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ARHGAP17 enhances 5-fluorouracil-induced apoptosis in colon cancer cells by suppressing Rac1

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Colon cancer is a common cause of death in the world, and its main cause of therapy failure is chemoresistance. Apoptosis is de-regulated in colon cancer and is one key mechanism of cancer treatment. We recently reported that reduced expression of ARHGAP17, a Rho GTPase activating protein, correlated with a poor prognosis of colon cancer patients. Here we investigated the role of ARHGAP17 in apoptosis induced by 5-fluorouracil (5-FU) in human colon cancer cells and in mouse xenograft tumor model. We observed a decreased protein level of ARHGAP17 in 5-FU resistant colon cancer cells (HCT116/5-FU and HCT8/5-FU). While ARHGAP17 knockdown attenuated apoptosis upon 5-FU treatment in HCT116 and HCT8, and ARHGAP17 overexpression in HCT116/5-FU and HCT8/5-FU cells increased apoptosis induced by 5-FU. We also found that ARHGAP17 knockdown led to a high level of active Rac1 in HCT116 and HCT8, but ARHGAP17 overexpression reduced active Rac1 in HCT116/5-FU and HCT8/5-FU cells. However, Rac1 inhibitor abolished the effect of ARHGAP17 knockdown, and Rac1 overexpression diminished the effect of ARHGAP17 overexpression on apoptosis induced by 5-FU. Apoptosis was also confirmed by cleaved Caspase-3 and cleaved PARP. Further, we observed that overexpression of ARHGAP17 promoted 5-FU-induced apoptosis and attenuated tumor growth *in vivo*. Collectively, our data indicate that ARHGAP17 sensitizes chemotherapy-resistant colon cancer cells to apoptosis induced by 5-FU, which is in part through suppressing Rac1.

Key words: ARHGAP17; apoptosis; Rac1; colon cancer; chemoresistance

Colon cancer is one leading cause of cancer-associated deaths around the world [1]. About half of people with colon cancer advance to the metastatic stage and patients with advanced-stage of (stage 3 and 4) colon cancer usually receive chemotherapy including oxaliplatin and 5-fluorouracil (5-FU) [2, 3]. However, one of the major causes of therapy failure is chemoresistance, which leads to tumor growth, metastasis, and ultimately the death of the patient [4]. There are a variety of mechanisms responsible for chemotherapy resistance, some of which exist before the course of therapy, while others develop during therapy. One common mechanism that colon cancer cells employ to survive is to evade apoptosis [4]. Apoptosis also called programmed cell death, safeguards tissue homeostasis, and it constitutes a series of biochemical events that are tightly regulated by a network of proteins. Poly (ADP-ribose) polymerase-1 (PARP-1), a chromatin-associated enzyme, can be activated by DNA nicks and breaks and other DNA damage [5]. In this process, caspase 8 and 9 are the initiators, while caspase 3 is one executioner. The initiator, such as caspase 9, can cleave caspase-3, which leads to the activation of caspase 3 [5]. Activated caspase 3 will subsequently cleave fundamental proteins, such as PARP-1. Collectively, these cleavage events lead to cell condensing and ultimately cell death. Many cancer treatments, including 5-FU chemotherapy, execute the anti-cancer effects through apoptosis induction [6]. Meanwhile, cancer cells can become resistant to treatment when they evade apoptosis [6].

ARHGAP17, also known for RICH 1, is one of the Rho GTPase activating proteins and is expressed broadly in human tissues, including the intestinal epithelium [7, 8]. ARHGAP17 maintains epithelial barrier integrity of mouse colon, as ARHGAP17 deficiency led to increased paracellular permeability and abnormal location of the junction complex in mice [9]. ARHGAP17 regulates tight junctions [10, 11] though modulating the activity of Rho GTPases, such as Cdc42 and Rac1 [7, 8]. Also, ARHGAP17 moderates the

tumor-suppressive role of Merlin through inactivating Rac1, and downregulating the Rac1- and Ras-MAPK pathways [10]. Rac1 inhibits apoptosis in intestinal epithelial cells [12], and active Rac1 (Rac1b) is upregulated in advanced stages of colon cancer [13]. Importantly, one recent study showed that overexpression of Rac1b confers resistance to chemotherapy in colon cancer by activating the NF-κB pathway [14], which highlights the importance of Rac1 in cancer treatment resistance. We observed that Rac1 activity was downregulated by overexpression of ARHGAP17 in colon cancer cells [15]. Also, mRNA expression of ARHGAP17 was reduced in the samples of colon cancer, and reduced ARHGAP17 expression correlates with poor outcomes of people with colon cancer [15]. In contrast, overexpression of ARHGAP17 suppressed tumor cell proliferation, as well as restricted cancer invasion to the lung through the Wnt/b-catenin signaling [15], which demonstrated the function of ARHGAP17 in suppressing tumor development as well as metastasis in colon cancer. This tumor suppressor function of ARHGAP17 was also confirmed in cervical cancer, where ARHGAP17 inhibited the proliferation of cervical cancer cells in part by suppressing the PI3K/AKT signaling [16]. However, the effect of ARHGAP17 on apoptosis in colon cancer or its role in chemotherapy resistance is not clear.

In the current research, we studied the impact of ARHGAP17 on apoptosis in colon cancer cells *in vitro*, and on tumor growth *in vivo*. We found that ARHGAP17 knockdown reduced 5-FU-induced apoptosis, while ARHGAP17 overexpression enhanced 5-FU induced apoptosis in 5-FU-resistant colon cancer cells, and impeded tumor growth in nude mice.

Materials and methods

Cell culture. Human colon cancer cell lines (HCT116 and HCT8) were bought from the Shanghai Cell Bank, Chinese Academy of Sciences (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Invitrogen. Cells were cultured in DMEM with 10% FBS and 1% penicillin/streptomycin mixture. The cells were maintained in a humidified incubator with 5% CO_2 at 37°C. Acquired 5-FU resistant HCT116 and HCT8 sublines (HCT116/5-FU) were developed through repeated, intermittent exposure to stepwise increasing doses of 5-FU (0.05, 0.1, and 0.2 μg/ml). All cell culture experiments were performed at least three times.

Lentiviral constructs for human ARHGAP17 or Rac1 were engineered with pLVX-puro vector (Clontech, Palo Alto, CA, USA). shRNAs silencing human ARHGAP17 (shARH-1, GGAUGAAGCUGGAAAUAAAGU; shARH-2, GCUGCUUUGGACUGUUCUACU) and a negative control shRNA (shNC, GGACGAGCUGUACAAGUAA) were cloned into the pLKO.1 vector (Addgene, Cambridge, MA, USA). Lentiviruses were produced following the protocol described in our previous article [15].

Western blotting. Western blotting was performed according to the procedure described in our previous study [15]. Antibodies again ARHGAP17 (ab74454), Rac1 (ab33186), cleaved PARP (ab32064), and cleaved Caspase-3 (ab32042) were ordered from Abcam (Cambridge, MA, USA). Antibody against GAPDH (#5174), a loading control, was from Cell Signaling Technology (Danvers, MA, USA).

Flow cytometry. Cell samples were harvested after 48 h of treatment, and apoptosis was determined with the Annexin V-FITC/PI apoptosis detection kit (Beyotime, China) following the product manual. Data were acquired using BD Biosciences Accuri C6 flow cytometer (Franklin Lakes, NJ, USA), and analyzed with FlowJo software (version 7.6.1, Tree Star, Ashland, OR, USA).

Determination of active Rac1. The Rac1 activity was determined with the detection kit for active Rac1 (Cell Signaling Technology) using the GST-PAK1-binding domain (GST-PBD) pull-down methods as described in the product protocol.

Implantation of tumor xenograft in nude mice. Experiments involving animals were performed in accordance with the institutionally animal welfare and were authorized by Shanghai Eighth's People Hospital (Shanghai, China). Nude mice were purchased from Shanghai Slac Laboratory Animal Co., Ltd. (Shanghai, China) and housed in a dedicated pathogen-free facility. Twenty-four mice, 5–6 weeks old, were divided into the ARHGAP17 overexpressing group and the Vector group with 12 mice per group. HCT116/5-FU cells stably expressing ARHGAP17 (oeARH) or Vector were created with puromycin selection. Cells were suspended in DMEM (serum-free) before injection into mice at a dose of 10^6 cells per mouse. At 12 days post-transplantation, 12 mice in each group were separated into two subgroups, randomly, (n=6 per group), and received an intraperitoneal injection with 20 mg/kg 5-FU or Vehicle (DMSO) twice a week. Tumor growth was monitored every three days. The tumor volume was measured with an established method [17]: tumor volume = $1/2 \times (\text{length} \times \text{width}^2)$ every three days post cell injection. 33 days after cell injection, mice were euthanized and the xenografts were harvested. The tumor samples were then used for the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-nick-end labeling (TUNEL) staining (Roche, Indianapolis, IN, USA) based on the product manual.

Data analysis. Data were analyzed using GraphPad Prism (La Jolla, CA, USA). The cell culture experiments were performed at least three times. Student's t-test (two groups), or one-way analysis of variance (ANOVA) (three or more groups) were used to determine differences, and $p<0.05$ values were determined as statically significant.

Results

ARHGAP17 enhances 5-FU-induced apoptosis in HCT116 and HCT8 cells. We observed that the protein

level of ARHGAP17 was remarkably lower in 5-FU-resistant cells (HCT116/5-FU and HCT8/5-Fu) in comparison with the colon cancer cell line (Figure 1A). This result suggests that ARHGAP17 might be involved in 5-FU resistance. In normal HCT116 and HCT8 cells, we successfully reduced the protein expression of ARHGAP17 using lentiviruses expressing shRNAs targeting human ARHGAP17 (shARH-1 and shARH-2, Figure 1B). We found that knockdown of ARHGAP17 in normal HCT116 and HCT8 cells statistically reduced apoptosis, which was induced by 48 h 5-FU treatment (0.2 μg/ml) (Figure 1D). Thus, ARHGAP17 knockdown attenuated apoptosis upon 5-FU treatment in HCT116 and HCT8 cells.

We next overexpressed ARHGAP17 in 5-FU resistant cells (HCT116/5-FU and HCT8/5-FU) (Figure 1C). Interestingly, overexpression of ARHGAP17 alone statistically increased the apoptosis rate upon 5-FU treatment (Figure 1E). In addition, ARHGAP17 overexpression further enhanced the apoptosis rate induced by 5-FU in HCT116/5-FU and HCT8/5-FU cells (Figure 1E). Our results suggest that ARHGAP17 overexpression may enhance the sensitivity of colon cancer cells to 5-FU.

ARHGAP17 enhances 5-FU-induced apoptosis by suppressing Rac1. We next explored the mechanisms by which ARHGAP17 augments 5-FU-induced apoptosis. In our former study, we observed that overexpression of ARHGAP17 inhibited Rac1 activity [15], which suggests that Rac1 may mediate the effect of ARHGAP17 on 5-FU-induced apoptosis. Knockdown of ARHGAP17 in HCT116 and HCT8 cells led to increased activity of Rac1 (Figure 2A). However, overexpression of ARHGAP17 in 5-FU-resistant cells suppressed Rac1 activity without altering the total protein level (Figure 2B).

In order to determine whether Rac1 mediates the function of ARHGAP17 in chemotherapy resistance, we used a Rac1 inhibitor NSC33766 to treat HCT116 cells. We found that NSC33766 significantly increased the apoptosis rate induced by 5-FU in HCT116 cells (Figure 2C). ARHGAP17 knockdown reduced the apoptosis rate, but inhibition of Rac1 with NSC33766 statistically restored the apoptosis rate (Figure 2C). We next examined whether Rac1 overexpression could mitigate the effect of ARHGAP17 on apoptosis induced by 5-FU. The protein level of Rac1 was confirmed in Rac1 overexpression cells (Supplementary Figure S1). We observed that in 5-FU-resistant HCT116 cells, ARHGAP17 overexpression led to increased apoptosis, but overexpression Rac1 reduced apoptosis induced by 5-FU (Figure 2D). However, Rac1 overexpression abolished the effect of ARHGAP17 overexpression on apoptosis induced by 5-FU (Figure 2D).

In addition to apoptosis detection by flow cytometry, we also determined the hallmark of apoptosis-cleaved Caspase-3 and cleaved PARP. We showed that ARHGAP17 knockdown resulted in markedly lower levels of cleaved Caspase-3 and cleaved PARP after 5-FU treatment, compared to the control in HCT116 cells (Figure 2E). Meanwhile, Rac1 inhibitor NSC33766 led to substantially elevated cleaved Caspase-3 and cleaved PARP after 5-FU treatment compared to vehicle (Figure 2E). However, Rac1 inhibition using NSC33766 attenuated the protein level of cleaved Caspase-3 and cleaved PARP after 5-FU treatment in ARHGAP17 knockdown cells (Figure 2E). On the other hand, in HCT116/5-FU cells, ARHGAP17 overexpression resulted in higher levels of cleaved Caspase-3 and cleaved PARP upon 5-FU treatment (Figure 2F). However, Rac1 overexpression diminished the effect of ARHGAP17 overexpression on cleaved Caspase-3 and cleaved PARP caused by 5-FU (Figure 2F). These data show that ARHGAP17 augments 5-FU-induced apoptosis by suppressing Rac1 in colon cancer cells.

Overexpression of ARHGAP17 augments 5-FU-induced apoptosis and attenuates tumor growth *in vivo*. The above data show overexpression of ARHGAP17 increased apoptosis upon 5-FU treatment in 5-FU-resistant cells, and we then confirmed such effect using *in vivo* model. HCT116/5-FU cells stably expressing ARHGAP17 or vector were injected into nude mice. Twelve days after transplantation, mice received 5-FU (20 mg/kg) or vehicle twice per week for therapy. We observed that overexpression of ARHGAP17 or 5-FU alone reduced the tumor growth rate from day 12 to 33 (Figure 3A). Correspondingly, overexpression of ARHGAP17 or 5-FU alone decreased the tumor size (Figure 3B) and weight (Figure 3C) on day 33 after cell transplantation. Interestingly, a combination of 5-FU and overexpression of ARHGAP17 statistically reduced the growth rate, tumor size, and tumor weight, compared to the controls (Figures 3A–3C). In the tumor section, with TUNEL staining, we detected statistically increased apoptosis in the ARHGAP17 overexpression and 5-FU group. Overexpression of ARHGAP17 further enhanced the apoptosis rate induced by 5-FU (Figure 3D). In addition, higher levels of cleaved Caspase-3 and cleaved PARP were observed in the ARHGAP17 overexpression and 5-FU group. A combination of 5-FU and overexpression of ARHGAP17 showed the highest levels of cleaved Caspase-3 and cleaved PARP (Figure 3E). Our data indicate that ARHGAP17 augments 5-FU induced apoptosis and reduces tumor growth *in vivo*.

Discussion

The main treatment for colon cancer is surgery, followed by chemotherapy for patients with advanced stages of the disease. Therapeutic 5-FU is widely used as a single agent or in combination chemotherapy [18]. However, less than 30% of people respond to 5-FU while using a single agent [19, 20], and about 50% of patients respond to treatment if 5-FU is administrated in a combination with agents such as oxaliplatin [21]. Thus, it is clinically important to identify people who are responding to chemotherapy and the subgroup patients who are not responding to chemo-

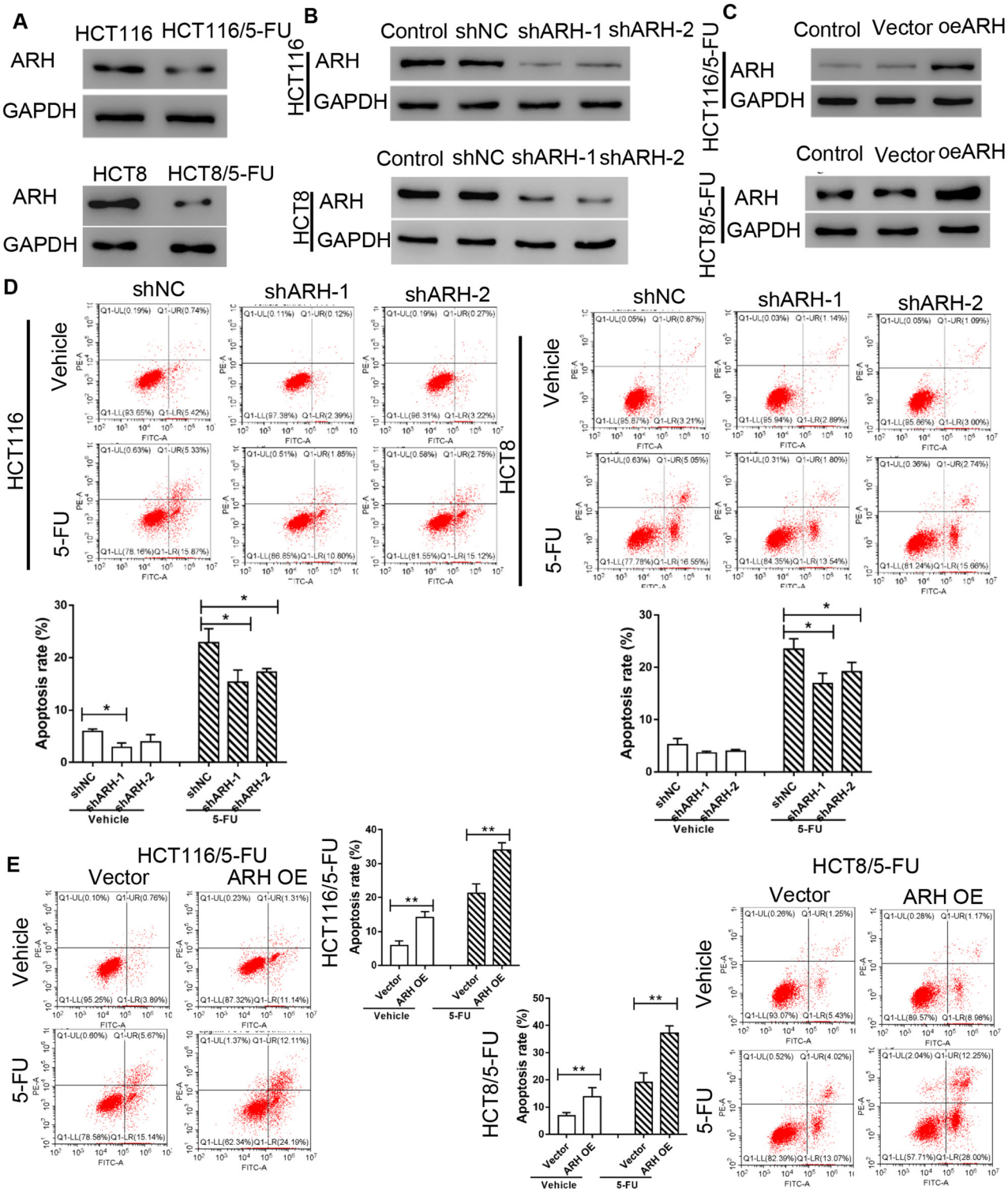

Figure 1. ARHGAP17 enhances 5-FU-induced apoptosis in colon cancer cells. A) The protein level of ARHGAP17 was downregulated by 5-FU in colon cancer cell lines. The protein level was detected with western blot and GAPDH served as a loading control. B) ARHGAP17 protein was downregulated by ARHGAP17 knockdown lentiviruses (shARH-1, shARH-2) in HCT116 and HCT8 cells. C) Overexpression of ARHGAP17 induced by ARHGAP17 expression lentivirus (oeARH) in HCT116/5-FU and HCT8/5-FU. D) Knockdown of ARHGAP17 by short hairpin RNAs (shARH-1, shARH-2) suppressed apoptosis induced by 0.2 μg/ml 5-FU in HCT116 and HCT8 cells. Cell apoptosis was detected by flow cytometry with the Annexin V-FITC/PI apoptosis detection kit. *p<0.05 vs. siNC. E) Overexpression of ARHGAP17 significantly enhanced apoptosis induced by 0.2 μg/ml 5-FU in HCT116/5-FU and HCT8/5-FU. Apoptosis was determined using the same methods as in D. **p<0.01 vs. vector.

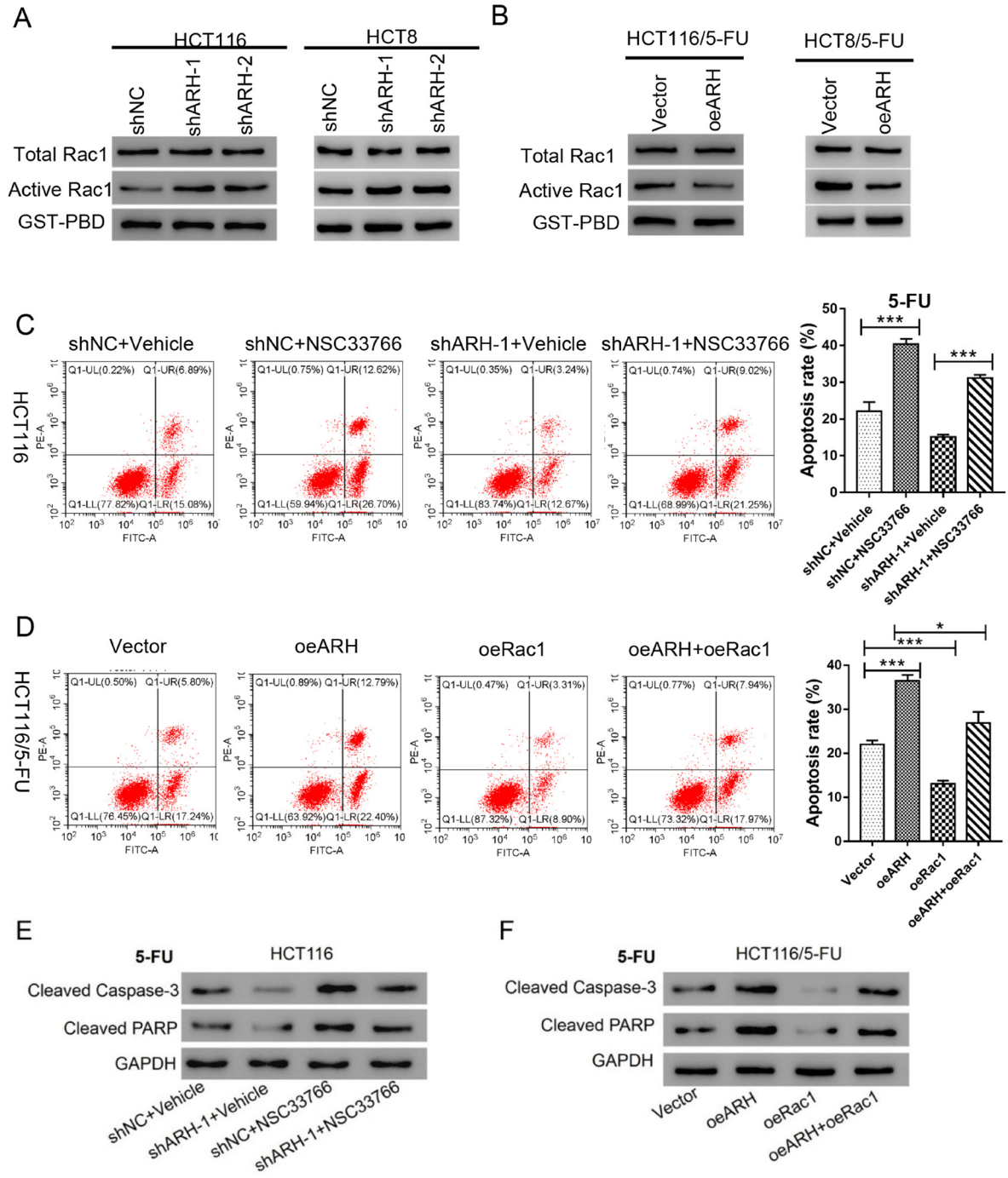

Figure 2. ARHGAP17 enhances 5-FU-induced apoptosis by damping the activity of Rac1. A) Knockdown of ARHGAP17 with short hairpin RNAs (shARH-1, shARH-2) is associated with a higher level of active Rac1 in HCT116 and HCT8 cells compared to control. B) Overexpression of ARHGAP17 is associated with a lower level of active Rac1 in HCT116/5-FU and HCT8/5-FU cells. Active Rac1 was detected by a kit using the company's protocol. The GST-PBD fusion protein and total Rac1 in the protein lysates were utilized as the quantitative controls. C) Inhibition of Rac1 with inhibitor NSC33766 significantly increased apoptosis induced by 5-FU in HCT116 cells with ARHGAP17 knockdown. HCT116 cells transfected with ARHGAP17 shRNA or control were incubated with Rac1 inhibitor NSC33766 or vehicle for 4 hours, before incubation with 0.2 μg/ml 5-FU for 48 hours. Apoptosis was then detected by flow cytometry. D) Overexpression of Rac1 attenuated apoptosis induced by 5-FU, but co-overexpression of ARHGAP17 significantly increased apoptosis induced by 5-FU. HCT116/5-FU cells were overexpressed with Rac1 or ARHGAP17 alone or together. E) Apoptosis markers cleaved Caspase-3 and cleaved PARP were downregulated by ARHGAP17 knockdown but were upregulated by Rac1 inhibitor NSC33766. Further, Rac1 inhibitor NSC33766 reduced Caspase-3 and cleaved PARP mediated by ARHGAP17 knockdown in 5-FU treated HCT116 cells. F) Cleaved Caspase-3 and cleaved PARP were upregulated by ARHGAP17 overexpression, but were attenuated by overexpression of Rac1. Co-expression of ARHGAP17 and Rac1 restored the level of cleaved Caspase-3 and cleaved PARP in HCT116/5-FU cells upon 5-FU treatment. ***p<0.001

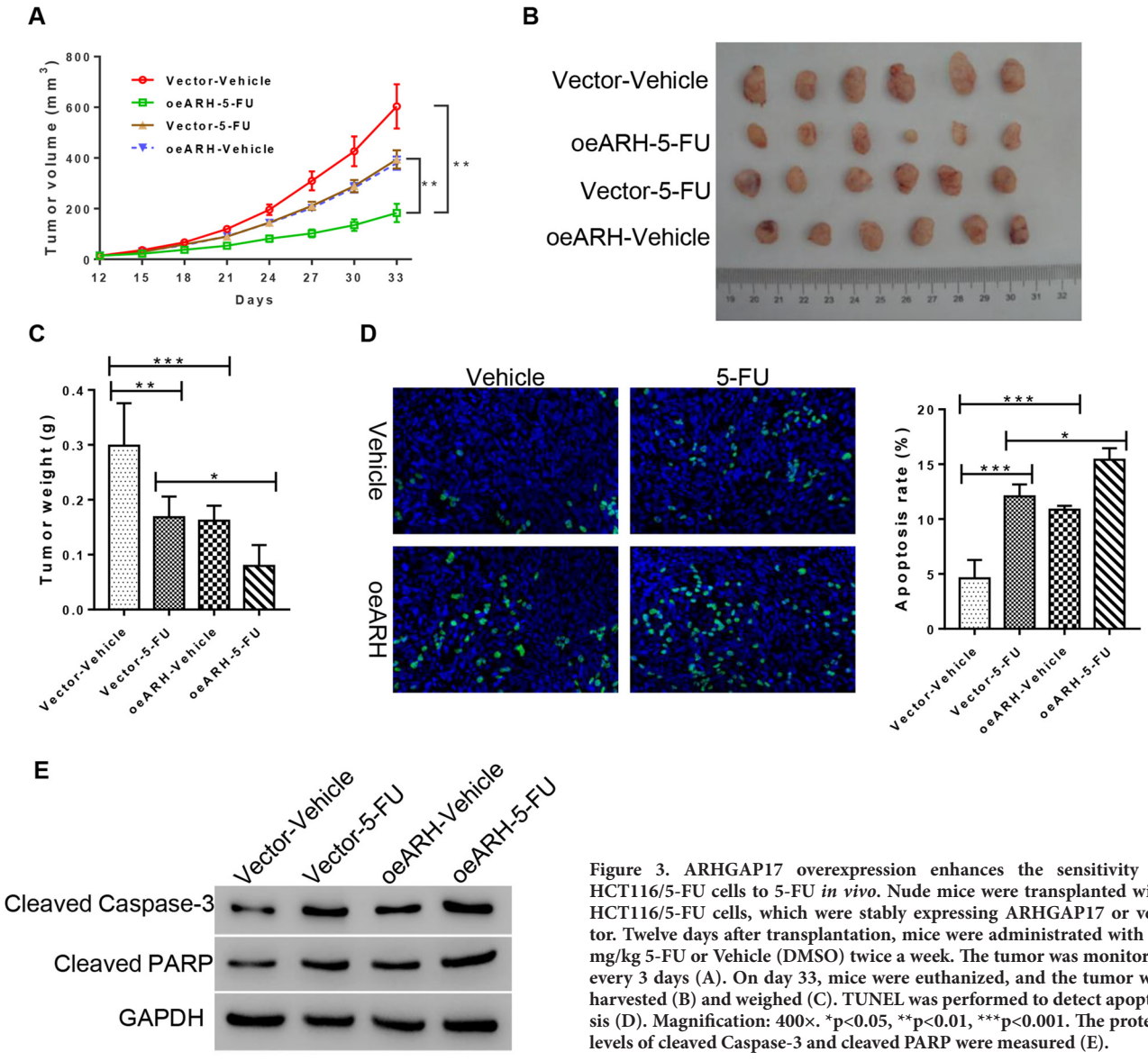

Figure 3. ARHGAP17 overexpression enhances the sensitivity of HCT116/5-FU cells to 5-FU *in vivo*. Nude mice were transplanted with HCT116/5-FU cells, which were stably expressing ARHGAP17 or vector. Twelve days after transplantation, mice were administrated with 20 mg/kg 5-FU or Vehicle (DMSO) twice a week. The tumor was monitored every 3 days (A). On day 33, mice were euthanized, and the tumor was harvested (B) and weighed (C). TUNEL was performed to detect apoptosis (D). Magnification: 400×. *p<0.05, **p<0.01, ***p<0.001. The protein levels of cleaved Caspase-3 and cleaved PARP were measured (E).

therapy. Accordingly, biomarkers that may direct chemotherapy treatment options for patients are highly needed. To our best knowledge, this is the first study to report that ARHGAP17 sensitizes the chemotherapy-resistant cells to 5-FU, promotes apoptosis, and thus inhibits tumor growth. Our data demonstrate the involvement of ARHGAP17 in chemotherapy resistance and suggests its potential usage in the treatment of colon cancer.

Many factors may contribute to 5-FU resistance, including deregulation of apoptosis [19, 22]. Our previous study has reported that ARHGAP17 overexpression inhibited Rac1 activity in colon cancer cells [15]. Rac1, the small GTPase, is critical in regulating cell apoptosis and survival. Active Rac1 impeded apoptosis in lymphoma cells upon treatment of cancer chemotherapy agents [23] by increasing Bad phosphorylation. Importantly, the expression of active Rac1 (Rac1b) was increased in advanced stages of colon cancer through enhancing the activity of the NF-κB pathway [14, 24]. Moreover, active Rac1 was also increased upon treatment with chemo agents, and Rac1 inhibitor could be used to prevent chemoresistance [14]. In this study, we showed that ARHGAP17 knockdown inhibited apoptosis induced by 5-FU, but Rac1 inhibitor NSC33766 statistically restored the apoptosis rate in colon cancer cells, demonstrating that ARHGAP17 enhances 5-FU-induced apoptosis by negatively regulating Rac1 activity. We also showed that ARHGAP17 overexpression increased apoptosis induced by 5-FU in its resistant colon cancer cells, but Rac1 overex-

pression diminished the effect of ARHGAP17 overexpression on apoptosis induced by 5-FU. It has been shown that 5-FU activated caspase-3 [25, 26], a main executor caspase that leads to the biochemical and morphological changes in the process of apoptosis. In this study, we found that ARHGAP17 overexpression enhanced the activation of caspase-3 in 5-FU resistant colon cancer cells induced by 5-FU, which correspondingly led to high levels of cleaved PARP, a marker of cells undergoing apoptosis. However, this enhancement by ARHGAP17 overexpression on caspase-3 activation was attenuated by Rac1 overexpression. These data support that ARHGAP17 promotes apoptosis induced by 5-FU by downregulating Rac1 activity in colon cancer cells. Additional studies are needed to investigate whether diminished ARHGAP17 and upregulated Rac1 activity contribute to chemotherapy resistance in other cancer cells.

In line with the *in vitro* findings, we reported that ARHGAP17 overexpression enhanced 5-FU-induced apoptosis and attenuated tumor growth in nude mice, suggesting ARHGAP17 may improve the sensitivity of colon cancer cells to chemotherapy treatment. We are aware that ARHGAP17 deficiency in mice did not change cell proliferation or apoptosis in the intestine [9], and that ARHGAP17 blocked apoptosis induced by mechanical stress in human periodontal ligament cells [27]. The discrepancy is likely due to the specific study context, and our focus is on chemotherapy resistance of colon cancer.

Overall, our present study reported that ARHGAP17 plays an important role in promoting apoptosis induced by 5-FU in colon cancer cells by suppressing Rac1. As ARHGAP17 overexpression sensitizes the chemotherapy-resistant colon cancer cells to 5-FU, and thus inhibits tumor growth, we speculate that ARHGAP17 may be used in conjunction with 5-FU to reduce treatment resistance, and enhance tumor cell sensitivity to apoptosis induced by chemotherapy in colon cancer.

Supplementary information is available in the online version of the paper.

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References

[1] FICHTNER M, BOZKURT E, SALVUCCI M, MCCANN C, MCALLISTER KA et al. Molecular subtype-specific responses of colon cancer cells to the SMAC mimetic Birinapant. Cell Death Dis 2020; 11: 1020. https://doi.org/10.1038/s41419-020-03232-z

[2] TSAI YJ, LIN JK, CHEN WS, JIANG JK, TENG HW et al. Adjuvant FOLFOX treatment for stage III colon cancer: how many cycles are enough? Springerplus 2016; 5: 1318. https://doi.org/10.1186/s40064-016-2976-9

[3] ANDRÉ T, BONI C, NAVARRO M, TABERNERO J, HICKISH T et al. Improved Overall Survival With Oxaliplatin, Fluorouracil, and Leucovorin As Adjuvant Treatment in Stage II or III Colon Cancer in the MOSAIC Trial. J Clin Oncol 2009; 27: 3109–3116. https://doi.org/10.1200/JCO.2008.20.6771

[4] SKARKOVA V, KRALOVA V, VITOVCOVA B, RUDOLF E. Selected Aspects of Chemoresistance Mechanisms in Colorectal Carcinoma-A Focus on Epithelial-to-Mesenchymal Transition, Autophagy, and Apoptosis. Cells 2019; 8: 234. https://doi.org/10.3390/cells8030234

[5] PONDER KG, BOISE LH. The prodomain of caspase-3 regulates its own removal and caspase activation. Cell Death Discov 2019; 5: 56. https://doi.org/10.1038/s41420-019-0142-1

[6] ZHANG L, YU J. Role of apoptosis in colon cancer biology, therapy, and prevention. Curr Colorectal Cancer Rep 2013; 9: 10.1007/s11888-013-0188-z. https://doi.org/10.1007/s11888-013-0188-z

[7] RICHNAU N, ASPENSTRÖM P. Rich, a Rho GTPase-activating Protein Domain-containing Protein Involved in Signaling by Cdc42 and Rac1. J Biol Chem 2001; 276: 35060–35070. https://doi.org/10.1074/jbc.M103540200

[8] HARADA A, FURUTA B, TAKEUCHI K-I, ITAKURA M, TAKAHASHI M et al. Nadrin, a Novel Neuron-specific GTPase-activating Protein Involved in Regulated Exocytosis. J Biol Chem 2000; 275: 36885–36891. https://doi.org/10.1074/jbc.M004069200

[9] LEE SY, KIM H, KIM K, LEE H, LEE S et al. Arhgap17, a RhoGTPase activating protein, regulates mucosal and epithelial barrier function in the mouse colon. Sci Rep 2016 27; 6: 26923. https://doi.org/10.1038/srep26923

[10] YI C, TROUTMAN S, FERA D, STEMMER-RACHAMIMOV A, AVILA JACQUELINE L et al. A Tight Junction-Associated Merlin-Angiomotin Complex Mediates Merlin's Regulation of Mitogenic Signaling and Tumor Suppressive Functions. Cancer Cell 2011; 19: 527–540. https://doi.org/10.1016/j.ccr.2011.02.017

[11] HEASMAN SJ, RIDLEY AJ. Mammalian Rho GTPases: new insights into their functions from in vivo studies. Nat Rev Mol Cell Biol 2008; 9: 690–701. https://doi.org/10.1038/nrm2476

[12] JIN S, RAY RM, JOHNSON LR. Rac1 mediates intestinal epithelial cell apoptosis via JNK. Am J Physiol Gastrointest Liver Physiol 2006; 291: G1137–1147. https://doi.org/10.1152/ajpgi.00031.2006

[13] JORDAN P, BRAZÃ£O R, BOAVIDA MG, GESPACH C, CHASTRE E. Cloning of a novel human Rac1b splice variant with increased expression in colorectal tumors. Oncogene 1999; 18: 6835–6839. https://doi.org/10.1038/sj.onc.1203233

[14] GOKA ET, CHATURVEDI P, LOPEZ DTM, GARZA ADL, LIPPMAN ME. RAC1b Overexpression Confers Resistance to Chemotherapy Treatment in Colorectal Cancer. Mol Cancer Ther 2019; 18: 957–968. https://doi.org/10.1158/1535-7163.MCT-18-0955

[15] PAN S, DENG Y, FU J, ZHANG Y, ZHANG Z et al. Tumor Suppressive Role of ARHGAP17 in Colon Cancer Through Wnt/β-Catenin Signaling. Cell Physiol Biochem 2018; 46: 2138–2148. https://doi.org/10.1159/000489543

[16] GUO Q, XIONG Y, SONG Y, HUA K, GAO S. ARHGAP17 suppresses tumor progression and up-regulates P21 and P27 expression via inhibiting PI3K/AKT signaling pathway in cervical cancer. Gene 2019; 692: 9–16. https://doi.org/10.1016/j.gene.2019.01.004

[17] JENSEN MM, JØRGENSEN JT, BINDERUP T, KJÆR A. Tumor volume in subcutaneous mouse xenografts measured by microCT is more accurate and reproducible than determined by 18F-FDG-microPET or external caliper. BMC Med Imaging 2008; 8: 16. https://doi.org/10.1186/1471-2342-8-16

[18] HAMMOND WA, SWAIKA A, MODY K. Pharmacologic resistance in colorectal cancer: a review. Ther Adv Med Oncol 2016; 8: 57–84. https://doi.org/10.1177/1758834015614530

[19] BRIFFA R, LANGDON SP, GRECH G, HARRISON DJ. Acquired and Intrinsic Resistance to Colorectal Cancer Treatment. In J. Chen (Eds.) Colorectal Cancer – Diagnosis, Screening and Management. London: IntechOpen; 2018, p. 128. ISBN 978-1-78923-101-4 https://doi.org/10.5772/intechopen

[20] WEIDLICH S, WALSH K, CROWTHER D, BURCZYNSKI ME, FEUERSTEIN G et al. Pyrosequencing-based methods reveal marked inter-individual differences in oncogene mutation burden in human colorectal tumours. Br J Cancer 2011; 105: 246–254. https://doi.org/10.1038/bjc.2011.197

[21] VIRAG P, FISCHER-FODOR E, PERDE-SCHREPLER M, BRIE I, TATOMIR C et al. Oxaliplatin induces different cellular and molecular chemoresistance patterns in colorectal cancer cell lines of identical origins. BMC Genomics 2013; 14: 480. https://doi.org/10.1186/1471-2164-14-480

[22] VAN DER JEUGHT K, XU HC, LI YJ, LU XB, JI G. Drug resistance and new therapies in colorectal cancer. World J Gastroenterol 2018; 24: 3834–3848. https://doi.org/10.3748/wjg.v24.i34.3834

[23] ZHANG B, ZHANG Y, SHACTER E. Rac1 Inhibits Apoptosis in Human Lymphoma Cells by Stimulating Bad Phosphorylation on Ser-75. Mol Cell Biol 2004; 24: 6205–6214. https://doi.org/10.1128/MCB.24.14.6205-6214.2004

[24] MATOS P, OLIVEIRA C, VELHO S, GONÇALVES V, DA COSTA LT et al. B-Raf(V600E) cooperates with alternative spliced Rac1b to sustain colorectal cancer cell survival. Gastroenterology 2008; 135: 899–906. https://doi.org/10.1053/j.gastro.2008.05.052

[25] ARESVIK DM, PETTERSEN RD, ABRAHAMSEN TG, WRIGHT MS. 5-Fluorouracil-induced Death of Jurkat T-Cells – A Role for Caspases and MCL-1. Anticancer Res 2010; 30: 3879–3887.

[26] CAN G, AKPINAR B, BARAN Y, ZHIVOTOVSKY B, OLSSON M. 5-Fluorouracil signaling through a calcium–calmodulin-dependent pathway is required for p53 activation and apoptosis in colon carcinoma cells. Oncogene 2013; 32: 4529–4538. https://doi.org/10.1038/onc.2012.467

[27] WANG L, YANG X, WAN L, WANG S, PAN J et al. ARHGAP17 inhibits pathological cyclic strain-induced apoptosis in human periodontal ligament fibroblasts via Rac1/Cdc42. Clin Exp Pharmacol Physiol 2020; 47: 1591–1599. https://doi.org/10.1111/1440-1681.13336

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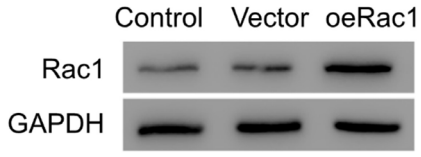
Supplementary Information



Supplementary Figure S1. HCT116/5-FU cells were transfected with Rac1 expression lentivirus (oeRac1) and control (Vector) lentivirus. Rac 1 protein level was detected with western blotting.