Int J Clin Exp Pathol 2018;11(2):577-585
www.ijcep.com /ISSN:1936-2625/IJCEP0067964

Original Article

Suppressive efficiency of RASSF1A in endometrial carcinoma via inhabiting estrogen receptor alpha expression and ERK pathway activation

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Received October 26, 2017; Accepted November 10, 2017; Epub February 1, 2018; Published February 15, 2018

Abstract: Introduction: Known as a tumor suppressor, the Ras association domain family 1 isoform A (RASSF1A) is implicated in many human cancers, such as endometrial carcinoma. There is little known about the tumor inhibitive effects of RASSF1A on endometrial carcinoma. The present study was designed to investigate the role of RASSF1A in HEC-1-A cells and to explore its potential mechanisms. Materials and methods: In this study, overexpression of RASSF1A was established by transfection the recombinant adenoviral RASSF1A in HEC-1-A cells. Cells viability was assessed by MTT assay and the apoptosis was analyzed using flow cytometry. Cell migration and invasion were measured in Transwell assay. The levels of ERα and PELP1 protein and extracellular regulated protein kinase (ERK) pathway activation were detected by Western blot. Results: RASSF1A over-expression could significantly inhibit the proliferation, migration and invasion of the HEC-1-A cells in transfection with RASSF1A group compared to that in transfection with control group, also induced apoptosis and suppressed the tumor growth after injection in nude mice. Moreover, overexpression of RASSF1A could inhibit the ERK signal pathway activation and decrease the ERα and PELP1 expression. Conclusion: Tumor suppressive efficiency of RASSF1A is exerted through the regulation of ERK pathway activation, ERα and PELP1 expression.

Keywords: Endometrial carcinoma, RASSF1A, estrogen receptor alpha, ERK pathway, HEC-1-A cells, apoptosis

Introduction

As we all know, the endometrial carcinoma is one of the most common and aggressive carcinoma in women worldwide [1]. With a very high potential for migration and invasion, the incidence and mortality rate of endometrial carcinoma is raising in the world [2]. Although several molecular targeting therapies and surgery have been applied in the treatment of endometrial cancer cases, the effects including the long-term survival for patients have not been satisfactory, and the prognosis is still poor [3, 4], Therefore, it is important to understand the deeper molecular mechanisms underlying the pathogenesis and progression of this cancer involved in proliferation, migration and invasion.

Ras association domain family 1 isoform A (RASSF1A), which is frequently known as a tumor suppressor gene, is decreased in endometrial carcinoma [5-7]. Previous studies have revealed the evidence that heterologous expression of RASSF1A could inhibit tumor cells proliferation and metastasis [8, 9]. RASSF1A is also associated with regulating the expression of estrogen receptor (ERα) and activation of extracellular regulated protein kinase (ERK) in tumor cells [10-12]. ERα is an important player in the proliferation, survival and differentiation of cells through regulating the activation of signal transduction pathways, such as ERK signal pathway [13-15]. PELP1/MNAR, as a new ERα co-activator, regulates ERα expression through several signal pathways and promotes tumor cells proliferation and apoptosis through activation of the Src/MAPK pathway but inhibit the PI3K/Akt pathway. However, it is not clear whether RASSF1A has effect on regulating these proteins includ-

ing ERK, ERα and PELP1 in endometrial carcinoma cells to suppress the tumor growth and metastasis. In previous studies, ERK phosphorylation was shown to be inhibited by RASSF1A overexpression in tumor cells and tissues, which suggested that RASSF1A plays an important role in regulating ERK activation. The significance of ERK, ERα and PELP1 expression in endometrial carcinoma with the RASSF1A component is currently unknown.

In this study, we have upregulated the expression of RASSF1A by transfection the recombinant adenoviral RASSF1A in HEC-1-A cells, to detect the suppressive effects of RASSF1A on proliferation, migration and invasion, and the regulation on ERK signal pathway activation, ERα and PELP1 expression was also further investigated.

Materials and methods

Reagents

Primary antibodies p-ERK and t-ERK were purchased from CST (Beverly, MA), RASSF1A, PELP1 and GAPDH were obtained from Abcam (Shanghai, China), and ERα was from Santa Cruz (Heidelberg, Germany). t-ERK was used for internal control to normalize the density of p-ERK.

Cell culture and adenovirus transfection

Human HEC-1-A cells were purchased from NTCC (Shanghai, China). The cells were cultured in McCoy's 5A supplement with 10% fetal bovine serum (FBS), under a condition of 5% CO_2, 37°C in the incubator. The recombinant adenoviral human RASSF1A (rAd-RASSF1A) was purchased from Bio Vector (Shanghai, China) then transfected into the HEC-1-A cells. rAd-Control was also purchased from Bio Vector to be used as control. All were performed according to the manufacturer's instructions.

Western blot analysis

Western blot analysis was performed as described previously [16]. Briefly, the proteins were extracted from the harvested cells with radio immunoprecipitation assay (RIPA). A total of 20 μg proteins were supplement and separated with 10% SDS-PAGE gel, then transferred onto a PVDF membrane (Millipore). The membrane with primary antibody was incubated overnight at 4°C. Signals were detected by ECL substrate (Bio-Rad, USA) and quantified using image analysis system Image J software.

Immunohistochemistry

Human RASSF1A antibody (1:200) in phosphate-buffered saline was incubated overnight at 4°C. Fluorescent microscope (Nikon, ECLIPSE 80i, Japan) was applied to detect and a CCD Spot camera was used to collect photos. The pictures were saved as TIF formats and processed by Adobe Photoshop 7.0.

MTT assay

HEC-1-A cells (Blank), transfected with control (rAd-Control) and transfected with RASSF1A (rAd-RASSF1A) were seeded in 96-well plates in triplicates (10^4 cells per well). Cell viability was detected using 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di-phenytetrazoliumromide (MTT) at a 450 nm wavelength measured by a 96-well plate reader to obtain the absorbance.

Flow cytometry assay for cell apoptosis

After transfected with RASSF1A or control for 48 hours, HEC-1-A cells were collected and washed three times with ice-cold PBS buffer. Cells were stained with 1 ml PI dye and incubated at 4°C for 30 min in a dark place. The apoptotic cells were analyzed by flow cytometry FACS Calibur (BD, Bioscience) according to the manufacturer's instructions. Flow cytometry excited wavelength with 488 nm, with a wave length of 515 nm through the filter to detect FITC fluorescence, another wave length greater than 560 nm filter to detect PI.

Cell migration and invasion assay

The cell migration and invasion assay was performed described as before [16]. HEC-1-A cells were transfected with RASSF1A or Control for 48 hours. After 0.1% gelatin incubation for an hour, cells (2×10^5) were seeded in upper chambers of transwells (Corning, MA), while 1×10^5 cells were also plated on the upper chamber percolated with Matrigel (BD Bioscience) coating for invasion assay, all the bottom chambers were added into 600 μl DMEM containing 10% FBS. After incubated for 24

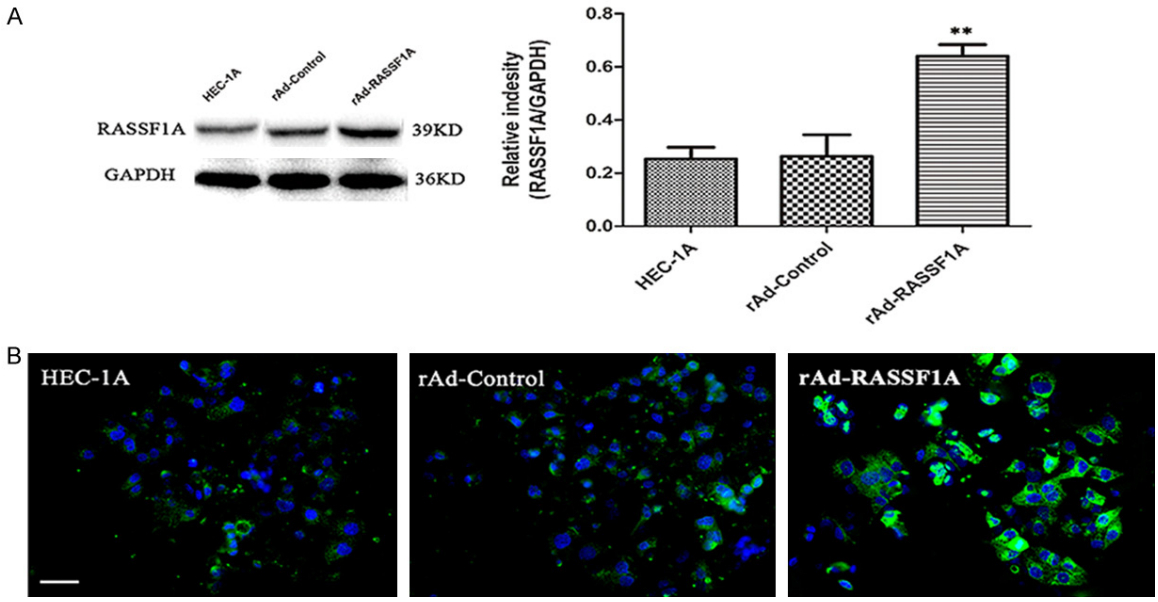

Figure 1. RASSF1A expression was upregulated in cells after transfected with rAd-RASSF1A. A: Western blot of RASSF1A protein level in HEC-1-A cells, cells transfected rAd-Control, cells transfected with rAd-RASSF1A. Data showed was the mean ± SD. **, $P<0.01$ *vs.* cells transfected rAd-Control. B: Immunofluorescence staining was showed for RASSF1A expression in HEC-1-A cells, cells transfected rAd-Control, cells transfected with rAd-RASSF1A. RASSF1A (green) and DAPI (blue). Bar = 50 μm.

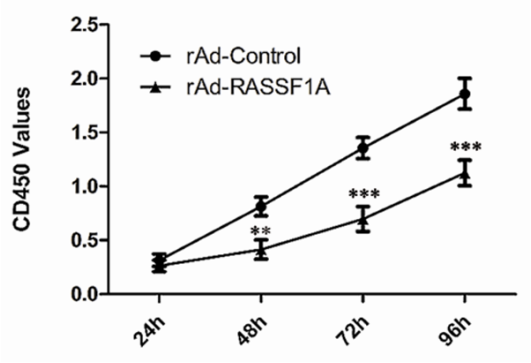

Figure 2. RASSF1A attenuates the proliferation of endometrial carcinoma cells. HEC-1-A cells viability was detected by MTT. Data are showed as mean ± SD. **, $P<0.01$ *vs.* cells transfected with rAd-Control.

hours, the upper chamber was removed and fixed by anhydrous ethanol for 10 minutes, stained with 0.2% crystal violet for 5 minutes. Finally the membranes of bottom chambers were removed gently and imaged with the microscope (Olympus).

Tumor growth assay

Twenty female BALB/c nude mice (22-25 g) were purchased from the Animal Department, China Medical University (Shenyang, China), and kept under a constant environment (12/12 h light/dark cycle). Mice were randomly divided into two groups (n = 10/group) and injected 1×10^6 HEC-1-A cells transfected with RASSF1A or Control in 50 μl of 1×PBS at the right flanks subcutaneously. The tumor size was measured with a micro caliper.

Statistical analysis

Data were presented as the mean ± the standard deviation (SD). Analysis between groups was assessed by Student's *t* test (GraphPad Prism5). Differences were termed statistically significant at $P<0.05$.

Results

The expression of RASSF1A in endometrial carcinoma cells after transfection

To determine whether the expression of RASSF1A was elevated in endometrial carcinoma cells after transfection with recombinant RASSF1A gene (rAd-RASSF1A), we detected the level of RASSF1A protein using western blot. As shown in **Figure 1A**, RASSF1A expression was significantly upregulated in cells after the transfection with rAd-RASSF1A compared to

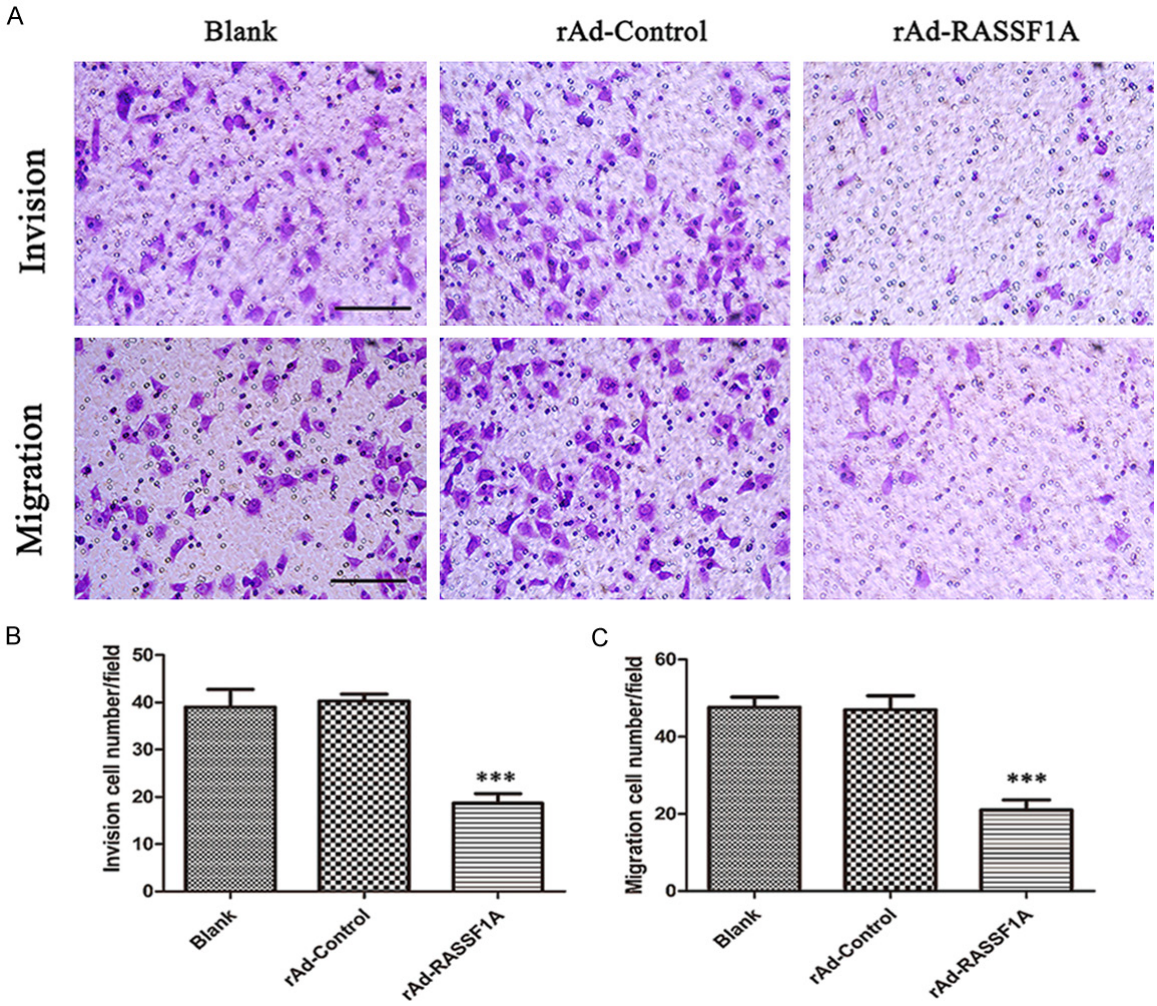

Figure 3. RASSF1A overexpression inhibits the migration and invasion of HEC-1-A cells. (A) Representative images of migration and invasion were shown. Bar = 50. (B) The analysis of migration and invasion (C) in HEC-1-A cells were measured. Data were shown as mean ± SD. ***, $P<0.001$ *vs.* cells transfected with rAd-Control.

the rAd-Control groups, which lack the endogenous expression of RASSF1A. Immunofluorescence staining also showed that HEC-1-A cells transfected with rAd-RASSF1A stained positively for RASSF1A (**Figure 1B**). Thus, we confirmed that the expression of RASSF1A was elevated in endometrial carcinoma cells after transfection with rAd-RASSF1A.

RASSF1A overexpression suppresses endometrial carcinoma cells proliferation

To determine the RASSF1A overexpression transfected with the recombinant RASSF1A, cell viability in HEC-1-A cells was detected by MTT assay. MTT assay showed that cell viability was significantly decreased at 48, 72 and 96 h in transfection with rAd-RASSF1A group compared to that in rAd-Control group, suggesting that RASSF1A could attenuate the proliferation of endometrial carcinoma cells (**Figure 2A**). The roles of RASSF1A on cells apoptosis were assessed by flow cytometry with annexin V-FITC/PI staining at 48 h after transfection with RASSF1A or Control. The percentage of apoptotic cells in rAd-RASSF1A group was significantly elevated compared to that in rAd-Control group or without transfection (10.8%±0.61 vs. 1.6±0.53 and 1.6±0.51; $P<0.05$; **Figure 6**).

RASSF1A overexpression attenuates the endometrial carcinoma cells migration and invasion

To evaluate the tumor-suppressed effects of RASSF1A, we also detected the migration and

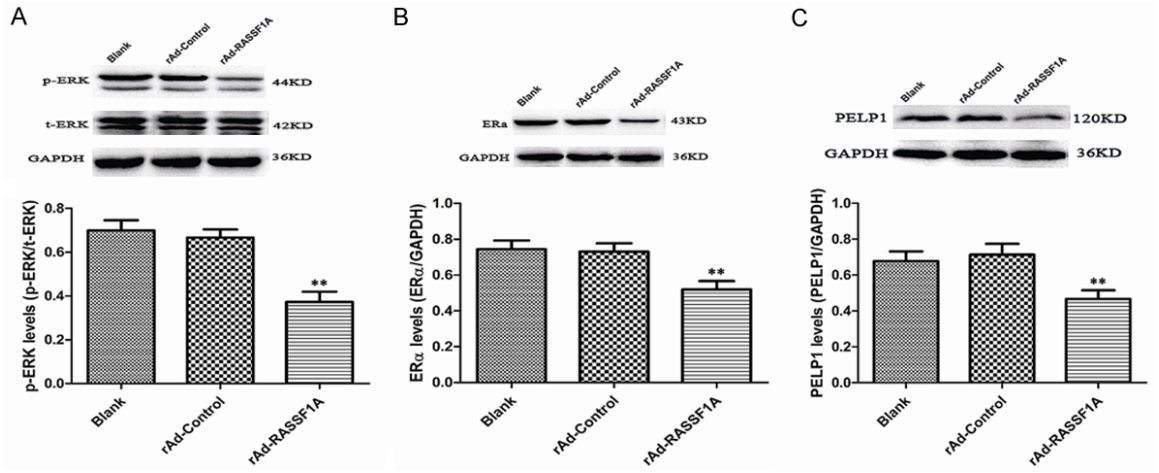

Figure 4. RASSF1A suppresses the expression of the p-ERK, ERα and PELP1. Western blot analysis was applied to detect the level of p-ERK (A), ERα (B) and PELP1 (C) protein in HEC-1-A cells transfected with blank, rAd-Control and rAd-RASSF1A respectively. Data were shown as mean ± SEM. **, *P*<0.01 *vs.* cells transfected with rAd-Control.

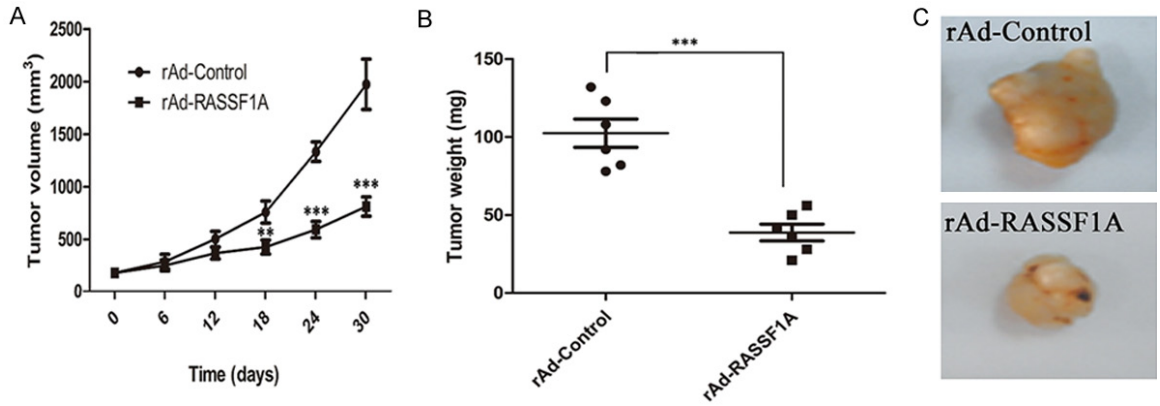

Figure 5. RASSF1A inhibits the tumor growth *in vivo*. A: Tumor volume in nude mice injected with HEC-1-A cells transfected with rAd-RASSF1A or rAd-Control was analyzed. B: Tumor weights in nude mice injected with HEC-1-A cells transfected with rAd-RASSF1A or rAd-Control was analyzed. C: The representative pictures of tumors were taken from nude mice. Data were shown as mean ± SEM. **, *P*<0.01; ***, *P*<0.001 *vs.* cells transfected with rAd-Control.

invasion potential of HEC-1-A cells. As shown in **Figure 3A**, overexpression of RASSF1A attenuated the migration and invasion of HEC-1-A cells after transfection with RASSF1A for 24 h in transwell assay, which was also supported by the migration and invasion cell number/field analysis in **Figure 3B** (*P*<0.05).

RASSF1A decreases ERα, PELP1 expression and inhibits the ERK signal pathway activation

To determine the effects of RASSF1A overexpression on ERα, PELP1 and ERK signaling activation, the western blot analysis was applied to reveal the expression of ERα, PELP1 and p-ERK. As shown in **Figure 4**, the p-ERK, ERα and PELP1 expression was significantly inhibited after rAd-RASSF1A transfection compared to the rAd-Control transfection in endometrial carcinoma cells, which suggesting that RASSF1A could play an important role in endometrial carcinoma via inhibiting the p-ERK, ERα and PELP1 expression.

RESSF1A inhibits the tumor growth of endometrial carcinoma in vivo

HEC-1-A cells with the rAd-RASSF1A or rAd-Control transfection were inoculated into the nude mice. As shown in **Figure 5**, the tumor volume was significantly small observed in rAd-RASSF1A transfection groups after 18 days

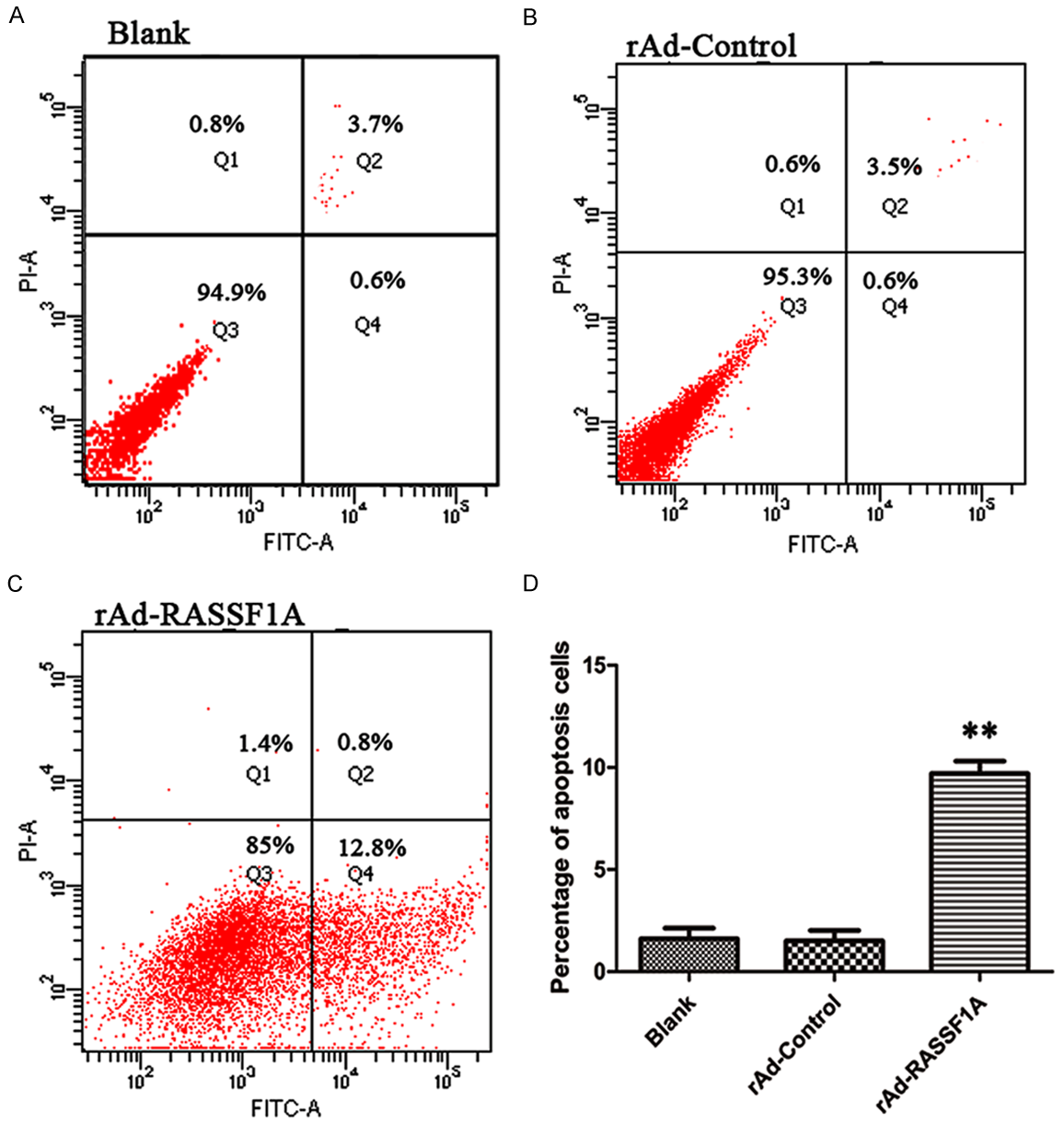

Figure 6. HEC-1-A cells apoptosis was assessed by flow cytometry of annexin V-FITC/PI staining. The blank (A) and the HEC-1-A cells transfected with rAd-RASSF1A (C) or rAd-Control (B) was showed. (D) The analysis of apoptosis cells was showed. Data are showed as mean ± SD. **, $P<0.01$ *vs.* cells transfected with rAd-Control.

compare with that in rAd-Control transfection groups ($P<0.05$). Moreover, a lower tumor weights also were detected in rAd-RASSF1A injected mice compared to rAd-Control injected mice ($P<0.05$). The results reveal that RASSF-1A could inhibit tumor growth in the xenograft HEC-1-A endometrial carcinoma cells.

Discussion

In this study, we have found that RASSF1A exerted a tumor suppressive activity in endometrial carcinoma cells. First, RASSF1A overexpression significantly inhibited the HEC-1-A cells viability and proliferation, induced apoptosis. Second, RASSF1A overexpression negatively regulated the HEC-1-A cells migration and invasion. Third, RASSF1A could inhibit the tumor growth in mice. A major founding of this study is that RASSF1A overexpression downregulated the ERα, PELP1 expression and ERK signal pathway activation, implying that there may be some dynamic relationships between the tumor suppressive effect of RASSF1A and

the expression of ERα, PELP1 and p-ERK, which should provide further evidence to be elucidate in the future.

RASSF1A expression has been reported to be downregulated in several human cancers and considered to be an important effector in the carcinogenesis and progression of the cancers [17]. Previous studies have demonstrated that RASSF1A is involved in the cell proliferation [18], migration and invasion [19], cell apoptosis and cell signaling pathway [20, 21]. Moreover, overexpression expression of RASSF1A can reduce tumorigenic transformation and tumorigenic feature of cervical [18], kidney [22], and prostate tumors [13]. However, there is little known about the suppressive role of RASSF1A in endometrial carcinoma cells. So we have constructed the recombinant adenoviral plasmid to elevate the expression of RASSF1A in HEC-1-A cells, which significantly suppressed the HEC-1-A cells proliferation and inhibited the tumor growth in nude mice. Accordance with previous studies [21, 23], our findings also revealed that overexpression of RASSF1A promoted apoptosis in HEC-1-A cells. Furthermore, RASSF1A overexpression also significantly inhibited the migration and invasion of HEC-1-A cells. Thus, the results demonstrate that the RASSF1A functions as a tumor suppressor via regulating the proliferation, apoptosis, migration and invasion in endometrial carcinoma cells.

The ERK signaling pathway, activated in variety of tumors, plays a crucial role in regulating the proliferation, survival and invasion of endometrial carcinoma cells [10, 24-26]. Other researchers have demonstrated that RASSF1A functioned as a tumor suppressor through regulating several signal pathways, such as inhibition of the Wnt/β-catenin signaling [16], ERK and AKT signaling [27]. In our study, western blot assay showed that RASSF1A overexpression significantly inhibited the ERK signal pathway activation in HEC-1-A cells. Overexpression of RASSF1A also was observed to inhibit the level of ERα and PELP1 protein, the important markers for prognosis and predictive of response to endocrine therapy in patients with endometrial carcinoma [28-31]. Thus, these results suggest that RASSF1A suppresses the proliferation and metastasis of endometrial carcinoma via inhibiting ERK signal pathway activation and ERα, PELP1 protein level.

In conclusion, RASSF1A functions as a tumor suppressor in endometrial carcinoma through suppressing the proliferation, migration and invasion, promoting apoptosis in HEC-1-A cells, and inhibiting tumor growth in nude mice, probably with relation to the ERK signaling pathway. These findings reveal a new potential therapeutic application of RASSF1A in anti-metastatic therapy for endometrial carcinoma.

Acknowledgements

This study is supported by Science and Technology Projects of Colleges and Universities in Shandong Province (No. J10LF09).

Disclosure of conflict of interest

None.

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References

[1] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in globocan 2012. Int J Cancer 2015; 136: E359-386.

[2] Kitchener HC, Trimble EL; Endometrial Cancer Working Group of the Gynecologic Cancer Intergroup. Endometrial cancer state of the science meeting. Int J Gynecol Cancer 2009; 19: 134-140.

[3] Oza AM, Elit L, Tsao MS, Kamel-Reid S, Biagi J, Provencher DM, Gotlieb WH, Hoskins PJ, Ghatage P, Tonkin KS, Mackay HJ, Mazurka J, Sederias J, Ivy P, Dancey JE, Eisenhauer EA. Phase ii study of temsirolimus in women with recurrent or metastatic endometrial cancer: a trial of the ncic clinical trials group. J Clin Oncol 2011; 29: 3278-3285.

[4] Ray-Coquard I, Favier L, Weber B, Roemer-Becuwe C, Bougnoux P, Fabbro M, Floquet A, Joly F, Plantade A, Paraiso D, Pujade-Lauraine E. Everolimus as second- or third-line treatment of advanced endometrial cancer: endorad, a phase ii trial of gineco. Br J Cancer 2013; 108: 1771-1777.

[5] Amin KS, Banerjee PP. The cellular functions of rassf1a and its inactivation in prostate cancer. J Carcinog 2012; 11: 3.
[6] Dammann R, Schagdarsurengin U, Seidel C, Strunnikova M, Rastetter M, Baier K, Pfeifer GP. The tumor suppressor rassf1a in human carcinogenesis: an update. Histol Histopathol 2005; 20: 645-663.
[7] Pfeifer GP, Dammann R. Methylation of the tumor suppressor gene rassf1a in human tumors. Biochemistry (Mosc) 2005; 70: 576-583.
[8] Fu L, Zhang S. Rassf1a promotes apoptosis and suppresses the proliferation of ovarian cancer cells. Int J Mol Med 2014; 33: 1153-1160.
[9] Zhou PH, Zheng JB, Wei GB, Wang XL, Wang W, Chen NZ, Yu JH, Yao JF, Wang H, Lu SY, Sun XJ. Lentivirus-mediated rassf1a expression suppresses aggressive phenotypes of gastric cancer cells in vitro and in vivo. Gene Ther 2015; 22: 793-801.
[10] Chen Q, Gao Q, Chen K, Wang Y, Chen L, Li XU. Curcumin suppresses migration and invasion of human endometrial carcinoma cells. Oncol Lett 2015; 10: 1297-1302.
[11] Porichi O, Nikolaidou ME, Apostolaki A, Arnogiannaki N, Papassideri I, Chatonidis I, Tserkezoglou A, Vorgias G, Kassanos D, Panotopoulou E. Isomorph expression of bag-1 gene, er and pr in endometrial cancer. Anticancer Res 2010; 30: 4103-4108.
[12] Zhang L, Wen K, Han X, Liu R, Qu Q. Adiponectin mediates antiproliferative and apoptotic responses in endometrial carcinoma by the adipors/ampk pathway. Gynecol Oncol 2015; 137: 311-320.
[13] Keshamouni VG, Mattingly RR, Reddy KB. Mechanism of 17-beta-estradiol-induced erk1 /2 activation in breast cancer cells. A role for her2 and pkc-delta. J Biol Chem 2002; 277: 22558-22565.
[14] Migliaccio A, Di Domenico M, Castoria G, de Falco A, Bontempo P, Nola E, Auricchio F. Tyrosine kinase/p21ras/map-kinase pathway activation by estradiol-receptor complex in mcf-7 cells. EMBO J 1996; 15: 1292-1300.
[15] Razandi M, Pedram A, Park ST, Levin ER. Proximal events in signaling by plasma membrane estrogen receptors. J Biol Chem 2003; 278: 2701-2712.
[16] Wang WG, Chen SJ, He JS, Li JS, Zang XF. The tumor suppressive role of rassf1a in osteosarcoma through the wnt signaling pathway. Tumour Biol 2016; 37: 8869-8877.
[17] Lim S, Yang MH, Park JH, Nojima T, Hashimoto H, Unni KK, Park YK. Inactivation of the rassf1a in osteosarcoma. Oncol Rep 2003; 10: 897-901.
[18] Feng L, Li J, Yan LD, Tang J. Rassf1a suppresses proliferation of cervical cancer cells. Asian Pac J Cancer Prev 2014; 15: 5917-5920.
[19] Dallol A, Agathanggelou A, Tommasi S, Pfeifer GP, Maher ER, Latif F. Involvement of the rassf1a tumor suppressor gene in controlling cell migration. Cancer Res 2005; 65: 7653-7659.
[20] van der Weyden L, Papaspyropoulos A, Poulogiannis G, Rust AG, Rashid M, Adams DJ, Arends MJ, O'Neill E. Loss of rassf1a synergizes with deregulated runx2 signaling in tumorigenesis. Cancer Res 2012; 72: 3817-3827.
[21] Yi M, Yang J, Chen X, Li J, Li X, Wang L, Tan Y, Xiong W, Zhou M, McCarthy JB, Li G, Xiang B, Xie H. Rassf1a suppresses melanoma development by modulating apoptosis and cell-cycle progression. J Cell Physiol 2011; 226: 2360-2369.
[22] Dreijerink K, Braga E, Kuzmin I, Geil L, Duh FM, Angeloni D, Zbar B, Lerman MI, Stanbridge EJ, Minna JD, Protopopov A, Li J, Kashuba V, Klein G, Zabarovsky ER. The candidate tumor suppressor gene, rassf1a, from human chromosome 3p21.3 is involved in kidney tumorigenesis. Proc Natl Acad Sci U S A 2001; 98: 7504-7509.
[23] Thaler S, Hahnel PS, Schad A, Dammann R, Schuler M. Rassf1a mediates p21cip1/waf1-dependent cell cycle arrest and senescence through modulation of the raf-mek-erk pathway and inhibition of akt. Cancer Res 2009; 69: 1748-1757.
[24] Hoshino R, Chatani Y, Yamori T, Tsuruo T, Oka H, Yoshida O, Shimada Y, Ari-i S, Wada H, Fujimoto J, Kohno M. Constitutive activation of the 41-/43-kda mitogen-activated protein kinase signaling pathway in human tumors. Oncogene 1999; 18: 813-822.
[25] Tong JS, Zhang QH, Wang ZB, Li S, Yang CR, Fu XQ, Hou Y, Wang ZY, Sheng J, Sun QY. Er-alpha36, a novel variant of er-alpha, mediates estrogen-stimulated proliferation of endometrial carcinoma cells via the pkcdelta/erk pathway. PLoS One 2010; 5: e15408.
[26] Wang Y, Zhu Y, Zhang L, Tian W, Hua S, Zhao J, Zhang H, Xue F. Insulin promotes proliferation, survival, and invasion in endometrial carcinoma by activating the mek/erk pathway. Cancer Lett 2012; 322: 223-231.
[27] Thaler S, Schmidt M, Schad A, Sleeman JP. Rassf1a inhibits estrogen receptor alpha expression and estrogen-independent signalling: Implications for breast cancer development. Oncogene 2012; 31: 4912-4922.
[28] Kaaks R, Lukanova A, Kurzer MS. Obesity, endogenous hormones, and endometrial cancer risk: a synthetic review. Cancer Epidemiol Biomarkers Prev 2002; 11: 1531-1543.

[29] Engelsen IB, Stefansson IM, Akslen LA, Salvesen HB. Gata3 expression in estrogen receptor alpha-negative endometrial carcinomas identifies aggressive tumors with high proliferation and poor patient survival. Am J Obstet Gynecol 2008; 199: 543, e541-547.

[30] Jongen V, Briet J, de Jong R, ten Hoor K, Boezen M, van der Zee A, Nijman H, Hollema H. Expression of estrogen receptor-alpha and -beta and progesterone receptor-a and -b in a large cohort of patients with endometrioid endometrial cancer. Gynecol Oncol 2009; 112: 537-542.

[31] Srijaipracharoen S, Tangjitgamol S, Tanvanich S, Manusirivithaya S, Khunnarong J, Thavaramara T, Leelahakorn S, Pataradool K. Expression of er, pr, and her-2/neu in endometrial cancer: a clinicopathological study. Asian Pac J Cancer Prev 2010; 11: 215-220.