

# *Sapindus* Saponin Inhibits Colorectal Cancer cell Proliferation and Metastasis by regulating microRNA-5590-3p expression

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## Zhang *et al.*: *Sapindus* Saponin in Colorectal Cancer Cells and Its Mechanism

This study explored *Sapindus* saponins role in the proliferation and metastasis of colorectal cancer cells and its possible mechanism. SW620 cells were divided into 8 groups; control group, *Sapindus* saponin-low group, *Sapindus* saponin-middle group, *Sapindus* saponin-high group, microRNA+negative control group, microRNA-5590-3p group, *Sapindus* saponin+anti-microRNA-negative control group and *Sapindus* saponin+anti-microRNA-5590-3p group. Cell proliferation and metastasis were determined using colony formation assay, wound healing assay, 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide assay and transwell assay. MicroRNA-5590-3p expression was detected by quantitative reverse transcription-polymerase chain reaction. Matrix metalloproteinases-2 and matrix metalloproteinases-9 protein levels were examined by Western blot. SW620 cell proliferation inhibition rate and microRNA-5590-3p expression were increased, while colony numbers, invaded cell numbers, wound healing rate, matrix metalloproteinases-2 and matrix metalloproteinases-9 protein levels were decreased in *Sapindus* saponin-low group, *Sapindus* saponin-middle group, *Sapindus* saponin-high group in a dose-dependent manner. Besides, microRNA-5590-3p overexpression increased SW620 cell proliferation inhibition rate, while suppressed colony numbers, invaded cell numbers, wound healing rate, matrix metalloproteinases-2 and matrix metalloproteinases-9 protein levels. Compared with the *Sapindus* saponin+anti-microRNA-negative control group, SW620 cell proliferation inhibition rate was reduced, while colony numbers, invaded cell numbers, wound healing rate, matrix metalloproteinases-2 and matrix metalloproteinases-9 protein levels were enhanced in the *Sapindus* saponin+anti-microRNA-5590-3p group. *Sapindus* saponin could repress colorectal cancer cell proliferation and metastasis by upregulating microRNA-5590-3p.

**Key words:** Colorectal cancer, *Sapindus* saponin, microRNA-5590-3p, matrix metalloproteinases, chemotherapy

Colorectal Cancer (CRC) is a common clinical malignant tumor<sup>[1,2]</sup>. Chemotherapy drugs and other drugs have great toxic and side effects, and patients are prone to develop drug resistance leading to poor treatment effect<sup>[3,4]</sup>. It is of great significance to find drugs that can inhibit CRC cell proliferation and metastasis. Traditional Chinese Medicine (TCM) has anti-tumor effects and can regulate CRC cell biological behaviors<sup>[5,6]</sup>. It has been reported that *Sapindus* saponin inhibits proliferation in lung cancer and hepatocellular carcinoma<sup>[7,8]</sup>. However, the effect of *Sapindus* saponin on CRC progression is still unknown.

MicroRNA (miRNA) is widely involved in regulating human disease processes<sup>[9,10]</sup>. Studies had shown that miR-5590-3p downregulation facilitated proliferation in prostate cancer cells<sup>[11]</sup>. Besides, miR-5590-3p had been confirmed to restrain cell invasion in ovarian cancer and renal cancer<sup>[12,13]</sup>. Therefore, miR-5590-3p may serve as a tumor suppressor. However, its role in CRC progression has not been elucidated.

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In this, we found that *Sapindus* saponin has a promotion effect on miR-5590-3p expression, but whether *Sapindus* saponin mediates CRC progression by increasing miR-5590-3p remains unclear. Basing on the above, we hypothesized that *Sapindus* saponin inhibited CRC cell functions by upregulating miR-5590-3p.

## MATERIALS AND METHODS

### Cell culture and grouping:

SW620 cells (Procell, Wuhan, China) were grown in Dulbecco's Modified Eagle Medium (DMEM) plus 10 % Fetal Bovine Saline (FBS) and 1 % dual-antibiotic (Invitrogen, Carlsbad, California, United States of America (USA)). Cells were treated with *Sapindus* saponin (Pushida, Wuhan, China) for 24 h<sup>[8]</sup>, and recorded as *Sapindus* saponin+low (25 µg/ml) group, *Sapindus* saponin+middle (50 µg/ml) group and *Sapindus* saponin+high (100 µg/ml) group. Normal cultured SW620 cells were used as control group. Lipofectamine 3000 (Invitrogen) was used for cell transfection. miR-5590-3p mimic and miR-NC were transfected into SW620 cells, recording as miR-NC group and miR-5590-3p group. Besides, SW620 cells were transfected with anti-miR-5590-3p and anti-miR-NC followed by treated with 100 µg/ml *Sapindus* saponin for 24 h, recording as *Sapindus* saponin+anti-miR-NC group and *Sapindus* saponin+anti-miR-5590-3p group.

### 3-[4,5-Dimethylthiazol-2-yl]-2,5 Diphenyl Tetrazolium Bromide (MTT) assay:

SW620 cells were inoculated on 96-well plates and incubated with MTT solution (Beyotime, Shanghai, China) followed by treated with Dimethyl Sulfoxide (DMSO). Absorbance at 490 nm was detected by microplate reader to count cell proliferation inhibition rate.

### Colony formation assay:

SW620 cells were cultured for 14 d in 6-well plates. After that, colonies were fixed and colony numbers were counted under a microscope.

### Wound healing assay:

After reached 90 % confluences in 6-well plates, SW620 cells were scratched by a 200 µl pipette tip. Wound area was observed under a microscope to count wound healing rate.

### Transwell assay:

SW620 cells were seeded into Matrigel-coated transwell upper chamber (Corning Inc., Corning, New York, USA), and complete medium were filled in lower chamber. Then, invaded cell numbers were counted under a microscope.

### Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR):

Extracted Ribonucleic Acid (RNA) was reverse-transcribed into complimentary Deoxyribonucleic Acid (cDNA), and PCR amplification was performed with SYBR Green (Takara, Dalian, China). miR-5590-3p expression was analyzed by  $2^{-\Delta\Delta C_t}$  method.

### Wesern blot:

Protein extracted by Radioimmunoprecipitation Assay (RIPA) buffer was transferred onto Polyvinylidene Difluoride (PVDF) membranes after separated by Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) gel. Membrane was incubated with anti-Matrix Metalloproteinase (MMP)-2, anti-MMP-9 or anti-Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) followed by treated with secondary antibody. Protein signals were displayed using Enhanced Chemiluminescence (ECL) reagents (Beyotime).

### Statistical analysis:

Measurement data were expressed as  $\bar{x} \pm s$ , and analyzed by Statistical Package for the Social Sciences (SPSS) 20.0 software. Comparison was assessed by Student's t-test or Analysis of Variance (ANOVA).  $p < 0.05$  was considered statistically significant.

## RESULTS AND DISCUSSION

HAs showed in Table 1, cell proliferation inhibition rate was increased, while colony numbers were decreased in the *Sapindus* saponin-low, *Sapindus* saponin-middle, and *Sapindus* saponin-high groups in a dose-dependent manner.

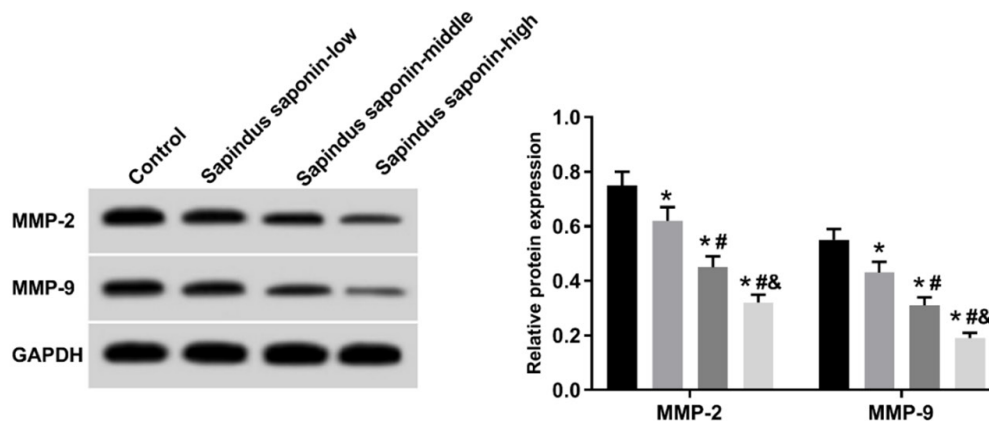
Moreover, MMP-2 and MMP-9 protein levels, wound healing rate and invaded cell numbers were reduced in the *Sapindus* saponin-low, *Sapindus* saponin-middle, and *Sapindus* saponin-high groups in a dose-dependent manner as shown in

fig. 1 and Table 2. miR-5590-3p expression was elevated in the *Sapindus* saponin-low, *Sapindus* saponin-middle and *Sapindus* saponin-high groups in a dose-dependent manner as shown in Table 3.

**TABLE 1: EFFECT OF *Sapindus* SAPONIN ON SW620 CELL PROLIFERATION ( $\bar{x}\pm s$ , n=9)**

| Group                          | Inhibition rate (%)       | Colony numbers             |
|--------------------------------|---------------------------|----------------------------|
| Control                        | 0.00±0.00                 | 111.70±10.26               |
| <i>Sapindus</i> saponin+low    | 24.57±2.07*               | 85.38±7.95*                |
| <i>Sapindus</i> saponin+middle | 45.13±5.02**              | 65.50±5.12 <sup>#</sup>    |
| <i>Sapindus</i> saponin+high   | 71.72±5.97** <sup>‡</sup> | 48.38±4.43** <sup>#‡</sup> |
| F                              | 513.020                   | 124.501                    |
| p                              | 0.000                     | 0.000                      |

Note: Compared to control group, \*p<0.05; compared to *Sapindus* saponin+low group, <sup>#</sup>p<0.05 and compared to *Sapindus* saponin+middle group, <sup>‡</sup>p<0.05



**Fig. 1: Effect of *Sapindus* saponin on metastasis-related protein expression in SW620 cells**

Note: (■): Control; (■): *Sapindus* saponin-low; (■): *Sapindus* saponin-middle and (■): *Sapindus* saponin-high

**TABLE 2: EFFECT OF *Sapindus* SAPONIN ON SW620 CELL MIGRATION AND INVASION ( $\bar{x}\pm s$ , n=9)**

| Group                          | Wound healing rate (%)    | Invaded cell numbers       |
|--------------------------------|---------------------------|----------------------------|
| Control                        | 72.71±5.09                | 127.41±11.23               |
| <i>Sapindus</i> saponin+low    | 55.81±4.69*               | 93.26±7.02*                |
| <i>Sapindus</i> saponin+middle | 42.39±3.43**              | 73.43±6.66 <sup>#</sup>    |
| <i>Sapindus</i> saponin+high   | 25.61±2.05** <sup>‡</sup> | 54.85±8.25** <sup>#‡</sup> |
| F                              | 225.313                   | 120.483                    |
| p                              | 0.000                     | 0.000                      |

Note: Compared to control group, \*p<0.05; compared to *Sapindus* saponin+low group, <sup>#</sup>p<0.05 and compared to *Sapindus* saponin+middle group, <sup>‡</sup>p<0.05

**TABLE 3: EFFECT OF *Sapindus* SAPONIN ON miR-5590-3p EXPRESSION IN SW620 CELLS ( $\bar{x}\pm s$ , n=9)**

| Group                          | miR-5590-3p              |
|--------------------------------|--------------------------|
| Control                        | 1.00±0.01                |
| <i>Sapindus</i> saponin+low    | 1.62±0.13*               |
| <i>Sapindus</i> saponin+middle | 2.41±0.21**              |
| <i>Sapindus</i> saponin+high   | 3.11±0.26** <sup>‡</sup> |
| F                              | 236.986                  |
| p                              | 0.000                    |

Note: Compared to control group, \*p<0.05; compared to *Sapindus* saponin+low group, <sup>#</sup>p<0.05 and compared to *Sapindus* saponin+middle group, <sup>‡</sup>p<0.05

miR-5590-3p mimic was used to overexpress miR-5590-3p expression in SW620 cells. Besides, miR-5590-3p overexpression decreased colony numbers, invaded cell numbers, wound healing rate, MMP-2 and MMP-9 protein levels, while elevated cell proliferation inhibition rate as shown in fig. 2 and Table 4.

Meanwhile, anti-miR-5590-3p was used to reduce

miR-5590-3p expression in *Sapindus* saponin-treated SW620 cells. Functional experiments showed that colony numbers, invaded cell numbers, wound healing rate, MMP-2 and MMP-9 protein levels were enhanced, while cell proliferation inhibition rate was reduced in *Sapindus* saponin+anti-miR-5590-3p group as shown in fig. 3 and Table 5.

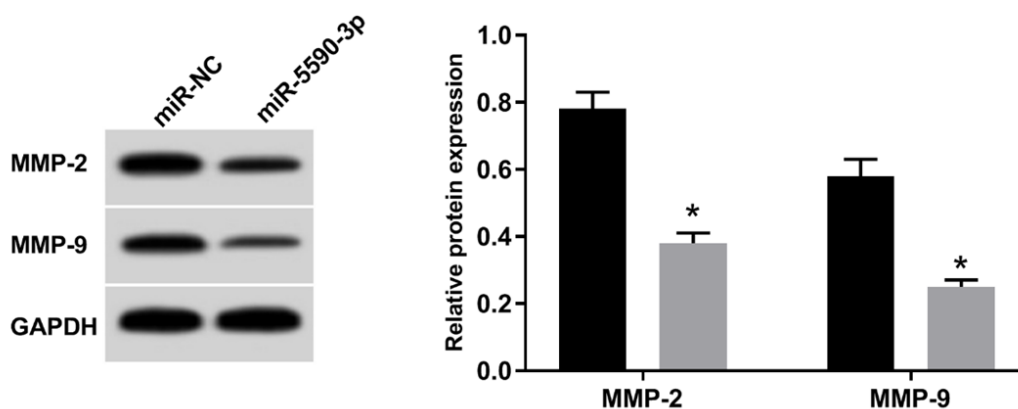


Fig. 2: miR-5590-3p overexpression regulated metastasis-related protein expression in SW620 cells

Note: (■): miR-NC and (■): miR-5590-3p

TABLE 4 miR-5590-3p OVEREXPRESSION REGULATED SW620 CELL PROLIFERATION AND METASTASIS ( $\bar{x} \pm s$ , n=9)

| Group       | miR-5590-3p | Inhibition rate (%) | Colony numbers | Wound healing rate (%) | Invaded cell numbers |
|-------------|-------------|---------------------|----------------|------------------------|----------------------|
| miR-NC      | 1.00±0.00   | 7.38±0.57           | 113.07±12.13   | 73.15±6.35             | 129.16±10.13         |
| miR-5590-3p | 3.02±0.27*  | 25.34±2.08*         | 53.76±4.62*    | 32.37±3.45*            | 60.68±4.96*          |
| t           | 22.444      | 24.983              | 13.708         | 16.929                 | 18.214               |
| p           | 0.000       | 0.000               | 0.000          | 0.000                  | 0.000                |

Note: Compared to miR-NC group, \*p<0.05

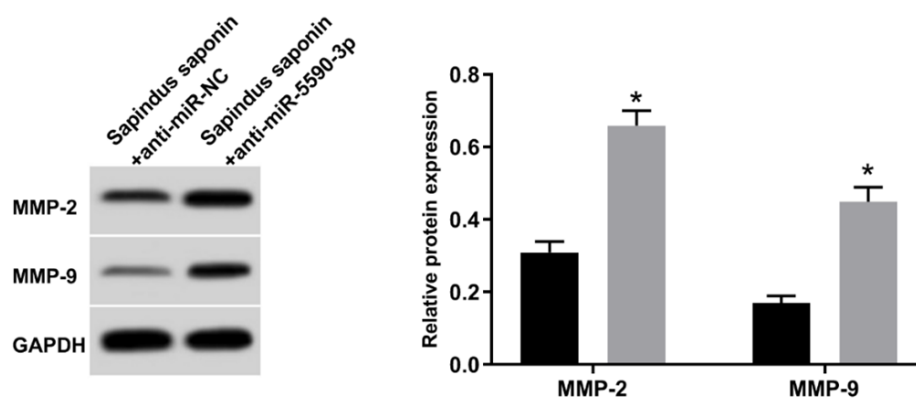


Fig. 3: miR-5590-3p inhibitor reversed *Sapindus* saponin-mediated metastasis-related protein expression in SW620 cells

Note: (■): *Sapindus* saponin+anti-miR-NC and (■): *Sapindus* saponin+anti-miR-5590-3p

**TABLE 5: miR-5590-3p INHIBITOR REVERSED *Sapindus* SAPONIN-MEDIATED SW620 CELL PROLIFERATION AND METASTASIS ( $\bar{x}\pm s$ , n=9)**

| Group                                    | miR-5590-3p | Inhibition rate (%) | Colony numbers | Wound healing rate (%) | Invaded cell numbers |
|--|-------------|---------------------|----------------|------------------------|----------------------|
| <i>Sapindus</i> saponin+anti-miR-NC      | 1.00±0.00   | 72.18±5.62          | 46.52±4.51     | 23.53±2.11             | 53.82±4.69           |
| <i>Sapindus</i> saponin+anti-miR-5590-3p | 0.43±0.04*  | 27.17±2.69*         | 89.28±6.58*    | 60.74±4.84*            | 106.86±11.85*        |
| t  | 42.75       | 21.672              | 16.081         | 21.142                 | 12.486               |
| p  | 0.000       | 0.000               | 0.000          | 0.000                  | 0.000                |

Note: Compared to *Sapindus* saponin+anti-miR-NC group, \*p<0.05

TCM may exert anti-CRC effects by regulating multiple signaling pathways or gene expression<sup>[14,15]</sup>. miRNAs are aberrantly expressed in CRC tissues and can affect CRC cell biological behaviors<sup>[16,17]</sup>. However, whether miRNAs can be a potential target for CRC treatment with TCM need to be further explored.

*Sapindus* saponin plays an important role in many cancers. *Sapindus* saponin could facilitate apoptosis in lung cancer cells<sup>[7]</sup>. Also, *Sapindus* saponin was found to inhibit proliferation in hepatocellular carcinoma cells<sup>[8]</sup>. However, *Sapindus* saponins role in CRC has been reported relatively little. Our data showed that SW620 cell proliferation was markedly reduced after treatment with *Sapindus* saponin, suggesting that *Sapindus* saponin could suppress CRC cell proliferation. MMP-2 and MMP-9 belong to matrix metalloproteinases, whose expression are up-regulated in CRC and can facilitate cell metastasis<sup>[18,19]</sup>. Here, *Sapindus* saponin was found to reduce wound healing rate, invaded cell numbers, MMP-2 and MMP-9 levels in SW620 cells, indicating that *Sapindus* saponin inhibited CRC cell metastasis.

miR-5590-3p is aberrantly expressed in many cancers<sup>[11,20]</sup>. miR-5590-3p overexpression suppressed proliferation in breast cancer<sup>[21]</sup>. miR-5590-3p upregulation inhibited renal carcinoma cell metastasis<sup>[22]</sup>. In this study, miR-5590-3p was upregulated in *Sapindus* saponin-treated SW620 cells. Meanwhile, miR-5590-3p overexpression reduced SW620 cell proliferation and metastasis, whereas its inhibition also reversed the effect of *Sapindus* saponin on SW620 cell functions, indicating that *Sapindus* saponin repressed CRC cell progression by promoting miR-5590-3p expression.

In conclusion, *Sapindus* saponin suppressed CRC cell proliferation and metastasis by upregulating

miR-5590-3p. However, whether *Sapindus* saponin can exert anti-cancer effects by regulating the other genes expression or signaling pathways needs to be further investigated.

### Conflict of interests:

The authors declared no conflict of interests.

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