:285-290.
37. Liu G, Yuan X, Zeng Z et al. Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. Mol Cancer 2006;5:67.
38. Pallini R, Ricci-Vitiani L, Montano N et al. Expression of the stem cell marker CD133 in recurrent glioblastoma and its value for prognosis. Cancer 2011;117:162-174.
39. Tamura K, Aoyagi M, Ando N et al. Expansion of CD133-positive glioma cells in recurrent de novo glioblastomas after radiotherapy and chemotherapy. J Neurosurg 2013;119:1145-1155.
40. Chen D. Tumor formation and drug resistance properties of human glioblastoma side population cells. Mol Med Rep 2015;11:4309-4314.