

# Retinoic acid receptor $\alpha$ facilitates human colorectal cancer progression via Akt and MMP2 signaling

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**Purpose:** Retinoic acid  $\alpha$  (RAR $\alpha$ ) is overexpressed in various tumors and facilitates cancer progression. Although RAR $\alpha$  has been shown to facilitate colorectal cancer (CRC) progression, more efforts to characterize mechanisms of RAR $\alpha$  in CRC are needed in order to develop better target-based drugs for tumor therapy.

**Methods:** RAR $\alpha$  expression in CRC was assessed by IHC, EdU, QPCR, Western blotting, dual-luciferase reporter assay and ChIP were performed to explore the role of RAR $\alpha$  in CRC and the mechanism involved.

**Results:** Here, we show an overexpression of RAR $\alpha$  in 73.5% (i.e., 25 of 34 human CRC specimens). RAR $\alpha$  knockdown decreased cell proliferation, migration, and invasion. Such phenotypic manifestations can be correlated to lowered activation of Akt and expression of PCNA (proliferating cell nuclear antigen) as well as MMP2 (matrix metalloproteinase). Mechanistically, RAR $\alpha$  facilitates CRC growth through Akt signaling activation to cause levels of PCNA to be upregulated. Furthermore, RAR $\alpha$  promotes migration and invasion of CRC cells by directly recruiting the *MMP2* promoter to enhance the expression of *MMP2*.

**Conclusions:** These findings demonstrate that CRC carcinogenesis is promoted by RAR $\alpha$  via an enhanced Akt signaling and by increasing *MMP2* transcription. CRC therapy can examine the use of RAR $\alpha$  as a prospective molecular target.

**Keywords:** colorectal cancer, RAR $\alpha$ , proliferation, PCNA, MMP2

## Introduction

Colorectal cancer (CRC) is the third most commonly cancer in males and the second in females, the third leading cause of cancer-related mortality all over the world.<sup>1</sup> In China, CRC morbidity and mortality increased to become the fifth most common cancer in 2015, and continues to rise as a considerable challenge to the nation's health.<sup>2</sup> Because of difficulty in early diagnosis of CRC,<sup>3</sup> the majority of patients are not diagnosed until the late stage.<sup>4</sup> This necessitates a rapid understanding of the molecular mechanism of CRC progression to devise new agents for therapy.

Retinoic acid receptors (RARs) are members of the superfamily of steroid/thyroid hormone nuclear receptors.<sup>5</sup> To date, three different RAR genes (*RAR $\alpha$* , *RAR $\beta$* , and *RAR $\gamma$* ) have been characterized.<sup>6</sup> *RAR $\alpha$*  is homologous to other RAR genes and has three major domains. RAR $\alpha$  plays diverse roles in many biological processes including carcinogenesis.<sup>7</sup> The fusion protein PML/RAR $\alpha$ , due to chromosomal translocation t (15;17) hinders the differentiation induced by RAR $\alpha$ , impedes the cells at the promyelocytic stage and increases hyperproliferation of

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blocked promyelocytes, which drives oncogenic alterations in acute promyelocytic leukemia cells.<sup>8,9</sup> Survival without relapse for breast cancer patients positive for estrogen receptor alpha (ER $\alpha$ ) is less when the levels of intratumoral RAR $\alpha$  protein are high.<sup>10</sup> All trans-retinoic acid (ATRA) ATRA regulates in part the innate immunity to protect liver via RAR $\alpha$ /Akt/Foxo1 pathway.<sup>11</sup> The levels of Apolipoprotein CIII secreted by human liver cell lines was inhibited by AM580: an agonist of RAR $\alpha$ .<sup>12</sup> AM580 causes a significant inhibition on the growth of endometrial cancer and breast tumor,<sup>13,14</sup> that indicates RAR $\alpha$  as a target in the therapeutic intervention of cancer. Research points out at a crucial involvement of RAR $\alpha$  in several cancers including CRC, breast cancer, leukemia, as well as, gastric cancer via several pathways such as ER $\alpha$ , p38 $\alpha$  MAPK, G protein alpha Q, Glycogen synthase kinase 3, beta/beta-catenin, nuclear factor kappa B and c-Jun N-terminal kinase.<sup>15–17</sup> RAR $\alpha$  is highly expressed in CRC tissues,<sup>18</sup> however, the possibility of metastasis to be modulated by RAR $\alpha$  to affect the progression of CRC is yet not established. The above evidence underscores the importance of the identification of RAR $\alpha$  targets, which may lead to strategies for developing improved anticancer drugs.

The current work showed overexpression of RAR $\alpha$  in human CRC specimens, that augmented the capability of these tumor cells to proliferate, invade and migrate through an activation of Akt and matrix metalloproteinase MMP2 signaling pathways.

## Materials and methods

### Reagents

Wnt3a was from R&D systems (Minneapolis, MN, USA). LY294002 was obtained from Sigma (St. Louis, MO, USA). 5-Ethynyl-2-deoxyuridine (EdU) was sourced from RiboBio (Guangzhou, China). Abcam Ltd. (Cambridge, United Kingdom) was the source of polyclonal antibodies against RAR $\alpha$ , Akt, as well as its phosphorylated form. Polyclonal antibody against  $\beta$ -actin was purchased from Cell Signaling Technology (Danvers, MA, USA). Monoclonal antibodies PCNA was procured from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Lipofectamine 2000, Goat anti-rabbit and anti-mouse secondary antibodies were sourced from Invitrogen (Carlsbad, CA, USA). Millipore (Billerica, MA, USA) provided the PVDF membrane. The EliVision Plus kit was obtained from Maixin Bio (Fuzhou, China).

### Tumor samples

A total of 34 patients with colorectal cancer subjected to resection at the First Affiliated Hospital of Xiamen University were the source of colorectal tissues that were paired tumorous and paracancerous. Informed consent was obtained from each patient. The protocol used was in accordance with the Institute Research Ethics Committee of the First Affiliated Hospital of Xiamen University in line with the 1975 Declaration of Helsinki.

### Details of plasmids

The pIRES2-EGFP vector was used to clone human RAR $\alpha$  (coding regions) via the *Xho I/Bam HI* sites to generate constructs for expression of RAR $\alpha$ . Construction of RNA interference vector targeting RAR $\alpha$  was described previously.<sup>18</sup> Lipofectamine 2000 was used to transfect 293T cells with pL3.7-shRAR $\alpha$  or control vector and lentivirus packaging plasmids (PMDL/VSVG/REV) in accordance with the instructions of the manufacturer. Following a 48 hr culture of the transfected cells at 37 °C, infection was done using a medium containing the virus. The promoter region of *MMP2* was constructed by inserting fragments into *Xho I/Kpn I* sites of the pGL3-basic vector. The *MMP2* promoter mutants were generated by site-directed mutagenesis.

### Culture of cells

The human CRC cell lines HT29, RKO and HCT116 were purchased from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). RPMI1640 medium (Hyclone, Logan, UT, USA) was used to grow HT29, while Dulbecco's modified Eagle's medium (DMEM) (Hyclone, Logan, UT, USA) was used for RKO and HCT116 culture. Supplements in both cases were 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA) and penicillin-streptomycin (100 U/mL). Culture conditions were a 5% CO<sub>2</sub> in a humidified incubator at 37 °C. Following transfection with control or RAR $\alpha$ , screening of monoclonal stable cell line was done to establish RKO and HCT116 transfections.

### Immunohistochemical studies (IHC)

IHC was done according to an earlier protocol.<sup>19</sup> A 1:200 dilution of anti-RAR $\alpha$  antibody was added to the CRC tissue sections that were embedded in paraffin. Following incubation at 4 °C overnight, corresponding secondary antibody was added and incubated at room temperature for 40 min. EliVision Plus Kit (Maixin Bio, Fuzhou,

China) was used to detect the slides in accordance with instructions of the manufacturer. Expression of RAR $\alpha$  protein was classified into four levels according to staining intensity of CRC tissues. While  $\pm$  and + are as low expression, and ++ and +++ are considered as high expression.

### Immunofluorescence (IF)

IF was performed as previously described.<sup>20</sup> After seeding cells on a glass slide and overnight culture, 4% paraformaldehyde was used to fix cells for 15 min. Triton X-100 (0.5%) was used for permeabilization of cells for 20 min. The blocked cells were incubated with 1:100 dilution of RAR $\alpha$  -primary antibody. This was followed by 1:100 of secondary antibodies conjugated to Alexa Fluor 647. Nuclei were stained with 1  $\mu$ g/mL DAPI. Leica TCS SP5 II laser confocal microscope (Leica, Barcelona, Spain) was used to record images.

### Labeling with 5-Ethynyl-2-deoxyuridine (EdU)

The proliferation of cells was studied using EdU assay in accordance with our previous work.<sup>18</sup>

### Assay for wound healing

A 10  $\mu$ L micropipette tip was used to scratch monolayer cells that were 80–90% confluent. After a fixed interval, double washing with PBS was done following which, 4% paraformaldehyde was used to fix cells for 20 min. Crystal violet was used to stain cells for half an hour. Images were captured using a Leica microscope.

### Transwell assay

For cell invasion assay, we used a 24-well transwell containing 8-mm pore size inserts coated with Matrigel (BD Biosciences, Franklin Lakes, New Jersey). 700  $\mu$ L medium was used to fill each well while the insert chamber was seeded with  $2 \times 10^4$  cells in 300  $\mu$ L medium. The invading cells were stained and visualized with a Leica microscope. ImageJ software was used to quantify the number of cells.

### Quantitative real-time PCR

RNeasy kit (Qiagen, Beijing, China) was used for extracting total RNA in accordance with the protocol of the manufacturer. ABI 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) was utilized for the assay. While RAR $\alpha$  and GAPDH primers have been earlier published,<sup>18</sup> the sequences of *MMP1*, *MMP2*, *MMP3* and *MMP9* can be issued on request.

### Dual-luciferase reporter assays

RT-PCR was used to amplify the promoter region of *MMP2* (nucleotides –1800 to +211 base pair) was from genomic DNA of a human followed by cloning into the pGL3-basic vector (Promega, Madison, Wisconsin). The assays were done in accordance with a procedure discussed earlier.<sup>18</sup>

### Gel zymography assay

The enzymatic activity of MMP2 was performed by gel zymography as previously described.<sup>21</sup>

### Western blotting

For an 8 hr exposure to LY294002 (20  $\mu$ M), cells were lysed with ice-cold RIPA buffer. Protein lysates in equal quantities were resolved on SDS-PAGE (10%) followed by transfer to PVDF membranes (Millipore, Temecula, CA, USA). Specific primary antibodies were used to incubate the membrane overnight at 4 °C, following which secondary antibodies conjugated to horseradish peroxidase were added. An enhanced chemiluminescence (ECL) system (Pierce, Rockford, IL, USA) was used to detect the signals.

### Chromatin immunoprecipitation assay (chip)

This was done in accordance with the protocol of Millipore (Billerica, MA, USA). The mean length of DNA was reduced as follows: 1% formaldehyde was added to RKO cells for 10 min at 37 °C followed by lysis and sonication (15 rounds of 9 seconds each). Protein A-agarose/salmon sperm DNA was applied as a pretreatment at 4 °C for 60 min. Next, incubation of negative control and tests was done with anti-mouse IgG and anti-RAR $\alpha$ , respectively at 4 °C, overnight. A 2 hr application of protein A-agarose was carried out to precipitate the complexes. Following washes, PCR was used to amplify the *MMP2* genomic region that flanked the potential binding areas of RAR $\alpha$ .

### Studies using animals

The protocols used were performed in accordance with animal protocols approved by the Laboratory Animal Center of Xiamen University. Twelve male BALB/c nude mice (4 weeks of age) were randomly assigned into 2 groups of 6 each that received either controls or shRAR $\alpha$ -RKO cells. Following sacrifice of mice 24 days after inoculation, tumors were subjected to photography and western blot processing. The volume of tumors was quantified with the equation:  $1/2 \times \text{length} \times \text{width}$ .<sup>2</sup>

## Statistical analysis

GraphPad Prism 6 (San Diego, CA, USA) was utilized for analysis with data of minimum of three independent experiments expressed as means  $\pm$  SEM of minimum 3 independent experiments. For comparing the 2 groups, One-way analysis of variance (ANOVA) was applied with statistical significance at  $P^* \leq 0.05$ .

## Results

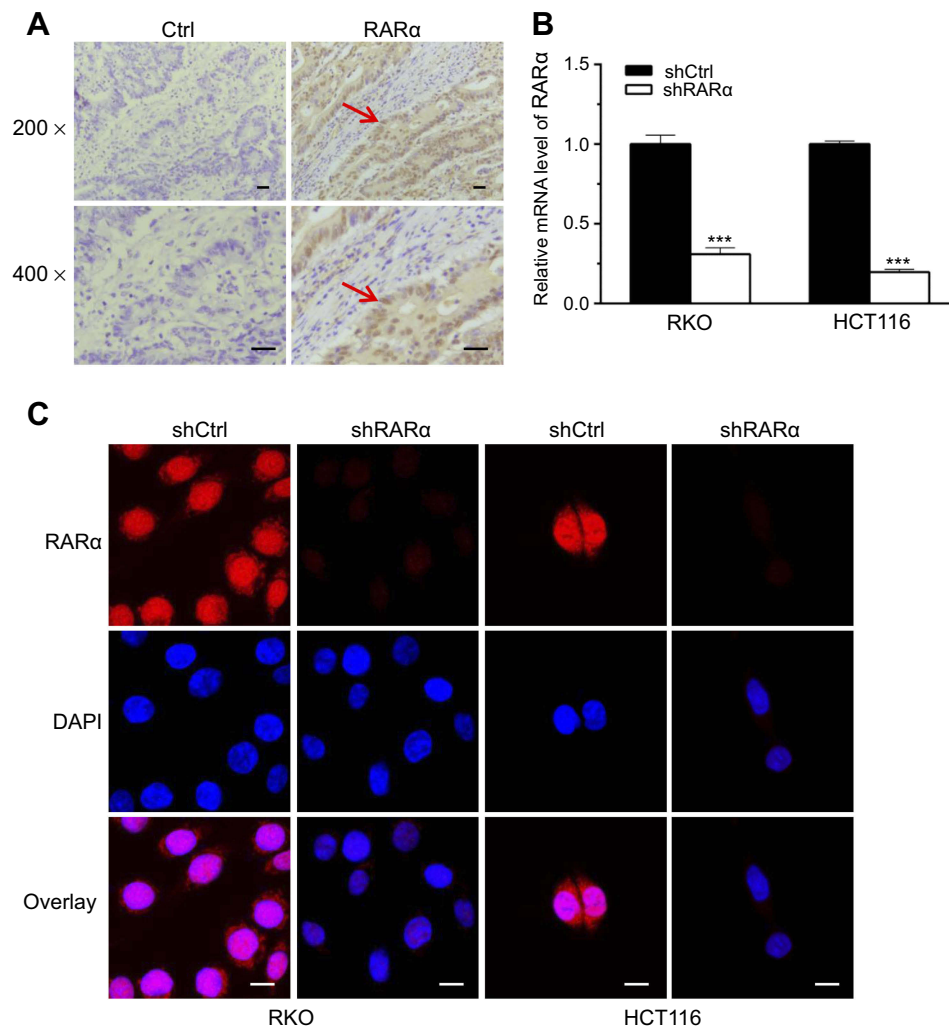
### Overexpression of RAR $\alpha$ human CRC samples

To determine the role of RAR $\alpha$  in CRC, IHC analysis was used to determine the protein levels of RAR $\alpha$  in a set of 34 pairs of human CRC specimens and the surrounding non-tumorous tissues (Figure 1A). RAR $\alpha$  was localized both in cytoplasm and in the nucleus of CRC. In comparison to

the surrounding non-tumorous tissues that surround 34 human CRC specimens, the study showed an overexpression of RAR $\alpha$  protein in 25/34 samples ie 73.5% of tissues. The results are in line with our previous study that involved a different platform for analysis and patient cohort.<sup>18</sup> This is suggestive of a putative role of RAR $\alpha$  in CRC carcinogenesis.

### Knockdown of RAR $\alpha$ causes suppression of proliferation of CRC cells

To assess RAR $\alpha$ 's role in the growth of CRC cells, stably RAR $\alpha$ -knockdown CRC cells were established. The expression of RAR $\alpha$  in CRC cells were shown in our previous studies.<sup>18</sup> RKO and HCT116 cells were stably transfected with p13.7 vector (shCtrl) or RAR $\alpha$ -knockdown vector p13.7-shRAR $\alpha$ , in which elevated

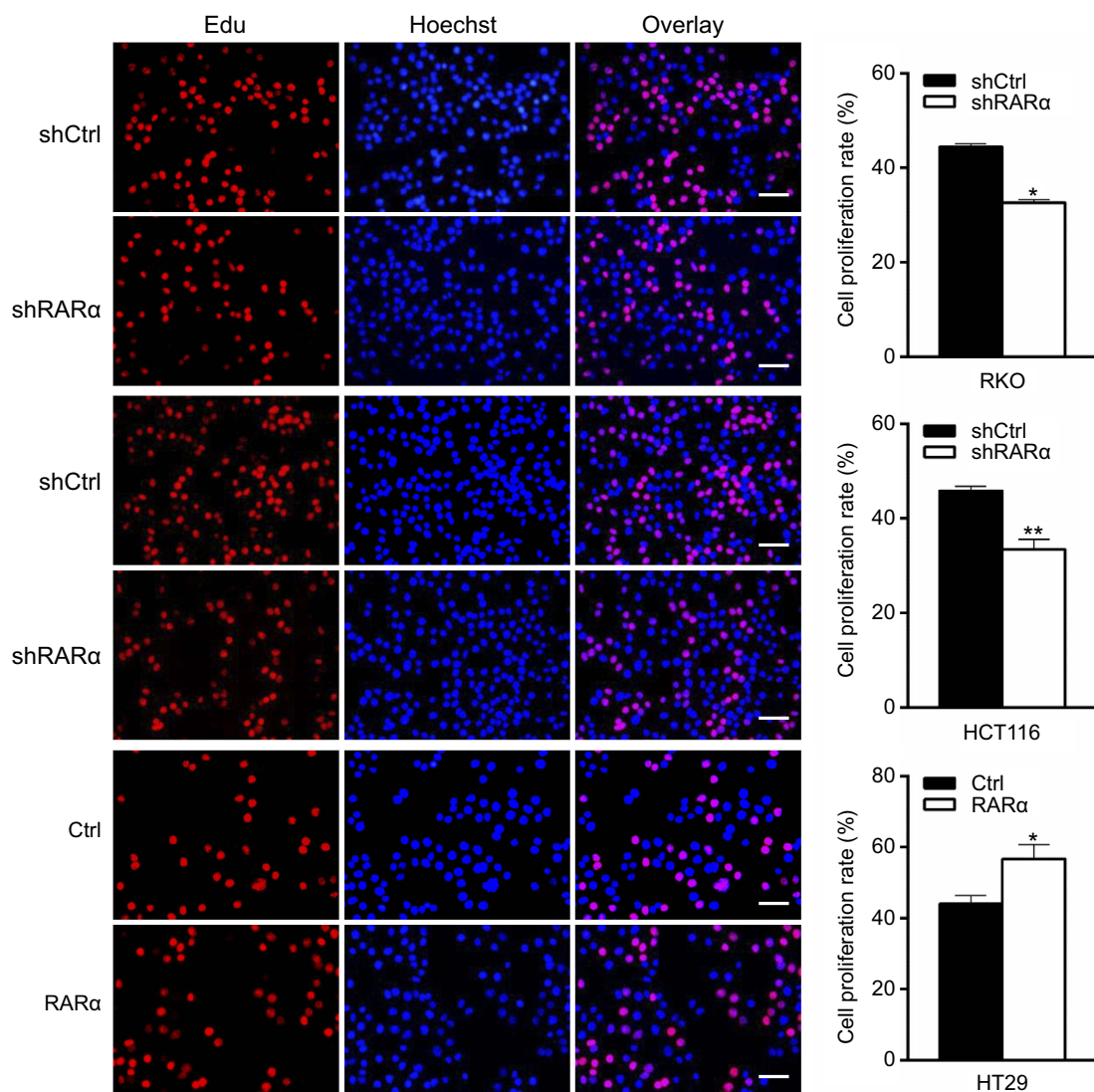


**Figure 1** Overexpression of RAR $\alpha$  in human CRC specimens and RAR $\alpha$ -knockdown cell model construction. (A) A representative photograph is shown of RAR $\alpha$  expression in CRC samples. Scale bar, 100  $\mu$ m. (B and C) Knockdown of RAR $\alpha$  in RKO and HCT116 as shown by QPCR and IF. Scale bar, 20  $\mu$ m. \*\*\* $P < 0.001$ .

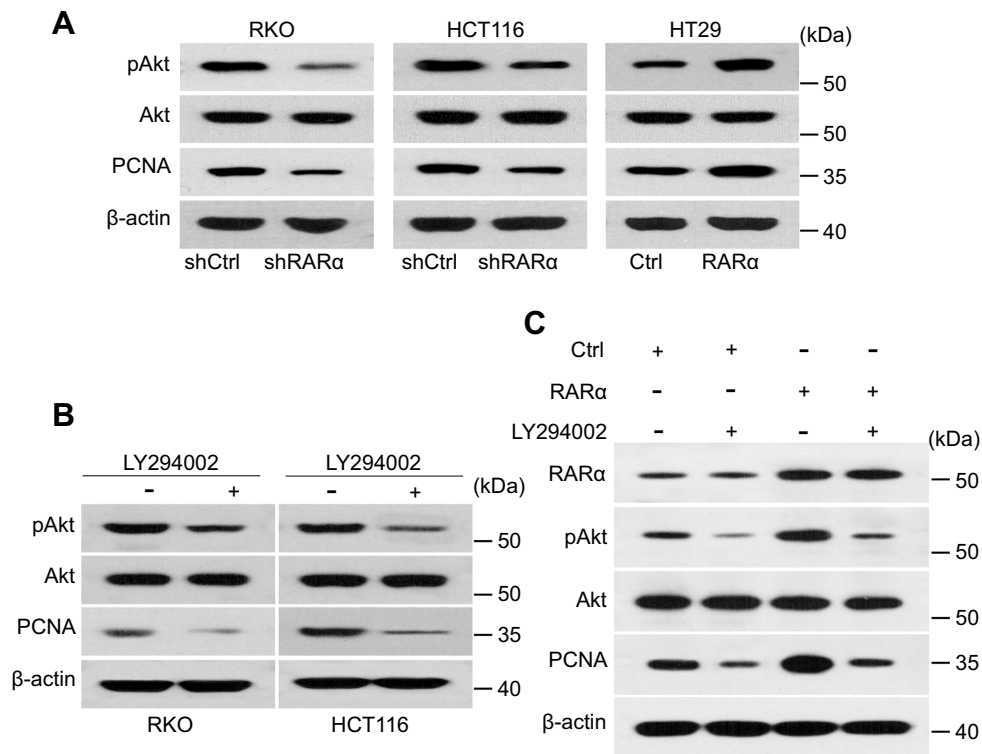
RAR $\alpha$  protein level was observed, whereas HT29 cells that express relatively less RAR $\alpha$  protein were transfected with pIRES2-EGFP vector (Ctrl) or RAR $\alpha$ -expression vector pIRES2-EGFP-RAR $\alpha$  (RAR $\alpha$ ). The mRNA and protein levels of RAR $\alpha$  in RAR $\alpha$ -knockdown cells were much lower than those in their respective control cells (Figure 1B and C), indicating that RAR $\alpha$  was effectively knockdown. Knockdown of RAR $\alpha$  significantly decreased cell proliferation in both RKO and HCT116 cells, along with a concomitant reduction of PCNA. Contrastingly, overexpression of RAR $\alpha$  in HT29 cells caused a significant increase in cell proliferation compared to those with Ctrl, with an increase of PCNA (Figures 2 and 3A). The observations corroborate the role of RAR $\alpha$  in the progress of CRC.

Research has shown the activation of PI3K/Akt signaling by RAR $\alpha$  by interacting with p85 $\alpha$  of PI3K.<sup>22</sup> Our next step was to measure the levels of phosphorylated Akt to see the effect of RAR $\alpha$  on the pathway. Consistent with previous studies, we found that RAR $\alpha$  knockdown suppressed the phosphorylated Akt at S473 (Figure 3A). PCNA was down-regulated in the shRAR $\alpha$  cells, as well as in cells treated with LY294002, specific inhibitors of Akt (Figure 3A and B). Meanwhile, PCNA expression was remarkably enhanced by RAR $\alpha$  overexpression and was abolished by the specific inhibitor of Akt (Figure 3C). These results confer support to the notion that RAR $\alpha$  promotes CRC cell proliferation through enhancing Akt signaling.

The xenograft tumor in nude mice was used to investigate the functional role of RAR $\alpha$  in regulating the growth



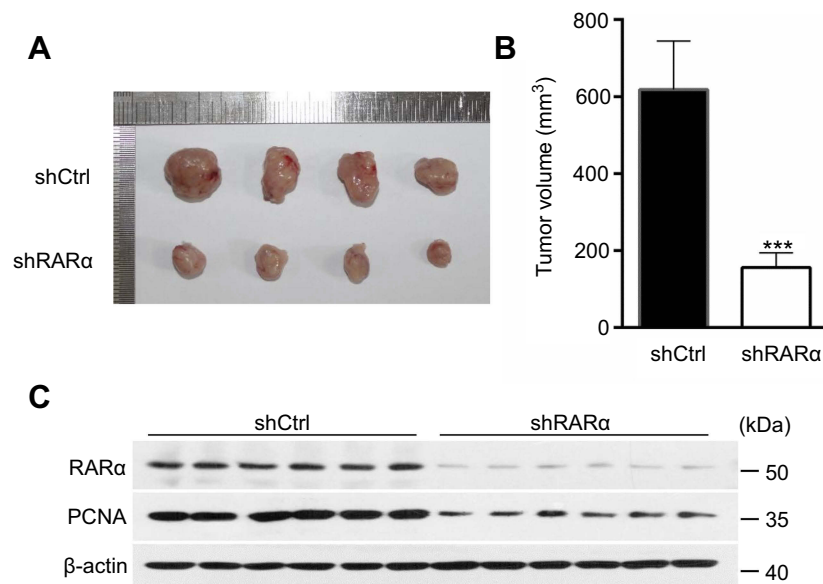
**Figure 2** Knockdown of RAR $\alpha$  suppresses CRC cell proliferation. Synthesis of DNA was assessed by 5-Ethynyl-20-deoxyuridine (EdU) assay in RAR $\alpha$ -knockdown or RAR $\alpha$ -overexpression cells. Scale bar, 100  $\mu$ m. (left). Quantification of 10 visions: at random to the right. Assays were independently repeated in triplicate. \* $P$ <0.05, \*\* $P$ <0.01.



**Figure 3** Knockdown of RAR $\alpha$  inhibits Akt signaling. **(A)** Western blotting to measure the amount of protein pAkt and PCNA in CRC cells. **(B)** LY294002 treatment decreased the expression of PCNA in RKO and HCT116 lines. **(C)** Expression of PCNA and Akt in RKO line subjected to various treatments. LY294002, 20  $\mu$ M.

of CRC *in vivo*. Nude mice received subcutaneous injections of RAR $\alpha$ -knockdown and control cells. Our results show that tumors formed by RAR $\alpha$  knockdown cells grow much slower than those formed by the control cells (Figure 4A). The mean volume of RAR $\alpha$ -knockdown

tumors was about fourfold smaller than that from control tumors (Figure 4B). Moreover, these knockdown tumors also displayed a significantly decreased level of PCNA (Figure 4C). Together, these results demonstrate that RAR $\alpha$  is a potent promoter of cancer cell growth.



**Figure 4** Knockdown of RAR $\alpha$  inhibits the tumor formation. **(A)** RAR $\alpha$  knockdown reduced cancer cell growth in nude mice (n=6) for 24 days. **(B)** The mean tumor volume in xenograft models in control and test. **(C)** Western blotting to measure the amount of RAR $\alpha$  and PCNA in tumor tissues of nude mice models. \*\*\*P<0.001.

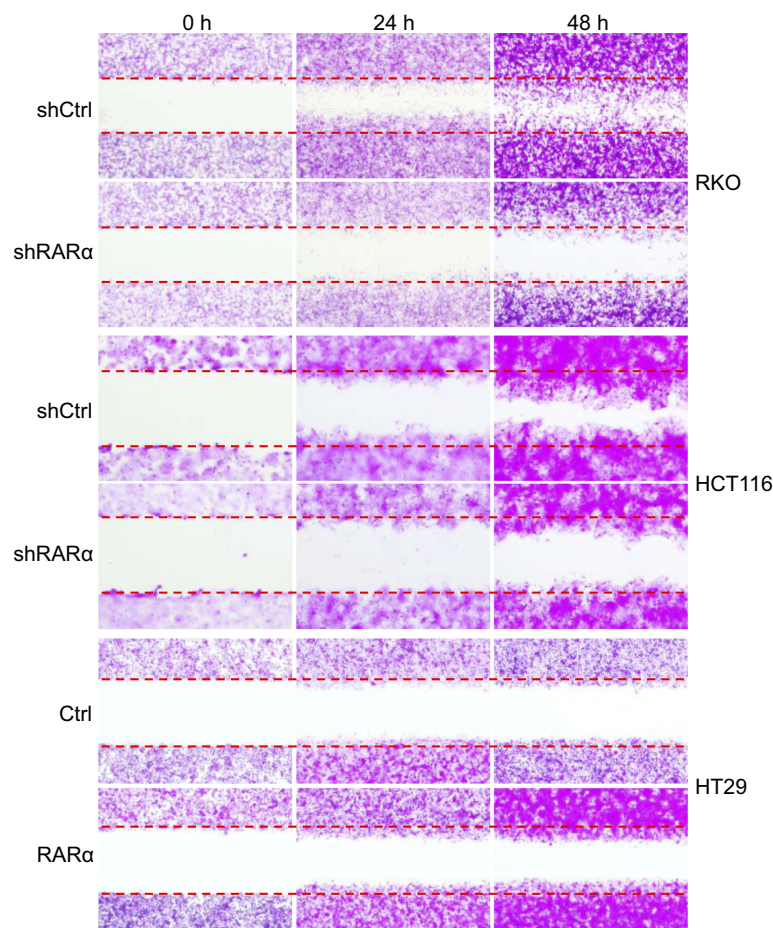
## RAR $\alpha$ knockdown hinders invasion and migration of CRC cell

It has been reported that RAR $\alpha$  is involved in metastasis of several types of cancers<sup>23–26</sup>. This led us to hypothesize that RAR $\alpha$  might involve in metastasis of CRC cells. In order to assess this, the ability of cells with RAR $\alpha$ -knockdown to migrate and invade was examined. Knockdown of RAR $\alpha$  inhibited the migration and invasion of RKO cells and HCT116 cells, whereas RAR $\alpha$  overexpression promoted HT29 cells migration and invasion (Figures 5 and 6A and B). These results indicate that the migrative and invasive potential of CRC cells is significantly reduced by RAR $\alpha$  knockdown and markedly increased by RAR $\alpha$  overexpression.

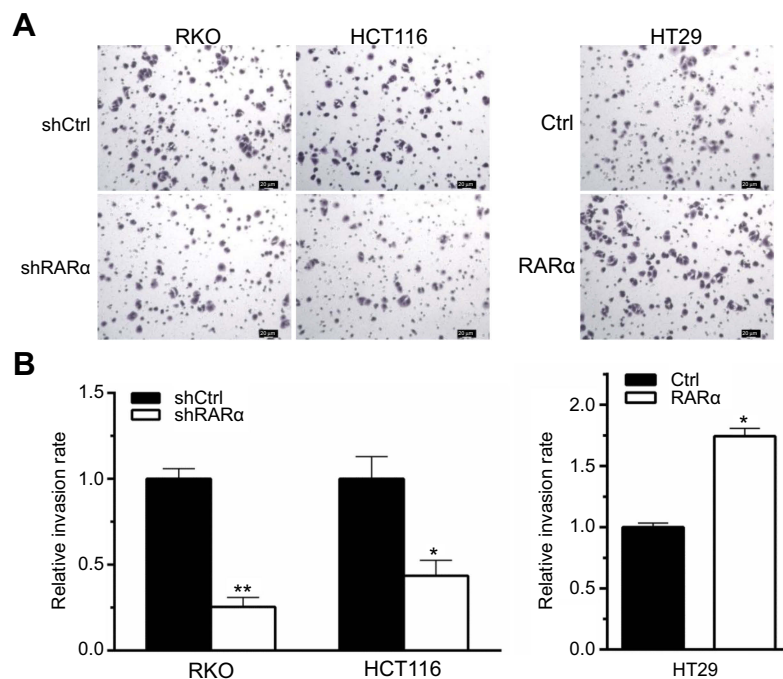
## RAR $\alpha$ increases the transcription of MMP2

To explore the mechanisms by which knockdown of RAR $\alpha$  inhibited metastasis of CRC cells, QPCR was

performed to test metastasis-associated gene expression. The mRNA expression of *MMP2*, but not *MMP1*, *MMP3* or *MMP9*, was decreased by knockdown and increased by overexpression of RAR $\alpha$  (Figure 7A). Furthermore, knockdown of RAR $\alpha$  reduced the enzyme activity of MMP2, while RAR $\alpha$  overexpression induced the enzyme activity (Figure 7B), a matrix metalloproteinase which plays an important role in the migration and invasion of a variety of tumor cells. Rescue of MMP2 expression in RAR $\alpha$ -knockdown RKO cells restored cell invasion (Figure S1). A search for the human *MMP2* gene promoter was the next step to study the role of RAR $\alpha$  on *MMP2* mRNA expression. A plasmid pGL3-basic with luciferase reporter was used to clone the sequence of the gene spanning 1.8 kb upstream to the ATG site at 211 bp downstream of the transcription start site. The activity of *MMP2* promoter increased significantly when RAR $\alpha$  was transfected into 293T cells while RAR $\alpha$ -targeted shRNA notably impaired the activity as determined by dual-luciferase



**Figure 5** Knockdown of RAR $\alpha$  reduces CRC cells migration. The representative images of wound healing: the migration of cells at 24 and 48 h are shown. Assays were independently repeated in triplicate.



**Figure 6** RAR $\alpha$  knockdown reduces CRC cells invasion. **(A)** Cell invasion was performed by transwell assays. Scale bar, 20  $\mu$ m. Representative images of indicated cells are shown. Assays were independently repeated in triplicate. **(B)** Quantitative analysis of invasion cells. \* $P$ <0.01, \*\* $P$ <0.001.

reporter assays (Figure 7C). A truncated fragment with P598 allowed for reporter activity dependent on equivalent RAR $\alpha$  (Figure 7D), that highlights the involvement of these positions in the activity of luciferase.

Analysis of P598 revealed a proximal RAR $\alpha$ -binding site (RARE) in the promoter region of *MMP2*. The proximal site was mutated and subjected to analysis to see if it was involved in RAR $\alpha$  associated activation of *MMP2*. Mutation of proximal RARE site removed the inhibitory effect of RAR $\alpha$  in RKO cells (Figure 8A), suggesting that this site might be responsible for RAR $\alpha$  binding in vivo. ChIP assay was conducted to see if RAR $\alpha$  could be recruited to the RARE site. The results showed that RAR $\alpha$  was recruited to the RARE in the promoter region of *MMP2*, and the recruitment was increased in the presence of exogenous RAR $\alpha$  (Figure 8B and C). Taken together, our findings establish that RAR $\alpha$  activates *MMP2* expression via direct binding to the *MMP2* promoter, which provides compelling evidence that *MMP2* is a direct target gene of RAR $\alpha$ .

## Discussion

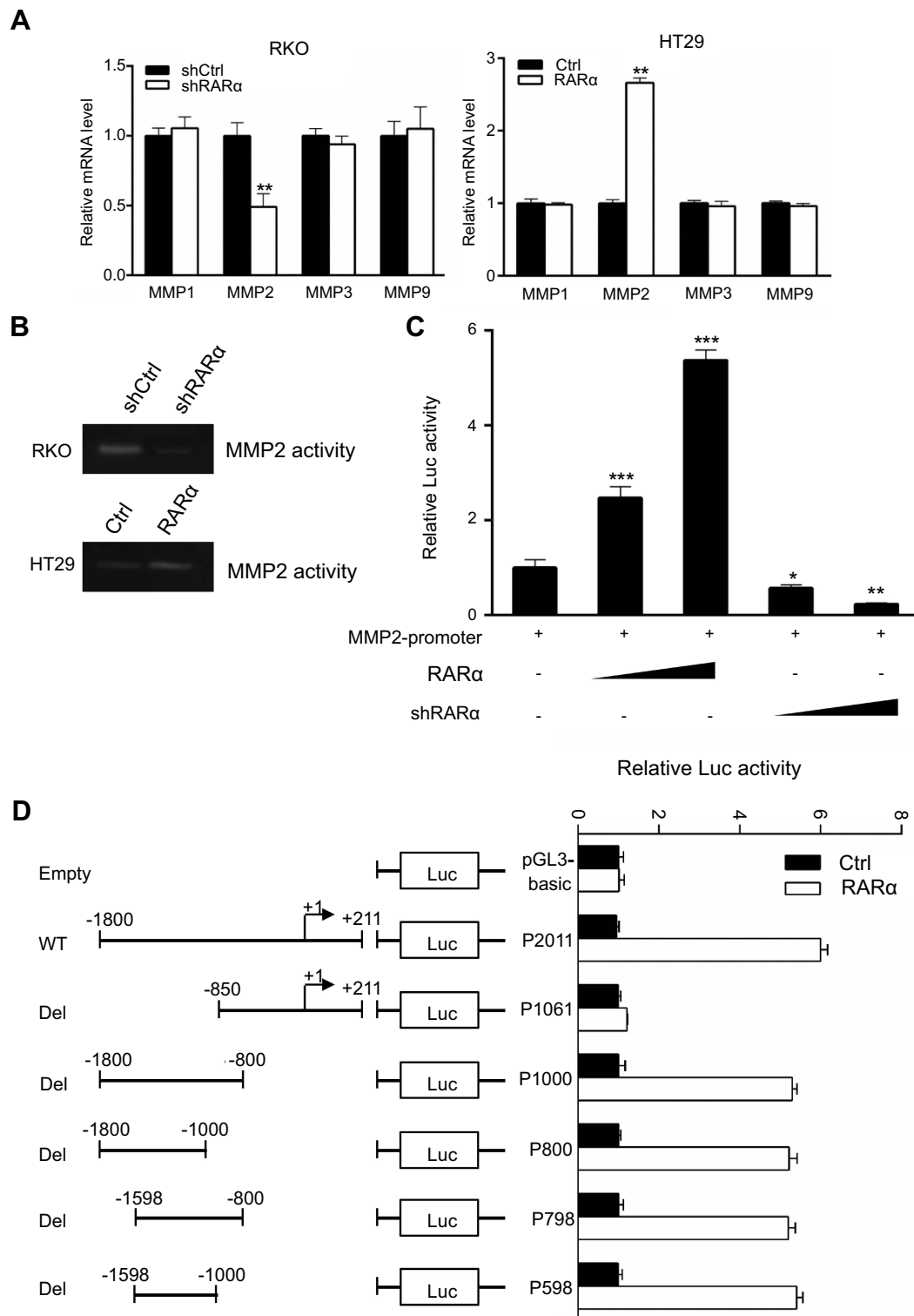
Accumulating evidence indicates that aberrant RAR $\alpha$  expression is a common phenomenon in multitude cancers, including leukemia, breast cancer, lung cancer, and gastric carcinoma.<sup>10,27–29</sup> Despite the reports of the promotional

role of RAR $\alpha$  in carcinogenesis of CRC or a potential marker of prognosis in CRC,<sup>18</sup> the exact role and expression are yet to be ascertained in CRC progression. This work confirmed an overexpression of RAR $\alpha$  on human CRC samples while knockdown of the protein significantly suppressed the proliferation of CRC cells and their migration and invasion. These findings demonstrate that RAR $\alpha$  plays an important role in CRC carcinogenesis.

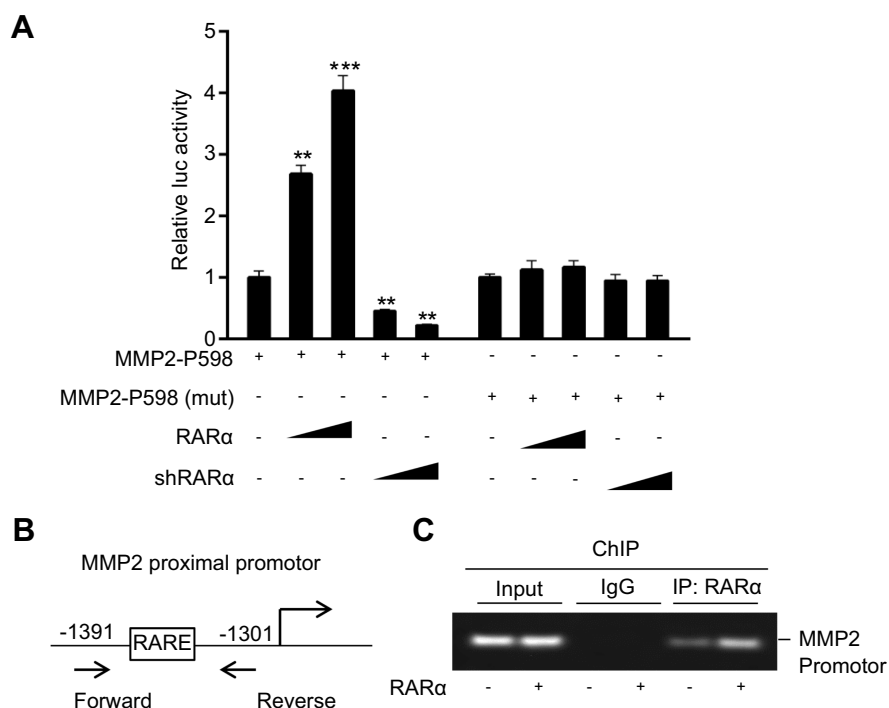
A basic feature in cancer cells is the sustenance of proliferative signaling.<sup>30</sup> PCNA, often overexpressed in cancer cells, is a multifunctional protein essential for DNA replication and repair.<sup>31</sup> In the present study, we observed that the expression of PCNA was significantly reduced by RAR $\alpha$  knockdown and increased by RAR $\alpha$  overexpression, indicating that RAR $\alpha$  promotes CRC growth by positively regulating the expression of PCNA. Since PCNA is the target gene of Akt signaling, which is frequently activated in cancer,<sup>32,33</sup> we were prompted to determine whether RAR $\alpha$  promotes CRC growth by activation of Akt signaling. Our present results demonstrated that RAR $\alpha$  promotes the growth of CRC cells through enhancing the expression of Akt-mediated expression of PCNA to accelerate proliferation.

It is increasingly apparent that research into the capability of cancer cells to migrate and invade has gained momentum dramatically over the past decade. MMPs, as





**Figure 7** RARα suppresses MMP2 transcription. **(A)** QPCR to determine the mRNA levels of MMPs in RARα-knockdown RKO and RARα-overexpressed HT29 and control cells. **(B)** The enzymatic activity of MMP2 were assessed by gel zymography assays. **(C)** MMP2 promoter activity was increased by overexpressing RARα and was decreased by the knockdown in 293T line. Normalization of firefly luciferase was done with reference to renilla luciferase. **(D)** Luciferase activity of various pGL3-MMP2 vectors (wild-type or deleted) whether exogenous RARα was present or absent in 293T cells. **(C and D)** A representative assay of independent and triplicate assays is displayed. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001.



**Figure 8** Recruitment of RAR $\alpha$  to the MMP2 proximal promoter. **(A)** Dual-luciferase activity (that was subjected to normalization with renilla luciferase) of RKO line. A representative assay of independent and triplicate assays is displayed. **(B)** A representation of proximal promoter of MMP2. Forward and reverse primers were utilized in ChIP assay. **(C)** The assay showed recruitment of RAR $\alpha$  to proximal promoter of MMP2 whether exogenous RAR $\alpha$  was present or not. \*\* $P < 0.05$ , \*\*\* $P < 0.01$ .

critical regulatory genes, play a crucial function in aiding invasion by tumors by destroying the basement membrane and extracellular matrix. MMP2 is considered an important enzyme in cancer cells to invade and metastasize,<sup>34</sup> with proof of these functions in cancers such as CRC.<sup>35</sup> A finding of noteworthy significance in this work is that knockdown of RAR $\alpha$  suppresses the expression of *MMP2* rather than other *MMPs*. However, how the expression of *MMP2* is controlled by RAR $\alpha$  has not yet been determined. Our study showed that RAR $\alpha$  was recruited to the promoter of *MMP2* to facilitate the expression of *MMP2*, and eventually promoted CRC invasion and metastasis.

These results collectively demonstrate that our study highlights the crucial role of RAR $\alpha$  in promoting the carcinogenesis of human CRC by enhancing cell proliferation and invasiveness, demonstrating that pharmacological targeting of RAR $\alpha$  is a promising approach for CRC treatment.

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## Disclosure

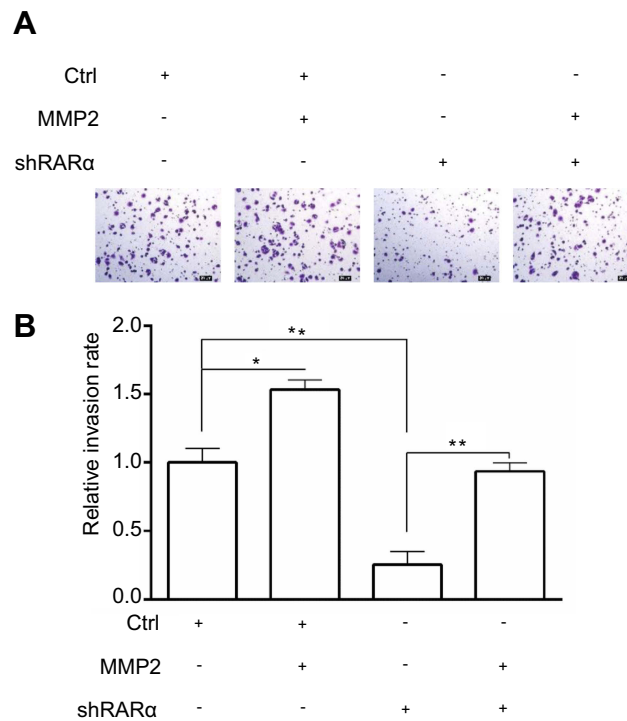
The authors report no conflicts of interest in this work.

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## Supplementary material



**Figure S1** Rescue of MMP2 expression in RAR $\alpha$ -knockdown RKO cells restored cell invasion

**Notes:** (A) Cell invasion was performed by transwell assays. Scale bar, 20  $\mu$ m. Assays were independently repeated in triplicate. (B) Quantitative analysis of invasion cells. \* $P < 0.01$ , \*\* $P < 0.001$ .

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