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RESEARCH ARTICLE

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RACK1 promotes the occurrence and progression of cervical carcinoma

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Abstract

Background: RACK1 has been identified as a multifunctional cytosolic protein, and plays a pivotal role in multiple biological responses involved in several kinds of tumors, while its effect in cervical cancer has not been well elucidated yet. The study aimed to investigate the role of RACK1 in cervical cancer occurrence and progression.

Methods: The expression of RACK1 in cervical specimens was measured by immunohistochemical staining and Western blot assay. Transgenic mice were used to detect the role of RACK1 in modulating tumorigenesis in vivo. Cervical carcinoma cell lines were used to explore the underlying mechanisms of RACK1 on the behaviors of tumor cells in vitro.

Results: We found that RACK1 expression was upregulated in cancer tissues compared with adjacent tissues, and its expression was gradually increased from cervictis, and cervical intraepithelial neoplasis (CIN) to carcinoma. Genetic overexpression of RACK1 facilitated tumor formation and growth in nude mice. Mechanism studies disclosed that RACK1 over-expression prolonged the G_0/G_1 phase by up-regulating the expression of cyclinD1, down-regulating p21 and p27 probably by modulating the phosphorylation of AKT.

Conclusions: Taken together, we concluded that RACK1 stimulates tumorigenesis and progression of cervical cancer via modulating the proliferation of tumor cells, implying that targeting RACK1 may serve as a promising method for cervical cancer therapy.

KEYWORDS
cervical carcinoma, progression, proliferation, RACK1, tumor cells

1 | INTRODUCTION

Cervical cancer (CC) is the fourth most common malignant tumors in women worldwide and is one of the leading cause of cancer-associated mortality.[1,2] Until now, curative surgery has remained the most effective treatment for early-stage cervical cancer, while cisplatin-based concurrent chemoradiotherapy is still the mainstay for advanced-stage disease.[3,4] Although the global mortality rate from cervical cancer has declined due to the advancement of treatment including HPV vaccines, surgery, radiation, chemotherapy, and cell immunotherapy, etc., cervical cancer-related death is continuing high in developing countries.[5] Therefore, the exploitation and development of superior and effective therapeutic agents remains crucial. Emerging evidence has demonstrated that certain genes and signal pathways

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dysregulation in tumors and involved in cell survival, proliferation, or cell-cycle progression, tend to exert as anticancer targets, with reference to clinical drugs focusing on HER2 and EGFR pathways.[6,7]

The receptor for activated C kinase (RACK1), originally identified as the receptor for activated protein C β, is a 36-kDa cytosolic protein that is composed of highly conserved WD-40 repeats (Trp-Asp).[8,9] It serves as a multifunctional scaffold protein and plays a functional role in a multiple of biological responses, including growth, migration and invasion of tumor cells.[8,10] There is evidence indicating that RACK1 is up-regulated in multiple tumor types and is considered as a promising marker in hepatocellular carcinoma, oral squamous cell carcinoma, melanoma, breast cancer, and non-small cell lung cancer.[11–15] As a matter of fact, several limited articles reported the role of RACK1 in CC in recent years[16,17]; however, due to the mechanisms of RACK1 involved in CC may be multifaceted, more available data are needed to elucidate its function and evaluate its clinical value in CC. Therefore, the aim of this study was to investigate the role of RACK1 in the occurrence and progression of cervical cancer.

2 | MATERIALS AND METHODS

2.1 | Patients and tissues

A total of 42 surgical cervical specimens were obtained from the Department of Pathology, Kunshan First People's Hospital during 2018–2020, among which 12 cases with cervical cancer, 24 cases with cervical intraepithelial neoplasia (CIN) in various stages, and six cases with cervicitis. The patients with cervical cancer had not previously received chemotherapy or radiation therapy. All histologic slides were re-evaluated by two experienced pathologists without discrepancy. Histologic grading and clinical stage were classified according to FIGO. The usage of human tissue samples was approved by the ethics committee of Kunshan First People's Hospital. All participants signed informed consent to donate their samples.

2.2 | Plasmids and cell lines

The plasmid encoding RACK1 was kindly presented by Gene Research Center, Shanghai Medical College of Fudan University. Three cervical cancer cell lines with different invasion abilities, including Siha and Hela (low invasion ability) and Caski (high invasion ability), were obtained from the Institute of Cell Biology of the Chinese Academy of Sciences (Shanghai, China). All cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (Gibco, Grand Island, USA), penicillin (100 IU/mL), and streptomycin (100 μg/mL) at 37°C in a humidified atmosphere with 5% CO_2. The above cervical cancer cell lines were transfected with pcDNA3.1-EGFP-RACK1 or pcDNA3.1-EGFP empty vector with Lipofectamine 2000 according to the manufacturer's protocol. Stable transfectants were identified and selected by flow cytometry.

2.3 | Western blot assay

Protein extracts of different samples including tissues and cells were first quantified using a BCA protein assay kit, mixed with Laemmli's loading buffer, applied to 10% SDS-PAGE, and then transferred onto a PVDF membrane. The blots were probed with the anti-RACK1, anti-GAPDH, anti-β-actin anti-cyclin D1, purchased from Santa Cruz Biotechnology, anti-p21, anti-p27, anti-p-Akt and anti-Akt antibodies purchased from Cell Signaling Technology, followed by the horseradish (HRP)-conjugated secondary antibody (1:5000); finally, the immunoreactions were visualized by ECL and exposed to X-ray film.

2.4 | Immunohistochemistry

Paraffin-embedded specimens were deparaffined in xylol and rehydrated with ethanol at graded concentrations of 70% to 100%, followed by washing with distilled water. Endogenous peroxidase activity was quenched by immersion in 3% hydrogen peroxide in methanol for 20 min. Then, slides were heated at 120°C in 0.1 M citric acid buffer for 5 min for antigen retrieval. Histological slides were then incubated with monoclonal anti-RACK1 antibody in a dilution of 1:200 overnight at 4°C, followed by incubation with biotinylated anti-rabbit antibody at room temperature for 30 min, visualization of bound antibodies was developed in 0.05% diaminobenzidine containing 0.01% hydrogen peroxidase.

2.5 | Flow cytometric analysis

Stably transfected cells (1×10^5/well) were cultured in six-well plates, trypsinized at a confluence of 80%–90%, and then harvested by centrifugation at 1200 rpm/min at 4°C for 5 min. Then, cells were fixed in 70% cold ethanol, resuspended in phosphate-buffered saline (PBS), and later treated with 50 μg/mL RNase in 0.1% BSA or 1 h at 37°C, followed by propidium iodide (PI) staining solution in dark. Cell-cycle distribution was analyzed by fluorescence-activated cell sorting (FACS; Becton Dickinson, San Jose, CA).

2.6 | Tumor formation in nude mice

The investigation of RACK1 tumorigenicity was carried out through an orthotopic nude mouse model. Cells stably transfected with a plasmid expressing RACK1 or an empty vector were injected into the bilateral flank of 5-week-old male nude mice. Each group contained 10 mice. Tumor formation was monitored regularly after 10 days after injection with an average interval of 3 days, using vernier calipers. Five weeks later, all mice were sacrificed with their tumors removed for weighing and measuring. Tumor volume was calculated by the formula: tumor volume (mm^3) = Length×Width^2/2 ($LW^2/2$). Animal experiments were carried out in accordance with the Animal Ethics Committee of the University of Jiangsu University.

2.7 | Statistical analysis

SPSS 26.0 statistical software (IBM Corp. Armonk, NY, USA) was used for data analysis. Results were presented as the mean ± SD. Comparison between the two groups was tested using an independent sample Student's 2-tailed *t*-test. One-way analysis of variance (ANOVA) was used for data comparison among multiple groups. For all values, $p < 0.05$ was considered to be statistically significant.

3 | RESULTS

3.1 | RACK1 is frequently upregulated in CC tissues and its expression was positively correlated with tumorigenesis

To investigate whether RACK1 was involved in CC tumorigenesis, we first examined the expression of RACK1 in various cervical tissues, as shown in Figure 1, RACK1 was higher expressed in cervical cancer tissues compared with their matched normal tumor-adjacent tissues, and its level gradually increased in normal cervix, cervicitis, increasing CIN stage and cancer tissues. These results indicated that RACK1 was involved in CC tumorigenesis.

3.2 | RACK1 over-expression accelerated tumor growth in nude mice

To examine the role of RACK1 on tumor growth in vivo, tumor xenografts in nude mice were also generated using CC cells stably transfected RACK1-overexpressed plasmid or empty plasmid. Then, tumor formation was observed daily, once the tumor was evident, the diameters of the tumor growth were measured at an interval of 3 days. As expected, overexpressed RACK1 tend to develop larger tumors compared with control ones (Figure 2). Taken together, it is reasonable to speculate that RACK1 may play a critical role in tumor progression.

3.3 | RACK1 over-expression accelerated cell cycle by regulating cell cycle proteins

To explore the potency of RACK1 on the proliferation of CC cells, we further measured the cell cycle distribution in RACK1-overexpressed CC cells using a flow cytometer (FCM). Results from FCM revealed that RACK1 overexpression promoted proliferation by increasing S-phase entry. To illuminate the underlying molecular mechanism of the RACK1-mediated accelerated cell cycle, we examined the expression of cyclins, including cell-cycle inhibitors p21 and p27, and

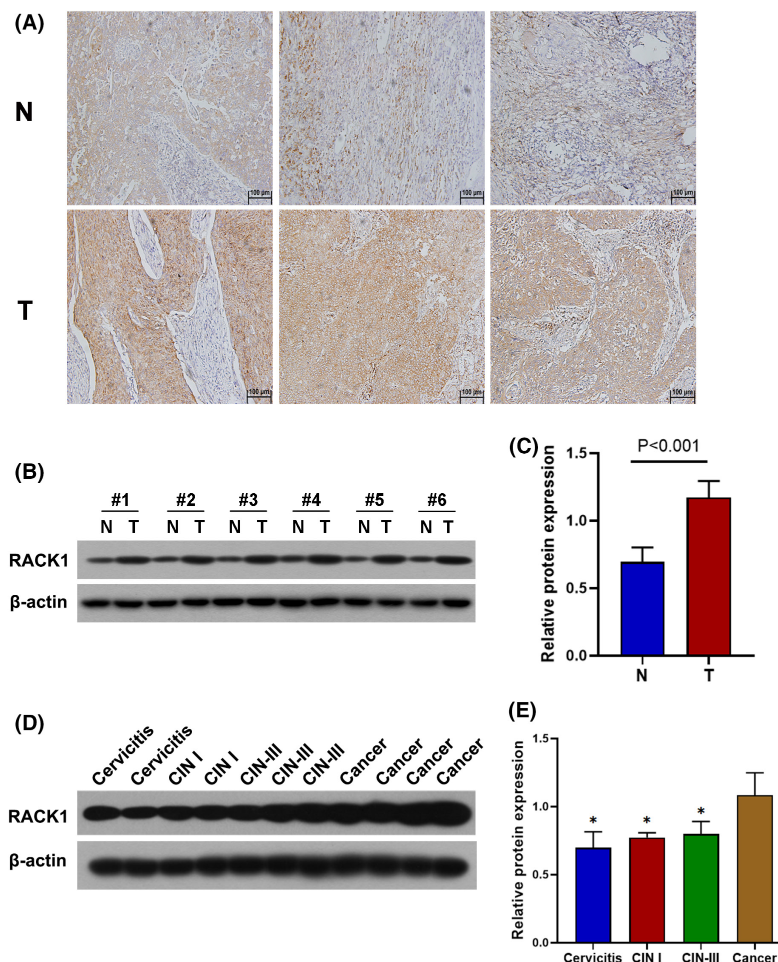

FIGURE 1 RACK1 is up-regulated in CC, and its expression was gradually increased from cervictis, cervical intraepithelial neoplasis to carcinoma. A, Representative immunohistochemistry staining of RACK1 expression in cervical tumor tissues (T) and their normal adjacent tissues (N); B and C, Representative western-blot assay of RACK1 and its quantitation with β-actin as internal control; D and E, Expression of RACK1 in cervical samples at cervicitis, different CIN staging and cancer tissues detected by western-blot assay, and its quantitation with β-actin as internal control. One-way analysis of variance (ANOVA) was used among multiple groups. Scale bars: 100 μm.

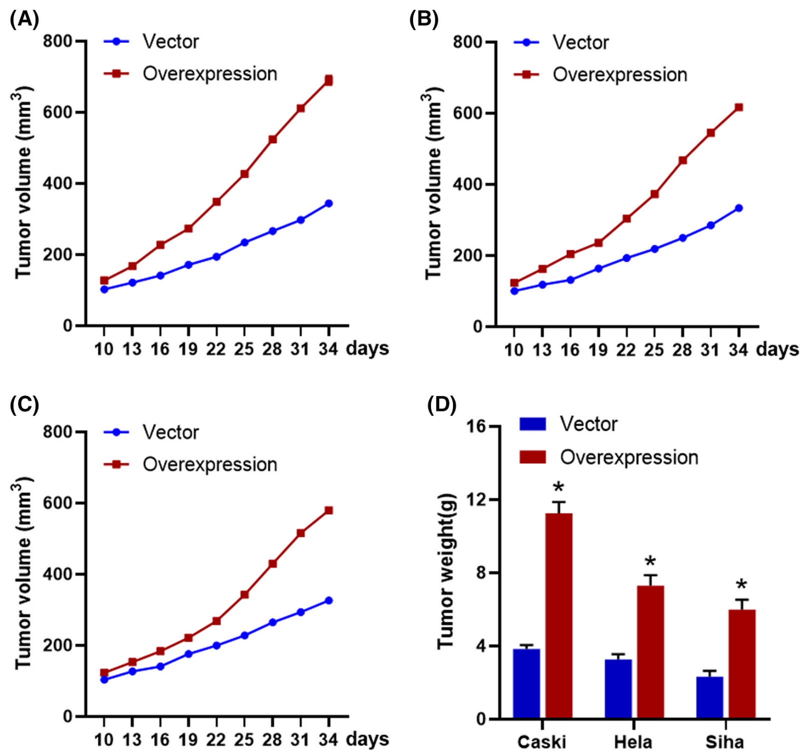

FIGURE 2 RACK1 expression accelerated tumor growth in vivo. Xenografts were generated using Caski, HeLa and SiHa cells stably expressing empty vector, or over-expressing RACK1. Tumor volume and weight was measured at indicated time points. A, B and C represented RACK1-overexpressing Caski, HeLa, SiHa cell, respectively; as expected, overexpression of RACK1 facilitated tumor formation and growth in nude mice, compared with control ones. D showed the tumor weight in three cervical cancer cells with or without RACK1 overexpression. Independent sample t test was used for data comparison between two groups. The experiment was repeated 6 times independently. $^{*}p<0.05$.

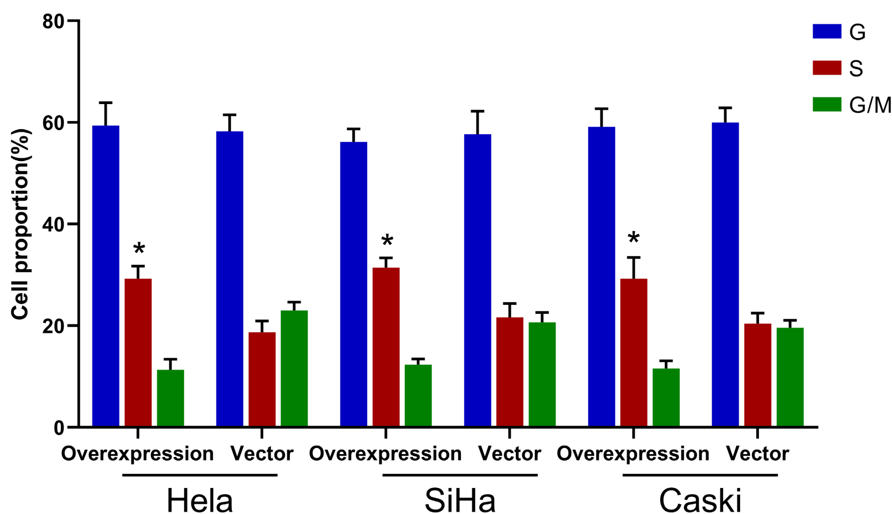

FIGURE 3 Analysis of cell cycle distribution by flow cytometry in CasKi, Hela and Siha cells. The experiment was repeated 3 times independently. $^{*}p<0.05$.

cell-cycle promoter cyclinD1. Western blot assay represented that p21 and p21 levels were decreased, compared with an increased expression of cyclinD1 in RACK1-overexpressing CC cells (Figures 3 and 4).

4 | DISCUSSION

Our findings suggested that RACK1 was upregulated in cervical cancer and its expression was positively correlated with tumor grading. RACK1 overexpression of RACK1 enhanced tumor formation and growth in an orthotopic nude mouse model. For mechanism, RACK1 functions as a cell cycle regulator by modulating the phosphorylation of AKT. Taken together, these results suggested that RACK1 might function as a cell cycle regulator, and targeting RACK1 may be a promising method for CC therapy.

RACK1 has been identified as a multifunctional scaffold protein by its involvement in multiple biological processes including tumorigenesis. Numerous studies have reported the relationship between RACK1 dysregulation and neoplasm, such as hepatocellular carcinoma, colon cancer, and non-small cell lung cancer.[12,18] In fact, there have been limited articles reporting the role of RACK1 in CC in recent years, while the results remain controversial. Wang et al. reported that the expression of RACK1 decreased in CC tissues in comparison with the adjacent noncancerous tissue, which is inconsistent with another two articles by Wu and Liao et al.[16,17,19] Wu et al.[17] revealed that RACK1 promoted CC cell invasion and lymph node metastasis via a galectin-dependent

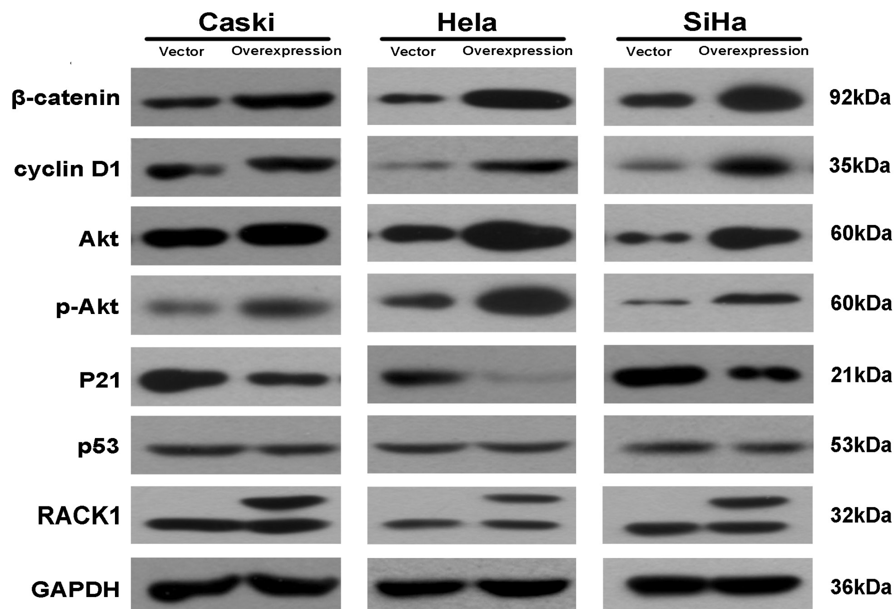

FIGURE 4 Western blot analysis showing the expression levels of β-catenin, cyclin D1, p21, p53, Akt, p-Akt in the RACK1 over-expressed cells compared with their vector control cells. The experiment was repeated 3 times independently.

manner, while Liao et al.[16] demonstrated that RACK1 promotes cell growth and invasion in CC. In the present study, we found that RACK1 was highly expressed in CC tissues compared with their normal tumor-adjacent tissues, and its level gradually increased in normal cervix, cervicitis, increasing CIN stage and cancer tissues, which is also confirmed by cell experiments. These results are consistent with most studies regarding this protein in multiple tumors, indicating that RACK1 was involved in CC tumorigenesis and development.

To investigate the underlying mechanisms of RACK1 influencing CC cells, we detected the expression of cell-related cyclin in RACK1 overexpressed CC cell lines. The results showed that RACK1 overexpression enhanced the cell cycle of CC cells by upregulating cyclin D1 and downregulating p21, while with no effect on TP53 (p53). TP53 is a well-established tumor suppressor, located on human chromosome 17p13.1, which is a negative regulator in the cell growth cycle. Mutations in TP53 occur in >50% of human tumors, it is generally believed that p53 overexpression is associated with tumor metastasis, recurrence and poor prognosis.[4,20] p21 was first identified as a downstream gene of p53, subsequent research showed that p21 functions as an important cell cycle regulator, involved in the process of cell growth, differentiation, aging and death.[21] Previous studies have established that p21 can arrest the cell cycle in the G1 phase by interacting with cyclin D/CDK.[22,23] In our study, over-expressed RACK1 led to p21 expression down-regulation without affecting the expression of p53, indicating that p21 functions in an independent way on p53 in the development of CC with RACK1 dysregulation. However, considering the versatility of RACK1 in different types of human tumors, further research studies to clarify its function are still needed.

In the current study, a series of experiments were carried out to investigate the role of RACK1 in the tumorigenesis and development of CC, the potential mechanisms were also explored. We concluded that RACK1 positively impacted cervical cancer progression, supported by both clinical and cell experiments.

The study has several limitations that must be acknowledged. First, we did not compare the correlation of RACK1 with previously proposed biomarkers like Ki67 to assess its clinical value. Second, there was a lack of prognosis data from clinical samples, so we cannot clarify its value in predicting clinical prognosis. Last but not least, tumorigenesis is modulated by the balance of cell proliferation, differentiation, and death, given that autophagy or necrosis may also lead to the death of tumor cells, more detailed mechanisms need further investigation.

5 | CONCLUSION

We demonstrated that RACK1 was upregulated in cervical cancer cells and clinical samples, and genetic overexpression of RACK1 significantly increased the proliferation of CC cells as a cell cycle promotor, which suggests that RACK1 can be used as a novel therapeutic target. However, as the study is still in the early stage of evaluating the role of RACK1 in CC, more studies are warranted to elucidate its mechanisms and clarify its clinical value in CC.

AUTHOR CONTRIBUTIONS

QL designed and conceived the research, DDY, XJL performed the experiments, analyzed data, and prepared the manuscript; XGZ, XJZ analyzed data, and revised the manuscript. The authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

All authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

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