RASSF1A regulates the abnormal cell proliferation in psoriasis via inhibition of YAP

Jinjing Jia (✉ 13310980167@163.com)

the second hospital of Guangzhou University of Chinese Medicine <https://orcid.org/0000-0003-2297-4394>

Ning Wang

Xi'an Jiaotong University Second Affiliated Hospital

Yan Zheng

Xi'an Jiaotong University Second Affiliated Hospital

Xiumei Mo

the second affiliated hospital of Guangzhou University of Chinese Medicine

Yu Zhang

the second affiliated hospital of Guangzhou University of Chinese Medicine

Siqi Ye

the second hospital of Guangzhou University of Chinese Medicine

Junfeng Liu

the second affiliated hospital of Guangzhou University of Chinese Medicine

Fenggen Yan

the second hospital of Guangzhou University of Chinese Medicine

Hongyi Li

the second affiliated hospital of Guangzhou University of Chinese Medicine

Dacan Chen

the second affiliated hospital of Guangzhou University of Chinese Medicine

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Abstract

Background: Psoriasis is a chronic, inflammatory skin disease with high incidence, treatment resistance, and high recurrence. Currently, the exact etiology and pathogenesis of psoriasis are unclear. The goal of this study was to characterize the effect of the upstream negative regulator RAS-association domain family 1A (RASSF1A) on Yes-associated protein (YAP) in psoriasis.

Methods: Skin lesions of 22 patients with psoriasis and 19 controls with normal skin tissue were used. Human epidermal keratinocytes were stimulated with M5 (IL-1 α , IL-17, IL-22, TNF- α , oncostatin M) to establish the psoriatic cell model. Methylation inhibitor 5-Aza-CdR was prepared at different concentrations (5, 10, 20 μ mol/L). Cells were infected with lentivirus vector overexpressing RASSF1A. Twenty-five 6–8-week-old female BALB/c mice were used to establish the psoriatic mouse model. Mice were randomly divided into five groups: control (Vaseline applied daily), psoriasis (imiquimod applied daily), and the three different 5-Aza-CdR concentrations (applied daily with imiquimod). Methylation-specific PCR (MSP) was used to detect RASSF1A methylation and immunohistochemistry was used to detect RASSF1A expression in skin lesions. After adding 5-Aza-CdR or lentivirus vector overexpressing RASSF1A, YAP expression, cell proliferation, cell cycle, apoptosis, inflammatory cytokines, and related signal pathway activity were investigated.

Results: As RASSF1A methylation level increased, its expression in patients with psoriasis and mice with skin lesions decreased. Addition of 5-Aza-CdR or lentivirus vector overexpressing RASSF1A increased the expression of RASSF1A, reduced the expression of YAP and inflammatory cytokines, cell proliferation, as well as AKT, ERK, STAT3, and NF- κ B signaling pathway activities, induced cell cycle arrest in G0/G1 phase, increased apoptosis, and improved skin lesions.

Conclusions: RASSF1A inhibited the proliferation of psoriatic cells, induced apoptosis, and reduced the expression of inflammatory factors by inhibiting YAP expression. Based on our findings, targeted drugs that can inhibit RASSF1A methylation and increase its expression may be useful in the treatment of psoriasis.

1. Introduction

Psoriasis is a chronic, inflammatory, and recurrent skin disease with a population incidence of 0.1–4%. Psoriasis affects all ages and genders equally^{1,2}. Due to the high incidence, treatment resistance, recurrence, and often visible skin lesions, psoriasis is considered a persistent skin disease that affects the physical and mental health of the patients^{3,4}. Currently, the exact etiology and pathogenesis of psoriasis are unclear. The over-proliferation of keratinocytes (KC) and shortening of cell cycle are important pathophysiological characteristics of psoriasis. Research shows that KC in psoriasis are apoptosis-resistant; it is, therefore, important to explore the causes of KC apoptosis imbalance and cell cycle acceleration while studying the pathogenesis of psoriasis⁵. In our previous study, we found that Yes-associated protein (YAP) was highly expressed in psoriatic lesions and was involved in its pathogenesis

through regulation of KC proliferation and apoptosis⁶. RAS-association domain family 1A (RASSF1A) is the most common negative regulator of RAS. It is expressed in almost all normal tissues, but is downregulated or absent in some tumor tissues and cell lines⁷.

The core of the kinase cascade of the Hippo signaling pathway includes Mst1/2, Sav1, Lats1/2, Mob1, YAP, and transcribed activator with PDZ binding motif, and the signaling transduction process is composed of a series of kinase cascade phosphorylation reactions. YAP is the downstream effector of this signaling pathway¹⁴, which regulates cell proliferation and apoptosis, and regulates the growth, development, and size of tissues and organs. When phosphorylated by upstream kinases at serine 127 site, pS127-YAP is inactivated and retained in the cytoplasm, so that cannot play its role as a transcription factor^{14,15}. However, the relationship between RASSF1A and the Hippo-YAP signaling pathway is still unclear. Studies show that *RASSF1A* rarely mutates, and its low or loss of expression is mainly due to hypermethylation of the CpG island in the promoter region⁸. RASSF1A can be used as a negative upstream regulator of YAP⁹⁻¹¹, which may play an important role in psoriasis pathogenesis. It is suggested that RASSF1A can be used as a promoter of the upstream kinase cascade to regulate the Hippo-YAP signaling pathway. Several research groups have found that in mammalian cells, RASSF1A can interact with Mst, the core of the kinase cascade in the Hippo-YAP signaling pathway. Furthermore, RASSF1A can interact with Mst1/2 through the SARAH domain^{9,10}. However, before 2007, the role of RASSF1A in combination with Mst1/2 was unclear. Matallanas et al.¹¹ found that the oncogene *Raf* can bind and inhibit the activity of Mst2, thereby inhibiting the apoptotic signaling pathway. RASSF1A inhibits the interaction between *Raf* and Mst2, leading to phosphorylation of Mst2, and further to phosphorylation of Lats1/2 and downstream YAP. Phosphorylated YAP binds to p73 in the nucleus and promotes the transcription of *PUMA*, which promotes apoptosis. In addition, RASSF1A also activates the Hippo signaling pathway to induce Fas, TNF- α , or BAX-mediated apoptosis. Knockdown of RASSF1A leads to a decrease in Mst1/2 activation and to blockage of apoptosis induced by Fas or TNF- α , indicating that RASSF1A and Mst1/2 belong to the same signaling pathway. This study examined RASSF1A expression and methylation in psoriasis, analyzed its correlation with YAP expression, and verified its effect on YAP expression, KC proliferation, cell cycle, and apoptosis by modifying RASSF1A methylation level *in vitro* and *in vivo*.

2. Materials And Methods

2.1 Patient samples

Skin lesions of 22 psoriasis patients (12 males and 10 females) were collected from the Department of Dermatology tissue bank at the Second Affiliated Hospital of Xi'an Jiaotong University from September 2016 to August 2019, and 19 normal skin tissues (10 males and 9 females) from cosmetic surgeries were selected as controls. The study was approved by the ethics committee of the Second Affiliated Hospital of Xi'an Jiaotong University. Specimens were obtained with informed consent, and were confirmed by pathomorphological examination. Tissue samples were paraffin-embedded and sectioned for

immunohistochemical staining. The results were classified as negative (-), weakly positive (+), moderately positive (++) and strongly positive (+++) after double blind examination by 2 pathologists. Additionally, 10 psoriasis samples and 10 healthy controls were frozen in liquid nitrogen for subsequent protein and methylation level detection.

2.2 Establishing the psoriatic cell model

Human epidermal KC (ScienCell) were routinely cultured in KC medium (ScienCell). We used 10 ng/mL M5 (mixture of IL-1 α , IL-17, IL-22, TNF- α and oncostatin M, PeproTech) to stimulate the cells for 48 h using the scheme for establishing a psoriatic cell model¹². Three cell groups, each treated with a different concentration of 5-Aza-CdR (5, 10, 20 μ mol/L; MedChemExpress), a widely used methylation inhibitor, were set-up after the cells were treated with M5.

2.3 Lentivirus infection

RASSF1A overexpression vector was constructed by Shenyang Wanleibio Technology. Cells were adjusted in the logarithmic growth period and infected with lentivirus according to the manufacturer's instructions. RASSF1A and YAP expression was detected 48 h after infection.

2.4 Establishing the psoriatic mouse model

Twenty-five 6–8-week-old female BALB/c mice (18–20 g) were selected and housed under specific pathogen-free conditions. Experiments were approved by the ethics committee of the Second Affiliated Hospital of Xi'an Jiaotong University. After 1 week of adaptive feeding, the mice were randomly divided into 5 groups: control, psoriasis, and three 5-Aza-CdR groups, each treated with a different concentration of 5-Aza-CdR. In the psoriasis group, 62.5 mg of imiquimod cream (Hubei Keyi Pharmaceutical Co., Ltd.) was applied daily to the back¹³. In the control group, equal amount of vaseline (Nanchang Baiyun Pharmaceutical Co., Ltd.) was applied daily. In the 5-Aza-CdR group, different concentrations (5, 10, 20 μ mol/L) of 5-Aza-CdR were prepared with dimethyl sulfoxide (DMSO) and applied with imiquimod. Seven days later, the mice were anesthetized with phenobarbital (Shenyang Wanleibio Co., Ltd) euthanized, and then skin lesions were taken.

2.5 Quantitative real-time PCR (qRT-PCR)

TRIzol (Invitrogen) was used to extract the total RNA from cells in each experimental group, and cDNA was obtained by reverse transcription. PCR primers were synthesized by Shanghai Biotechnology Co., Ltd.

The primer sequence is shown in Supplemental Table S1. The standard curve for each group was obtained by qRT-PCR, and the CT value was computer-analyzed. After standardization with glyceraldehyde-3-phosphate dehydrogenase (GAPDH), relative expression was calculated with the $2^{-\Delta\Delta CT}$ method.

2.6 Western blot

Total protein was extracted and quantified. Twenty-five micrograms of protein was separated by 10% g polyacrylamide gel electrophoresis (PAGE), and then transferred to a nitrocellulose membrane. Skim milk 5% was used to block non-specific antigen binding, and then primary antibodies were added (RASSF1A, Abcam, ab23950; YAP, Cell Signaling Technology, #4912; pS127-YAP, Cell Signaling Technology, #13008; Cyclin A, Santa Cruz, sc-596; Cyclin B1, Proteintech, 55004-1-AP; Cyclin D1, Santa Cruz, sc-246; Cyclin E, Santa Cruz, sc-25303; CDK1, Wanleibio, WL02373; cleaved-caspase-3, Wanleibio, WL02348; Bcl-2, Wanleibio, WL01556; BAX, Proteintech, 50599-2-Ig; p73, Wanleibio, WL01604; p53, Santa Cruz, sc-126; p21, Wanleibio, WL0362; AKT, Wanleibio, WL0003b; p-AKT, Wanleibio, WLP001a; ERK, Wanleibio, WL01864; p-ERK, Wanleibio, WLP1512; STAT3, Wanleibio, WL03207; p-STAT3, Wanleibio, WLP2412; NF- κ B P65, Wanleibio, WL01980; β -actin, Santa Cruz, sc-47778; Histone H3, Wanleibio) and incubated at 4 °C overnight. Next, secondary antibodies were added and incubated at room temperature for 1 h, and finally exposed on X-ray film.

2.7 Detection of RASSF1A gene promoter methylation

Tissue DNA was extracted according to the manufacturer's instructions (Bioteke). Methylation of RASSF1A promoter was detected by methylation-specific PCR (MSP). Flowing treatment of DNA samples with EZ DNA methylation kit (ZYMO), PCR assay, and agarose gel analysis were performed. Primer sequences of methylated RASSF1A (M-RASSF1A) and unmethylated RASSF1A (U-RASSF1A) are provided in Supplemental Table S2.

2.8 Cell proliferation assay

MTT assay was used to assess cell proliferation. Cells in the logarithmic growth period were inoculated on a 96-well plate with 5×10^3 cells/ 200 μ L culture medium per well. After 24, 48, and 72 h of culture, 20 μ L of MTT was added into each well. After 4 h of incubation in the dark, 150 μ L DMSO was added into each well and shaken for 10 min to fully dissolve the crystals. The 490 nm absorbance value of each well was measured by a microplate reader. Five technical replicates were set-up for each group.

2.9 Cell cycle

Cells were digested according to the cell cycle kit manufacturer's instructions (Jiangsu Kaiji Biotechnology Co., Ltd.), washed twice with precooled phosphate buffered saline (PBS), and fixed overnight with 75% ethanol at -20 °C. Then, 100 µg/mL RNaseA and 50 µg/mL propidium iodide (PI) staining solution were added, and cells were incubated at 37 °C in the dark for 30 min. Cell cycle was measured through flow cytometry (FACSCalibur, BD Biosciences).

2.10 Cell apoptosis

Cells were digested according using a cell apoptosis kit following the manufacturer's instructions (Jiangsu Kaiji Biotechnology Co., Ltd.) and were washed twice with precooled PBS. Cells ($1-5 \times 10^5$) suspended in 500 µL binding buffer were collected, stained with 5 µL Annexin V-FITC and 5 µL PI staining solution, and then incubated at room temperature in the dark for 5–15 min. Cell apoptosis was measured through flow cytometry.

2.11 ELISA

Using the ELISA Kit's instructions (Lianke Biotech), supernatant or mouse serum samples were detected by standard ELISA. Optical density (OD) 450 nm values were then obtained using a spectrophotometer to draw the standard curve. The concentrations of IL-1 β , IL-6, IL-17, and IL-23 were then measured.

2.12 Statistical analysis

All data are presented as mean $\pm$ standard error of the mean (SEM). SPSS 19.0 software was used for statistical analysis. Pearson chi-square and Mann-Whitney *U* tests were used to analyze the immunohistochemistry results. Student *t*-test was used for comparisons between 2 groups. One-way analysis of variance (ANOVA) was used for experiments with more than 2 groups and least significant difference (LSD) test was used as a post-hoc for multiple comparisons. $P < 0.05$ was considered statistically significant.

3. Results

3.1 Expression and methylation of RASSF1A in psoriasis

Immunohistochemistry results showed that the rate of RASSF1A protein expression was 78.95% (15/19) in normal skin tissues and 50.00% (11/22) in psoriatic lesions. The difference was statistically significant (Table 1, Fig. 1a). The qRT-PCR and western blot results showed that RASSF1A mRNA and protein

expression in normal skin was higher than that in psoriatic lesions (Fig. 1b, c). Results of MSP assays performed for methylation detection showed that the methylation level of RASSF1A increased gradually in normal skin tissue, normal tissue beside psoriatic lesions, and psoriatic tissues, indicating that low expression may be due to promoter methylation (Fig. 1d).

3.2 Validation of RASSF1A overexpression efficiency and effect on YAP expression

To confirm the effect of RASSF1A overexpression on YAP expression, we infected cells with a RASSF1A overexpressing lentivirus. After 48 h of lentivirus infection, RNA and protein were extracted to verify the RASSF1A overexpression efficiency. Compared with those in the control group (Ctrl) and the group infected with empty virus vector (BLK), RASSF1A mRNA and protein were overexpressed in the group infected with RASSF1A overexpression lentivirus. RASSF1A overexpression reduced YAP expression and induced its phosphorylated protein pS127-YAP expression (Fig. 1e and Supplemental Fig. S1a, b).

3.3 Effect of methylation inhibitor 5-Aza-CdR on RASSF1A and YAP expression in psoriatic cells

5-Aza-CdR is a methylation inhibitor that can inhibit the methylation of RASSF1A gene and induce its expression. MSP results showed that different concentrations of 5-Aza-CdR reduced RASSF1A methylation level in psoriatic cells induced by M5 dose-dependently (Fig. 1f). Western blot showed that 5-Aza-CdR dose-dependently increased RASSF1A expression in psoriatic cells, with the level not returning to pre-M5 levels. Simultaneously, 5-Aza-CdR gradually reduced YAP expression and induced pS127-YAP expression in M5-induced psoriatic cells. RASSF1A overexpression in psoriatic cells had the similar effect with 5-Aza-CdR (Fig. 1g).

3.4 Effect of RASSF1A overexpression and 5-Aza-CdR on cell proliferation

To verify RASSF1A overexpression upon lentivirus infection or upon treatment with the methylation inhibitor 5-Aza-CdR, MTT assay was performed. Results showed that cell proliferation increased at all time points after adding M5, consistent with the observation in the abnormal psoriasis cell proliferation model. After adding 5-Aza-CdR, cell proliferation was inhibited, and was more obvious with increasing 5-Aza-CdR concentration, but did not return to pre-M5 level. The proliferation ability of cells also increased following BLK infection and incubation with M5, but there was no significant difference compared with the uninfected group. However, after RASSF1A overexpression, the proliferation ability of cells decreased significantly but did not return to pre-M5 level (Fig. 2a).

3.5 Effect of RASSF1A overexpression and 5-Aza-CdR on cell cycle

PI staining and flow cytometry were used to detect cell cycle after RASSF1A was overexpressed through lentivirus infection or through treatment with the methylation inhibitor 5-Aza-CdR. Results showed that the cell proportion decreased in the G0/G1 phase and increased in the S phase after adding M5, indicating that the proportion of cells in active replication increased. This was consistent with the abnormal psoriasis cell proliferation model (Fig. 2a). Adding 5-Aza-CdR increased the G0/G1 phase ratio and decreased the S phase ratio, and this was more obvious with increasing 5-Aza-CdR concentration, but did not return to pre-M5 level. There was no significant difference in cell cycle distribution between the BLK transfected group and the non-transfected group. However, after RASSF1A overexpression, the G0/G1 phase proportion increased and S phase proportion decreased, but did not return to pre-M5 level. Although G2/M ratio also changed, there was no statistical difference (Fig. 2b and Supplemental Fig. S1c). Western blot showed that the expression of cyclin A, cyclin B1, cyclin D1, cyclin E, and CDK1 increased at different levels when M5 was added. Their expression decreased in a dose-dependent manner when 5-Aza-CdR was added, and RASSF1A overexpression had the same effect (Fig. 2c). Cell cycle regulation is complicated because of different regulating factors that are involved in each cell cycle. Our results showed that altering RASSF1A expression affected the cell cycle process of psoriatic cells by affecting the expression of each cell cycle regulation factor.

3.6 Effect of RASSF1A overexpression and 5-Aza-CdR on cell apoptosis

V-FITC/PI double staining and flow cytometry were used to detect cell apoptosis after RASSF1A was overexpressed through lentivirus infection or through treatment with the methylation inhibitor 5-Aza-CdR. Results showed that KC apoptosis rate decreased when M5 was added, and increased when 5-Aza-CdR or RASSF1A was added, but did not return to pre-M5 level (Fig. 2d and Supplemental Fig. S1d). Further, western blot results showed that the expression of apoptosis-related proteins, cleaved-caspase-3 (c-caspase-3), BAX, p53, and p21 decreased, while that of Bcl-2 and p73 increased. RASSF1A overexpression or 5-Aza-CdR methylation inhibitor partially restored their expression at different levels (Fig. 2e).

3.7 Effect of methylation inhibitor 5-Aza-CdR on psoriatic mouse model

Our results *in vitro* confirmed that RASSF1A overexpression through lentivirus infection or through treatment with the methylation inhibitor 5-Aza-CdR could inhibit cell proliferation and induce G0/G1 cell cycle arrest and apoptosis of M5-induced psoriatic cell model. To verify the results *in vivo*, we established imiquimod-induced psoriatic mouse model. The skin of psoriatic model group (Pso) mice was thickened with erythema and scales, while the skin of Ctrl mice was normal, indicating that the model induction was successful. The erythema and scales on the mouse's back decreased, and the skin lesions became thinner with increasing 5-Aza-CdR concentration (Table 2, Fig. 3a, b).

3.8 Effect of methylation inhibitor 5-Aza-CdR on YAP and RASSF1A expression in psoriatic mouse model

Immunohistochemistry confirmed that RASSF1A expression in the Pso was lower than that in the Ctrl in mice similar to that in the human samples (Fig. 1a). The addition of 5-Aza-CdR increased the positive rate of RASSF1A expression, and the trend was more obvious with increasing 5-Aza-CdR concentration. The semiquantitative analysis results showed that the intensity of RASSF1A expression in the epidermis of the Ctrl group was significantly different from that in the Pso group ($P=0.032$). Moreover, the expression of YAP in the Pso group was higher than that in the Ctrl group. The positive rate of YAP expression decreased after adding 5-Aza-CdR, and the trend was more obvious with increasing 5-Aza-CdR concentration. The semiquantitative analysis of expression intensity showed that RASSF1A expression in the Ctrl group was significantly higher than that in the Pso group ($P=0.009$), and there was a significant difference between the Pso group and 10 μM 5-Aza-CdR group ($P=0.031$) or 20 μM 5-Aza-CdR group ($P=0.013$) (Table 3, Fig. 4a, b). MSP confirmed 5-Aza-CdR could reduce RASSF1A methylation (Fig. 4c), and western blot confirmed 5-Aza-CdR could induce RASSF1A expression, while reducing YAP expression and driving its phosphorylated protein pS127-YAP in a dose-dependent manner (Fig. 4d).

3.9 Effects of RASSF1A overexpression and 5-Aza-CdR on the expression of cytokines

To detect changes in different cytokines after RASSF1A overexpression through lentivirus infection or through treatment with the methylation inhibitor 5-Aza-CdR *in vitro* and *in vivo*, ELISA was performed. Results showed that the concentrations of IL-1 β , IL-6, IL-17, and IL-23 in the supernatant of the psoriatic model of cells or mouse serum increased. RASSF1A overexpression or addition of 5-Aza-CdR in cells and topical 5-Aza-CdR administration on the back of mice reduced the expression of the cytokines to different levels, but the levels did not return to the Ctrl level (Fig. 5a, b). The results suggested that RASSF1A may reduce the expression of these cytokines to a certain extent to reduce the inflammatory response in psoriasis.

3.10 The effect of RASSF1A overexpression and 5-Aza-CdR on the expression of various signaling pathways

To explore which signaling pathways are involved in the effect of RASSF1A on psoriasis, the expression of the key members of AKT, ERK, STAT3, and NF- κ B pathways was detected through western blotting. Results showed that the expression of p-ERK, p-STAT3, NF- κ B P65 in the nucleus (NF- κ B P65[n]) increased and that of NF- κ B P65 in the cytoplasm (NF- κ B P65[p]) decreased with M5 addition to KC cells under conditions where the total AKT, ERK, and STAT3 were basically unchanged, indicating increased activities of these signaling pathways. However, RASSF1A overexpression or addition of methylation

inhibitor 5-Aza-CdR reduced the expression of p-ERK, p-STAT3, NF- κ B P65 (n) and increased that of NF- κ B P65 (p), with not so obvious changes in the expression of p-AKT (Fig. 5c).

4. Discussion

Our previous study showed that YAP expression increased in psoriasis patients and mouse model of psoriasis skin lesions. *In vitro*, after YAP knockdown, the proliferation of the KC cell line HaCaT slowed down, the cell cycle was blocked in the G0/G1 phase and cell apoptosis increased⁶. However, it is still unknown that which upstream regulator regulate its expression.

In this study, we found that the expression of RASSF1A decreased whereas its methylation increased in psoriasis lesions compared to those in normal skin tissues. So we speculated that there might be a relationship between the expression of RASSF1A and YAP. As it was assumed that low or loss of RASSF1A expression is mainly caused by hypermethylation of the CpG island in the promoter region⁸, we overexpressed RASSF1A by inhibiting its methylation or by lentivirus infection. After RASSF1A was overexpressed through lentivirus infection or through treatment with the methylation inhibitor 5-Aza-CdR, the expression of YAP was found to be downregulated. Simultaneously, the proliferation rate of the M5-induced psoriatic cells decreased; the cell cycle was blocked in the G0/G1 phase and the mRNA expression of the cell cycle regulators cyclin A, cyclin B1, cyclin D1, cyclin E, and CDK1 was decreased. Further, under this same condition, apoptosis increased and the expression of the apoptosis-promoting proteins c-caspase-3, BAX, p53, and p21 increased, while that of the apoptosis-inhibiting protein Bcl-2 decreased. The study of related signaling pathways showed that the expression of key molecules in the ERK, STAT3, and NF- κ B signaling pathways was downregulated to varying degrees, with not so obvious changes in the AKT signaling activity, after RASSF1A overexpression. *In vivo* study of the imiquimod-induced psoriatic mice confirmed that the methylation inhibitor 5-Aza-CdR could improve skin lesions, increase RASSF1A expression, and reduce YAP expression at the same time. Meanwhile, levels of IL-1 β , IL-6, IL-17, and IL-23 in the supernatant of the psoriatic model of cells or mouse serum decreased, indicating the suppression of inflammatory response. The changes in the psoriatic cell model after RASSF1A overexpression were consistent with those observed after YAP knockdown⁶. As overexpression of RASSF1A inhibited YAP expression in both cell and animal experiments, we believe that RASSF1A can inhibit psoriatic cell proliferation, induce apoptosis, and improve the expression of inflammatory factors by inhibiting YAP expression.

5. Conclusions

Our study found that as RASSF1A methylation level increased, its expression in psoriasis patients and mice with skin lesions decreased. Adding methylation inhibitor 5-Aza-CdR or RASSF1A overexpression lentivirus inhibited cell proliferation, induced cell cycle arrest at G0/G1 phase, increased apoptosis, reduced the expression of inflammatory cytokines and activity of AKT, ERK, STAT3 and NF- κ B signaling pathways, and inhibited the expression of YAP. Increasing RASSF1A expression through methylation inhibitors may provide new directions, ideas, and strategies for developing new drug therapies for treating

psoriasis. However, the specific regulation process of the Hippo-YAP signaling pathway, particularly the influence of upstream factors such as Mst1/2, Sav1, Lats1/2 and Mob1 in the core of the kinase cascade, needs further study.

Abbreviations

RASSF1A: RAS-association domain family 1A; YAP: Yes-associated protein; MSP: Methylation specific PCR; KC: keratinocytes; DMSO: dimethyl sulfoxide; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; PAGE: polyacrylamide gel electrophoresis

Declarations

Ethics approval and consent to participate

The study was approved by the ethics committee of the Second Affiliated Hospital of Xi'an Jiaotong University (2018-2096).

Consent for publication

Written informed consent for tissue collection and use was obtained from all patients.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

JJ and Y.Z. designed the experiments. JJ, YZ, SY, and WN performed the experiments. JL and FY analyzed data. JJ, HL and XM wrote the manuscript. DC is the supervisor of the whole project. All authors read and approved the final manuscript.

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Tables

Table 1. RASSF1A expression in psoriasis and normal skin tissues

Group	N	Expression Grade				Positive Rate (%)
		-	+	++	+++	
Normal	19	4	5	4	6	78.947
Psoriasis	22	11	6	4	1	50.000*

* $P < 0.05$ compared with normal skin.

Table 2. Thicknesses of the skin tissues of mice

Group	N	Skin thickness (μm)
Ctrl	5	23.21 $\pm$ 2.66
Pso	5	169.76 $\pm$ 21.36
5 μM 5-Aza-CdR	5	156.65 $\pm$ 11.58
10 μM 5-Aza-CdR	5	129.68 $\pm$ 12.08*
20 μM 5-Aza-CdR	5	71.10 $\pm$ 7.07***

* $P < 0.05$, *** $P < 0.001$ vs. Pso.

Table 3. Results of immunohistochemistry of RASSF1A and YAP in mouse skin lesions

	Group	N	Expression Grade				Positive Rate (%)
			-	+	++	+++	
RASSF1A	Ctrl	5	1	0	1	3	80
	Pso	5	4	1	0	0	20
	5 μM 5-Aza-CdR	5	3	2	0	0	40
	10 μM 5-Aza-CdR	5	2	3	0	0	60
	20 μM 5-Aza-CdR	5	2	2	1	0	60
	Ctrl	5	4	1	0	0	20
YAP	Pso	5	0	1	1	3	100 ^{##}
	5 μM 5-Aza-CdR	5	1	2	1	1	80
	10 μM 5-Aza-CdR	5	2	2	1	0	60
	20 μM 5-Aza-CdR	5	3	2	0	0	40*

$P < 0.01$ vs. Ctrl * $P < 0.05$ vs. Pso.

Figures

Figure 1

Expression and methylation of RASSF1A in the skin lesions and effect of RASSF1A overexpression lentivirus transfection and methylation inhibitor 5-Aza-CdR. a Immunohistochemistry of RASSF1A in psoriasis and normal skin tissues (bar length = 600 μ m). b RASSF1A mRNA expression in psoriasis and normal skin tissues. c RASSF1A protein expression in psoriasis and normal skin tissues. d RASSF1A methylation in psoriasis and normal skin tissues. e RASSF1A, YAP and pS127-YAP protein expression after RASSF1A overexpression lentivirus transfection. f RASSF1A methylation after adding methylation inhibitor 5-Aza-CdR. g RASSF1A, YAP and pS127-YAP protein expression in M5 induced psoriatic cells after adding methylation inhibitor 5-Aza-CdR or RASSF1A overexpression lentivirus. M=methylated RASSF1A, U=unmethylated RASSF1A. ***P<0.001 vs. Normal.

Figure 2

Effect of overexpression of RASSF1A and 5-Aza-CdR on cell proliferation, cell cycle and apoptosis. a Cell proliferation rate 24, 48, and 72 h after adding methylation inhibitor 5-Aza-CdR or transfecting RASSF1A overexpression lentivirus. b The cell cycle profiles were analyzed 48 h after adding methylation inhibitor 5-Aza-CdR or transfecting RASSF1A overexpression lentivirus. c Cell cycle regulators were analyzed by western blot 48 h after adding methylation inhibitor 5-Aza-CdR or transfecting RASSF1A overexpression

lentivirus. d The cell apoptosis profiles were analyzed 48 h after adding methylation inhibitor 5-Aza-CdR or transfecting RASSF1A overexpression lentivirus. e Apoptosis-related proteins were analyzed by western blot 48 h after adding methylation inhibitor 5-Aza-CdR or transfecting RASSF1A overexpression lentivirus. *P<0.05, **P<0.01, ***P<0.001 vs. M5; ##P<0.01, ###P<0.001 vs. M5+BLK.

Figure 3

Effect of methylation inhibitor 5-Aza-CdR on psoriatic mouse model. a General pictures of mice. b HE staining for histopathological features (bar length = 100 μ m).

Figure 4

Effect of methylation inhibitor 5-Aza-CdR on methylation or expression of RASSF1A and YAP in psoriatic mouse model. a Immunohistochemistry of RASSF1A expression (bar length = 50 μ m). b Immunohistochemistry of YAP expression (bar length = 50 μ m). c RASSF1A methylation. M=methylated

RASSF1A, U=unmethylated RASSF1A. d RASSF1A, YAP and pS127-YAP protein expression in the skin tissues of the mice.

Figure 5

Effect of overexpression of RASSF1A and 5-Aza-CdR on the expression of cytokines or signaling pathways. a IL-1 β , IL-6, IL-17, and IL-23 in the cell culture supernatant 48 h after adding methylation inhibitor 5-Aza-CdR or transfecting RASSF1A overexpression lentivirus in M5 induced psoriatic cell model.

b IL-1 β , IL-6, IL-17, and IL-23 in mouse serum. c Protein expression of key members of the AKT, ERK, STAT3, and NF- κ B signaling pathways. *P<0.05, **P<0.01, ***P<0.001 vs. M5; ###P<0.001 vs. M5+BLK.

Supplementary Files

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