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Original Article

Significant decrease of a master regulator of genes (REST/NRSF) in high-grade squamous intraepithelial lesion and cervical cancer

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ABSTRACT

Background: The repressor element 1-silencing transcription factor (REST) is a regulator of gene expression, and the Ras association domain family member 1 A (RASSF1A) is an important tumor suppressor gene involved in cancer development. Although extensive characterization of the roles of REST and RASSF1A in cancer development have been reported in cellular models, the link between them and their possible role in the development of squamous intraepithelial lesions (SIL) and squamous cell carcinoma (SCC) of the cervix have not been explored. The aim of this study was to evaluate the expression of REST and RASSF1A in cervical cytological and histological samples from patients diagnosed with SIL or SCC and in CC-derived cell lines.

Methods: We analyzed the expression of REST and RASSF1A by immunocyto/histochemistry in cervical samples from patients ($n = 271$) and in cancer cell lines. Data analyses were performed using the Kruskal–Wallis test and generalized linear models.

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Results: We identified binding sites for REST in *RASSF1A* and observed a significant reduction in REST and *RASSF1A* nuclear expression in samples from patients with high-grade SIL (HSIL) and SCC. For REST, we observed an average decrease of 334 and 423 r.u.d. for HSIL ($n = 21$) and SCC ($n = 18$) compared with non-LSIL ($n = 72$), whereas for RASSF1A, this decrease was 126 and 217 r.u.d., respectively ($p < 0.001$).
Conclusion: Our results provide evidence of the altered expression of REST and RASSF1A in SIL and SCC, with a significant decrease in HSIL, SCC, and SCC-derived cell lines; findings that can contribute to the diagnosis, prognosis, and post-treatment follow-up of patients diagnosed with SIL or SCC.

At a glance of commentary

Scientific background on the subject

Cervical cancer is one of the main types of cancer in women, to which new biomarkers are needed. REST is a master regulator associated with the regulation of multiples targets as RASSF1A and, have been proposed as a potential biomarker in several types of cancer.

What this study adds to the field

A significant reduction in the nuclear expression of REST was observed in high-grade squamous intraepithelial lesion and squamous cell carcinoma derived from cervical cytology and tissues. Also, we reported binding sites for REST in the promoter and introns of RASSF1A.

Cervical cancer (CC) is the fourth most common cancer among women worldwide and seventh in the overall population, with 569,847 new cases diagnosed in 2018 [1]. The incidence and mortality rates reported in Mexico were 82,090 and 46,173, respectively [2]. It has been reported that approximately 80% of CC cases diagnosed in Central and South America were squamous-cell carcinoma (SCC) [3]. Certain well-defined progressive premalignant stages can lead to the development of SCC. Cytologically, these stages are identified as low-grade and high-grade squamous intraepithelial lesions, LSIL and HSIL, respectively; histologically, they are identified as low-grade and high-grade cervical intraepithelial neoplasia, CIN 1 and CIN 2/3, respectively [4,5]. The main cause of CC is persistent infection with high-risk human papillomavirus (HR-HPV), which is identified in approximately 95% of malignant cervical lesions. The integration of viral DNA into the host cell genome interferes with gene regulation, activates oncogenes, and deactivates tumor suppressor genes. In addition, viral DNA integration promotes cell proliferation rates and increases the risk of developing SIL/CIN [6].

Functional changes in the cellular cycle induced by HR-HPV occur through abnormal expression of proteins such as Ki-67, $p16^{INK4a}$, MCM2/TOP2A (ProEx C), lam-5, and others [7–9]. Though several biomarkers have been proposed for the diagnosis of SIL and SCC, integrative analysis is necessary. This analysis must include molecular abnormalities, such as chromosomal anomalies, DNA mutations, alterations in cell cycle checkpoints, and abnormal expression of oncogenes and tumor suppressor genes, allowing for improvement in the diagnosis, treatment monitoring, and detection of women with a potential risk of developing CC.

Several studies have reported on the aberrant expression of the repressor element-1 silencing transcription factor (REST), also known as the neuron-restrictive silencer factor, a master regulator of neuronal gene expression, implicated in cell metabolism and neurogenesis. REST is expressed ubiquitously in mammals and represses the expression of neuronal genes in non-neuronal tissues. REST regulates motility, angiogenesis, apoptosis, cell division, protein synthesis, and maintenance and suppression of neuronal differentiation, by binding to its cognate DNA sequence, the repressor element-1 (RE-1), a conserved 21-bp sequence, widely found in human and mouse genes [10–12]. A bioinformatics-based genome-wide study revealed several REST target genes in humans, based on the altered expression of REST [13], indicating its potential clinical importance. Several studies have reported an aberrant expression of REST in various cancers and benign tumors, such as leiomyomas [14–21]. In some tumors, aberrant expression of REST is associated with increased aggressiveness, early recurrence, and transformation phenotype [22].

In contrast, RASSF1A (Ras-association domain family member 1A) is involved in the activation of apoptosis, microtubule stabilization, and suppression of inflammatory mediators linked to NF-κB and tumor suppressor genes [23,24]. Previously, the loss of its function due to promoter methylation has been reported in approximately 37 types of cancers, including brain, breast, cervical, and lung. All these instances correlated with advanced tumors and poor prognosis [25]. Particularly, *RASSF1A* promoter methylation has been reported in SCC and adenocarcinoma [26].

The importance of REST corresponds with its capacity to regulate a broad range of genes involved in cancer development. However, for SCC of the cervix there is no evidence indicating that REST regulates biomarkers, like *RASSF1A*, which have been associated with oncogenic HPV in invasive CC [27]. Although data on REST functions exist, alterations in REST expression and REST-mediated deregulation of genes implicated in cervical carcinogenesis are unknown. In this work, we performed *in silico* and immunocyto/histochemical analyses to evaluate REST expression in SIL and SCC of the cervix, the usefulness of REST in the prognosis of cervical cancer, and the possible link between REST with *RASSF1A* dysregulation.

Material and methods

A search for RE1 sequences in RASSF1A: in-silico analysis

Gene sequences used in this study were downloaded from the NCBI nucleotide and gene database (https://www.ncbi.nlm.nih.gov/nuccore/; https://www.ncbi.nlm.nih.gov/gene/). The RE-1 sequences in *RASSF1A* were analyzed using Pairwise Sequence Alignment in the Matcher (EMBOSS) server in the EMBL-EBI (https://www.ebi.ac.uk/Tools/psa/emboss_matcher/) [28]. p53 union site was previously reported [29]. CpG island content was analyzed at Data Base of CpG island and analytical tool (DBCAT: http://dbcat.cgm.ntu.edu.tw/).

Participants and samples

Cervical cytology results and tissue samples from 271 and 73 patients respectively, 16 to 76 years of age, and residents of the state of Guerrero, Mexico, were studied. Cytological specimens were provided by the Cervical Cancer Screening Service of the Autonomous University of Guerrero, and biopsies were provided by the Hospital General Dr. Raymundo Abarca-Alarcón, Chilpancingo, Guerrero, Mexico, and by the State Institute of Cancerology Dr. Arturo Beltran Ortega, Acapulco, Guerrero, Mexico. Histopathological diagnosis was performed by a pathologist. The Bethesda system and cervical intraepithelial neoplasia classification were used for cytological and histological diagnosis, respectively [4,30]. The Ethics Committee of the Autonomous University of Guerrero approved the experimental protocols, and each participant gave an informed written consent, in accordance with the Declaration of Helsinki.

Exfoliated cells were collected from the ectocervix and the endocervix, by scraping the ectocervix with an Ayre spatula and sampling the endocervix with a cytobrush, respectively, to ensure that the cytological material was obtained from the transformation zone. Cytological samples were preserved using a preservative solution liquid-PREP™ (LGM International Inc., Melbourne, FL, USA) for at least 1 h prior to processing. Liquid-based cytology specimens were processed according to the manufacturer's protocol liquid-PREP™. Thereafter, cleaning solution was added, and the sample was concentrated by centrifugation at 1000 *g* for 10 min. The supernatant was discarded, and a cellular base solution was added to the cell pellet. The samples were vortexed, ~50 μL aliquots were placed on clean glass slides, and fixed with 95% ethanol. Fixed specimens were stained using the Pap test and results were interpreted by a trained professional.

REST and RASSF1A expression in cervical samples

Immunocytochemistry (ICC): The streptavidin biotin peroxidase-based immunocytochemical method was performed using the CytoScan HRP/DAB cell detection system (Cell Marque Corporation, Hot Springs, AR, USA). The antibodies used included anti-REST (Abcam, Cambridge Science Park, Cambridge, UK) and anti-RASSF1A (Santa Cruz Biotechnology, Dallas, TX, USA). The liquid-base cytology slides and tissues were subjected to antigen retrieval with ImmunoDNA Retriever Citrate (Bio SB, Santa Barbara, CA, USA) for 3 and 9 min, respectively, at 120 °C. Samples were incubated with the primary antibody (REST 1:500 and RASSF1A 1:200) for 2 h; thereafter, with biotin and streptavidin peroxidase. The reaction was developed using the chromogen 3,3′-diaminobenzidine (DAB) and counterstained with Mayer's hematoxylin. *Immunohistochemistry (IHC)*: For immunohistochemistry-using tissue sections, 3-μm thick paraffin sections were cut from a paraffin block, deparaffinized with xylene, and IHC-stained, in a manner similar to that used for ICC. Localization: we evaluated nuclear expression of REST and cytoplasmic expression of RASSF1A. As negative controls, cytological or tissue samples were processed without the primary antibody and as positive controls samples were processed with the $p16^{INK4}$ antibody.

We performed the histoscore (H-score) calculation to evaluate cut-off values for the positive expression of REST in IHC, considering the staining intensity and the percentage of positive cells, as previously reported [31]. The percentage of positive tumor cells per slide (0–100%) was multiplied by the dominant intensity pattern of staining (1 = negative or trace; 2 = weak; 3 = moderate; and 4 = intense); therefore, the overall score ranged from 0 to 400. Specimens with scores of 0–200, 201–300, and 301–400 were classified as negative/low, intermediate, or high expression of REST, respectively.

REST and RASSF1A expression in cell lines

Cell lines used in this study included human keratinocyte (HaCaT) (CLS, Eppelheim, Germany), C33A, SCC-derived cells, non-HPV (ATCC®, Manassas, VA, USA), SiHa, SCC-derived cells, HPV-16-infected cells (ATCC®, Manassas, VA, USA), and HeLa, adenocarcinoma-derived cells, HPV-18-infected cells (ATCC®, Manassas, VA, USA). All cell lines were grown on coverslips, fixed with 3% paraformaldehyde, and processed using immunocytochemistry in the same manner as cervical cytology samples.

REST and RASSF1A mRNA and protein level in several types of cancers: in silico analysis

We downloaded mRNA and protein expression data for REST (https://www.proteinatlas.org/ENSG00000084093-REST/pathology) and RASSF1A (https://www.proteinatlas.org/ENSG00000068028-RASSF1/pathology) in different cancers, from The Cancer Genome Atlas database of the Human Protein Atlas. The mRNA numbers were expressed as number fragments per kilobase of exon per million reads (FPKM).

Data analysis

Densitometric analyses were performed by obtaining 40× photos of the nuclei, 50 cells per tissue, or cytological/cell line. Brightness and contrast were adjusted using the ArcSoft PhotoStudio software. Subsequently, the integrated density and area of each nucleus were quantified using the ImageJ software [32]. In each sample, the background obtained on account of hematoxylin was subtracted from the densitometry value. The average value for each sample was calculated as relative units of densitometry (r.u.d.).

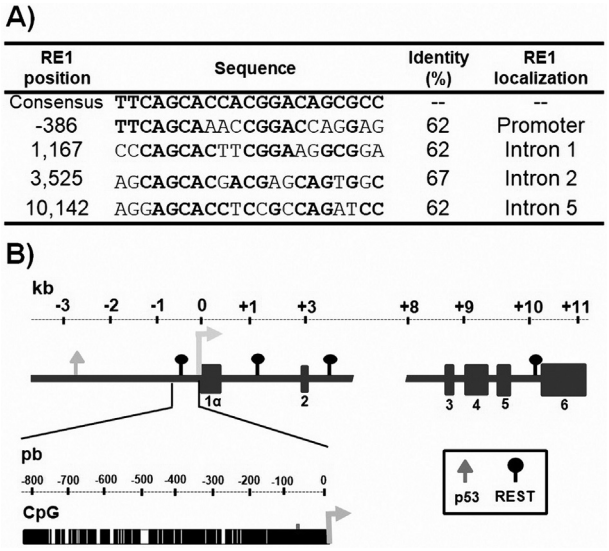

Fig. 1 Search for RE-1 in the *RASSF1A*. A) Characteristics of RE-1 sites in the *RASSF1*. Position of RE-1 sites in the gene, aligned sequences, and scores are shown. B) Schematic representation of *RASSF1* (drawn to scale) and RE1 sequences. Start site of transcription (gray arrow), exons (black boxes), introns (thin lines). The promoter region of *RASSF1* is magnified to show the presence of CpG islands (white lines).

Expression values for REST and RASSF1A were summarized in geometric means because the data did not show normal distribution, after performing the normal distribution analysis using the Shapiro–Wilk test. Comparison between groups was performed using Kruskal–Wallis test followed by Dunn's test of multiple pairwise comparisons. Regression coefficient (β) and 95% confidence interval were calculated using a generalized linear model (GLM), assuming a normal (Gaussian) dispersion [33]. Although the expression values for REST and RASSF1A showed a non-normal distribution, the residual analysis of the GLM in this study reveals that residuals distributed approximately to normal distribution. Residuals versus individual predictors show dependencies of variance (result not shown). The model was adjusted for HPV risk, because for this variable significant variation in REST and RASSF1A expression levels were identified. Notably, age was not included in the GLM model, because age did not significantly correlate with REST and RASSF1A [Fig. S1, Table S1]. The histoscore results were compared between the groups using a chi-square (X^2) test. The analysis was performed using the Stata v.13 software.

Results

RASSF1 contains RE1 sequences for REST binding

REST is a master regulator of gene expression and binds to RE1 sequences in DNA. In the first approach, we searched for RE1 sequences and CpG islands in the *RASSF1A* promoter. *In silico* analyses in the Matcher (EMBOSS) server at the EMBL-EBI webpage, showed four RE1 sites, one located in the *RASSF1A* promoter (position−386), and three in introns (positions 1167, 3525, and 10142) [Fig. 1A]. In addition, CpG island surrounding the RE1, located in the *RASSF1A* promoter, was found [Fig. 1B]. Even though these predictions must be confirmed experimentally, our results suggest a molecular link between REST and *RASFF1*.

Expression of REST and RASSF1A in SIL and cervical cancer

The loss of REST and RASSF1A expression has been reported in different cancers. However, these alterations have not been reported in CC. To analyze REST and RASSF1A expression, we performed ICC in cervical samples from patients without SIL (non-SIL), LSIL, HSIL, and SCC. We identified significant reduction ($p < 0.001$) in REST expression in HSIL and SCC compared to non-SIL. Moreover, using Dunn's test of multiple pairwise comparisons, we identified significant differences in REST expression between non-SIL and LSIL ($p = 0.005$), LSIL and HSIL ($p < 0.001$), and LSIL and SCC ($p < 0.001$), but not between HSIL and SCC ($p = 0.218$). Similar results were observed for RASSF1A expression, where significant decrease was seen, corresponding to the advance of SIL and the development of cancer ($p < 0.001$). On the other hand, a decreased expression of REST

Table 1 Comparison of REST and RASSF1A expression levels in cervical samples from patients with squamous intraepithelial lesions and squamous cell carcinoma.

Group, *n* (%)	REST				RASSF1A			
Immunocytochemistry	Expression level, r.u.d.	P[a]	β (95% CI)[b]	P[b]	Expression level, r.u.d.	P[a]	β (95% CI)[b]	P[b]
N-SIL, 72 (26.6)	443 ± 10.2	Ref	Ref.		276 ± 4.9	Ref	Ref.	
LSIL, 160 (59.0)	474 ± 6.9	0.005	4.4 (−23.5, 32.3)	0.759	236 ± 4.8	<0.001	−18.8 (−37, −1.1)	0.038
HSIL, 21 (7.8)	141 ± 5.6	<0.001	−334 (−376, −292)	<0.001	132 ± 6.0	<0.001	−126 (−151, −101)	<0.001
SCC, 18 (6.6)	44 ± 8.3	<0.001	−423 (−467, −379)	<0.001	37 ± 5.4	<0.001	−217 (−243, −191)	<0.001

Data are geometric mean ± standard error.

[a] Dunn's test.

[b] Generalized linear model [family(gaussian) link(identity)] adjusted for HPV type by oncogenic risk (negative, low-risk, high-risk, and multiple infection); β = regression coefficient and 95% CI = confidence interval. Abbreviations: r.u.d.: Relative units of densitometry; N-SIL: Non-squamous intraepithelial lesion; LSIL: Low grade squamous intraepithelial lesion; HSIL: High grade squamous intraepithelial lesion; SCC: Squamous cell carcinoma.

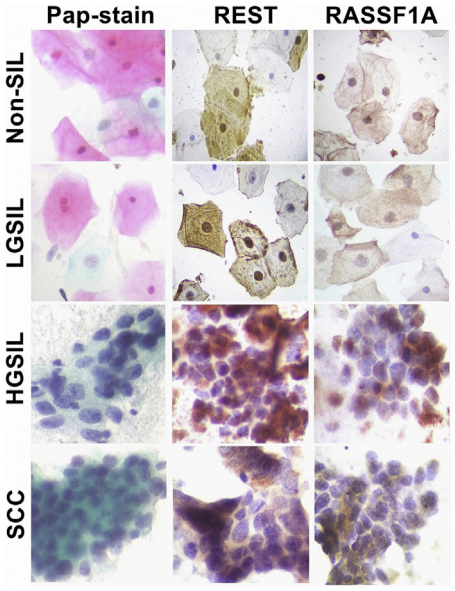

Fig. 2 REST and RASSF1A expression using immunocytochemistry. Representative images (40× amplification) of Papanicolaou (Pap) smear test and immunostaining, using antibodies against REST and RASSF1A. Pap staining was performed for morphological observation of each lesion. Abbreviations used: LSIL: Low-grade squamous intraepithelial lesions (binucleation, perinuclear halo, and karyomegaly); HSIL: High-grade squamous intraepithelial lesions and SCC: Squamous cell carcinoma (for both lesions: karyomegaly, hyperchromatic nuclei, and granular chromatin).

Table 2 REST expression in cervical intraepithelial neoplasia (CIN) and squamous-cell carcinoma (SCC).

REST histoscore	Histological diagnosis			p value[a]
	CIN 1, n (%)	CIN 2/3, n (%)	SCC, n (%)	
Negative or low (0–200)	8 (34.8)	15 (71.4)	23 (79.3)	<0.001
Intermediate (201–300)	6 (26.1)	6 (28.6)	6 (20.7)	
High (301–400)	9 (39.1)	0 (0.0)	0 (0.0)	
Total	23	21	29	

[a] Chi-square (X^2) test.

and RASSF1A is observed with the increase in the grade of SIL to SCC compared to non-SIL. Using generalized linear models, a significant decrease in REST and RASSF1A expression in HSIL and SCC was identified. For REST we found an average decrease of 334 and 423 r.u.d. for HSIL and SCC, compared with non-LSIL, while for RASSF1A this decrease was 126 and 217 r.u.d., respectively [Table 1, Fig. 2].

To analyze the expression and cellular localization of REST, we performed IHC in biopsy specimens of CIN 1, CIN 2/3, and SCC. A significant reduction of nuclear REST expression was noted in some cells of the basal layer in CIN 1, and cytoplasmic accumulation was observed in CIN 2/3 (76.2%) and SCC

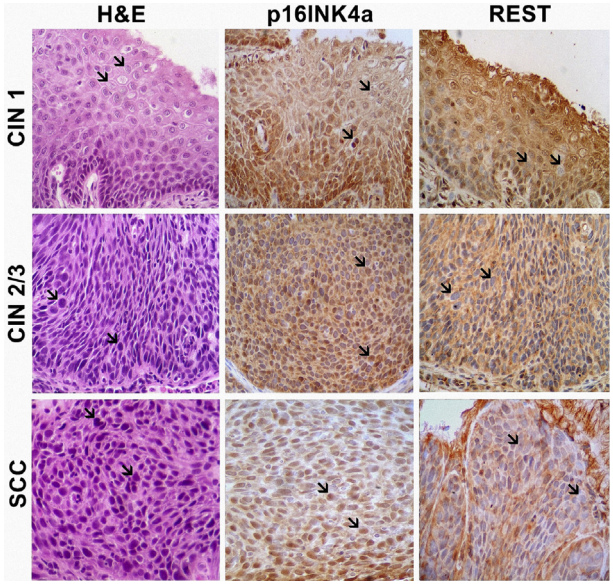

Fig. 3 REST expression using immunohistochemistry. Representative histological samples of REST immunostaining (40× amplification) in cervical intraepithelial neoplasia (CIN) 1 and 2/3 and squamous cell carcinoma (SCC). $p16^{INK4a}$ expression was used as a positive control, whereas hematoxylin–eosin (and not antibody) staining was used as a negative control for immunostaining.

(72.4%). The loss of REST in SCC was observed in all cells, particularly in the pleomorphic nuclei of the tumor nests present in the cervical stroma. Using the histoscore method, we identified negative or low REST expression in 34.8%, 71% and 79.3% of samples with CIN 1, CIN 2/3 and SCC, respectively [Table 2, Fig. 3].

Genotyping of human papillomavirus (HPV) was done using INNO-LiPA, according to the manufacturer's instructions (Fujirebio, Malvern, PA, USA). Among patients who did not have SIL, 37.5% ($n = 27$) were negative for HPV infection, 8.3% ($n = 6$) had low-risk HPV (LR-HPV), 37.5% ($n = 27$) had high-risk HPV (HR-HPV), and 16.7% ($n = 12$) had multiple infection (MI). Among patients with LSIL, 2.5% ($n = 4$) had LR-HPV, 91.3% ($n = 146$) had HR-HPV, and 6.3% ($n = 10$) had MI. In contrast, among patients with HSIL, 81% ($n = 17$) presented HR-HPV and 19% ($n = 4$) had MI. Among patients with SCC, 83.3% ($n = 15$) had HR-HPV and 16.7% ($n = 3$) had MI. A significant decrease in RASSF1A expression was seen between HR-HPV ($p = 0.014$) and MI ($p = 0.034$) compared with LR-HPV. However, no relationship was observed with different viral types for REST (data not shown).

REST and RASSF1A expression in cervical cancer cell lines

To corroborate the loss of REST and RASSF1A expression in cervical cancer, we performed an ICC analysis in CC-derived cell lines (C33A, SiHa, and HeLa) and a healthy keratinocyte cell line (HaCaT) was used as control. The loss of REST expression was more evident in SCC-derived cell lines (C33A and SiHa) than in adenocarcinoma derived cells (HeLa), while reduced expression of RASSF1A was seen in all cancer cell

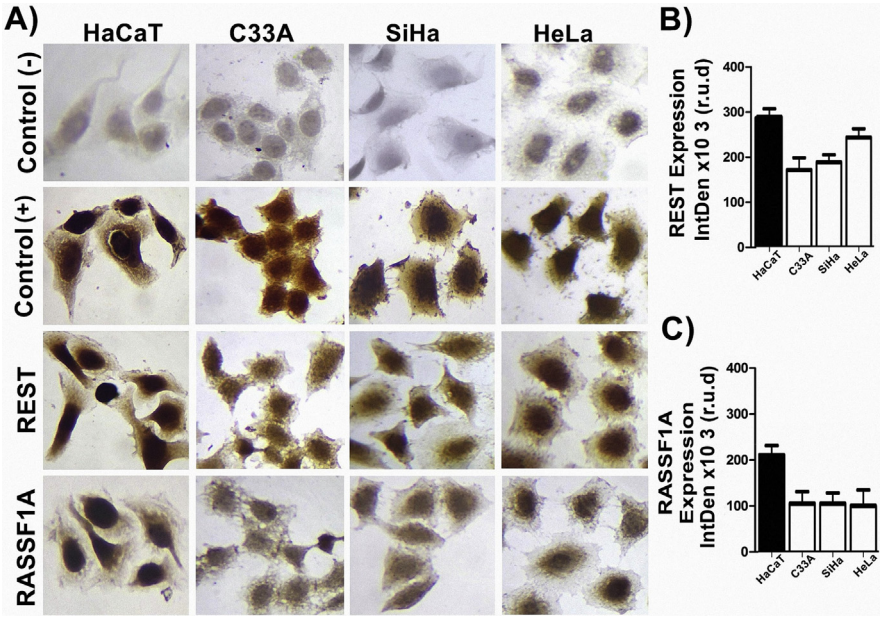

Fig. 4 REST and RASSF1A expression in cervical cancer cell lines. (A) Immunostaining of REST and RASSF1A in HaCat, C33A, SiHa and HeLa cells. $p16^{INK4a}$ expression was used as a positive control, whereas only biotin/streptavidin (but not antibody) was used as a negative control for immunostaining. (B) Densitometry analyses were performed using the ImageJ software.

lines [Fig. 4]. Notably, these results were consistent with those observed in SCC.

REST *and* RASSF1A *mRNA and protein in different types of cancer*

Finally, we performed an integral analysis with 15 different types of cancer, to evaluate the expression levels reported for mRNA and protein of REST and RASSF1A. All data were collected from The Cancer Genome Atlas database of the Human Protein Atlas. We identified a significant reduction in REST expression level in 13 of the 15 analyzed cancers, with a correlation between mRNA and protein levels. For RASSF1A, change in protein levels was observed in 14 of the 15 analyzed cancers, whereas low levels of mRNA were observed in all the analyzed cancers, which does not correlate with protein levels [Fig. S2].

Discussion

In the present study, we analyzed the expression patterns of REST and its possible relation with RASSF1A in SIL/CIN and SCC, in cervical tissue samples obtained from women in southern Mexico and CC-derived cell lines. Cervical cancer is the fourth most common cancer among women worldwide, and its primary cause is HR-HPV infection. Therefore, it is important to study molecules that are important for diagnosis, post-treatment follow-up, and identification of at-risk women. Several studies have shown aberrant expression of REST in several types of cancers, such as small cell lung cancer, medulloblastoma, breast cancer, glioblastoma multiforme, prostate cancer, and cholangiocellular carcinoma. In these cases, it has been associated with apoptosis inhibition, increase in the cellular proliferation, aggressiveness, and recurrence [14–20]. However, most of this data has been obtained from cellular models or includes a limited number of samples. In contrast, *RASSF1A* promoter hypermethylation has been reported as an early event in several types of cancers, such as breast [34], ovarian [35], endometrial [36], and cervical [37], and has been related with prognostic, surveillance, and treatment response. To our knowledge, this is the first report on REST protein level in SIL/CIN and SCC tissue samples and cervical cancer cell lines. Additionally, we evaluated the possible relationship between REST levels and RASSF1A levels, a protein related to cancer prognosis.

The use of samples in different stages of cancer progression from early premalignant lesions to cervical cancer provided a broad view of REST and RASSF1A expression, before the manifestation of cancer. We found a significant reduction in the expression of REST and RASSF1A in women with LSIL, HSIL or SCC compared to non-SIL, and we propose that the determination of the expression of both proteins may be useful in monitoring SIL and SCC prognosis. The loss of nuclear REST in early lesions, seen in the time-course analysis of cervical carcinogenesis, suggests that REST could trigger molecular events leading to carcinogenesis. In this regard, conclusive evidence has been reported on the role of REST as a tumor suppressor [17], and its altered expression is noted in almost all analyzed cancers. Moreover, the loss of nuclear REST in tissue samples suggests a reduction of neuronal gene expression, as demonstrated in neuroendocrine cancers. Notably, we noticed increased cytoplasmic REST in CIN 2/3 and SCC samples, which has been reported previously in lung cancer cell lines [38] and warrants further analysis. During neurogenesis, REST is degraded by the proteasome or expressed as its cytoplasmic isoforms, allowing neuronal gene expression and differentiation of neuronal cell lineages [39].

The presence of the RE1 sequence in *RASSF1A* suggested a transcription repression mediated by REST. Previously, loss of RASSF1A expression has been reported in several types of cancer [34–37]; particularly in cervical cancer, in which a significant difference between non-SIL, LSIL, HSIL and SCC has been reported [40]. This has also been linked to cancer development, progression, metastasis, and poor prognosis [37]. The loss of RASSF1A function is due to an allelic deletion [41] and hypermethylation of its promoter [42]. It is known that inactivation of tumor suppressor genes is involved in various cellular pathways (such as cell cycle, apoptosis, or genome maintenance). During carcinogenesis, its inactivation is mediated by DNA hypermethylation one of the main mechanisms mediated by REST [43].

An important factor for CC development is HR-HPV infection. HPV can integrate its genome into the genome of the host, producing oncoproteins that regulate cell expression, inhibit apoptosis, and cause cell proliferation [44]. We found no association between REST and RASSF1A expression with HPV genotypes or with the integrated viral state. Previously, a link between increase of p53 and decrease of RASSF1A expression [29], and HPV-16 E6/E7 expression with aberrant methylation and RASSF1A expression, has been reported [44]. In this study, we did not find a relation between REST and RASSF1A protein expression levels. Therefore, it is necessary to explore other mechanisms involved in the transcriptional regulation of RASSF1A, and it is like to that reported for E6/E7 oncoproteins of HPV 16 and p53 [44,45].

Altogether, our results demonstrate significant reduction in REST and RASSF1A expression in SIL/CIN and SCC. Moreover, our results suggest the need for an extensive study on the effect of reduced REST and RASSF1A expression and their link with HR-HPV infection. This study reports for the first-time aberrant REST expression in SIL/CIN and contributes to the design of new strategies for diagnosis, prognosis, and post-treatment follow-up of pre-malignant lesions and SCC. In addition, our study identified the presence of RE1 sites in *RASSF1A* and corroborated altered RASSF1A expression in cervical cytology samples. Finally, though we have presented convincing data for reduced REST expression in HSIL and SCC, the role of REST in promoting cervical tumorigenesis warrants further study.

Conclusion

Our results provide evidence of a possible molecular link between REST and RASSF1A, which needs to be analyzed further in other cellular models. The results also revealed a significant decrease in REST and RASSF1A expression levels, which corresponded with the progression of SIL/CIN to SCC. Our results indicate that these biomolecules could contribute to the development of cervical cancer and be useful in the diagnosis, prognosis, and post-treatment follow-up of SIL and SCC.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bj.2020.08.012.

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