Research Article

Low \textit{REST} Expression Indicates a Biomarker of Poor Prognosis in Patients with Renal Cell Carcinoma

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It was initially found that neural-restrictive silencer factor/repressor 1-silencing transcription factor (\textit{REST}) is a transcriptional repressor of neuronal genes in nonneuronal cells. However, it is reported to be abundantly expressed in various types of aggressive cancer cells. In this study, we evaluated the expression patterns of \textit{REST} in renal cell carcinoma and found that its expression is lower in tumor tissues compared to normal tissues. The chi-square test showed that the low \textit{REST} expression was closely related to patients’ clinicopathologic parameters, including the pathologic stage and survival status. ROC curve showed that \textit{REST} had excellent clinical diagnostic prospect. In addition, patients with low \textit{REST} expression had poor over survival (OS) and relapse-free survival (RFS). Univariate and multivariate Cox regression analysis confirmed that the low \textit{REST} expression was an independent predictor of poor prognosis in renal cell carcinoma. Gene set enrichment analysis identified P53 pathway, reactive oxygen species pathway, glycolysis, DNA repair, cholesterol homeostasis, and MYC targets V2 enriched with low \textit{REST} expression phenotype. These results suggested that \textit{REST} may be a novel biomarker for the diagnosis and prognosis of renal cell carcinoma in clinical applications.

1. Introduction

Renal cell carcinoma (KIRC), a common urinary system tumor, accounts for 2% to 3% of human malignant tumors [1–3]. It has been reported that 90 percent of patients had been diagnosed with KIRC [4, 5]. In many countries, the incidence and case fatality of KIRC are steadily increasing [6, 7]. Although the significant progress had been made in diagnosis and treatment, the patient’s prognosis is still worse. In recent years, with the further research in tumor molecular biology, targeted therapy has become a new diagnosis and treatment strategy in current clinical applications [8]. Therefore, the search for new molecular targets is extremely important for the clinical diagnosis, treatment, and prognostic monitoring of KIRC.

\textit{RE} 1-silencing transcription factor (\textit{REST}), also known as neural-restrictive silencing factor (NRSF), is a zinc-finger transcription factor that inhibits target gene transcription by recruiting transcription coinhibitors such as histone deacetylase (HDACs) [9–11]. Moreover, \textit{REST} can serve as a hub for the recruitment of multiple chromatin-modifying enzymes, revealing the interdependencies between enzymes
that influence gene regulation [12]. In addition, REST inhibits the expression of neuroendocrine-related genes during neuronal differentiation [13–15]. As a result, REST was initially regarded as the primary regulator of neurogenesis. Recent studies have reported that REST can inhibit the occurrence of tumors, and REST gene deletion or mutation is closely related to the occurrence of many tumors such as small-cell lung cancer [16], prostate cancer [17], and ovarian cancer [18].

In the current study, our group focused on the relationship between the REST expression and clinicopathological features, diagnostic value, and prognostic assessment of patients with KIRC. We compared the REST mRNA expression between cancer patients and healthy individuals and analyzed the application prospect and diagnostic significance of the REST expression in KIRC. In addition, we investigated the association between the REST expression and the clinicopathologic features, including OS and RFS. The results revealed that the REST expression is an independent risk factor for poor survival, suggesting that REST may be a valuable diagnostic and prognostic biomarker for KIRC.

2. Materials and Methods

2.1. Dataset Mining and Database Collection. We first obtained RNAseq of REST and clinical information of KIRC patients from The Cancer Genome Atlas (TCGA) dataset. RNAseq was converted to RSEM by estimating the log2 (x + 1) normalized counts which are used for subsequent analysis by selecting R software (version 4.0.1) [19].

2.2. Data Analysis. The data was analyzed by the program package in the R software. The box plot showed the mRNA expression of REST in the KIRC dataset through ggplot2 visual analysis. The chi-square test was used to evaluate the correlation between the REST expression and clinical characteristics of KIRC patients. The receiver operating characteristic (ROC) curve was used to evaluate the diagnostic value of expression through the pROC software. Kaplan-Meier survival curves were performed to compare OS and RFS in different groups of patients. Risk regression models were used to perform univariate and multivariate analysis to evaluate the prognostic value of the REST expression. P < 0.05 is considered statistically significant.

2.3. Gene Set Enrichment Analysis (GSEA). In order to detect the distribution of predefined genomes and determine the potential mechanism to influence the effect of the REST expression on the prognosis of KIRC patients, we opted for GSEA (version 4.0.3). This analysis was performed through the “h.all.v7.2.symbols.gmt” gene set in the Molecular Signatures database [20]. Gene sets with a normal P value <0.05 were regarded as significantly enriched.

3. Results

3.1. The Patient Clinical Characteristics and Expression of REST in KIRC. Through using R software, clinical data of 373 patients were collected from the TCGA database, including the patient’s age, gender, histological type, histologic grade, histologic stage, and TNM classification, as well as radiation therapy, residual tumor, vital status, and relapse-free survival (Table 1). Subsequently, we analyzed the expression pattern of REST. As shown in Figure 1, REST was significantly higher in normal tissues than tumor tissues (P = 2.20 × 10^-16), which indicated that the expression of REST is downregulated in KIRC. Additionally, differences in the REST expression were observed according to patient histological grade (P = 0.00153), pathological stage (P = 0.000102), T classification (P = 0.000292), N classification (P = 0.0000724), and vital status (P = 8.18 × 10^-8).

3.2. The Diagnostic Significance of the REST Expression and Relationship between Clinical Characteristics in KIRC.
We previously showed that the REST expression was significantly downregulated in KIRC. To evaluate the diagnostic significance of the REST expression, ROC curve was established. We found that the REST expression had excellent diagnostic value overall (AUC = 0.861; Figure 2).

Subsequently, we analyzed the diagnostic value of the REST expression in different stages of KIRC, including stage I cancer (AUC = 0.826), stage II cancer (AUC = 0.864), stage III cancer (AUC = 0.906), and stage IV (AUC = 0.901). Subsequently, we divided patients into two groups (high expression and low expression) according to the ROC curve threshold (Figure 2(a)). As shown in Table 2, the low REST expression was significantly associated with patient age (P = 0.00500), histologic grade (P = 0.0260), pathologic stage (P = 0.0140), T classification (P = 0.0260), M classification (P = 0.00400), and vital status (P = 0.000).

3.3. The Effect of the Low REST Expression for OS in Patients with KIRC. We used survival analysis to evaluate the effect of the REST expression on the overall survival (OS) of kidney cancer patients. As shown in Figure 3, Kaplan-Meier survival curves shown that the low REST expression significantly decreased the patient’s OS (P < 0.000100). In addition, we also observed that male
Figure 2: Diagnosis value of REST expression in KIRC. The ROC curves of REST expression in cancerous vs. normal tissues was generated. Cancerous vs. normal liver tissues was analyzed in different stages of KIRC.

Table 2: Associations between the clinicopathologic variables and REST expression.

| Parameters   | Variables | Numbers | High Prop (%) | Low Prop (%) | $X^2$ | $P$ value |
|--------------|-----------|---------|---------------|--------------|-------|-----------|
| Age          | ≥55       | 362     | 60            | 56.60        | 302   | 70.73     | 7.773      | 0.005      |
|              | <55       | 171     | 46            | 43.40        | 125   | 29.27     |            |            |
| Gender       | Male      | 345     | 68            | 64.15        | 277   | 64.87     | 0.0193     | 0.890      |
|              | Female    | 188     | 38            | 35.85        | 150   | 35.13     |            |            |
| Histologic grade | G1       | 14      | 4             | 3.77         | 10    | 2.36      |            |            |
|              | G2        | 230     | 52            | 49.06        | 178   | 41.98     |            |            |
|              | G3        | 205     | 39            | 36.79        | 166   | 39.15     | 11.006     | 0.026      |
|              | G4        | 76      | 8             | 7.55         | 68    | 16.04     |            |            |
|              | GX        | 5       | 3             | 2.83         | 2     | 0.47      |            |            |
|              | I         | 269     | 67            | 63.21        | 202   | 47.31     |            |            |
| Pathologic stage | II       | 56      | 12            | 11.32        | 44    | 10.30     | 10.566     | 0.014      |
|              | III       | 124     | 16            | 15.09        | 108   | 25.29     |            |            |
|              | IV        | 84      | 11            | 10.38        | 73    | 17.10     |            |            |
|              | M0        | 422     | 95            | 89.62        | 327   | 76.94     |            |            |
|              | M1        | 79      | 11            | 10.38        | 68    | 16        | 11.002     | 0.004      |
|              | MX        | 30      | 0             | 0            | 30    | 7.06      |            |            |
|              | N0        | 240     | 48            | 45.28        | 192   | 44.97     |            |            |
| N classification | N1       | 17      | 2             | 1.89         | 15    | 3.51      | 0.7340     | 0.693      |
|              | NX        | 276     | 56            | 52.83        | 220   | 51.53     |            |            |
|              | T1        | 274     | 68            | 64.15        | 206   | 48.24     |            |            |
|              | T2        | 68      | 12            | 11.32        | 56    | 13.11     | 9.267      | 0.026      |
| T classification | T3       | 180     | 24            | 22.64        | 156   | 36.54     |            |            |
|              | T4        | 11      | 2             | 1.89         | 9     | 2.11      |            |            |
|              | Dead      | 161     | 13            | 12.26        | 148   | 34.66     |            |            |
|              | Survival  | 372     | 93            | 87.74        | 279   | 65.34     | 20.204     | 0.0001     |
patients with low REST expression had a shorter OS ($P = 0.0180$), and female patients ($P = 0.000590$). Subgroup analysis found that the low REST expression significantly affects patient OS in G1/G2 ($P = 0.0120$), G3/G4/GX ($P = 0.00530$), stage I/II ($P = 0.027$), stage III/IV ($P = 0.0330$), T1 ($P = 0.0200$), T3 ($P = 0.0170$), N0 ($P = 0.0230$), N1/NX ($P = 0.00120$), and M0 ($P < 0.000100$). Subsequently, we selected potential variables that were significant in univariate analysis to conduct multivariable Cox analysis (Table 3). We found that low REST is an
Table 3: Univariate and multivariate analysis of over survival in patients with KIRC.

|                  | Hazard ratio | Univariate analysis CI95 | P value | Hazard ratio | Multivariate analysis CI95 | P value |
|------------------|--------------|--------------------------|---------|--------------|--------------------------|---------|
| Age              | 1.89         | 1.30-2.75                | 0.001   | 1.53         | 1.03-2.26                | 0.033   |
| Gender           | 1.04         | 0.75-1.43                | 0.826   |              |                          |         |
| Histologic grade | 2.06         | 1.71-2.47                | 0.0001  | 1.56         | 1.25-1.94                | 0.0001  |
| Pathologic stage | 1.96         | 1.71-2.24                | 0.0001  | 2.04         | 1.40-2.97                | 0.0001  |
| M classification | 2.47         | 1.94-3.19                | 0.0001  | 0.82         | 0.50-1.35                | 0.431   |
| N classification | 0.86         | 1.07-1.01                | 0.063   |              |                          |         |
| T classification | 2.07         | 1.74-2.46                | 0.0001  | 0.80         | 0.55-1.18                | 0.260   |
| REST             | 1.30         | 1.15-1.46                | 0.0001  | 1.20         | 1.04-1.39                | 0.010   |

3.4. The Effect of the Low REST Expression for RFS in Patients with KIRC. We have previously shown that the low REST expression predicts a poor prognosis for OS among KIRC patients. To assess the expression in KIRC, and its expression gradually decreased with patients’ higher historical level and tumor level. In addition, our results showed that the low REST expression had poor OS and RFS. Univariate and multivariate Cox regression analysis confirmed that REST was an independent predictor of poor prognosis among KIRC patients.

Previous studies have reported that REST is highly expressed in a variety of tumors, including glioma, neuroblastoma, and medulloblastoma [22–24]. However, the expression of REST in KIRC has been rarely reported. In this study, we observed that the REST expression is low in cancerous tissues, which contradicts other findings about the REST expression in tumors, suggesting that the REST expression is complex in tumors. Interestingly, we also found that the REST expression gradually downregulated as histologic grade increasing from G1 to G4, as histologic stage increased from I to IV and as T classification increased from T1 to T3. The reason for the slightly higher expression in patients with GX and T4 is unclear, but this may be due to the limited samples from advanced cancer.

REST is a key target oncogenic transformation and neural differentiation and inhibits transcription by regulating chromatin structure or inhibiting underlying transcription mechanisms [25–27]. During neuron development, REST is the main transcriptional repressor of neuron-specific genes and plays an important role in nonneuron and neuronal progenitor cells through histone deacetylation, chromatin remodeling, methylation, and other mechanisms [28–31]. Recent studies have confirmed that REST is closely related to carcinogenesis and cancer progression [32]. In this study, we observed that the low REST expression gradually decreased with the increase of degree of malignant tumor, which indicated that REST may be an important regulatory gene for tumor occurrence and development. In addition, ROC curve analysis provided evidence that REST can be developed as a biomarker for the diagnosis of KIRC.

Although the association of REST with various cancer types has been reported, the mechanism by which REST plays a role in cancer progression and tumorigenesis is still unclear. Studies have verified that decreased REST expression promotes epithelial cell transformation [33]. In ovarian cancer, REST regulates the growth and survival of tumor cells via the regulation of mTOR signaling [34].
In addition, the REST expression is closely related to the depth of malignant tumor invasion, TNM stage, and local lymph node metastasis, and the patients with high REST expression had a worse overall survival in medulloblastoma [35]. These indicate that REST can be used as a drug target and a new prognostic factor for medulloblastoma. In contrast, our findings suggest that the REST expression in kidney cancer patients is associated with patient OS and
Table 4: Univariate and multivariate analysis of relapse-free survival in patients with KIRC.

|                      | Hazard ratio | CI95        | P value | Hazard ratio | CI95        | P value |
|----------------------|--------------|-------------|---------|--------------|-------------|---------|
| Age                  | 1.33         | 0.93-1.91  | 0.117   | 1.31         | 1.04-1.64  | 0.020   |
| Gender               | 0.77         | 0.54-1.10  | 0.155   |              |             |         |
| Histologic grade     | 1.97         | 1.62-2.38  | 0.0001  | 1.31         | 1.04-1.64  | 0.020   |
| Pathologic stage     | 2.42         | 2.07-2.83  | 0.0001  | 2.62         | 1.84-3.80  | 0.0001  |
| M classification      | 3.42         | 2.69-4.34  | 0.0001  | 1.08         | 0.66-1.78  | 0.762   |
| N classification      | 1.03         | 0.87-1.22  | 0.730   |              |             |         |
| T classification      | 2.34         | 1.94-2.83  | 0.0001  | 0.73         | 0.51-1.05  | 0.092   |
| REST                 | 1.34         | 1.18-1.52  | 0.0001  | 1.21         | 1.04-1.41  | 0.014   |

Table 5: Gene set enrichment analysis in phenotype low among KIRC.

| Name                                      | ES   | NES   | NOM P value |
|-------------------------------------------|------|-------|-------------|
| HALLMARK_P53_PATHWAY                      | 0.44 | 1.82  | 0.000       |
| HALLMARK_REACTIVE_OXYGEN_SPECIES_PATHWAY  | 0.61 | 1.70  | 0.007       |
| HALLMARK_GLYCOLYSIS                       | 0.48 | 1.65  | 0.033       |
| HALLMARK DNA_REPAIR                       | 0.47 | 1.64  | 0.028       |
| HALLMARK CHOLESTEROL_HOMEOSTASIS          | 0.47 | 1.61  | 0.019       |
| HALLMARK MYC_TARGETS_V2                   | 0.57 | 1.55  | 0.041       |

ES: Enrichment score; NES: normalized enrichment score; NOM: nominal.

Figure 5: Gene set enrichment plots. GSEA results showing differential enrichment of genes related to P53 pathway, reactive oxygen species pathway, glycolysis, DNA repair, cholesterol homeostasis, and MYC targets V2 in KIRC cases with low REST expression.
RFS. These data suggested that REST may serve as a potential marker for adjuvant diagnosis, efficacy, and prognosis assessment of KIRC.

To our knowledge, this is the first report on the correlation between REST expression and clinical features and prognosis prediction in KIRC patients based on the TCGA database. Our study revealed that REST had good clinical diagnostic value and is an independent risk factor for poor prognosis in KIRC patients. However, in the future, the structural network and specific mechanism between REST downregulation and shortened survival time of kidney cancer patients still need to be improved, so as to provide better treatment strategies for KIRC patients.

Data Availability
TCGA-KIRC dataset used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest
The authors declare that they have no conflicts of interest.

Authors’ Contributions
Chaoxiang Lv and Yuanguo Li contributed equally to this work.

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References
[1] G. Courthod, M. Tucci, M. Di Maio, and G. V. Scagliotti, “Papillary renal cell carcinoma: a review of the current therapeutic landscape,” Critical Reviews in Oncology/Hematology, vol. 96, no. 1, pp. 100–112, 2015.
[2] I. Richter and J. Dvorák, “Treatment of Metastatic Renal Cell Carcinoma,” Klinicka Onkologie, vol. 31, no. 2, pp. 110–116, 2018.
[3] N. Tariq, N. Mamoon, A. Haroon, Z. Ali, and I. N. Ahmad, “Role of immunohistochemistry in subtyping renal cell carcinomas with overlapping morphological features,” Journal of Ayub Medical College, Abbottabad, vol. 30, no. 3, pp. 325–332, 2018.
[4] R. E. Gray and G. T. Harris, “Renal cell carcinoma: diagnosis and management,” American Family Physician, vol. 99, no. 3, pp. 179–184, 2019.
[5] Q. Wang, H. Zhang, Q. Chen, Z. Wan, X. Gao, and W. Qian, “Identification of METTL14 in kidney renal clear cell carcinoma using bioinformatics analysis,” Disease Markers, vol. 2019, Article ID 5648783, 2019.
[6] M. A. Perezella, R. Dreicer, and M. H. Rosner, “Renal cell carcinoma for the nephrologist,” Kidney International, vol. 94, no. 3, pp. 471–483, 2018.
[7] Y. T. Shih, Y. Xu, C. R. Chien et al., “Rising economic burden of renal cell carcinoma among elderly patients in the USA: part II-an updated analysis of SEER-Medicare data,” Pharmacoeconomics, vol. 37, no. 12, pp. 1495–1507, 2019.
[8] F. D. C. Guerra Liberal, J. M. O’Sullivan, S. J. McMahon, and K. M. Prise, “Targeted alpha therapy: current clinical applications,” Cancer Biotherapy & Radiopharmaceuticals, vol. 35, no. 6, pp. 404–417, 2020.
[9] M. Shimojo, J. H. Lee, and L. B. Hersh, “Role of zinc finger domains of the transcription factor neuron-restrictive silencer factor/repressor element-1 silencing transcription factor in DNA binding and nuclear localization,” The Journal of Biological Chemistry, vol. 276, no. 16, pp. 13121–13126, 2001.
[10] Y. Nakano, M. C. Kelly, A. U. Rehman et al., “Defects in the alternative splicing-dependent regulation of REST cause deafness,” Cell, vol. 174, no. 3, pp. 536–548.e21, 2018.
[11] A. S. Alshawali, H. Wurdak, I. C. Wood, and J. E. Ladbury, “Histone deacetylase inhibitors induce medulloblastoma cell death independent of HDACs recruited in REST repression complexes,” Molecular Genetics & Genomic Medicine, vol. 8, no. 10, p. e1429, 2020.
[12] L. Ooi and I. C. Wood, “Chromatin crosstalk in development and disease: lessons from REST,” Nature Reviews. Genetics, vol. 8, no. 7, pp. 544–554, 2007.
[13] M. Tateno, W. Ukai, E. Hashimoto, H. Ikeda, and T. Saito, “Implication of increased NRSF/REST binding activity in the mechanism of ethanol inhibition of neuronal differentiation,” Journal of Neural Transmission (Vienna), vol. 113, no. 3, pp. 283–293, 2006.
[14] A. C. Ravanpay, S. J. Hansen, and J. M. Olson, “Transcriptional inhibition of REST by NeuroD2 during neuronal differentiation,” Molecular and Cellular Neuroscience, vol. 44, no. 2, pp. 178–189, 2010.
[15] H. J. Kim, A. M. Denli, R. Wright et al., “REST regulates non-cell-autonomous neuronal differentiation and maturation of neural progenitor cells via secretogranin II,” Journal of Neuroscience, vol. 35, no. 44, pp. 14872–14884, 2015.
[16] M. Shimojo, Y. Shudo, M. Ikeda, T. Kobashi, and S. Ito, “The small cell lung cancer-specific isoform of RE1-silencing transcription factor (REST) is regulated by neural-specific Ser/Arg repeat-related protein of 100 kDa (nSR100),” Journal of Biological Chemistry, vol. 276, no. 16, pp. 13126, 2001.
[17] M. Tateno, W. Ukai, E. Hashimoto, H. Ikeda, and T. Saito, “Implication of increased NRSF/REST binding activity in the mechanism of ethanol inhibition of neuronal differentiation,” Journal of Neural Transmission (Vienna), vol. 113, no. 3, pp. 283–293, 2006.
[18] A. C. Ravanpay, S. J. Hansen, and J. M. Olson, “Transcriptional inhibition of REST by NeuroD2 during neuronal differentiation,” Molecular and Cellular Neuroscience, vol. 44, no. 2, pp. 178–189, 2010.
[19] H. J. Kim, A. M. Denli, R. Wright et al., “REST regulates non-cell-autonomous neuronal differentiation and maturation of neural progenitor cells via secretogranin II,” Journal of Neuroscience, vol. 35, no. 44, pp. 14872–14884, 2015.
[20] M. Shimojo, Y. Shudo, M. Ikeda, T. Kobashi, and S. Ito, “The small cell lung cancer-specific isoform of RE1-silencing transcription factor (REST) is regulated by neural-specific Ser/Arg repeat-related protein of 100 kDa (nSR100),” Molecular Cancer Research, vol. 11, no. 10, pp. 1258–1268, 2013.
[21] A. Flores-Morales, T. B. Bergmann, C. Lavallee et al., “Proteogenomic characterization of patient-derived xenografts highlights the role of REST in neuroendocrine differentiation of castration-resistant prostate cancer,” Clinical Cancer Research, vol. 25, no. 2, pp. 595–608, 2019.
[22] S. Gao, X. Zhao, B. Lin, Z. Hu, L. Yan, and J. Gao, “Clinical implications of REST and TUBB3 in ovarian cancer and its relationship to paclitaxel resistance,” Tumour Biology, vol. 33, no. 5, pp. 1759–1765, 2012.
[23] Y. Jiao, Z. Fu, Y. Li, W. Zhang, and Y. Liu, “Aberrant FAM64A mRNA expression is an independent predictor of poor survival in pancreatic cancer,” PLoS One, vol. 14, no. 1, p. e0211291, 2019.
[24] A. Subramanian, P. Tamayo, V. K. Mootha et al., “Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles,” Proceedings of the
[21] D. Nam, "Effect of the absolute statistic on gene-sampling gene-set analysis methods," Statistical Methods in Medical Research, vol. 26, no. 3, pp. 1248–1260, 2017.

[22] P. Zhang, J. D. Lathia, W. A. Flavahan, J. N. Rich, and M. P. Mattson, "Squelching glioblastoma stem cells by targeting REST for proteasomal degradation," Trends in Neurosciences, vol. 32, no. 11, pp. 559–565, 2009.

[23] J. Liang, P. Tong, W. Zhao et al., "The REST Gene Signature Predicts Drug Sensitivity in Neuroblastoma Cell Lines and Is Significantly Associated with Neuroblastoma Tumor Stage," International Journal of Molecular Sciences, vol. 15, no. 7, pp. 11220–11233, 2014.

[24] P. Lawinger, R. Venugopal, Z. S. Guo et al., "The neuronal repressor REST/NRSF is an essential regulator in medulloblastoma cells," Nature Medicine, vol. 6, no. 7, pp. 826–831, 2000.

[25] T. F. Westbrook, G. Hu, X. L. Ang et al., "SCFβ-TRCP controls oncogenic transformation and neural differentiation through REST degradation," Nature, vol. 452, no. 7185, pp. 370–374, 2008.

[26] Q. R. Kong, B. T. Xie, H. Zhang et al., "RE1-silencing Transcription Factor (REST) Is Required for Nuclear Reprogramming by Inhibiting Transforming Growth Factor β Signaling Pathway," Journal of Biological Chemistry, vol. 291, no. 53, pp. 27334–27342, 2016.

[27] J. M. Zullo, D. Drake, L. Aron et al., "Regulation of lifespan by neural excitation and REST," Nature, vol. 574, no. 7778, pp. 359–364, 2019.

[28] R. D’Alessandro and J. Meldolesi, "In PC12 cells, expression of neurosecretion and neurite outgrowth are governed by the transcription repressor REST/NRSF," Cellular and Molecular Neurobiology, vol. 30, no. 8, pp. 1295–1302, 2010.

[29] Y. Shudo, M. Shimojo, M. Fukunaga, and S. Ito, "Pituitary adenylyl cyclase-activating polypeptide is regulated by alternative splicing of transcriptional repressor REST/NRSF in nerve injury," Life Sciences, vol. 143, pp. 174–181, 2015.

[30] A. W. Bruce, I. J. Donaldson, I. C. Wood et al., "Genome-wide analysis of repressor element 1 silencing transcription factor/neuron-restrictive silencing factor (REST/NRSF) target genes," Proceedings of the National Academy of Sciences, vol. 101, no. 28, pp. 10458–10463, 2004.

[31] S. Rockowitz and D. Zheng, "Significant expansion of the REST/NRSF cistrome in human versus mouse embryonic stem cells: potential implications for neural development," Nucleic Acids Research, vol. 43, no. 12, pp. 5730–5743, 2015.

[32] M. T. Epping, A. Lunardi, D. Nachmani et al., "TSPYL2 is an essential component of the REST/NRSF transcriptional complex for TGF _β_ signaling activation," Cell Death & Differentiation, vol. 22, no. 8, pp. 1353–1362, 2015.

[33] S. K. Singh, M. N. Kagalwala, J. Parker-Thornburg, H. Adams, and S. Majumder, "REST maintains self-renewal and pluripotency of embryonic stem cells," Nature, vol. 453, no. 7192, pp. 223–227, 2008.