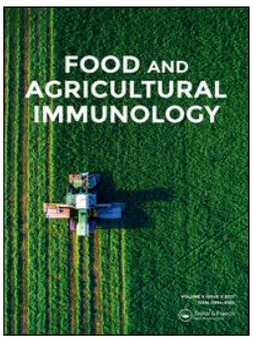

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Genistein inhibits AOM/DSS-induced colon cancer by regulating lipid droplet accumulation and the SIRT1/FOXO3a pathway in high-fat diet-fed female mice

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ABSTRACT

Obesity is one of the risk factors associated with colon cancer. In this study, we investigated the effect of genistein on colon cancer in obese mice and its underlying mechanism. Colon cancer was induced by azoxymethane/dextran sulfate sodium injection in Kun Ming mice, and they were fed with regular or high-fat diet (HFD). Genistein-rich diet (50, 150 and 450 mg/kg) decreased body weight gain, serum TC, TG, LDL-C levels, whereas it increased serum HDL-C level and lipase activity under HFD conditions. Genistein reduced mRNA levels of fat metabolism-related genes, including *Fas* and *Acc1*, and decreased levels of lipid droplet-related proteins, including perilipin, ADRP and TIP-47. Furthermore, genistein decreased PPAR-γ expression, whereas it increased SIRT1 and FOXO3 levels in colon tissue. Thus, genistein prevented the development of colon cancer by inhibiting abnormal fatty acid biosynthesis via regulation of the SIRT1/FOXO3a pathway in HFD-fed female mice.

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KEYWORDS
Genistein; obesity; lipid droplets; colon cancer; SIRT1/FOXO3a pathway

1. Introduction

The incidence and mortality of colon cancer, a common malignant digestive system tumour, is on the rise worldwide because of several predisposing factors such as high-fat diet, sedentary lifestyle, and lack of health-care resources (Issa & Noureddine, 2017; Shen, Wang, Dong, Xiang, & Liu, 2016). Preventing the transformation of normal cells into cancerous cells can reduce the probability of high-risk groups from developing colon cancer (Yamaji et al., 2011). Several reports showed that obese people are at higher risk than healthy weight individuals for developing colon cancer (Divella, De Luca, Abbate, Naglieri, & Daniele, 2016). Accumulated lipid droplets (LDs) in obese individuals provide the energy required for cancer cell proliferation (Qi et al., 2013).

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Analysis of human colon adenocarcinoma cell lines and colon cancer biopsy samples showed increase in the expression of adipose differentiation-related protein (ADRP), which is a major structural protein associated with LDs (Pfisterer et al., 2014). Furthermore, inhibition of LD formation correlates with reduced cancer cell proliferation *in vitro* (Penrose et al., 2016; Qi et al., 2013).

In Asian countries, the incidence and mortality associated with colon tumour is lower than that in the United States and other developed countries (Pudenz, Roth, & Gerhauser, 2014), which could be related to the consumption of soy food-rich diet by Asians (Kallay et al., 2002; Qi, Weber, Wasland, & Savkovic, 2011). Indeed, several studies have shown that isoflavones, a prominent component of soy, possesses anticancer properties (Pudenz et al., 2014). Dietary intake of isoflavones is estimated to range from 15 to 200 mg/day in Asian countries such as China, Japan, and Singapore (Cassidy & Faughnan, 2000; Chen et al., 1999; Seow et al., 1998). In contrast, less than 3 mg/day isoflavones are ingested in western societies (Keinan-Boker et al., 2002).

Genistein, a major component of soybean isoflavones, possesses antioxidant, anti-inflammatory, and anti-tumourigenic activities and has generated extensive research interest. Recent data indicate that genistein was able to attenuate estrogen-deficiency-induced obesity, white adipose tissue inflammation and hepatic lipogenesis (Shen et al., 2019). Dietary genistein ameliorated hyperlipidemia in hamsters via reducing the abdominal fat weight and decreasing serum and hepatic lipid contents (Tang, Zhang, Zhao, & Zhang, 2015). Previously, we have shown that genistein can induce apoptosis of colon cancer HT-29 cells *in vitro* by inhibiting LDs accumulation via regulation of lipid metabolism-associated genes (Liang et al., 2018). Our previous paper found that genistein reduced azoxymethane/dextran sulfate sodium (AOM/DSS)-induced colonic cancer by regulating the PI3 K/AKT/FOXO3 pathway that closely related to cell apoptosis (Song et al., 2018). This research investigate the effect of gensitein on regulation of lipid metabolism and reversing negative effects of obesity on colon cancer. Thus, AOM/DSS-induced colon neoplasms with high-fat diet (HFD) was constructed with Kun Ming (KM) mice in this research.

2. Materials and methods

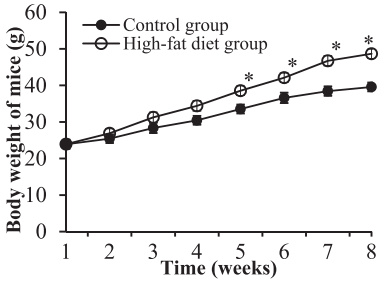
2.1. Animals

Female KM mice (4-week-old), purchased from the Centre of Laboratory Animal Science Academy of Military Medicine Sciences (Beijing, China), were housed in the laboratory of animal experiment at the Academy of National Food and Strategic Reserves Administration. Mice were caged individually and were maintained under controlled temperature (22 ± 2°C), humidity and airflow condition, with a 12-h on/off light cycle.

2.2. Experimental design

After 7 days of acclimatization, 140 female KM mice were divided into two groups. One group ($n = 50$) was provided regular diet (RD, 13.9 kJ/g, 4.5% fat, w/w), whereas the other group ($n = 90$) was provided HFD for 8 weeks (Figure 1(A)). The HFD ingredients included 66.8% common feed, 10% egg yolk, 12% lard, 1% cholesterol, 0.2% bile salt, and 10% sucrose, which was provided by the Animal Department of Academy of Military

A



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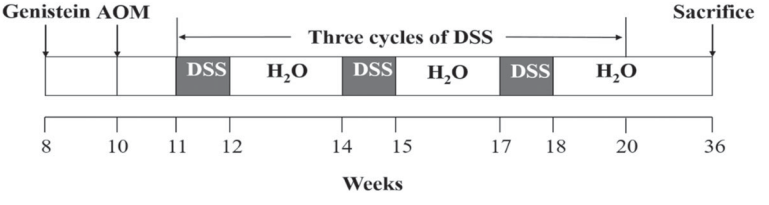

C

Group	Diets	Treatments
A	Regular diets (RD)	RD control
B		AOM/DSS
C		AOM/DSS + 50 mg/kg genistein
D		AOM/DSS + 150 mg/kg genistein
E		AOM/DSS + 450 mg/kg genistein
F	High-fat diets (HFD)	HFD control
G		50 mg/kg genistein
H		150 mg/kg genistein
I		450 mg/kg genistein
J	High-fat diets (HFD)	AOM/DSS
K		AOM/DSS + 50 mg/kg genistein
L		AOM/DSS + 150 mg/kg genistein
M		AOM/DSS + 450 mg/kg genistein

Figure 1. (A) High-fat diets significantly increased the body weight of mice at week 8. $^{*}p < 0.05$ indicates significantly different from the control group. (B) Experimental design. Obesity was induced by HFD and colon cancer was induced by AOM/DSS in mice. After administration of genistein for 2 weeks, colonic tumours were induced by single intraperitoneal injection of AOM (12 mg/kg body weight) and three cycles of 2.5% DSS in drinking water. Mice were treated DSS for 1 week, and then were feed water for 2 weeks. Mice were sacrificed at 26 weeks after AOM injection, and samples were collected. (C) Groups of mice with different treatments ($n = 10$ per group).

Medical Sciences. Mice were kept under SPF conditions with different diets and water *ad libitum*. After 8 weeks, mice with at least 20% higher body weight than the average body weight of the RD group were classified into 8 subgroups ($n = 10$), and other extra mice were sacrificed. Thus, all selected mice were divided into 13 groups ($n = 10$) as shown in Figure 1(C). Three doses of genistein (50, 150 and 450 mg/kg) (Solarbio, Beijing, China) were added to the diets, and mice were feed with the respective diets. After 2 weeks, colonic tumours were induced by a single intraperitoneal injection of AOM (12 mg/kg body weight) (Sigma-Aldrich, St. Louis, MO, USA). Starting 1 week after injection, mice in groups B–E and J–M were administered 2.5% (w/v) DSS (MP Biomedicals, Solon, OH, USA) in drinking water for 1 week. Then feed water for 2 weeks, and this process was repeated 3 times (Figure 1(B)). Mice were allowed to develop tumours for 26 weeks following AOM injection. Tumourigenicity was calculated based on the tumour number in the colon tissue.

All guidelines and experimental procedures in the entire animal trial were approved by the animal ethics committee of the Academy of National Food and Strategic Reserves Administration and were in agreement with the guidelines of experimental animal management of People's Republic of China (Documentation number 55, 2001, Ministry of Health of PR China).

2.3. Serum biochemistry profiles

At the 36 weeks, blood samples were taken from orbital venous by capillary tube under anesthesia after a 12-h overnight fasting, and centrifuged at 4,000 g for 10 min to obtain serum. Then all animals were sacrificed. Serum triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C) levels and lipase activity were determined using a spectrophotometry-based kit, following the manufacturer's instructions (Comin Biotechnology Co. Ltd, Suzhou, China).

2.4. mRNA expression analysis

Total colonic tissue RNA was extracted using the Trizol reagent (TransGen Biotech, Beijing, China) according to manufacturer's instructions. RNA was further purified using the RNeasy kit (Qiagen, Duesseldorf, Germany). The concentration of extracted RNA from each group was adjusted to 200 ng/μL based on the absorbance value at 260 nm. Five micrograms of RNA was reverse transcribed using the Easy Script first strand cDNA synthesis super mix (TransGen Biotech). Each round of amplification included 40 cycles, where a cycle consisted of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 120 s. The RT–PCR kit was used to detect mRNA levels of fatty acid synthase (*Fas),* acetyl Co-A carboxylase *(Acc1),* and *Gapdh* according to the manufacturer's instructions. Finally, the RT–PCR bands were scanned and the relative density value was calculated with respect to the *Gapdh* band density. The primer sequences used were as follows: for *Fas*, 5′-GATGATGAGTCGGAGCCTGG-3′ (forward) and 5′-CCTACCAGGTTGGCATGGTT-3′ (reverse); for *Acc1*, 5′-CTGTACAGCGCACCTGAGAG-3′ (forward) and 5′-CCAAGGCTGAAGACCTGAGTT-3′ (reverse); for *Gapdh*, 5′-GGACTCATGGTATGAGAGCTGG-3′ (forward) and 5′-GATGGCATGGACTGTGGTCT-3′ (reverse).

2.5. Western blot analysis

Colonic mucosal samples were used for protein analysis. Briefly, the mucosal samples were washed with serum-free media, followed by extraction of total and fractionated proteins using the cell lysis buffer (Cell Signaling Technology, Danvers, MA, USA). The lysates were centrifuged at 12,000 × *g* for 20 min at 4°C. Protein concentration was determined using the Bradford assay (Bio-Rad, Hercules, CA, USA), and equal amounts of proteins were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (Bio-Rad). After blocking, specific antibodies against perilipin, ADRP, tail-interacting protein of 47 kilo Daltons (TIP-47), peroxisome proliferator activated receptor gamma (PPAR-γ), SIRT1, FOXO3, and β-actin from ABclonal Biotechnology Co. Ltd (Wuhan, China) were used to probe the blots. Finally, each protein was detected using an enhanced chemiluminescence system (GE Healthcare, USA). The images were digitized (Chemidoc, Bio-Rad, Milan, Italy) and the area of each band was quantified using the computerised imaging system and the Quantity One software (Bio-Rad). Relative optical density (arbitrary units) was obtained after normalizing to the control groups and β-actin band intensities in each series. Each test was performed in triplicate.

2.6. Statistical analysis

The results were expressed as the means ± standard deviation (SD) of triplicate measurements using SPSS 18.0. The statistical significance of data was determined using one-way analysis of variance (ANOVA), followed by Dunnett's contrast, and P value < 0.05 was considered significant.

3. Results

3.1. Effect of genistein on body weight in mice

In RD group, body weight decreased by 11.19% at the time of sacrifice with AOM/DSS treatment ($p < 0.05$), and body weight decreased by 7.81%, 4.39% and 1.13% after treatment with 50, 150 and 450 mg/kg of genistein, respectively (Table 1). In HFD without

Table 1. Effects of genistein on body weight in mice.

Treatment	Group	Genistein (mg/kg diet)	Initial weight (g)	Final weight (g)	Growth rate/%
RD	A	0	39.00 ± 1.41	38.50 ± 1.89	−1.27
RD+	B	0	39.58 ± 2.29	35.15 ± 1.22$^{\#}$	−11.19*
AOM/DSS	C	50	39.70 ± 1.49	36.60 ± 0.99$^{\#}$	−7.81*
	D	150	39.95 ± 2.01	38.20 ± 1.46	−4.39*
	E	450	39.75 ± 2.59	39.31 ± 2.10	−1.13
HFD	F	0	48.94 ± 2.77	51.30 ± 2.62	4.8*
	G	50	47.42 ± 2.54	47.62 ± 1.00$^{¥}$	0.4
	H	150	48.28 ± 2.68	47.37 ± 2.09	−2.0*
	I	450	47.54 ± 1.99	45.32 ± 1.52$^{¥}$	−4.7*
HFD+	J	0	47.32 ± 2.61	41.90 ± 2.13	−11.45*
AOM/DSS	K	50	48.12 ± 2.31	43.82 ± 1.89	−8.98*
	L	150	48.67 ± 1.89	45.50 ± 1.98	−6.51*
	M	450	48.92 ± 2.87	50.16 ± 2.21$^{\$}$	2.41*

Results represent means ± SD ($n = 10$). RD: Regular diet; HFD: High-fat diet.
*$p < 0.05$ vs. growth rate of A group.
$^{\#}p < 0.05$ vs. final weight of A group.
$^{¥}p < 0.05$ vs. final weight of F group.
$^{\$}p < 0.05$ vs. final weight of J group.

Table 2. Effects of genistein on serum TC, TG, HDL-C, LDL-C levels and lipase activity of mice at the 36 weeks.

Treatment	Group	Genistein (mg/kg diet)	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	Lipase activity (U/ml)
RD	A	0	2.04 ± 0.19^{a}	0.63 ± 0.50^{a}	2.57 ± 0.53^{a}	0.32 ± 0.028^{a}	1.11 ± 0.037^{a}
RD+AOM/DSS	B	0	2.08 ± 0.13^{a}	0.69 ± 0.12^{b}	2.60 ± 0.08^{a}	0.31 ± 0.019^{a}	1.15 ± 0.031^{a}
	C	50	1.86 ± 0.16^{a}	0.66 ± 0.07^{ab}	2.48 ± 0.29^{a}	0.31 ± 0.02^{a}	1.18 ± 0.063^{a}
	D	150	1.83 ± 0.31^{a}	0.62 ± 0.09^{a}	2.69 ± 0.29^{a}	0.31 ± 0.024^{a}	1.21 ± 0.057^{a}
	E	450	1.80 ± 0.23^{a}	0.58 ± 0.10^{c}	3.16 ± 0.18^{b}	0.30 ± 0.018^{a}	1.23 ± 0.039^{b}
HFD	F	0	6.13 ± 0.99^{b}	1.35 ± 0.42^{d}	1.92 ± 0.12^{c}	0.91 ± 0.042^{b}	1.60 ± 0.019^{c}
	G	50	4.62 ± 0.41^{c}	0.95 ± 0.11^{d}	1.97 ± 0.15^{c}	0.90 ± 0.041^{b}	1.62 ± 0.047^{c}
	H	150	4.60 ± 0.34^{cd}	0.99 ± 0.15^{d}	2.22 ± 0.20^{ab}	0.89 ± 0.072^{c}	1.67 ± 0.028^{d}
	I	450	4.03 ± 0.23^{d}	0.93 ± 0.07^{e}	2.17 ± 0.18^{ab}	0.82 ± 0.062^{c}	1.66 ± 0.027^{d}
HFD+AOM/DSS	J	0	5.73 ± 1.28^{b}	1.42 ± 0.24^{d}	1.92 ± 0.08^{c}	0.89 ± 0.024^{c}	1.56 ± 0.05^{c}
	K	50	4.71 ± 0.32^{bc}	1.02 ± 0.19^{d}	2.03 ± 0.06^{c}	0.83 ± 0.056^{c}	1.60 ± 0.02^{c}
	L	150	4.53 ± 0.18^{cd}	0.99 ± 0.07^{de}	2.07 ± 0.06^{b}	0.82 ± 0.028^{c}	1.64 ± 0.036^{d}
	M	450	3.97 ± 0.41^{e}	0.89 ± 0.05^{e}	2.34 ± 0.05^{ab}	0.76 ± 0.054^{d}	1.70 ± 0.031^{d}

Results represent means ± SD ($n = 10$). Different superscripts indicated significant differences within a column ($p < 0.05$, $n = 10$). RD: Regular diet; HFD: High-fat diet.

AOM/DSS treatment group, body weight decreased by 2.0% and 4.7% at the time of sacrifice after treatment with 150 and 450 mg/kg genistein, respectively ($p < 0.05$). These results indicated that genistein exhibited antiobesity effect in mice. In HFD + AOM/DSS groups, body weight were decreased by 11.45%, 8.98% and 6.51% with 0, 50, and 150 mg/kg genistein, respectively, while increased by 2.41% with 450 mg/kg genistein treatment ($p < 0.05$). These results showed that AOM/DSS treatment decreased the body weight gain induced by HFD, while genistein can reverse this process to some extent.

3.2. Effect of genistein on serum lipid profiles and lipase activity in mice

Under RD + AOM/DSS treatments, TG level was decreased by 15.94% ($p < 0.05$), whereas HDL-C level and lipase activity were increased by 21.54% and 6.96% ($p < 0.05$), respectively, after administration of 450 mg/kg genistein (Table 2). Compared with RD control group, the levels of TC, TG and LDL-C, and lipase activity were increased by 200.49%, 114.29%, 184.38% and 44.14% ($p < 0.05$), respectively, whereas HDL-C level was decreased by 25.29% ($p < 0.05$) in HFD without AOM/DSS treatment group. Under HFD without AOM/DSS treatment, administration of 450 mg/kg genistein significantly decreased TC, TG, and LDL-C levels by 34.26%, 31.11% and 9.89% ($p < 0.05$), respectively, whereas HDL-C level and lipase activity were significantly increased by 13.02% and 3.75% ($p < 0.05$), respectively. Under HFD + AOM/DSS treatments, genistein decreased TC, TG, and LDL-C levels, and increased HDL-C level and lipase activity in a dose-dependent manner.

3.3. Effect of genistein on AOM/DSS-induced colon cancer in mice

In this study, AOM/DSS were used to induce colon cancer in mice. AOM/DSS induced 50% tumour incidence in mice with RD, which increased to 80% under HFD treatment (Figure 2). Genistein treatment decreased tumourigenicity of mice in RD and HFD groups in a dose-dependent manner.

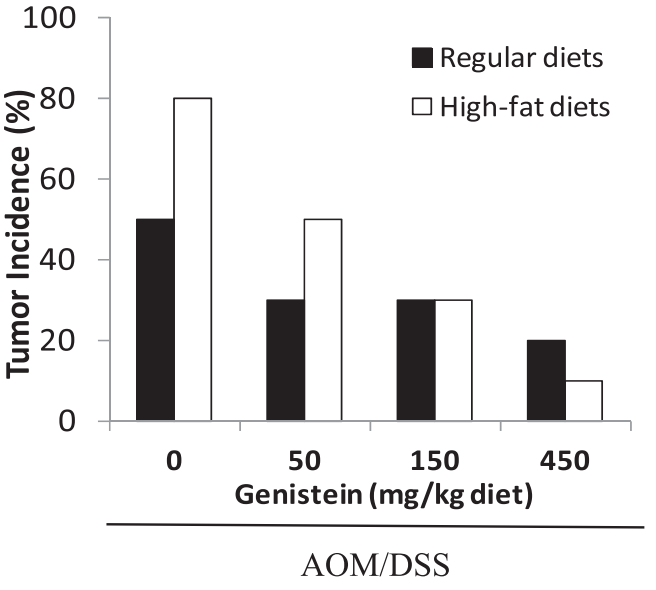

Figure 2. Genistein decreased the tumourigenicity of colon cancer with regular diet or high-fat diet treatments in mice ($n = 10$ per group).

3.4. Effect of genistein on the mRNA levels of fat metabolism-related genes in mice

Our data showed that there were 4.45, 5.01 and 5.60 times increase of *Fas* mRNA levels by RD + AOM/DSS, HFD, and HFD + AOM/DSS treatments ($p < 0.05$), respectively (Figure 3). The mRNA levels of *Acc1* were increased by 2.19, 1.95 and 4.06 times by RD + AOM/DSS, HFD, and HFD + AOM/DSS treatments ($p < 0.05$), respectively. Genistein treatments significantly reduced *Fas* and *Acc1* mRNA levels ($p < 0.05$) under RD + AOM/DSS, HFD, and HFD + AOM/DSS conditions in a dose-dependent manner. After administration of 450 mg/kg genistein, the decrease of *Fas* mRNA levels were 67.2%, 86.0% and 45.0% in RD + AOM/DSS, HFD, and HFD + AOM/DSS groups, respectively, and the decrease of *Acc1* mRNA levels were 36.1%, 64.6% and 51.1%, respectively.

3.5. Effect of genistein on the expression of LD-related proteins in mice

The PAT family proteins, including perilipin, ADRP, and TIP-47, are involved in intracellular LD formation and their metabolism. We observed that both AOM/DSS and HFD treatments significantly increased ADRP, TIP-47, and perilipin levels in colon tissue ($p < 0.05$) (Figure 4). The increase of ADRP expression were 1.41, 1.56 and 2.02 times by RD + AOM/DSS, HFD, and HFD + AOM/DSS treatments, respectively. The increase for TIP-47 expression were 2.51, 3.01 and 2.85 times by RD + AOM/DSS, HFD, and HFD + AOM/DSS treatments, respectively, and for perilipin were 1.78, 2.56 and 2.98 times, respectively. Genistein supplementation decreased the expression of PAT family proteins, especially at 450 mg/kg, under RD + AOM/DSS, HFD, and HFD + AOM/DSS treatments. After administration of 450 mg/kg genistein,

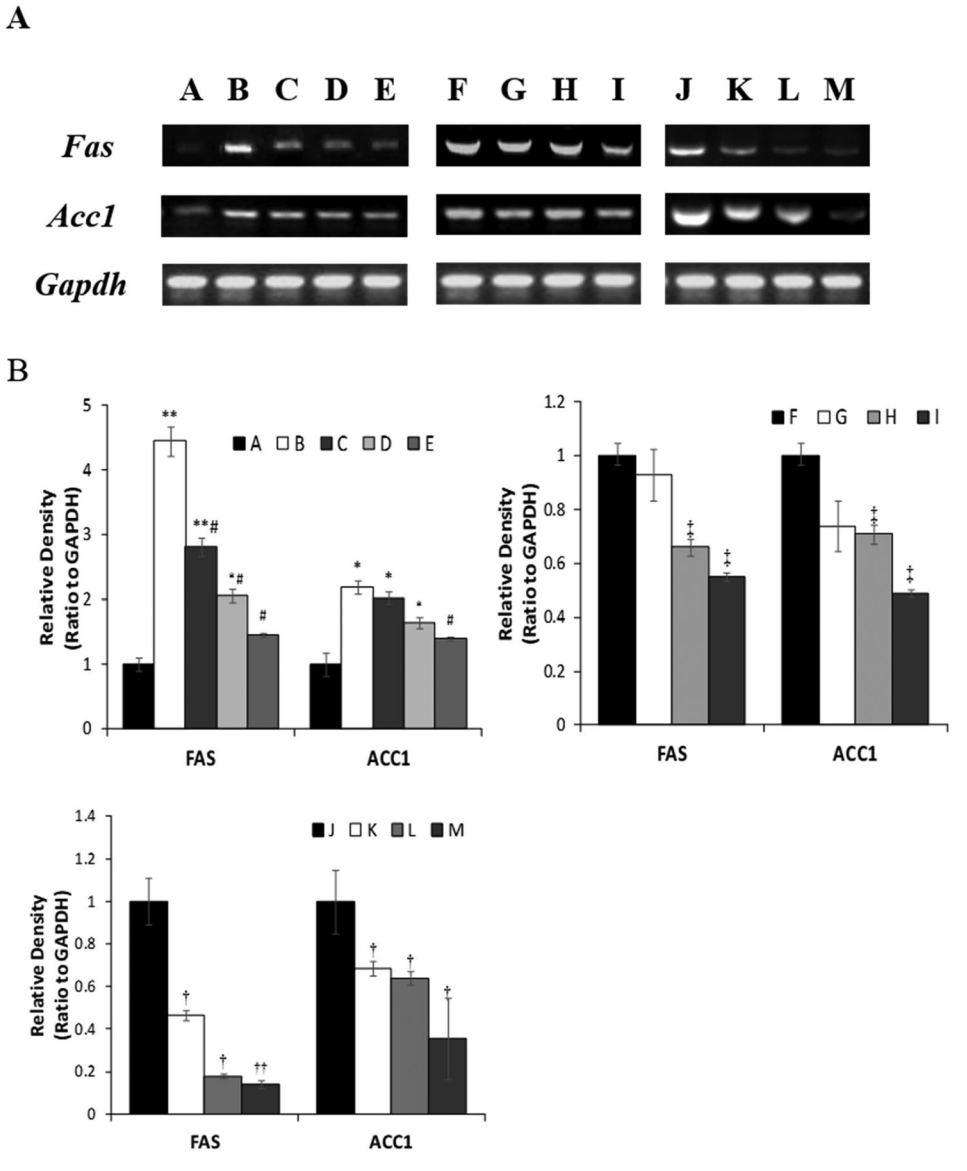

Figure 3. Effect of genistein on mRNA levels of fat metabolism-associated genes in mice. (A) *Fas* and *Acc1* mRNA levels in the mucosa of colon tissue were determined by RT-PCR. (B) Quantified mRNA levels were normalized to that of GAPDH. The data shown are representative of three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$ indicate significantly different from the RD control group; $^{\#}p < 0.05$ indicates significantly different from the RD + AOM/DSS group; $^{\ddagger}p < 0.05$ indicates significantly different from the HFD control group; $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$ indicate significantly different from the HFD + AOM/DSS group. A: Regular diet (RD control); B: Regular diet + AOM/DSS; C: Regular diet + AOM/DSS + 50 mg/kg genistein; D: Regular diet + AOM/DSS + 150 mg/kg genistein; E: Regular diet + AOM/DSS + 450 mg/kg genistein; F: High-fat diet (HFD control); G: High-fat diet + 50 mg/kg genistein; H: High-fat diet + 150 mg/kg genistein; I: High-fat diet + 450 mg/kg genistein; J: High-fat diet + AOM/DSS; K: High-fat diet + AOM/DSS + 50 mg/kg genistein; L: High-fat diet + AOM/DSS + 150 mg/kg genistein; M: High-fat diet + AOM/DSS + 450 mg/kg genistein.

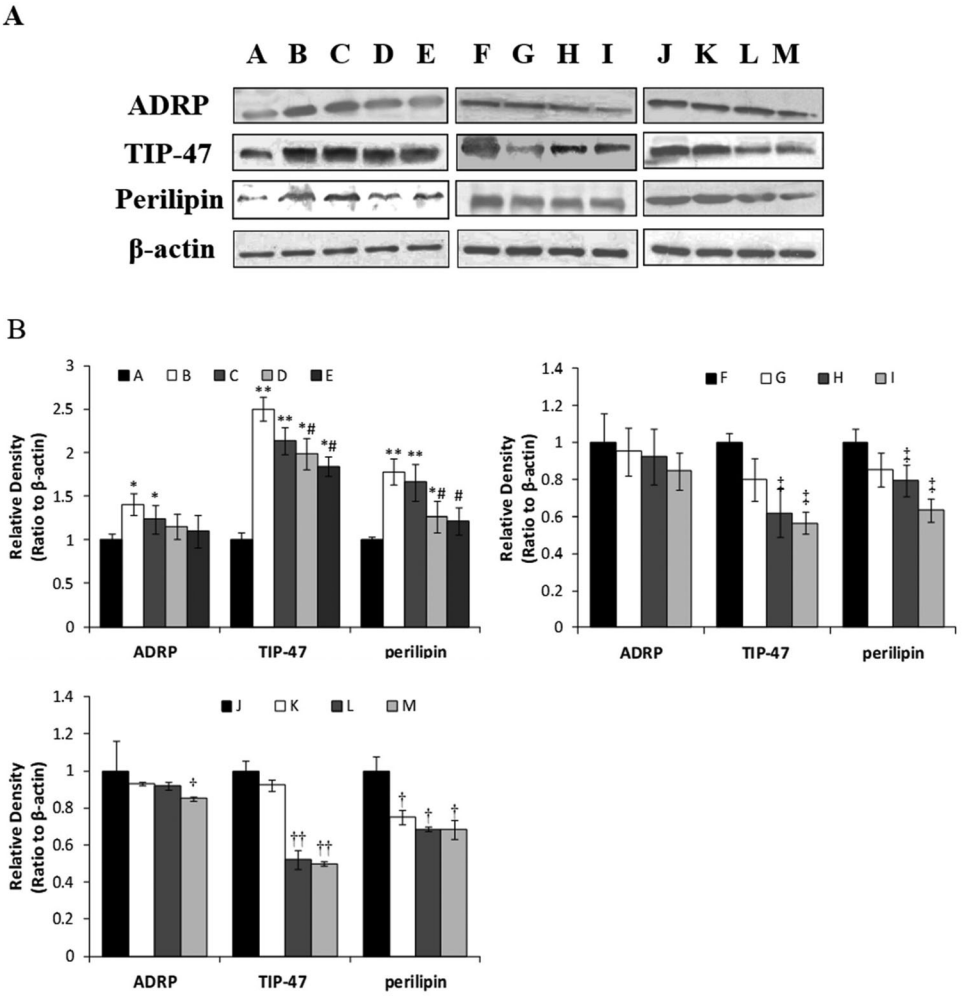

Figure 4. Effect of genistein on the expression of PAT family proteins in mice. (A) Western blot analysis of ADRP, TIP-47, and perilipin expression in the mucosa of colon tissue. (B) Band densities were quantified by densitometry analysis. Data are presented after normalization to β-actin level. The data shown are representative of three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$ indicate significantly different from the RD control group; $^{\#}p < 0.05$ indicates significantly different from the RD + AOM/DSS group; $^{\ddagger}p < 0.05$ indicates significantly different from the HFD control group; $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$ indicate significantly different from the HFD + AOM/DSS group. A: Regular diet (RD control); B: Regular diet + AOM/DSS; C: Regular diet + AOM/DSS + 50 mg/kg genistein; D: Regular diet + AOM/DSS + 150 mg/kg genistein; E: Regular diet + AOM/DSS + 450 mg/kg genistein; F: High-fat diet (HFD control); G: High-fat diet + 50 mg/kg genistein; H: High-fat diet + 150 mg/kg genistein; I: High-fat diet + 450 mg/kg genistein; J: High-fat diet + AOM/DSS; K: High-fat diet + AOM/DSS + 50 mg/kg genistein; L: High-fat diet + AOM/DSS + 150 mg/kg genistein; M: High-fat diet + AOM/DSS + 450 mg/kg genistein.

the decrease of ADRP expression were 22.1%, 15.4% and 31.8% in RD + AOM/DSS, HFD, and HFD + AOM/DSS groups, respectively, the decrease of TIP-47 expression were 26.7%, 43.3% and 50.0%, respectively, and the decrease of perilipin expression were 31.4%, 36.4% and 31.8%, respectively.

3.6. Effect of genistein on the expression of PPAR-γ, SIRT1, and FOXO3 in mice

We observed significant increase in PPAR-γ expression by 3.14, 2.18 and 4.12 times in RD + AOM/DSS, HFD, and HFD + AOM/DSS treatments, respectively, and decrease in FOXO3 expression by 43.6% and 42.1% in RD + AOM/DSS and HFD + AOM/DSS treatments ($p < 0.05$), respectively (Figure 5). After administration of 450 mg/kg genistein, the

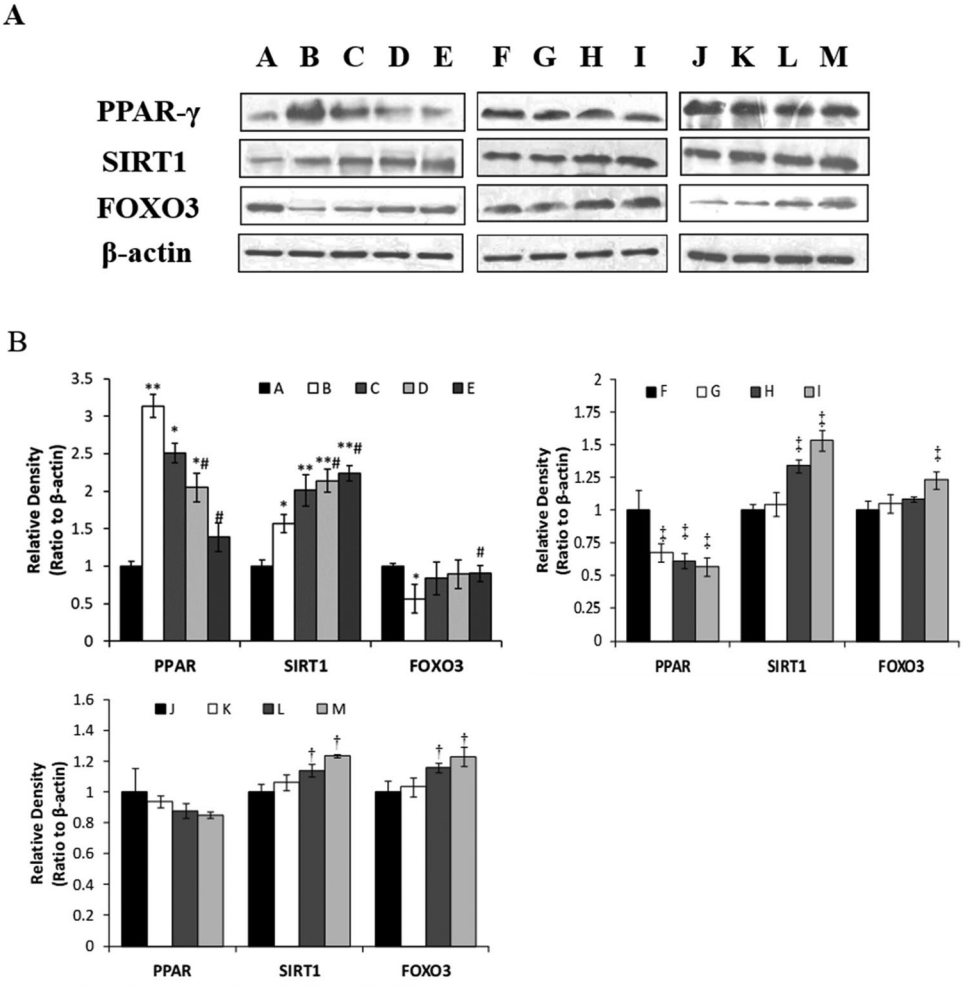

Figure 5. Effect of genistein on the expression of PPAR-γ, SIRT1 and FOXO3 levels in mice. (A) Western blot analysis was performed to determine PPAR-γ, SIRT1, and FOXO3 expression in mucosa of colon tissue. (B) Band densities were quantified by densitometry analysis. Data are presented after normalization to β-actin level and are representative of three independent experiments. *$p < 0.05$, **$p < 0.01$ indicate significantly different from the RD control group; $^{\#}p < 0.05$ indicates significantly different from the RD + AOM/DSS group; $^{\ddagger}p < 0.05$ indicates significantly different from the HFD control group; $^{\dagger}p < 0.05$ indicates significantly different from the HFD + AOM/DSS group. A: Regular diet (RD control); B: Regular diet + AOM/DSS; C: Regular diet + AOM/DSS + 50 mg/kg genistein; D: Regular diet + AOM/DSS + 150 mg/kg genistein; E: Regular diet + AOM/DSS + 450 mg/kg genistein; F: High-fat diet (HFD control); G: High-fat diet + 50 mg/kg genistein; H: High-fat diet + 150 mg/kg genistein; I: High-fat diet + 450 mg/kg genistein; J: High-fat diet + AOM/DSS; K: High-fat diet + AOM/DSS + 50 mg/kg genistein; L: High-fat diet + AOM/DSS + 150 mg/kg genistein; M: High-fat diet + AOM/DSS + 450 mg/kg genistein.

increase of FOXO3 were 29.5%, 23.2% and 23.3% in RD + AOM/DSS, HFD, and HFD + AOM/DSS groups, respectively, and the decrease of PPAR-γ were 55.8%, 43.2% and 14.9%, respectively. Furthermore, SIRT1 levels were significantly increased ($p < 0.05$) under RD + AOM/DSS, HFD, and HFD + AOM/DSS treatments after administration of 150 and 450 mg/kg genistein.

4. Discussion

Genistein possesses anticancer properties, and its mechanism of action has been the subject of considerable interest. In this study, we investigated whether and how genistein supplementation inhibited AOM/DSS-induced colon cancer in HFD-fed female mice. Our results showed that a genistein-supplemented diet alleviated HFD-induced obesity in mice. This is consistent with a previous study that genistein could reduce in the serum levels of TC, TG, and LDL-C and increase in HDL-C level (Zhang et al., 2018). Lipase is involved in LD degradation in mammalian cells, which inhibits obesity (Smirnova et al., 2006). We observed that genistein significantly increased lipase activity under RD + AOM/DSS, HFD, and HFD + AOM/DSS treatments. Genistein could inhibit cell proliferation via PI3K/AKT pathway and target FOXO3 in several cancers (Chan et al., 2018; de la Parra et al., 2016). In our previous study, genistein prevented the colonic neoplasms growth by modulating the PI3 K/FOXO3/AKT signaling pathway in mice and HT-29 cell (Song et al., 2018; Qi et al., 2011). LD was regarded as storage depots by which colon cancer cells support their high fuel demands during tumor progression (Liu, Luo, Halim, & Song, 2017; Liang et al., 2018). These results together are consistent with the idea that genistein promotes fat degradation, which decreases the accumulation of LD to inhibit cell proliferation in AOM/DSS-induced colon cancer in mice.

Obesity is characterized by enhanced accumulation of LDs in adipose tissue, liver, and skeletal muscle (Liang et al., 2018; Rinnankoski-Tuikka et al., 2014; Wang et al., 2018). LDs harbour different members of the PAT family proteins on their surfaces, which are composed of perilipin, ADRP, TIP-47, S3-12, and OXPAT (Bickel, Tansey, & Welte, 2009). We observed that genistein significantly decreased the AOM/DSS and HFD-induced expression of ADRP, TIP-47, and perilipin. *Fas* plays an important role in fatty acid biosynthesis (Baenke, Peck, Miess, & Schulze, 2013). Increased *Fas* expression provides a survival advantage to colorectal cancer cells by upregulating cellular respiration, whereas *Fas* knockdown inhibits cancer cell growth and migration (Li et al., 2017). ACC, including ACC1 and ACC2, generates malonyl-CoA, a substrate for fatty acid biosynthesis (Tong, 2005). ACC1 is present in the cytoplasm of all cells, but it is enriched in lipogenic tissues, which is important site of fatty acid synthesis. Studies suggest that tumour cells obtain most of their fatty acids by *de novo* synthesis, which depends on increased expression of fatty acid biosynthetic enzymes for producing fatty acids in large quantities (Mohammadzadeh et al., 2014). In this study, genistein reduced *Fas* and *Acc1* mRNA levels under RD + AOM/DSS, HFD, and HFD + AOM/DSS treatments. Taken together, our results indicated that genistein induced fat degradation by regulating the transcription and translation of genes involved in fatty acid biosynthesis and consequent inhibition of colon tumor progression.

PPAR-γ is involved in the regulation of adipogenesis, energy balance, and lipid biosynthesis (Grygiel-Gorniak, 2014; Liu et al., 2019). Upregulation of LD-associated proteins

such as members of the PLIN family, CIDEC, CIDEA, HILPDA, FITM1, FITM2, and G0S2 by PPAR-γ has been considered as a mechanistic link between lipid uptake and regulation of lipid storage capacity (Montserrat & Kersten, 2017). Mice with dyslipidemia have high PPAR-γ level, whereas PPAR-γ inhibition can prevent HFD-induced hyperlipidemia and obesity (Wang et al. 2017; Wang, Shi, Joyce, Wang, & Feng, 2017). SIRT1, a protein deacetylase, represses PPAR-γ and inhibits fatty acid biosynthesis (Picard et al., 2004). In this study, genistein-rich diets decreased PPAR-γ expression and increased SIRT1 expression in mice. Thus, these results partially validate the observation that genistein reduces PPAR-γ level by upregulating SIRT1 and confirms the anti-LD activity of genistein. This study showed that HFD aggravated AOM/DSS-induced colon cancer compared with RD and it became harder for genistein to reverse the harmful effects on colon cancer with HFD treatments. For example, the decrease of PPAR-γ were not significant by genistein in HFD + AOM/DSS groups, it can be considered that the dual influences of HFD and AOM/DSS eliminated the PPAR-γ reduction by genistein.

SIRT1 is also known to play key roles in adaptive responses of cells to various oxidative stressors by deacetylating and activating several forkhead-type transcription factors (FOXO), including FOXO3 (Boskovic et al., 2019; Das, Mitrovsky, Vasanthi, & Das, 2014). FOXO3 regulates the expression of specific target genes involved in control of cell cycle progression, mitosis, or induction of apoptosis (Coomans de Brachène & Demoulin, 2016). In human colon cancer cells, FOXO3 negatively regulates proliferation during tumourigenesis, and loss of FOXO3 activity promotes tumour growth (Liu et al., 2018; Qi et al., 2011). Our results showed significant upregulation of SIRT1 and FOXO3 by genistein in both obesity and colon cancer conditions. We previously observed

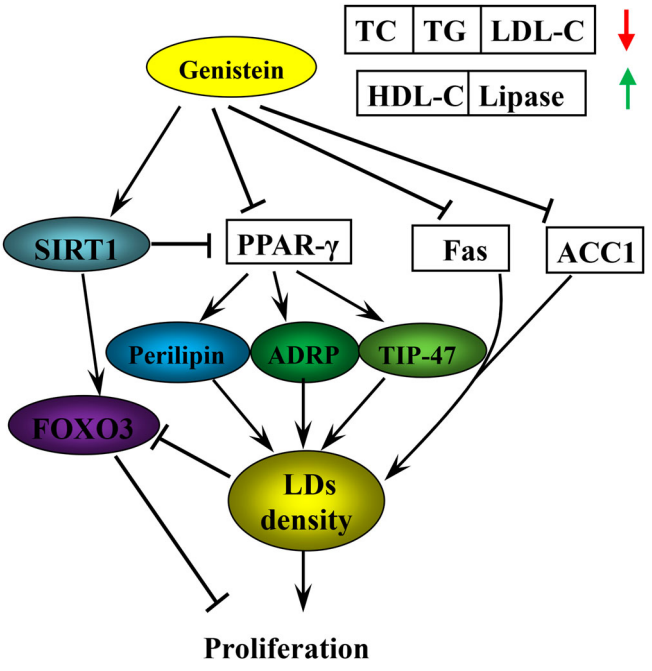

Figure 6. Mechanism is potentially involved in genistein-mediated prevention of colon cancer by regulating lipid droplet accumulation and the SIRT1/FOXO3a pathway in high-fat diet-fed mice.

that stimulation of LD density in human colon cancer cells led to a PI3K-dependent loss of FOXO3 (Qi et al., 2013). Genistein had been found induce apoptosis of colon cancer cells by inhibiting the accumulation of LDs (Liang et al., 2018). This indicated that genistein regulated the SIRT1/FOXO3a signalling pathway to exerting negative effects on LD accumulation, which inhibited colon cancer (Figure 6).

5. Conclusion

Our results demonstrated that genistein supplementation prevented development of obesity and ameliorated dyslipidemia caused by HFD in female mice. These effects were mediated via suppression of genes encoding LD- and fatty acid biosynthesis-associated proteins, which finally inhibited progression of AOM/DSS-induced colon cancer. These findings provided new evidence for mechanisms of genistein preventing colon cancer.

Disclosure statement

No potential conflict of interest was reported by the authors.

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