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Favourable effects of grape seed extract on intestinal epithelial differentiation and barrier function in IL10-deficient mice

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Abstract

The impairment in the rate of cell proliferation and differentiation leads to a negative consequence on the renewal of the intestinal epithelium, which is the aetiological factor of a number of digestive diseases. Grape seed extract (GSE), a rich source of proanthocyanidins, is known for its beneficial health effects. The present study evaluated the beneficial effects of GSE on colonic cell differentiation and barrier function in IL10-deficient mice. Female mice aged 6 weeks were randomised into two groups and given drinking-water containing 0 or 0·1 % GSE (w/v) for 12 weeks. GSE supplementation decreased serum TNF-α level and intestinal permeability, and increased the colonic goblet cell density that was associated with increased mRNA expression of mucin (*Muc*)-2. Immunohistochemical analyses showed lower accumulation of β-catenin in the crypts of colon tissues of the GSE-supplemented mice, which was associated with a decreased mRNA expression of two downstream effectors of Wingless and Int (Wnt)/catenin signalling, myelocytomatosis oncogene protein (*Myc*) and cyclin D1 (*Ccnd1*). Consistently, GSE supplementation decreased the number of colonic proliferating cell nuclear antigen-positive cells, a well-known cell proliferation marker, and a weakened extracellular signal-regulated kinases 1 and 2 (ERK1/2) signalling. In summary, these data indicate that supplementation of 0·1 % GSE for 12 weeks improved gut barrier function and colonic cell differentiation in the IL10-deficient mice probably via inhibiting Wnt/β-catenin pathway.

Key words: Grape seed extract: Polyphenol: Gut epithelium barrier: Catenin: IL10

The gut epithelial integrity and barrier function has been a central predisposing factor in the pathogenesis of inflammatory bowel disease (IBD) and its related gastrointestinal diseases, food allergies, type 1 diabetes and other autoimmune diseases[1–6]. Gut epithelium is constantly renewing, which extensively involves epithelial cell proliferation and differentiation. Disruption of this process impairs epithelial barrier function, which underlies many digestive-related diseases. In IBD, such as Crohn's disease and ulcerative colitis, the gastrointestinal tract has been found to be damaged due to extensive mucosal hyperplasia, inflammatory reactions and abnormal expression of MHC class II molecules in the intestinal epithelial cells[4].

Intestinal cell proliferation and differentiation are regulated by various signalling pathways and mediators[7]. Bone morphogenetic protein 2 (Bmp2) functions to promote the overall epithelial differentiation; hairy and enhancer of split 1 (Hes1) regulates the differentiation of enterocytes; atonal homolog 1 (Atoh1) is essential for the formation of secretory cells; and Krueppel-like factor 4 (Klf4) is one of the factors important for the development of goblet cells[7]. Growth arrest and DNA damage (GADD45) inducible protein levels are elevated under stresses in order to arrest the cell cycle and promote cell survival[8], and proliferating cell nuclear antigen (PCNA) is a commonly used marker of cell proliferation[9]. In addition to the above-mentioned pathways and mediators, the Wingless and Int (Wnt) signalling is one of the most fundamental mechanisms driving the proliferation of intestinal epithelial cells, and alters cell fate commitment and tissue homeostasis. The Wnt/β-catenin signalling regulates cell proliferation through mediating the transcription of many target genes including cyclin D1 (*Ccnd1*) and myelocytomatosis oncogene protein (*Myc*)[10,11]. Dysregulation of the Wnt/β-catenin pathway has been implicated in IBD-associated carcinogenesis as well as colorectal cancer[12,13]. *Ccnd1* expression was up-regulated in active ulcerative colitis- and ulcerative colitis- related

Abbreviations: AMPK, AMP-activated protein kinase; DPPH, 2, 2-diphenyl-1-picrylhydrazyl; ERK1/2, extracellular signal-regulated kinases 1 and 2; GADD45, growth arrest and DNA damage; GSE, grape seed extract; IBD, inflammatory bowel disease; PCNA, proliferating cell nuclear antigen; phos, phosphorylated; Wnt, Wingless and Int.

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carcinogenesis[14]. The aminosalicylate mesalazine drug exerts chemopreventive effects to IBD through inhibition of the Wnt/β-catenin signalling[15]. Polyphenols down-regulate the Wnt/β-catenin signalling in colonic cancer cells[16,17]; however, relatively less is known about their effects on the proliferation of gut epithelial cells *in vivo*.

Grape seed extract (GSE) is a heterogeneous mixture of polyphenols (anthocyanidins, catechins and their derivatives) extracted from grape seed and skin. A body of evidence has shown that GSE has anti-inflammatory, antioxidant, cardio-protective, hepatoprotective, neuroprotective, anti-diabetic, anti-carcinogenic and anti-ageing effects[18–26]. Our previous study using 1% (w/w) GSE supplementation attenuated intestinal inflammation and ameliorated the colitis symptom in IL10-deficient mice[27]. In a different study, 0·5% (w/w) GSE supplementation has been shown to reduce the cell proliferation rate in the small intestine of the APC knockout mice[28]. In addition, 0·1% GSE supplementation has been shown to increase the intestinal occludin content and reduce the faecal neutrophil protein calprotectin level in mice fed with a high-fat diet[29]. IL10-deficient mice develop spontaneous enterocolitis similar to human Crohn's disease[30], and have been widely used to study the mucosal structure damage and barrier function impairment[31,32]. Using this mouse model, the objective of the present study was to test whether a lower dose (0·1% GSE) compared with that of our previous study (1% GSE) has beneficial effects, and to further explore the mechanistic changes linking GSE to improved gut epithelial function.

Materials and methods

Grape seed extract

The GSE product (Gravinol-S™) was purchased from OptiPure Chemco Industries, Inc. As per the company product specification sheet, the GSE product contains a minimum of 95% flavonols, of which 82% are oligomeric proanthocyanidins and 12% being the highly active monomeric proanthocyanidins.

Animal care and experimental design

All animal procedures were approved by the Washington State University Animal Care and Use Committee. The homozygous IL10-deficient mice (B6.129P2-*Il10*^{tm1Cgn}/J) were purchased from The Jackson Laboratory and were bred in the Experimental Animal Laboratory Unit at the Washington State University. Mice were housed in a temperature-controlled room with a 12 h light–12 h dark cycle, and had free access to food (2018 Teklad Global 18% Protein Rodent Diet) and drinking-water. Female mice aged 6 weeks were randomised into two groups (n 10 per group) and were given drinking-water containing 0 or 0·1% GSE (w/v) for 12 weeks[29]. Wild-type mice were not included in this study, because our previous study has shown that GSE supplementation has little effect on the wild-type mice[27]. To avoid potential confounding effects of sex, only female mice were used in the present study. Water was changed on a daily basis to avoid the possible oxidation of functional compounds in GSE. During the feeding trial, mice were housed individually for the measurement of feed intake

Table 1. Primer sequences used in the present study

Gene name	Accession no.	Product size	Direction	Sequence (5′–3′)	Source
Atoh1	NM_007500.4	108 bp	Forward	GTGCGATCTCCGAGTGAGAG	The present study
			Reverse	GGGATAAGCCCCGAACAACA	
Bmp2	NM_007553.3	83 bp	Forward	TGCTTCTTAGACGGACTGCG	The present study
			Reverse	CTGGGGAAGCAGCAACACTA	
Myc	NM_001177353.1	190 bp	Forward	ATGATGATGTTCTTGATGAAGGTCTC	The present study
			Reverse	GGCTCTGCTCTCCATCCTATGTT	
Ccnd1	NM_007631.2	166 bp	Forward	TCAAGTGCGTGCAGAAGGAGATT	The present study
			Reverse	TGCAGGCGGCTCTTCTTCAAG	
Gadd45a	NM_007836.1	130 bp	Forward	CTGCAGAGCAGAAGACCGAA	The present study
			Reverse	GGGTCTACGTTGAGCAGCTT	
Gadd45b	NM_008655.1	80 bp	Forward	TGTGCATAAGTCAGCGGAGG	The present study
			Reverse	ATGTGCTGTAGCTGCGAAGT	
Gadd45g	NM_011817.2	137 bp	Forward	CCCTCCGCACTCTTTTGGAT	The present study
			Reverse	CAGCAGAAGTTCGTGCAGTG	
Hes1	NM_008235.2	86 bp	Forward	CAACACGACACCGGACAAAC	The present study
			Reverse	TTCTTGCCCTTCGCCTCTTC	
Klf4	NM_010637.3	75 bp	Forward	CAGGATTCCATCCCCATCCG	The present study
			Reverse	GAGAGGGGACTTGTGACTGC	
Muc1	NM_001018017.2	108 bp	Forward	CAGCCAGCGCCTGCCTGAAT	The present study
			Reverse	GCACTGTGAGGAGCAGCAGCA	
Muc2	M94132.1	138 bp	Forward	CCTGCCGACACCTGCTGCAA	The present study
			Reverse	ACACCAGTAGAAGGGACAGCACCT	
Muc3	AB038784.1	130 bp	Forward	CTCGTGTTGCCATTGCCTCTCTCG	The present study
			Reverse	CTGCAGGTTGCCTCCAGGTTCAGA	
Pcna	NM_011045.2	130 bp	Forward	TGGTAGTTGTCGCTGTAGGC	The present study
			Reverse	ATCAGGCGTGCCTCAAACAT	
Tubb2a	NM_009450.2	146 bp	Forward	TCTACAACCAGCACCATGCGCG	The present study
			Reverse	AGCTGCAAGTCACTGTCGCCA	

Atoh1, atonal homolog 1; *Bmp2*, bone morphogenetic protein 2; *Myc*, myelocytomatosis oncogene protein; *Ccnd1*, cyclin D1; *Gadd45*, growth arrest and DNA damage; *Hes1*, hairy and enhancer of split 1; *Klf4*, Krueppel-like factor 4; *Muc*, mucin; *Pcna*, proliferating cell nuclear antigen; *Tubb2a*, β-tubulin.

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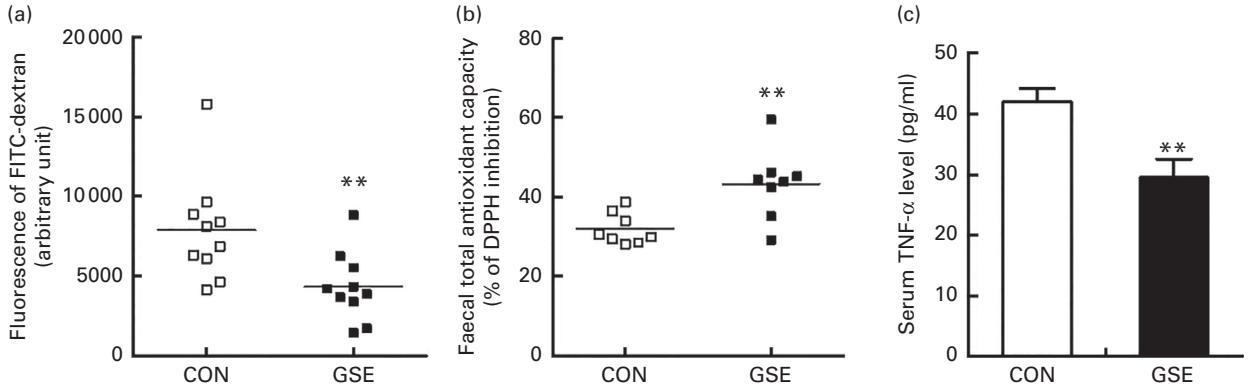

Fig. 1. Intestinal permeability, faecal total antioxidant capacity and serum TNF-α level in mice fed a control (CON) or grape seed extract (GSE) supplemented drinking-water. (a) *In vivo* intestinal permeability measured with permeability tracer fluorescein isothiocyanate (FITC)-labelled dextran; (b) faecal total antioxidant capacity; (c) serum TNF-α level. Values are means (n 8–10), with individual values (a and b), or with standard errors represented by vertical bars (c). **Mean value was significantly different from that for CON group ($P<0·01$).

and water consumption. There was no difference in the amount of water drunk per mouse between treatment groups (3·5–4 ml/mouse per d). This equates to 140–160 mg/kg per d for an adult mouse (approximately 25 g), which converts to 680–780 mg of polyphenols for a 60 kg human as per the published formula[33]. This indicates that the GSE concentration used in the present study was also the equivalent of the consumption of 180–205 ml of red wine (3·8 g/l total polyphenols)[34] per d for a 60 kg adult.

In vivo *intestinal permeability*

At the 10th week of the feeding trial, *in vivo* intestinal permeability was measured as per the published method[35]. Briefly, mice that had been fasted for 6 h were administered with fluorescein isothiocyanate-dextran (500 mg/kg body weight; Sigma-Aldrich). After 1 h of administration, blood sample was collected from the orbital sinus and centrifuged at 12 000 g for 5 min at 4°C. The resulting serum was diluted 1:3 with PBS (pH 7·4), and the fluorescence intensity was measured using the Synergy H1 Hybrid Multi-Mode Microplate Reader (BioTek Instruments) at an excitation wavelength of 485 nm and an emission wavelength of 535 nm.

Faecal total antioxidant capacity assay and serum TNF-α measurement

Faecal samples from the 11th week of the feeding trial were powdered in liquid N_2 and then homogenised in 80 % methanol solution. Scavenging activities of the faecal

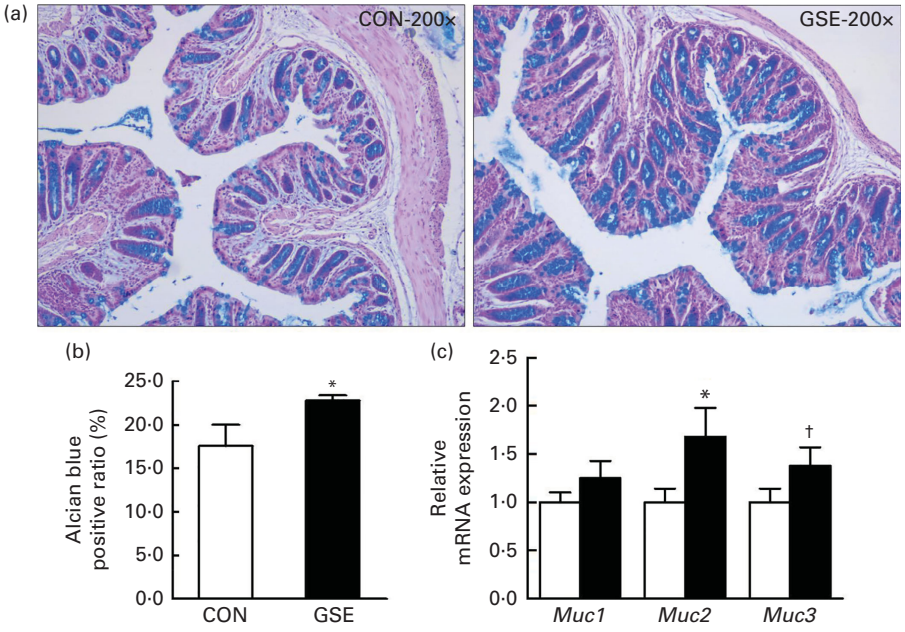

Fig. 2. Goblet cell density, mucin (*Muc*) and differentiation transcription factors mRNA expression in the colon of mice fed a control (CON; □) or grape seed extract (GSE; ■) supplemented drinking-water. (a) Representative images of Alcian blue staining of goblet cells, original magnification at 200 × ; (b) quantification of Alcian blue staining; (c) mRNA expression of *Muc1*, *Muc2* and *Muc3*. Values are means (n 8), with their standard errors represented by vertical bars. *Mean value was significantly different from that for CON group ($P<0·05$). †Mean value was marginally significantly different from that for CON group ($P<0·1$). A colour version of this figure can be found online at http://www.journals.cambridge.org/bjn

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homogenate against the stable free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH; Sigma-Aldrich) were assayed according to the published methods[36,37] with modification. Briefly, 50 μl of diluted faecal samples or 80 % (v/v) methanol (Blank) were mixed with 150 μl of 60 μM-DPPH solution. The absorbance at 517 nm was monitored during a 2 h incubation period using the Synergy H1 Hybrid Microplate Reader (BioTek Instruments). All experiments were performed in triplicate, and the percentage of decrease in DPPH reading was calculated using the following equation:

$$\%\ \text{of inhibition} = (1 - \text{OD}_{\text{sample}}/\text{OD}_{\text{blank}}) \times 100,$$

where $\text{OD}_{\text{sample}}$ and OD_{blank} indicate the absorbance of faecal sample and methanol blank at a wavelength of 517 nm, respectively.

The serum collected at necropsy (at the end of the 12th week of the feeding trial) was used for analysing the level of TNF-α using the mouse TNF-α instant ELISA kit (eBiosciences) according to the manufacturer's protocol.

Tissue collection

On the day of necropsy, mice were anaesthetised intraperitoneally with tribromoethanol (250 mg/kg body weight), followed by cervical dislocation. The colon section was dissected. A 5 mm segment of colon was fixed in freshly prepared 4 % (w/v) paraformaldehyde (pH 7·0), processed and embedded in paraffin. The remaining colon tissue was cut opened longitudinally, rinsed in PBS, frozen in liquid N_2 and stored at −80°C for further biochemical analyses.

Histological examination

For pathobiological examination, paraffin-embedded colonic gut tissues were sectioned at 5 μm thickness and subjected to haematoxylin–eosin staining. Each colonic section was scored in a blinded fashion following a method published previously[38], with some modifications. Briefly, the severity of epithelial hyperplasia (scored from 0 to 3), the intensity and severity of inflammation (scored as 0–4), and the extent of mucosal hyperplasia (scored as 0–4) and extent of inflammation (scored as 0–4) were scored individually. The summation of

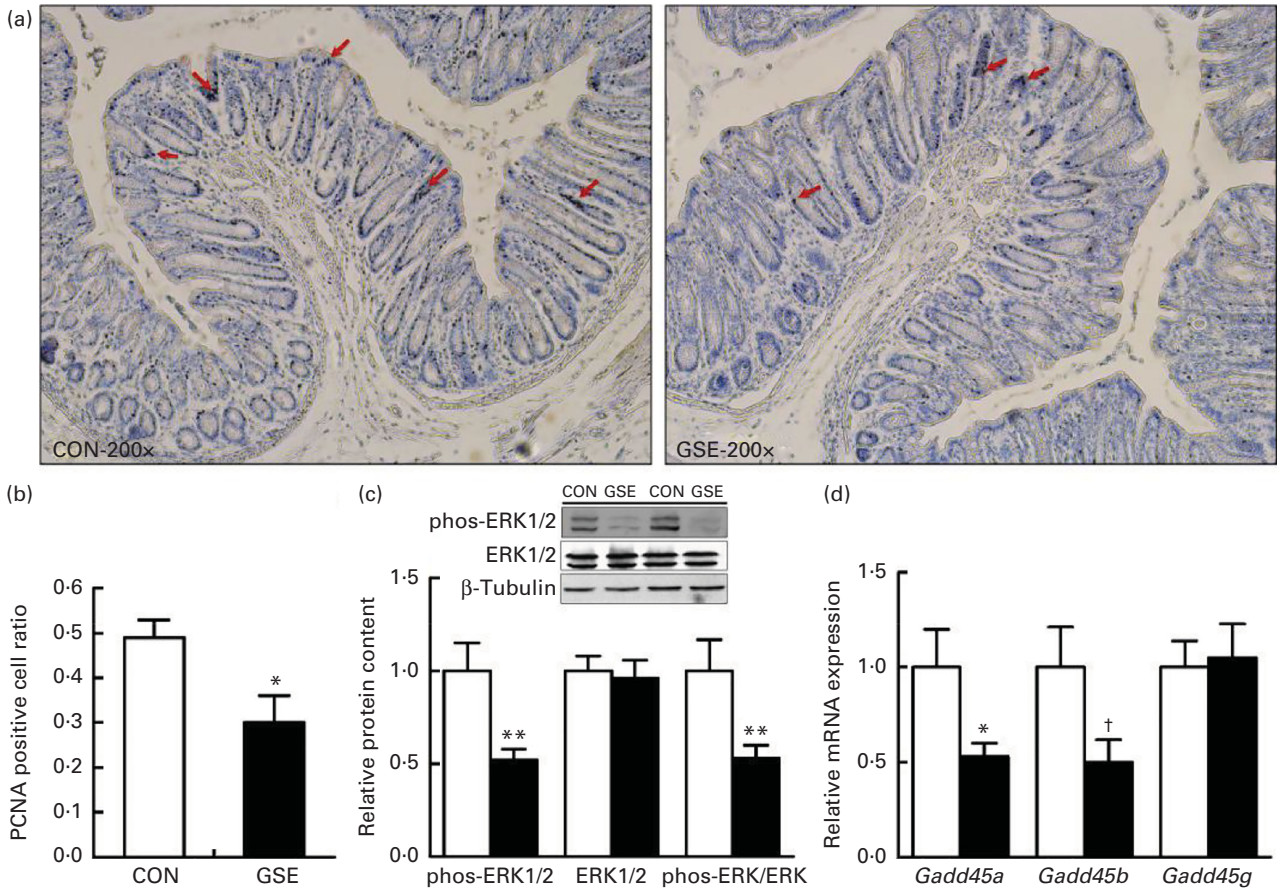

Fig. 3. Colonic cell proliferation in the colon of mice fed a control (CON; □) or grape seed extract (GSE; ■) supplemented drinking-water. (a) Representative images of proliferating cell nuclear antigen (PCNA) staining, original magnification at 200 ×, dark brown dots indicate PCNA staining signal as pointed by red arrows; (b) ratio of PCNA-positive cells; (c) mitogen-activated protein kinases/extracellular signal-regulated kinases 1 and 2 (ERK1/2) immunoblotting (top: representative western blot images; bottom: quantification data); (d) growth arrest and DNA damage (*Gadd45*) mRNA expression. Values are means (n 8), with their standard errors represented by vertical bars. Mean value was significantly different from that for CON group: *$P<0·05$, **$P<0·01$. †Mean value was marginally significantly different from that for CON group ($P<0·1$). A colour version of this figure can be found online at http://www.journals.cambridge.org/bjn

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these scores provided a total colonic inflammatory score ranging from 0 to a maximum of 15 per colonic section, with 0 being a normal tissue and 15 being the most extensive/severe disease symptoms.

For goblet cell staining, paraffin-embedded colonic tissues were sectioned at 5 μm thickness, deparaffinised and hydrated, and then stained with Alcian blue (pH 2·5)[39], which stains goblet cells blue. Colonic tissue sections (five fields per section, 3–4 sections per animal, *n* 8) were randomly selected for the quantification of goblet cell:total area ratio using the Image J 1.30v software (split colour channels; National Institute of Health).

Immunohistochemical staining

Immunohistochemical staining was conducted as described previously[9,40]. Briefly, colonic tissue sections were deparaffinised and hydrated, followed by antigen retrieval before incubation with anti-PCNA (Santa Cruz Biotech, Inc.), anti-β-catenin (Cell Signaling Technology) or anti-phosphorylated (phos)-β-catenin^{Ser552} (Cell Signaling Technology) antibody. Signal was visualised using the Vectastain ABC and DAB kits (Vector Laboratories). After counterstaining with haematoxylin, images were taken using the Leica DM2000 LED light microscope (200×; Leica Microsystems, Inc.). The cell proliferation index was expressed as the PCNA-positive cells/total cells in the crypts. Phos-β-catenin^{Ser552} staining was expressed as the phos-β-catenin^{Ser552} positive crypts/100 crypts. β-Catenin staining intensity was scored semi-quantitatively from 0 (normal tissue) to 5 (intensive staining) by a trained examiner in a blinded manner, four sections per animal, eight mice per dietary group.

Quantitative RT-PCR analysis

Total RNA was extracted from powdered large intestine tissues using the TRIzol® Reagent (Sigma-Aldrich), treated with DNase I (Qiagen) and purified with RNeasy Mini kit (Qiagen). Complementary DNA was synthesised with the iScript™ complementary DNA synthesis kit (Bio-Rad). SYBR Green Master Mix (Bio-Rad) was used in all PCR. The amplification efficiency was 0·90–0·99. The quantitative RT-PCR conditions were 95°C, 3 min; 35 cycles of 95°C for 10 s, 56°C for 10 s and 72°C for 20 s. At the end of each run, dissociation melt curves were obtained to confirm the purity of PCR products. The PCR products were further confirmed by electrophoresis and sequencing. β-Tubulin (*Tubb2a*) was used as the housekeeping gene. The primer sequences are listed in Table 1.

Immunoblotting analyses

Immunoblotting analyses were conducted as described previously[40]. The band density of target protein was

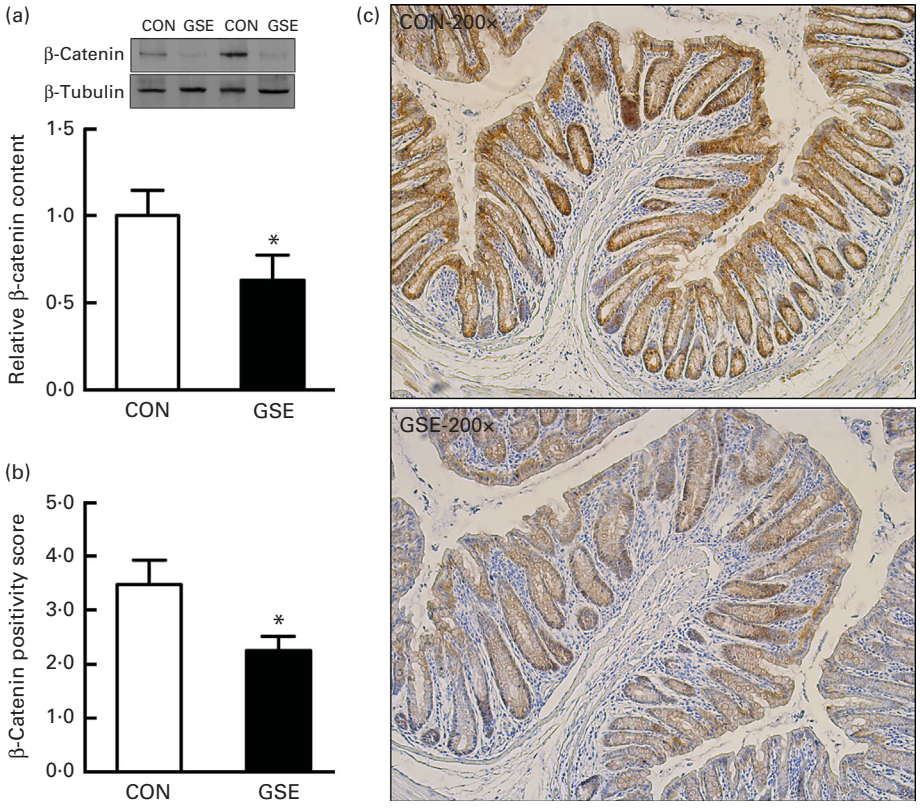

Fig. 4. Wingless and Int (Wnt)/β-catenin signalling in the colon of mice fed a control (CON) or grape seed extract (GSE) supplemented drinking-water. (a) Immunoblotting of β-catenin; (b) β-catenin immunohistochemical staining; (c) representative image of β-catenin staining, original magnification at 200×. Values are means with their standard errors represented by vertical bars (*n* 8). *Mean value was significantly different from that for CON group ($P<0·05$). A colour version of this figure can be found online at http://www.journals.cambridge.org/bjn

normalised to the β-tubulin. Antibodies against phos-AMP-activated protein kinase (phos-AMPK), AMPK, phos-extracellular signal-regulated kinases 1 and 2 (phos-ERK1/2), ERK1/2, β-catenin were purchased from Cell Signaling Technology. Anti-β-tubulin antibody was purchased from Sigma-Aldrich. Horseradish peroxidase-conjugated anti-rabbit and anti-mouse secondary antibodies were purchased from Cell Signaling Technology.

Statistical analysis

Data were analysed as a complete randomised design using General Linear Model of Statistical Analysis System (2000). Data are presented as means with their standard errors. A significant difference was considered as $P<0{\cdot}05$.

Results

Effect of dietary grape seed extract on intestinal permeability, total antioxidant capacity, serum TNF-α level and histological score

There was no difference in the overall feed intake or body weight gain between the two groups after 12 weeks of feeding trial (data not shown). GSE supplementation significantly decreased the *in vivo* intestinal permeability (Fig. 1(a)). In addition, GSE supplementation increased the faecal total antioxidant capacity (Fig. 1(b)) and reduced the serum TNF-α level (Fig. 1(c)). However, GSE supplementation has no significant effect on the pathological score (data not shown).

Dietary grape seed extract enhanced colonic goblet cell differentiation

GSE supplementation increased the number of goblet cells (blue stained, Fig. 2(a) and (b)) associated with enhanced mRNA expression of mucin (*Muc*)-*2*, a major secretory mucin, and a tendency of increased *Muc3*, while had no effect on the mRNA expression of *Muc1* (Fig. 2(c)). However, interestingly, no differences in the mRNA expression of *Bmp2*, *Hes1*, *Atoh1* and *Klf4* were observed between the control and the GSE groups (data not shown).

Grape seed extract supplementation decreased cell proliferation

GSE supplementation reduced the number of PCNA-positive cells per crypt (Fig. 3(a) and (b)) and had a trend to decrease the mRNA expression of *pcna* in the control group (1·00 (SEM 0·19)) compared with the GSE group (0·63 (SEM 0·09)) ($P < 0{\cdot}10$). In agreement, dietary GSE significantly down-regulated mitogen-activated protein kinases/ERK growth signalling as indicated by reduced phos-ERK1/2/ERK1/2 level in the colonic tissues of GSE compared with that in control mice (Fig.3(c)). Additionally, GSE supplementation decreased the mRNA expression of *Gadd45a* ($P<0{\cdot}05$) and had a tendency to decrease *Gadd45b* ($P<0{\cdot}1$) (Fig. 3(d)).

Dietary grape seed extract diminished Wingless and Int/β-catenin and AMP-activated protein kinase signalling

In line with the decreased epithelial cell proliferation, GSE supplementation reduced the β-catenin protein content

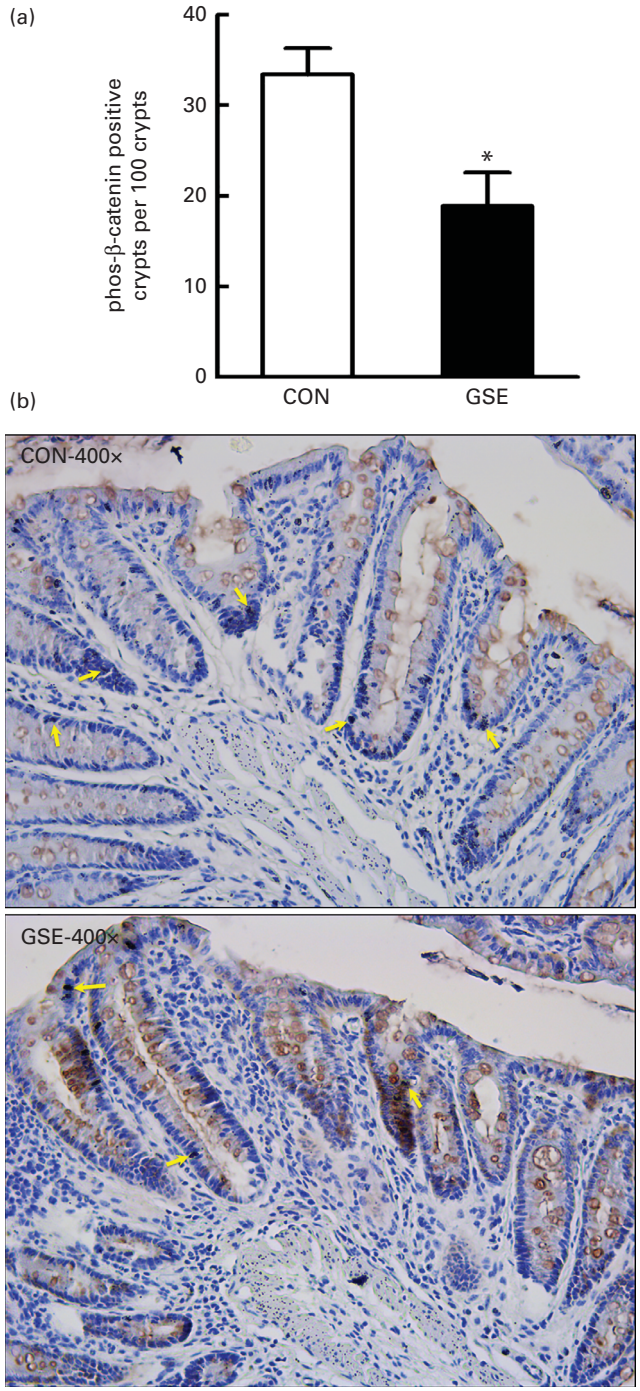

Fig. 5. Immunohistochemical staining of phosphorylated (phos)-β-catenin^{Ser552} in the colon of mice fed a control (CON) or grape seed extract (GSE) supplemented drinking-water. (a) Quantification data; (b) representative images, original magnification at 200×. Values are means with their standard errors represented by vertical bars (n 8). *Mean value was significantly different from that for CON group ($P<0{\cdot}05$). Yellow arrows point to the phos-β-catenin^{Ser552}-positive cells. A colour version of this figure can be found online at http://www.journals.cambridge.org/bjn

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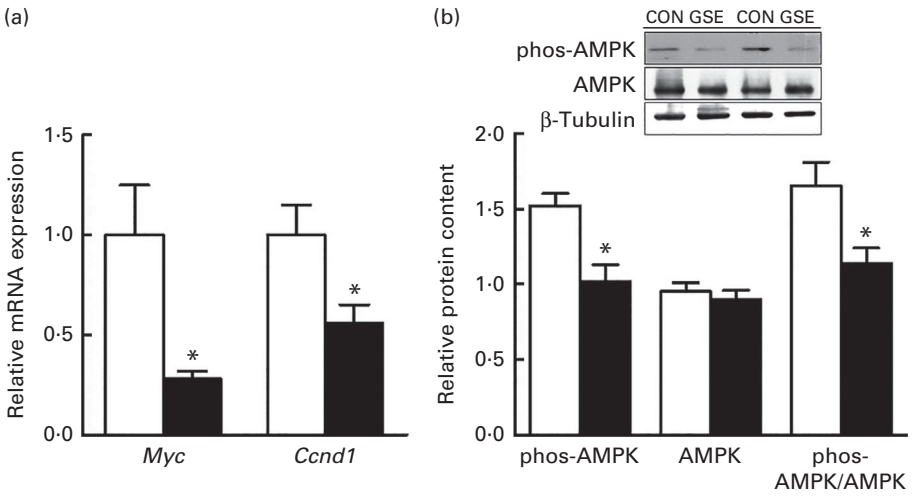

Fig. 6. AMP-activated protein kinase (AMPK) signalling, cyclin D1 (*Ccnd1*) and myelocytomatosis oncogene protein (*Myc*) mRNA expression in the colon of mice fed a control (CON; □) or grape seed extract (GSE; ■) supplemented drinking-water. (a) mRNA expression of *Ccnd1* and *c-Myc* (b) Immunoblotting of AMPK. Values are means with their standard errors represented by vertical bars (n 8). *Mean value was significantly different from that for CON group ($P<0·05$).

(Fig. 4(a)), nuclear accumulation of β-catenin (Fig. 4(b) and (c)) and phos-β-catenin^{Ser552} (Fig. 5). Consistently, β-catenin signalling target molecules, the mRNA expression of *Ccnd1* and *Myc*, were decreased by dietary GSE supplementation (Fig. 6(a)). In agreement with decreased nuclear accumulation of phos-β-catenin^{Ser552}, the AMPK activity was reduced in the GSE-supplemented mice (Fig. 6(b)).

Discussion

GSE is an extract of polyphenols, mainly catechins, proanthocyanidins and their derivatives. Accumulating evidence points to the beneficial role of GSE in intestinal inflammation and IBD, primarily mediated by proanthocyanidins[23,41,42]. Our previous study has found that dietary 1% GSE supplementation reduces disease indices of IBD[27]. Intragastrical administration of GSE at a dose of 400 mg/kg reduced the qualitative histological severity score in the proximal colon of dextran sulphate sodium-induced colitis in rats[23]. In the present study, we tested the effectiveness of a lower dose (0·1%) of GSE supplementation on gut inflammation, and found that GSE supplementation decreased the serum TNF-α level and increased the total antioxidant activity, which are consistent with our previous study[27]. However, no significant differences in the pathological scores were observed between mice with/without 12 weeks of 0·1% GSE supplementation, indicating that this low dose of GSE supplementation was insufficient to improve the gut pathological changes in the IL10-deficient mice.

A balanced rate of cell proliferation and cell differentiation is essential to maintain the intestinal epithelial homeostasis. Hyperproliferation in the intestinal tract has been observed in ulcerative colitis in both human subjects and mice[43,44]. The present study found that 0·1% of GSE supplementation reduced the colonic cell proliferation, which was consistent with the previous studies showing that GSE decreased cell proliferation in the intestinal tract of APC$^{min/+}$ mice[28] and azoxymethane-induced Fischer 344 Rats[45]. Inflammation is known to induce GADD45 expression[46]. Along with the decreased serum TNF-α level, GADD45 expression was down-regulated in the GSE-supplemented mice, indicating that the beneficial effects of GSE might be associated with its anti-inflammatory effect.

The goblet cell is one of the four cell lineages that are derived from the intestinal stem cells. Goblet cells secrete mucins and are one of the markers that can be used for studying cell differentiation[47]. The depletion of goblet cells in the large intestine is a characterised feature of IBD[48,49]. In the present study, we found that both goblet cells and *Muc2* expression were enhanced in GSE-supplemented mice, indicating that GSE might result in enhanced intestinal cell differentiation, but could exert a protective effect on epithelium due to stimulation of innate immunity, given no difference was found in the expression of factors regulating lineage-specific cell differentiation including *Bmp2*, *Hes1* *Atoh1* and *Klf4*.

Mechanically, Wnt signalling plays a crucial role in cell proliferation and tissue homeostasis[50]. Wnt signalling has the highest expression in the early stage of the proliferative compartment and decreases as the cells move upwards into the differentiating area[51], indicating that the Wnt pathway is the dominant force behind the proliferative activity of the intestinal epithelium. In the present study, we found that GSE supplementation decreases the nuclear accumulation of β-catenin, the expression of its target genes and the ERK1/2 signalling[52]. These data are in consistent with the previous studies, showing that polyphenol compounds could attenuate aberrantly activated Wnt/β-catenin signalling[53]. Polymeric black tea polyphenols inhibit 1,2-dimethylhydrazine-induced colorectal carcinogenesis by inhibiting cell proliferation via the Wnt/β-catenin pathway[54]. Furthermore, dietary green tea polyphenol supplementation inhibits the intestinal tumorigenesis in APC knockout mice partly through the down-regulation of β-catenin nuclear expression[55]. Recently, green tea

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polyphenol, (-)-epigallocatechin-3-gallate, has been shown to be toxic to human skin cancer cells by inactivation of β-catenin[56]. These data suggested that the inhibition of β-catenin signalling is one of the possible mechanisms underlying the beneficial effects of GSE.

AMPK is a serine/threonine protein kinase that plays an important role in maintaining cellular energy balance. In the present study, AMPK activity was suppressed in the GSE-supplemented mice associated with a decreased phosphorylation of β-catenin at Ser^{552}. This result is in line with our previous study, showing that AMPK phosphorylates β-catenin at Ser^{552}, which stabilises β-catenin[57].

In conclusion, 0·1% GSE supplementation reduced cell proliferation, enhanced cell differentiation and improved barrier function in IL10-deficient mice probably through inhibiting the Wnt/β-catenin pathway.

Acknowledgements

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The authors' contributions are as follows: G. Y. and M. J. Z. participated in the study design, data acquisition, analysis and interpretation of the data; G. Y., Y. X. and H. Z. conducted the research; G. Y., M. J. Z. and M. D. contributed to the writing and revision of the manuscript.

None of the authors has any conflicts of interest to declare.

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