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Inhibition of green tea polyphenol EGCG((−)-epigallocatechin-3-gallate) on the proliferation of gastric cancer cells by suppressing canonical wnt/β-catenin signalling pathway

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ABSTRACT

(−)-Epigallocatechin-3-gallate (EGCG), the major polyphenol in green tea, could affect carcinogenesis and development of many cancers. However, the effects and underlying mechanisms of EGCG on gastric cancer remain unclear. We found that EGCG significantly inhibited proliferation and increased apoptosis of SGC-7901 cells *in vitro*. The decreased expressions of p-β-catenin(Ser552), p-GSK3β(S9) and β-catenin target genes were detected in SGC-7901 cells after treated by EGCG. XAV939 and β-catenin plasmid were further used to demonstrate the inhibition of EGCG on canonical Wnt/β-catenin signalling. Moreover, EGCG significantly inhibited gastric tumour growth *in vivo* by inhibiting Wnt/β-catenin signalling. Taken together, our findings establish that EGCG suppressed gastric cancer cell proliferation and demonstrate that this inhibitory effect is related to canonical Wnt/β-catenin signalling. This study raises a new insight into gastric cancer prevention and therapy, and provides evidence that green tea could be used as a nutraceutical beverage.

Introduction

Gastric cancer is a frequent cancer and the major cause of malignant disease morbidity and mortality worldwide. The incidence of gastric cancer in developing countries is particularly high (Jemal et al. 2011). Gastrectomy remains the main therapeutic approach for patients with localised gastric carcinoma (Koizumi et al. 2008; Chen et al. 2016). However, overall 5-year survival after curative gastrectomy is only about 30% even with dramatic advances in the diagnosis and treatment (Sasako et al. 2011). Thus, developing new strategies for preventing and inhibiting gastric cancer cell proliferation is of paramount importance for developing effective therapies.

β-Catenin staining is abnormally upregulated in gastric cancer specimens, and its expression is associated with tumour recurrence and poor disease-free survival (Sereno et al. 2012). β-Catenin binds E-cadherin to the actin cytoskeleton via α-catenin. Dysfunction in the E-cadherin/β-catenin complex is associated with decreased cell differentiation and increased invasiveness and metastasis (Czyzewska et al. 2010). In addition, β-catenin also has important functions in cell signalling via the Wnt pathway (Clevers & Nusse 2012). Increasing evidence suggests that deregulation of Wnt/β-catenin signalling which is frequently found in gastric cancers, is an important clinicopathological character of gastric cancer (Zhang & Xue 2008). Therefore, identifying new prodrugs targeting Wnt/β-catenin signalling are significant for developing effective gastric cancer therapies.

Green tea (*Camellia sinensis*) is one of the most popular beverages worldwide, particularly in China (Ogunleye et al. 2010; Singh et al. 2011). Tea polyphenols are ubiquitous in green tea and regular consumption is associated with a reduced risk of a number of chronic diseases, including cancers and cardiovascular and neurodegenerative diseases. High intake of green tea is correlated with decreased morbidity due to primary gastric cancer (Shirakami et al. 2012). The most abundant polyphenol in green tea is (−)-epigallocatechin gallate (EGCG) (Henning et al. 2013). The chemistry and anticancer effects of EGCG are well documented (Yang et al. 2011b). Its activities related to preventing cancers include inhibiting various protein kinases, DNA methyltransferases, and epidermal growth factor receptor signalling (Yang and Wang 2011a). *In vivo* animal studies have demonstrated its cancer-preventing effects in different organs, such as the liver, lung, mammary glands and colon (Crespy & Williamson 2004). Nevertheless, how EGCG may

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inhibit the proliferation of gastric cancer cells has not been clearly established *in vivo* or *in vitro*.

In this study, we investigated the inhibitory effects of EGCG on gastric cancer cells and the primary mechanism involved *in vitro* and *in vivo*.

Materials and methods

Reagents and antibodies

EGCG (purity $= 95\%$) was obtained from Sigma-Aldrich (St. Louis, MO). XAV939 was purchased from Selleck Chemicals (Houston, TX). Anti-GSK-3β and anti-p-β-catenin (Ser552) were purchased from CST Technology Inc. (Danvers, MA). Anti-β-catenin and anti-proliferating cell nuclear antigen (PCNA) were obtained from Abcam (Cambridge, MA). Anti-cyclinD1, anti-Bcl-2, anti-p-GSK3β (S9), anti-β-actin, and anti-laminA were obtained from Bioworld Technology Inc. (St. Louis, MO). All of these primary antibodies were used at 1:1000–1:2000. Secondary antibodies (goat anti-rabbit horse radish peroxidase (HRP) or goat anti-mouse HRP) were purchased from Vazyme Biotech (Nanjing, China) and used at 1:10,000.

The β-catenin and control plasmids were obtained from Addgene (Cambridge, MA). Transfection was performed by from Vazyme Biotech Co., Ltd. (Nanjing, China). The pTOP Flash and pFOP Flash reporter plasmids were obtained from Upstate Biotechnology (Lake Placid, NY).

Western blot assay

SGC-7901 cells were collected and lysed in extraction buffer for 1 h on ice. The lysates were centrifuged at 13,000 rpm for 15 min. Protein in the supernatants was quantified, and equal quantities of protein in the samples were separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis under specific conditions. Then the proteins were electro-blotted onto PVDF membranes using a standard procedure. After blocking with 5% bovine serum albumin in phosphate-buffered saline (PBS) for 1.5 h at 37 °C, the membranes were incubated with primary antibodies overnight at 4 °C, followed by HRP-conjugated secondary antibodies for 1 h at 37 °C. The immunoreactive protein blots were visualised with a Tanon 5200 Chemiluminescence imaging system (Shanghai, China).

Real-time quantitative polymerase chain reaction

Total RNA was isolated from SGC-7901 cells using TRIzol® Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The quality and the concentration of total RNA were assessed by the absorbance at 260 nm (A_{260})/A_{280} ratio using a BioPhotometer (Eppendorf, Hamburg, Germany). Total RNA (1 μg) was reverse transcribed to cDNA using the First-strand cDNA Synthesis Super Mix. Quantitative real-time polymerase chain reaction (qPCR) was conducted following the protocol supplied with the Themo SYBR Green Master Mix kit. The relative amount of target mRNA was determined using the comparative threshold (Ct) method by normalising target mRNA Ct values to those for GAPDH (ΔCt). The primer sequences were as follows:

GAPDH: (F) 5′-TGGTATCGTGGAAGGACTCA-3′;

(R) 5′-CAGTAGAGGCAGGGATGATG-3′

c-myc: (F) 5′-ATCACCAGCAACAGCAGAGC-3′;

(R) 5′-GCAACATAGGACGGAGAGCA-3′

c-jun: (F) 5′-TGACTGCAAAGATGGAAACG-3′;

(R) 5′-CCAGGTTCAAGGTCATGCTC-3′

ccnd1: (F) 5′-ATTTGCACACCTCTGGCTCT-3′;

(R) 5′-TGTGGTCCCTACCCTCCATA-3′

Colorimetric MTT assay

Cells (4000–5000 cells/well) were incubated in 96-well plates in a final volume of 100 μL medium and cultured for 12 h at 37 °C. Then the cells were treated with various EGCG concentrations (5–80 μM) for the indicated durations (24 and 48 h). Cell viability was measured using the MTT assay following the manufacturer's protocol. Absorbance was measured at 570 nm using the Universal Microplate Reader (BioTek Instruments Inc., Winooski, VT). Percentage cytotoxicity was determined as follows: ty $= [1 - (A_{570}$ of test sample)/(A_{570} of control sample)] $\times 100\%$. The IC_{50} was taken as the concentration that caused 50% inhibition of cell proliferation and was calculated using SAS statistical software (SAS Institute, Cary, NC). All assays were performed five times.

Cell cycle and apoptosis assays

Cells were plated into 6-well tissue culture plates at approximately 2×10^5 cells/well and starved in serum-free medium for 6 h to be synchronised. Then they were treated with various concentrations of EGCG. After 24 h incubation, they were harvested and resuspended with PBS. Apoptosis-mediated cell death was examined in the different groups using double staining with recombinant FITC-conjugated Annexin-V and propidium iodide (PI), according to the manufacturer's protocol for the Annexin-V-FITC Apoptosis Detection

Kit (KeyGen, Nanjing, China). Cells for the cell cycle assay were trypsinised, washed with PBS, and fixed in 95% ethanol at 4 °C overnight, followed by incubation with RNase and staining with PI. Finally, the cells were analysed by Accuri C6 Software flow cytometry (Becton Dickinson, San Jose, CA).

Dual-luciferase reporter assay

The TOP-FLASH and FOP-FLASH luciferase reporter constructs were used to analyse β-catenin transcriptional activity. The TOP-Flash (with three Tcf-binding site repeats) or FOP-Flash (with three mutated Tcf-binding site repeats) plasmids were transfected into SGC-7901 cells, using the PRL-TK vector as the internal control reporter. Renilla luciferase activity was used as an internal control to normalise transfection efficiency. The cells were treated with 15, 30, or 45 μM EGCG for 24 h. Then luciferase activity was measured with the dual Luciferase Reporter Gene Assay Kit (TransGen Biotech, Beijing, China). TCF activity was calculated as fold activity (TOP/FOP Luc).

Nude mouse tumour xenograft model

Male nude mice (weight, 20 ± 2 g) were maintained at 25 ± 5 °C under a 12 h light/dark cycle and 45–55% humidity. The mice were observed for wellbeing, body weight, toxicity and survival. All animal experiments were carried out in accordance with the institutional ethics committee guidelines. SGC-7901 cells were resuspended at 5×10^6 cells/0.2 mL and transplanted subcutaneously into nude mice using a no. 7 injector needle. The mice were divided randomly into four groups ($n = 8$/group) when tumour volume reached 100 mm^3. Each group was treated with various EGCG concentrations or solvent through the caudal vein. The treatment was performed once every 2 days for 21 days. Body weights and tumour volumes were measured throughout the experiment. The mice were euthanised at the end of the experiment and the tumours were removed, measured, and weighed individually. Tumour volumes were calculated as: volume = (length × width^2)/2.

Immunohistochemistry

Immunohistochemistry (IHC) staining was performed to study β-catenin expression in SGC-7901 tumours. Tumour tissues were fixed in 4% formalin, embedded in paraffin, and cut into 4 μm sections. After blocking with 10% goat serum for 1.5 h at room temperature, the sections were incubated with anti-β-catenin at 37 °C overnight. The slides were washed three times in PBS and

then incubated with 1:5000 anti-rabbit HRP-conjugated secondary antibodies for 1 h at room temperature. An IHC kit (Maixin, Fujian, China) was used according to the manufacturer's instructions. Immunoreactivity was visualised under a Nikon microscope (Tokyo, Japan).

DAPI staining

SGC-7901 cells were plated onto 12-well plates and treated with 0, 15, 30, or 45 μM EGCG for 48 h. The cells were collected, washed with PBS, and fixed with methanol for 15 min at −20 °C. Then the cells were stained with DAPI to visualise apoptosis. The cells were examined and photographed using a Nikon A1R-A1 laser-based confocal microscope.

Statistical analysis

Data are presented as means ± standard deviations of triplicates. All data were compared by one-way analysis of variance or Student's t-test using SPSS software (SPSS Inc., Chicago, IL). A p-value <0.05 was considered significant.

Results

EGCG inhibits proliferation of SGC-7901 cells

SGC-7901 cells are a poorly differentiated cell line and were used to the study the inhibitory effects of EGCG. EGCG (Figure 1(a)) was dissolved in sterile distilled water. The cells were incubated with EGCG in medium supplemented with 2% foetal bovine serum FBS, superoxide dismutase (5 U/mL), and catalase (30 U/mL) to prevent auto-oxidation of EGCG and production of superoxide and hydrogen peroxide. We first assessed viability of SGC-7901 cells treated with EGCG by the MTT assay. As shown in Figure 1(b), EGCG inhibited growth of SGC-7901 cells with IC$_{50}$ values of 28.87 ± 1.46 μM (24 h) and 17.35 ± 1.24 μM (48 h). Therefore, 15, 30, and 45 μM EGCG were used for all the subsequent experiments. Then the colony formation assay was used to further assess the viability. Colony formation was markedly reduced by 72 h exposure to EGCG compared to the control (Figure 1(c,d)). Taken together, viability was significantly inhibited in cells treated with EGCG (15, 30, and 45 μM).

EGCG induces apoptosis and G0/G1 cell cycle arrest in SGC-7901 cells

DAPI staining showed that the nuclei of cells changed from uniformly dispersed to pyknotic shaped after a
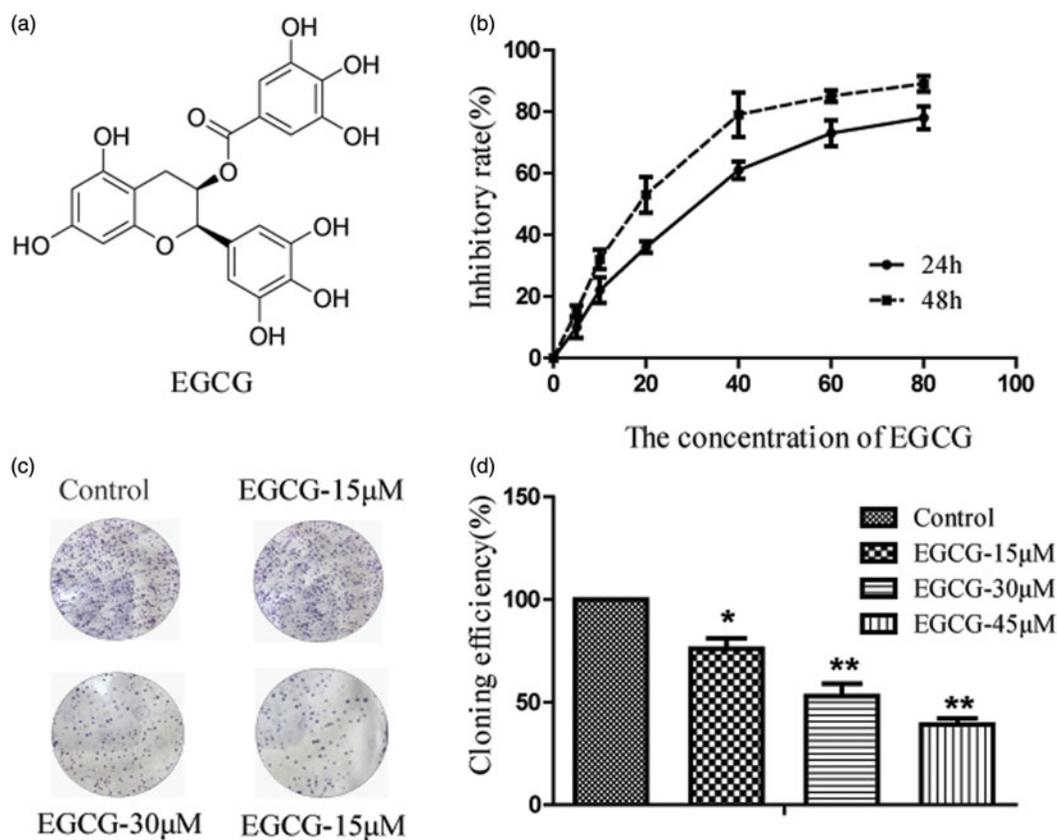
Figure 1. EGCG inhibits proliferation of SGC-7901 cells. (a) The chemical structure of EGCG. (b) SGC-7901 cells were treated with different concentrations of EGCG (15, 30, and 45 μM). Cell viability was determined by the MTT assay. (c) A macrograph of SGC-7901 cell clone formation. (d) Cloning efficiency (%) of SGC-7901 cells. Each value is mean ± standard deviation and is representative of results obtained from three independent experiments. Asterisks (*$p < 0.05$ and **$p < 0.01$ versus control group) indicate a significant difference compared to the appropriate control cells.

24 h treatment with 15, 30, and 45 μM EGCG. This morphological change indicates cells in apoptosis (Figure 2(a)). The cell cycle analysis revealed that SGC-7901 cells were arrested in the G1 phase after treatment with 30 and 45 μM EGCG, accompanied by a significant reduction in the number of S-phase cells (Figure 2(b)). The rate of apoptosis-induced cell death increased significantly after the EGCG treatment, as determined by Annexin-V/PI assay (Figure 2(c)). Western blot demonstrated that EGCG dose-dependently reduced the expression levels of PCNA, cyclin D1, and the apoptosis-associated protein Bcl-2 (Figure 2(d,e)). Taken together, EGCG inhibited SGC-7901 cell proliferation by inducing G0/G1 cell cycle arrest and cell apoptosis.

EGCG inhibits activation of canonical β-catenin signalling in SGC-7901 cells

β-Catenin is a crucial Wnt/β-catenin signalling transcription factor that regulates tumour cell proliferation in many cancers. Previously reported evidence has shown that β-catenin is overexpressed in gastric cancer. We determined whether EGCG could suppress canonical β-catenin signalling in SGC-7901 cells. The results of the β-catenin TOP/FOP luciferase reporter assay indicated that 15, 30, and 45 μM EGCG effectively inhibited activation of β-catenin (Figure 3(a)). The 30 μM EGCG concentration was typically chosen for the following experiments. The expression of p-β-catenin (Ser552) significantly decreased in cells treated with 30 μM EGCG, compared to that in control SGC-7901 cells (Figure 3(b)). Phosphorylation of β-catenin at Ser552 is associated with nuclear accumulation and transcriptional activation of its target genes. The protein levels of p-GSK-3β (S9) were downregulated, indicating suppressed activation of β-catenin. EGCG decreased nuclear translocation of β-catenin in SGC-7901 cells (Figure 3(c)), and also significantly downregulated the transcription of downstream β-catenin signalling genes, including ccnd1, c-myc, and c-jun (Figure 3(d)). These findings suggest that canonical Wnt/β-catenin signalling was suppressed by EGCG.
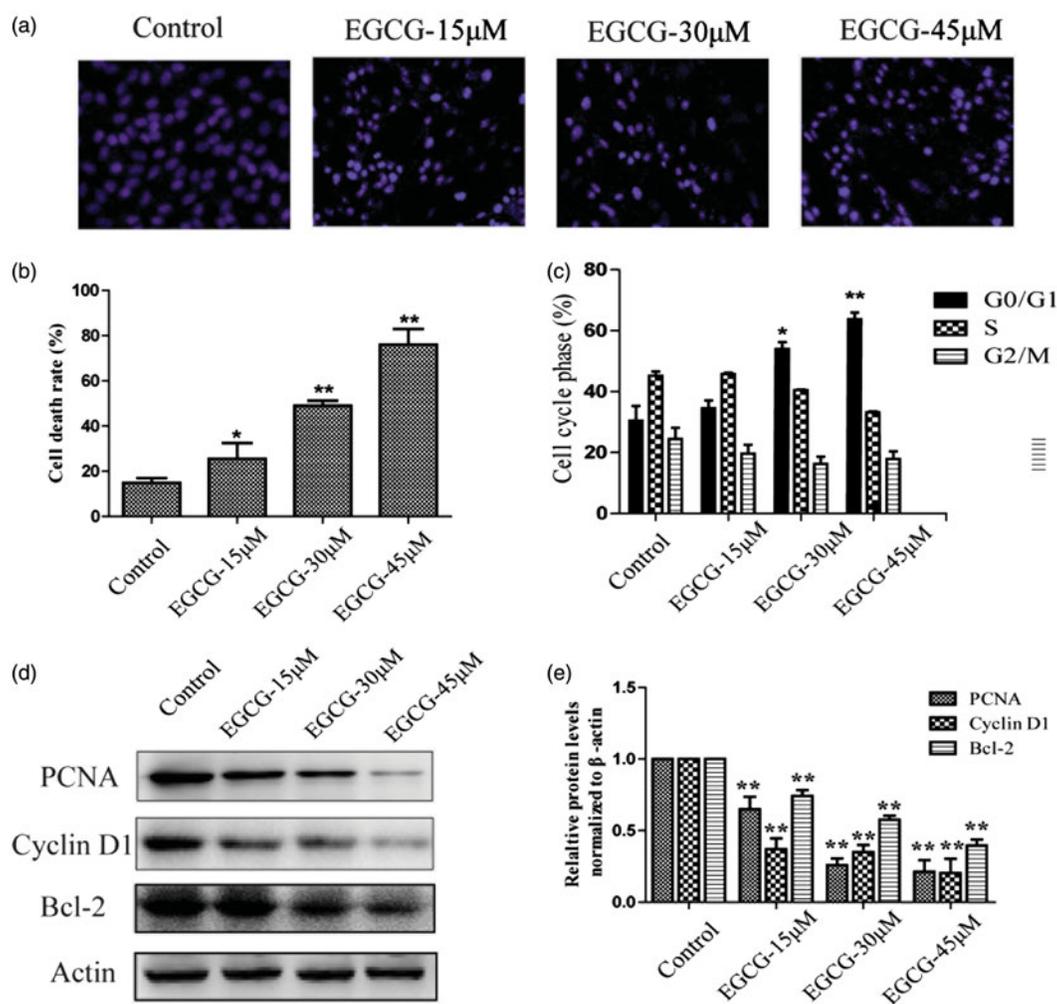
Figure 2. EGCG induces apoptosis and cell-cycle arrest in the G0/G1 phase, and inhibits viability of SGC-7901 cells. (a) SGC-7901 cells were treated with 15, 30, and 45 μM EGCG for 24 h, SGC-7901 cell nuclei were stained with DAPI and the cells were photographed at 200 × magnification. (b) Apoptosis of SGC-7901 cells was analysed by flow cytometry (FCM). Histograms of death rates were quantified to show early and late apoptosis. (c) The cell cycle phase was determined by FCM following PI staining. Cells were treated with different EGCG concentrations for 24 h. Different percentages of the three cell phases (G0/G1, S, and G2/M) are shown. (d) Total PCNA, Bcl-2, and cyclin D1 protein expression levels were assessed by Western blot (e) normalised to that of β-actin. Each value is mean ± standard deviation of at least three independent experiments. *$p < 0.05$ and **$p < 0.01$ versus control.

EGCG suppresses the proliferation of gastric cells by inhibiting activity of the wnt/β-catenin signalling pathway

XAV939 and β-catenin overexpression were used to explore whether EGCG could suppress the proliferation of SGC-7901 cells by inhibiting canonical Wnt/β-catenin signalling. XAV939, which is a selective and potent Wnt/β-catenin inhibitor, stimulates degradation of β-catenin by stabilising axin, a concentration-limiting component of the destruction complex, resulting in reduced nuclear accumulation of β-catenin. As shown in Figure 4(a), XAV939 significantly downregulated β-catenin expression. EGCG combined with XAV939 did not further inhibit β-catenin activity (Figure 4(c)) or proliferation of SGC-7901 cells (Figure 4(d)). On the

other hand, the opposite effect occurred when SGC-7901 cells were transfected with the β-catenin overexpressing plasmid. Overexpressing β-catenin (Figure 4(b)) increased β-catenin activity, whereas EGCG reversed this effect. At the same time, the increased proliferation ability induced by the β-catenin overexpressing plasmid was also reversed by EGCG (Figure 4(d)). Taken together, these results show that EGCG restrained proliferation of gastric cancer cells by inhibiting activation of canonical Wnt/β-catenin signalling.

EGCG inhibits gastric cancer growth by blocking β-catenin signalling in vivo

SGC-7901 cells were injected subcutaneously into male nude mice. The mice were divided randomly into four
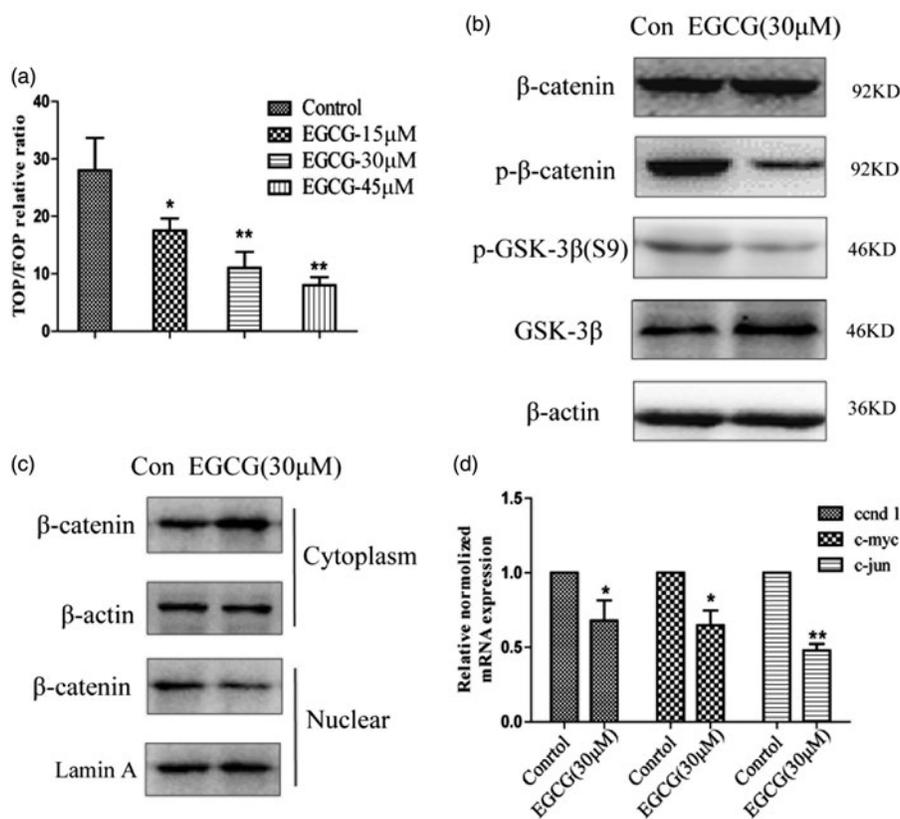
Figure 3. β-Catenin activation in SGC-7901 cells is inhibited by EGCG. (a) β-catenin TOP/FOP luciferase reporter assay of SGC-7901 cells exposed or not to EGCG (15, 30 and 45 μM). The luciferase activity was normalised to Renilla activity. (b) β-Catenin, p-β-catenin (ser552), GSK3β, and p-GSK3β (S9) protein levels were detected by Western blot. (c) The nuclear translocation of β-catenin was inhibited by 30 μM EGCG. (d) ccnd1, c-myc, and c-jun mRNA expression levels were measured in SGC-7901 cells using real-time PCR. GAPDH was used as an endogenous housekeeping gene. Results are representative of three independent experiments. *$p < 0.05$ and **$p < 0.01$ versus control.

groups ($n = 8$/group) when tumour volume reached 100 mm^3 (about 5 days after transplantation). Each group was injected intravenously with various EGCG concentrations (0.5(L), 1(M), or 2(H) mg/kg) in the caudal vein once every 2 days for 21 days. Treated tumours were significantly smaller than those in controls (Figure 5(a)). No changes in BW were observed (Figure 5(b)), suggesting that EGCG was not toxic. The 1 mg/kg EGCG group started to show significant inhibition of tumour growth on day 17 (Figure 5(c)). Mice were sacrificed at the end of the experiment, and the tumours were removed, measured, and weighed. As shown in Figure 5(d), tumour weights were notably reduced, and β-catenin expression decreased, in the EGCG-treated groups compared to controls (Figure 5(e)). In addition, the Western blot results of tumour tissues showed that ECGG significantly decreased the level of PCNA, which is related to cell proliferation (Figure 5(f)). In conclusion, our data demonstrate that EGCG inhibits tumour growth by inducing differentiation of cancer cells through β-catenin signalling *in vivo*.

Discussion

Gastric cancer remains the most common cancer and is the leading cause of cancer mortality worldwide (Rahman et al. 2014). The highest incidence and mortality of gastric cancer are registered in north-eastern Asian countries, including China, Japan, and Korea, accounting for more than half of the world total (Sugano 2015). Current therapeutic approaches include surgery alone or combined with chemotherapy and radiotherapy, but outcomes remain poor. Therefore, there is a strong urgency to find new therapeutic strategies to treat advanced gastric cancer (Jia & Cai 2016). For this reason, more attention is being paid to therapeutic uses of natural products, due to their high efficacy and few adverse effects (Han et al. 2015; Jeong et al. 2016).

Specific dietary supplements have shown potential effects to prevent chronic diseases, including cancers. Green tea has been a popular beverage worldwide, particularly in China, for thousands of years. Evidence supports the *in vitro* and *in vivo* anticancer effects of
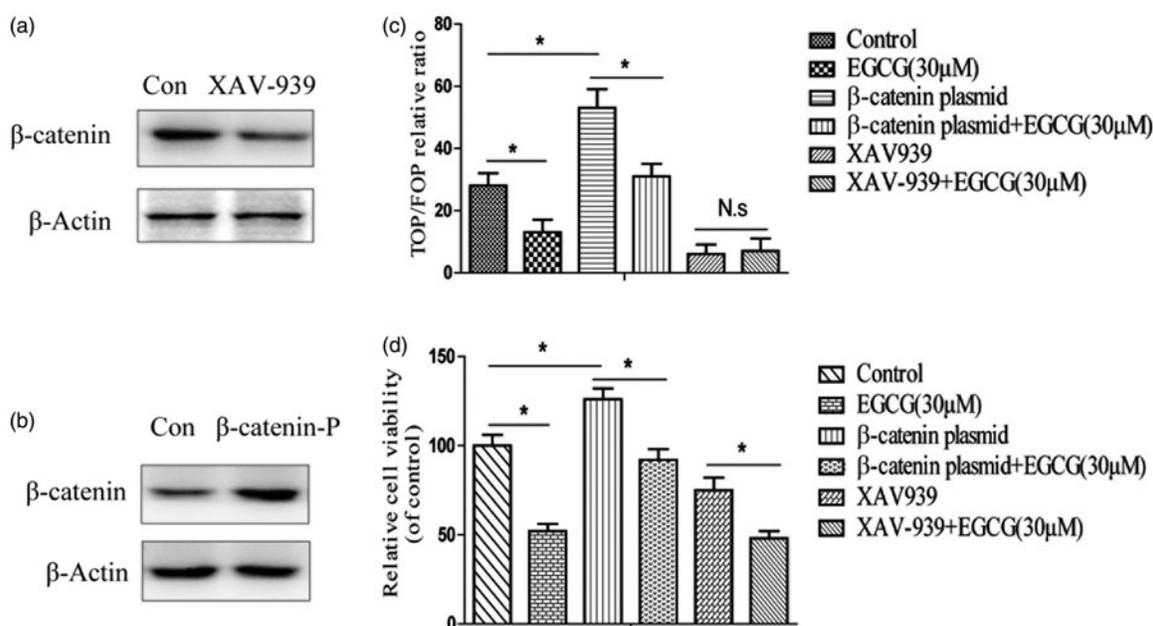
Figure 4. EGCG suppresses Wnt/β-catenin signalling pathway activity. The β-catenin signalling inhibitor XAV939 (a) and a β-catenin overexpressing plasmid (b) were used in SGC-7901 cells, and β-catenin proteins levels were measured. (c) β-catenin TOP/FOP luciferase reporter assays were conducted with or without XAV939 and the β-catenin overexpressing plasmid. Luciferase activity was normalised to Renilla activity. (d) After the SGC-7901 cells were treated with or without XAV939 and the β-catenin overexpressing plasmid, cell viability was determined by the MTT assay. Data (mean ± standard deviation) are representative of at least three independent experiments. $*p < 0.05$ and $**p < 0.01$ versus control.

EGCG in green tea (Bhatti et al. 2013). The protective effects of tea extract have been demonstrated in lung, digestive tract, bladder, liver, prostate, breast and skin cancers (Patel et al. 2008; Henning et al. 2013), and high intake of green tea is correlated with decreased morbidity from primary gastric cancer. In this study, we hypothesised that EGCG may inhibit carcinogenesis and the development of gastric cancer.

Activation of Wnt/β-catenin signalling contributes to carcinogenesis in a subset of cancers (Ochoa-Hernandez et al. 2012; Polakis 2012; Sherwood 2015). Increasing evidence shows that Wnt/β-catenin signalling and β-catenin expression and localisation are correlated with gastric cancer (Clements et al. 2002; Nusse 2005; Pan et al. 2008). We initially investigated the inhibitory effects of EGCG on human gastric cancer SGC-7901 cells and found that EGCG inhibited cell proliferation and induced G0/G1 cell cycle arrest and apoptosis. In addition, EGCG inhibited tumour growth in a nude mouse tumour xenograft model.

EGCG exhibits significant chemopreventive properties against cancer by modulating Wnt/β-catenin signalling (Oh et al. 2014). However, the underlying mechanism remains unclear. EGCG has been shown to block the Wnt/β-catenin pathway by inducing the HMG-box protein 1 transcriptional repressor without affecting β-catenin level in breast cancer cells (Kim et al. 2006). Yet, Dashwood et al. (2005) demonstrated

that EGCG activates β-catenin signalling in skin cancer cells by increasing phosphorylation of β-catenin on Ser33/37 residues through the CK1/GSK-3β signalling pathway. In contrast, EGCG inhibits GSK-3β activity, resulting in decreased β-catenin phosphorylation in HT29 colon cancer (Gwak et al. 2009) and genetically engineered HEK293 cells, in which the Wnt/β-catenin pathway is robustly activated by Wnt3a-CM. EGCG downregulates β-catenin through a GSK-3β-independent proteasomal degradation pathway (Oh et al. 2014). In our study, we demonstrated for the first time that EGCG inhibited Wnt/β-catenin signalling, which depended on reduced GSK3β-mediated β-catenin degradation and increased β-catenin nuclear translocation.

β-Catenin is the major mediator of canonical Wnt signalling and is degraded through interactions with axin, adenomatous polyposis coli, and the protein kinase GSK-3 in cells not exposed to a Wnt signal. When the canonical Wnt signal is activated, inactivation of GSK-3β via phosphorylation at Ser9 increases the cytoplasmic pool of the β-catenin protein. Accumulation of cytoplasmic β-catenin results in its translocation to the nucleus where it binds to the Tcf/LEF transcriptional factor (Wodarz & Nusse 1998). This complex regulates transcription of multiple target genes, including c-myc and cyclin D1, PCNA, and matrix metalloproteinases (Logan & Nusse 2004; Gough 2012; Zou & Salinas 2014; Guo et al. 2015).
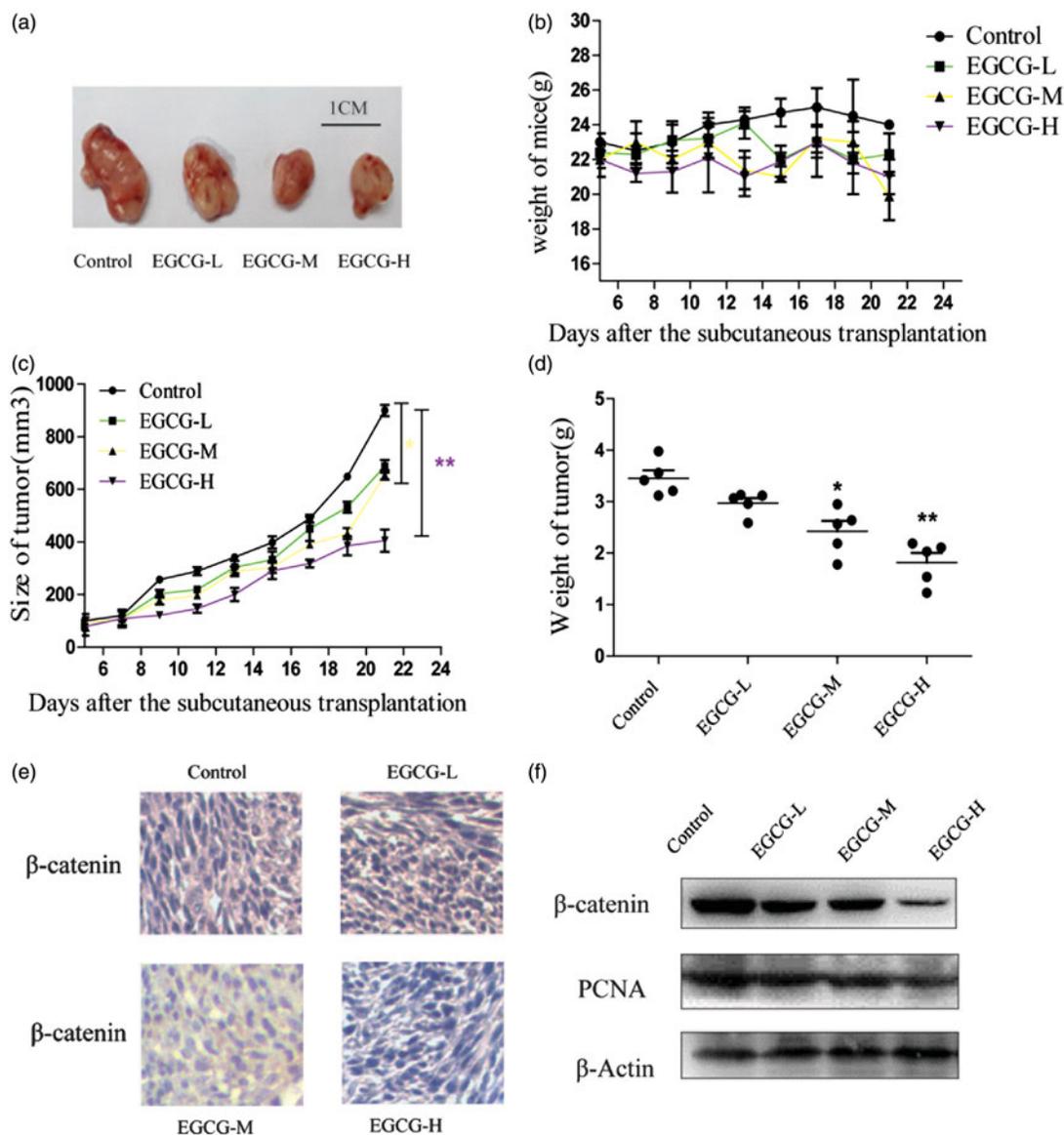
Figure 5. EGCG inhibits gastric cancer growth by blocking β-catenin signalling *in vivo*. (a) Tumours were measured in the control and the three EGCG-treated (15, 30, and 45 μM) groups. (b) Weight changes in nude mice in the four groups were measured for 21 days. (c) Tumour sizes of nude mice were measure with Vernier callipers for 21 days during treatment with EGCG. (d) Weights of tumours from tumour-bearing mice. (e) β-Catenin expression was measured by immunohistochemistry. (f) Western blot indicates the expression of β-catenin and PCNA. Data are mean ± standard deviation ($n = 3$). *$p < 0.05$, **$p < 0.01$ versus control.

The expression of p-β-catenin (Ser552) was inhibited when SGC-7901 cells were treated with EGC G (30 μM), and the β-catenin TOP/FOP luciferase reporter assay also showed suppressed β-catenin activation. Subsequently, XAV939 and a β-catenin overexpressing plasmid were used in SGC-7901 cells to demonstrate regulation of β-catenin signalling by EGCG. The decrease in Ser552 expression in response to EGCG was tested by Western blot. Its phosphorylation is associated with its nuclear accumulation and transcriptional activation of its target genes. To assess the effects of EGCG on the inhibition of β-catenin activation, we measured GSK-3β and p-GSK-3β expression (S9) and found that EGCG (30 μM)

prominently decreased the level of p-GSK-3β (S9) protein. β-Catenin was not degraded when GSK-3β was phosphorylated at Ser9, and expression of the β-catenin protein increased in the cytoplasm, indicating that EGCG inhibited p-GSK-3β (S9) leading to the inhibition of β-catenin activation.

Conclusions

In conclusion, we found that EGCG suppressed proliferation of SGC-7901 cells and tumour cells in a nude mouse xenograft model by inhibiting the activation of canonical Wnt/β-catenin signalling. These results may help with gastric cancer prevention and therapy and

provide evidence that green tea can be used as a nutraceutical and functional drink.

Disclosure statement

Funding information

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