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Preparation of nanoparticles of β-cyclodextrin-loaded scutellarein anti-tumor activity research by targeting integrin $\alpha_v\beta3$

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Abstract

Background: The problems associated with the poor water solubility of anticancer drugs are one of the most important challenges in achieving effective cancer therapy. The present study was designed to evaluate the effect of scutellarein on human colon cancer cells in vitro by using a target $\alpha_v\beta3$ novel scutellarein (Scu)-loaded niosome nanoparticle (β-CD-CL-Scu-cRGD).

Results: β-CD-CL-Scu-cRGD has a diameter of 140.2 nm and zeta potential of − 11.3 mV with constant physicochemical stability. The MTT assay showed both Scu and β-CD-CL-Scu-cRGD caused a decrease in cell proliferation and viability of LoVo, but β-CD-CL-Scu-cRGD showed better activity in vitro. Colony formation assay and flow cytometry assay showed that β-CD-CL-Scu-cRGD has a better effect on cell proliferation and apoptosis. In vivo, animal experimental results showed that β-CD-CL-Scu-cRGD can significantly inhibit tumor growth, and the bodyweight of mice decreases during the treatment of scutellarein and its derivatives. β-CD-CL-Scu-cRGD could inhibit the protein levels of Ki67 and αvβ3, thereby inhibiting tumor growth.

Conclusions: Although further in vitro and in vivo studies are necessary, our results suggested that β-CD-CL-Scu-cRGD could be an outstanding carrier to deliver Scu for potential therapeutic approaches into colon cancer.

Keywords: Scutellarein, Nanoparticles, Colon cancer, β-Cyclodextrin, $\alpha_v\beta3$

Introduction

Colon cancer is one of the most common malignant tumors of the digestive system in clinical practice, and its incidence ranks the third in the world, which seriously endangers people's life and health (Torre et al. 2016; Jemal et al. 2011). At present, the clinical treatment of colon cancer is usually based on comprehensive treatment, including radiotherapy, surgery, and Chinese medicine adjuvant chemotherapy (Wang et al. 2012). Scutellarein (5, 6, 7, 4′-tetrahydroxy-flavone) is an active monomer component isolated from traditional Chinese medicines with anti-inflammatory and analgesic properties such as *Erigeron breviscapus* and *Oroxylum indicum* (Goh et al. 2005). Scutellarein has neuroprotective and anti-inflammatory effects (Goh et al. 2005; Sung et al. 2015; Sang

et al. 2015). It also has good activity in anti-lung cancer, fibrosarcoma, and other cancers, but research on colon cancer is rarely reported (Cheng et al. 2014). However, the therapeutic potential of scutellarein is limited due to its low bioavailability as well as poor water solubility and absorption.

Drug-carrying nanoparticles increase the permeability of biofilms and can be swallowed by macrophages as foreign bodies to target sites where ligands, antibodies, and enzyme substrates are attached (Sahab-Negah et al. 2020). Nanoparticles are highly dispersed and have a large surface area, which is conducive to increasing the contact area between the drug and biofilm at the absorption site and improving the absorption and bioavailability of the drug (Sánchez et al. 2003). Increasing the local concentration of the drug reduces the concentration of the rest of the body, thus greatly reducing the systemic toxicity of the drug (Ubrich et al. 2004). Cyclodextrin is a kind of non-toxic, water-soluble, and can be chemically modified cyclic oligosaccharide, which is connected by 6–8 D-glucopyranose units (Memisoglu-Bilensoy et al. 2005). β-Cyclodextrin (β-CD) can increase the solubility and stability of fat-soluble drugs, promote the absorption and bioavailability of drugs, cover up the bad smell of drugs, and reduce or eliminate the toxicity of drugs (Memisoglu-Bilensoy et al. 2005; Racuciu et al. 2007). Cyclodextrin derivatives and their polymers can combine with drugs in physical or chemical ways, so it had been widely studied in the field of nano-drug loading.

Integrin $\alpha_v\beta3$ is highly expressed on the surface of neovascular endothelial cells and a variety of tumor cells, which plays an important role in the process of cell adhesion, proliferation, differentiation, metastasis, and apoptosis (Brooks et al. 1994; Ruoslahti 2002). It also plays an important role in angiogenesis and tumor migration and invasion. Artificial cyclic RGD peptides (arginine-glycine-aspartate) have a very high affinity with $\alpha_v\beta3$ integrin, and the use of these cyclic RGD peptides can improve tumor-targeted therapy (Liu 2009; Dijkgraaf et al. 2006). RGD targeting integrins ligand research has become a hot spot in tumor pathology and pharmacology.

The purpose of this study is to introduce a nanoparticle that was composed of β-cyclodextrin and Scu and decorated with cRGD peptide as a targeted drug delivery system for anti-tumor therapeutics. The physical and chemical properties and anti-tumor activity in vitro and in vivo of β-CD-CL-Scu-cRGD were evaluated.

Materials and methods

Materials

β-Cyclodextrin was purchased from (www.best-reagent.com) (ChengDu, China). ε-caprolactone and stannous isooctanoate were purchased from Aladdin (Shanghai, China). (EDC/NHS) was purchased from Sigma (San Francisco, American). cRGD peptide was synthesized by China Peptides Co. Ltd (Shanghai, China). Indole green fluorescent probe IR780 was provided by Xian ruixi Biological Technology Co. Ltd (Xi'an, China). Scutellarein was provided by Must Bio-Technology Co. Ltd (ChengDu, China).

Synthesis of nanoparticles coated with β-cyclodextrin-wrapped scutellarein

Synthesis of β-CD-CL

β-CD (227.3 mg, 0.2 mmol) and ε-caprolactone (4.65 mL, 42 mmol) were added into a 50-mL flask with three necks, slowly adding 10 mL of *p*-xylene under magnetic stirring

to make the reaction substance was completely dissolved. After dissolution, under the protection of N_2, add ($Sn(Oct)_2$ (0.1% w/w)) as the catalyst and react for 36 h at 120 °C. When the reaction was completed, add 15 mL dichloromethane to the reaction product, and then drop it to 300 ml ice ether at a constant speed. There will be a lot of precipitation, and filter to obtain filter cake. Put it in a vacuum drying oven at 40 ℃ for 24 h until constant weight, and the product β-CD-CL was 3 g.

β-CD-CL nanoparticles with IR780 was prepared by ultrasonic emulsification

10 mg scutellarein was added to 1 mL of chloroform and methanol solution (3:1) and fully dissolved. Then, 100 mg of carrier material β-CD-CL was precisely weighed and fully dissolved in 3 mL chloroform until it was clear and transparent. 0.5 mg IR780 powder was added to the above β-CD-CL chloroform solution and fully dispersed. Scutellarein solution was mixed with β-CD-CL trichloromethane solution as oil phase, and then under vortex oscillation condition 10 mL (polyvinyl alcohol) PVA aqueous solutions (3%) was added into it. At last, ultrasonic emulsification was performed by ultrasonic breaker (100 W, 5 min, ice bath). The ultrasonic solution was placed in a fume hood and stirred overnight to remove the organic solvent. After the volatilization of organic solvent, β-CD-CL nanoparticles were ultrafiltrated with a 100-kDa ultrafiltration tube to remove the residual PVA. Finally, the nanoparticles were resuspended with MES buffer at pH 6.5.

Surface modification of β-CD-CL nanoparticles with cRGD by carbodiimide method

19.2 mg EDC and 11.5 mg NHS were added into 10 mL of the above Scu-IR780-NPs solution, incubated at room temperature for 2 5 min, then removed the by-products of the EDC/NHS reaction through a 100-kDa ultrafiltration tube, and add a PB solution containing 10 mg cRGD (pH 7.4); reacted at room temperature for 4 h. Finally, the unreacted cRGD was removed by ultrafiltration several times, then resuspended and dispersed in ultrapure water.

Characterization techniques

FT-IR spectra were obtained by Thermo Fisher Scientific, NICOLET IS50 (America). 1H-NMR spectra were obtained by Bruker Avance 250 (Germany) instruments. Transmission electron microscopy TEM (JEOL JEM-2100 microscope operating at 120 kV and linear resolution of 2.5 Å) was used to observe the morphology of the nanoparticles. The morphology of β-CD-CL-Scu-cRGD under 40,000 × magnification was observed with a transmission electron microscope (TEM).

The molecular weight of the polymer β-CD-CL was determined by gel permeation chromatography (Waters1515, Waters) (MA, USA). Particle size and zeta potential were detected by DLS (Brookhaven Instruments) (USA).

Entrapment efficiency (EE%) and drug loading (DL%)

Scutellarein was detected by HPLC at 0.02, 0.04, 0.06, 0.08, and 0.1 mg/mL to make a calibration curve. The drug contained in β-CD-CL-Scu-cRGD dissolved by methanol demulsification. The content of the drug was determined by HPLC, and drug

encapsulation efficiency (EE%) and drug loading (DL%) content were calculated according to the standard curve. The equations used were as follows:

$$\mathrm{EE\%} = \frac{\text{Weight of drug in nanoparticles}}{\text{Weight of feed drug}} \times 100,$$

$$\mathrm{DL\%} = \frac{\text{Weight of drug in nanoparticles}}{\text{Weight of nanoparticles}} \times 100.$$

In vitro stability of β-CD-CL-Scu-cRGD

The β-CD-CL-Scu-cRGD (100 mg) was dissolved in 14 mL tetrahydrofuran. Then, 2 mL of the β-CD-CL-Scu-cRGD solution was diluted fourfold using PBS and RPMI1640 medium and incubated at 37 °C. The tetrahydrofuran with β-CD-CL-Scu-cRGD was divided into seven equal parts according to the above method. At every given time point (1, 2, 3, 4, 5, 6, 7, day), 1 mL of the sample solution was collected from the PBS and RPMI1640 medium, excess acetonitrile was added to remove the ingredients from medium, followed by centrifugation, then the size and zeta potential of β-CD-CL-Scu-cRGD were measured by using TEM and DLS as scribed above.

In vitro anticancer activity assay

Cell culture

LoVo cell from National Collection of Authenticated Cell Cultures was maintained in RPMI1640 containing 10% FBS (Gibco, USA), penicillin (100 μg/mL), glutamine (100 μg/mL), and incubated in a humid atmosphere at 37 °C with 5% CO_2.

MTT assay

LoVo cells of the logarithmic growth phase were seeded into a 96-well culture plate (5000 cells/well) and medicine administration was conducted after 24 h of cell culture. 24 and 48 h after medicine administration, added 20 μL MTT (5 mg/mL) to each well, and incubated for 4 h at 37 °C with 5% CO_2. Then, the culture medium was aspirated and discarded. 150 μL of DMSO was added to each well, shaken to dissolve totally, the absorbance value of each well measured on an enzyme-linked immunoassay with a wavelength of 490 nm to calculate the cell proliferation rate.

Colony formation assay

The cells at logarithmic growth stage were pretreated to prepare cell suspension. Inoculate 200 cells per well in a 6-well plate containing 4 mL of complete medium. Then, gently rotate to disperse the cells evenly. Place the 6-well plate in an incubator at 37 °C and 5% CO_2. Change the medium every 3 days, and the drug concentration (40 μg/mL) remains unchanged. After 2 weeks, discard the culture medium, and carefully immerse the plate with PBS. Add 5 mL of pure methanol to fix the cells for 15 min. Aspirate and discard methanol before adding the appropriate amount of Giemsa staining solution. Finally, slowly wash away the dyeing solution with running water, and air dry.

Flow cytometry assay

The cells are seeded in a 6-well plate at 300,000 per well. The concentrations of the drugs Scu, β-CD-CL-Scu, and β-CD-CL-Scu-cRGD were both (40 μg/mL), and incubated for 24 h. All cells were collected and stained with Annexin V-FITC and PI (MultiSciences). Cell apoptosis was determined by flow cytometry using annexin V and propidium iodide (PI) double-staining technique for phophatidylserine membrane redistribution. The apoptotic cells were measured by BD Caliber flow cytometry (Becton Dickinson, USA).

Animal experiments

A nude mouse model was used to monitor the effect of β-CD-CL-Scu-cRGD on tumor progression. Male Balb/c nude mice (5 weeks old) were purchased from Dashuo Experimental Animal Co., Ltd. (Chengdu, China). All mice were raised under the animal welfare regulations and protocols approved by the Animal Care and Use Committee of the Affiliated Hospital of Southwest Medical University. LoVo cells (1×10^7 cells/mouse) were injected subcutaneously into the axillary area of the right forelimb of Balb/c nude mice to produce tumors. After the tumor takes shape, the mice were randomly divided into 4 groups ($n = 4$), and β-CD-CL-Scu (10 mg/kg), β-CD-CL-Scu-cRGD (10 mg/kg) and Scu (10 mg/kg) were injected intraperitoneally.

The control group received the same dose of saline treatment at the same time. 24 days later, the mice were killed by cervical dislocation, and the tumor weight and volume of each mouse were evaluated.

Histological analysis—H&E staining

Transplanted tumor samples were fixed in 4% neutral formaldehyde solution, dehydrated in ethanol (concentration gradient), treated with xylene, paraffin-embedded, sectioned, and stained with H&E (Solarbio, China). Histopathological analysis was performed under the microscope, and images were obtained for further analysis.

Immunohistochemical analysis

Graft tumors were fixed with 4% neutral formaldehyde solution, dehydrated in a gradient of ethanol concentration, and treated with xylene and paraffin embedding. Paraffin sections were routinely dewaxed into the water, antigen repaired and cooled at room temperature, sealed with 10% goat serum (Gibco, USA), and diluted primary antibody anti-Ki67 (1:500, monoclonal, ab15580, abcam, UK), anti-integrin $\alpha_V\beta3$ (1:300, monoclonal, ab179473, abcam, UK) incubated overnight at 4 °C. After 12 h, the sections were incubated with biotinylated secondary antibody for 30 min at room temperature, and color developed with DAB. The staining procedure was repeated by adding H&E and sections were closed with neutral gum. Images were observed and photographed with a KEYENCE microscope.

Statistical analysis

Statistical data were presented as mean ± standard deviation (SD), and analyses w carried using SPSS software (19.0 revision, IBM, Chicago, IL, USA). Graphs were

obtained by GraphPad Prism software (6.0 revision, La Jolla, CA, USA). One-way ANVOA was used to compare the group differences. $P < 0.05$ was considered statistically significant.

Results

β-CD-CL polymer characterization of ^{1}H-NMR

To develop an effective scutellarein delivery system, we constructed a scutellarein-loaded niosome nanoparticle using the β-CD-CL as package material (Fig. 1A). The polymer of β-CD-CL was characterized by ^{1}H-NMR analyses. ^{1}H-NMR information revealed that there are obvious proton peaks at 5.74, 5.69, 4.83, 4.46, 4.34, 3.98, 3.63, 3.36, 2.27, 1.53, 1.29. However, the signals at 3.98, 2.27, 1.53, and 1.29 ppm are significantly stronger than

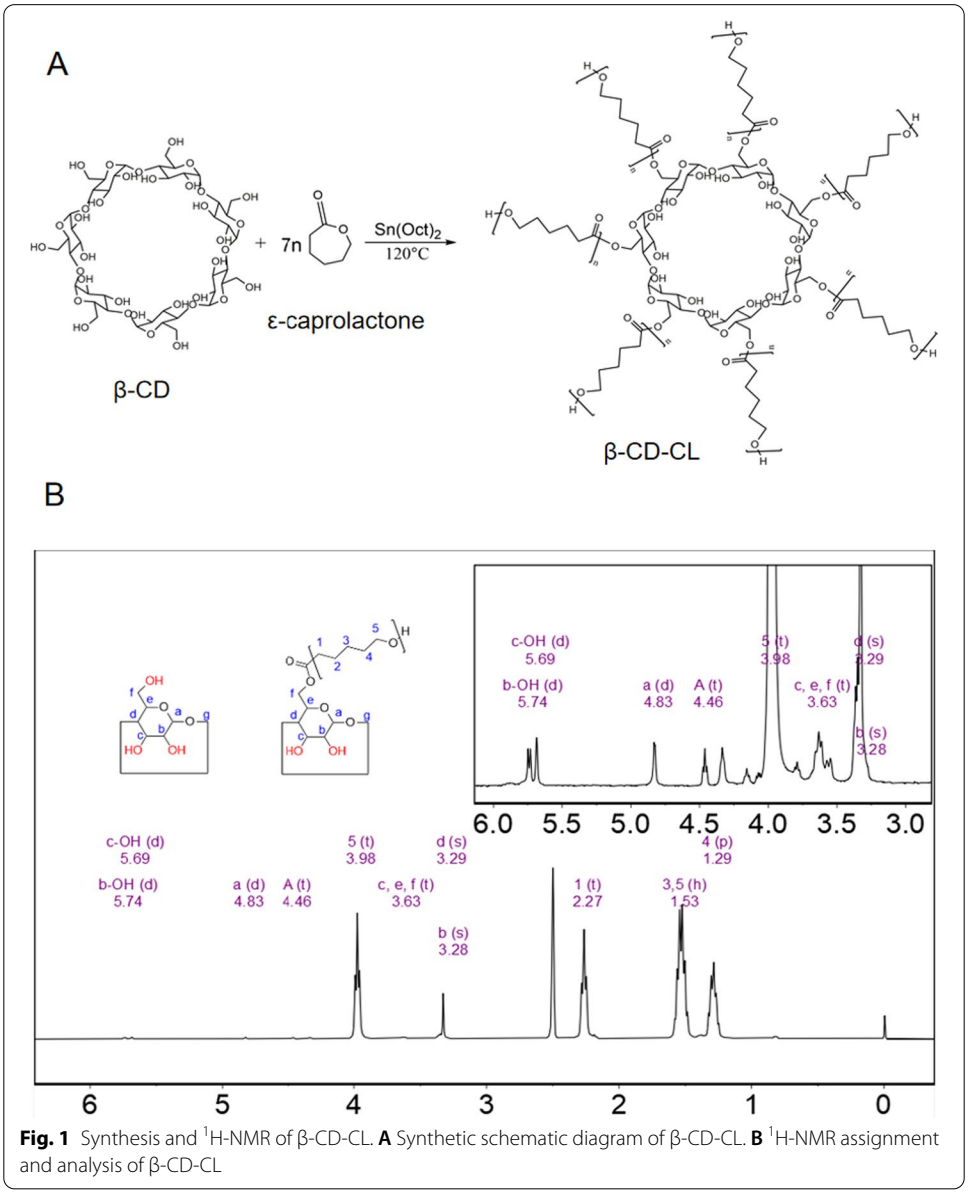


Fig. 1 Synthesis and ^{1}H-NMR of β-CD-CL. **A** Synthetic schematic diagram of β-CD-CL. **B** ^{1}H-NMR assignment and analysis of β-CD-CL

other signals. The target compound is mainly composed of β-cyclodextrin and polycaprolactone (PCL) fragments. Among these, the signals at 5.74 and 5.69 ppm correspond to the protons on the two hydroxyl groups facing inward on the cyclodextrin structure, and the signals at 4.83, 3.63, and 3.36 ppm correspond to the cyclodextrin structural formula contains protons on the methylene and methine groups, and the stronger signals at 3.98, 2.27, 1.53, and 1.29 ppm correspond to the protons on the methylene group in PCL (Fig. 1B). The peak corresponding to the target compound can be found in the NMR spectrum, indicating the formation of the product.

Synthesis schematic diagram of β-CD-CL-Scu-cRGD

This article reviews the synthesis process and delivery route of scutellarein carried by nanoparticles, and the targeting of cRGD peptides to inhibit tumor cell growth. Using β-CD-CL to load scutellarein improves stability and efficiency. Scutellarein is encapsulated in a niosome shell. The anti-tumor activity of scutellarein loaded with nanoparticles can effectively reduce the vitality, proliferation, migration, and invasion of human colon cancer. The targeted nanoparticles β-CD-CL-Scu-cRGD were synthesized by β-CD-CL according to the process in Fig. 2.

β-CD-CL polymer characterization of FT-IR

FT-IR was used to further verify the synthesis of β-CD-CL. The terminal hydroxyl (–O–H) of β-CD is 3392.5 cm^{-1}, the asymmetric stretching vibration peak (C–H) of the methylene group on β-CD is 2928.9 cm^{-1}, and the in-plane bending vibration peak (C–H) of the methylene group is 1417.4 cm^{-1}. The asymmetric stretching vibration peak of the (C–O–C) bond on β-CD is 1028.6 cm^{-1}. The infrared spectrum of β-CD-CL shows the ester group stretching vibration peak on β-CD-CL C=O is 1726.7 cm^{-1}, and the stretching vibration peaks of the methylene group in the structural unit are 2946.8 cm^{-1} and 2867.5 cm^{-1}. In addition, the hydroxyl stretching vibration peak (O–H) on β-CD-CL is 3439.9 cm^{-1}, and the intensity of the hydroxyl characteristic peak is greatly weakened

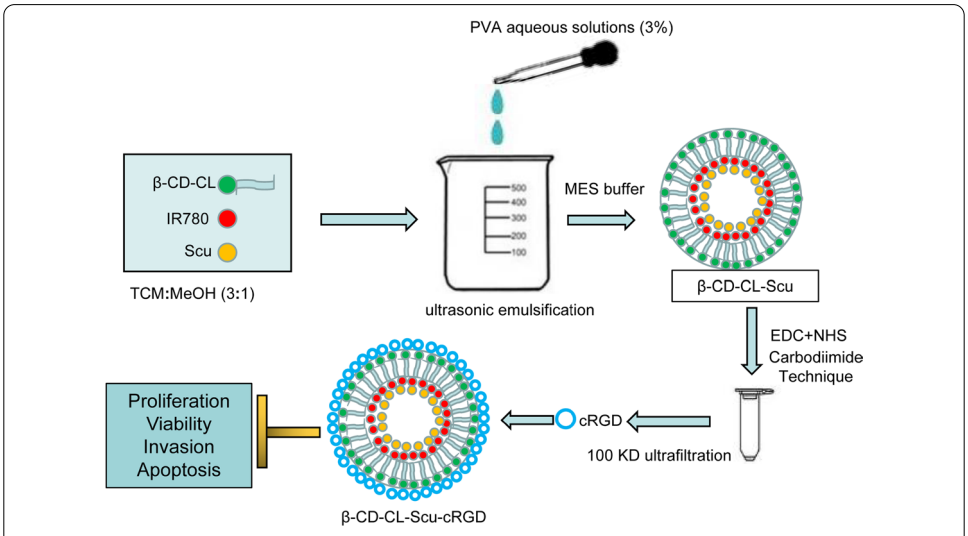


Fig. 2 Schematic diagram of β-CD-CL-Scu-cRGD synthesis

due to the substitution of the primary hydroxyl groups on β-CD. The FT-IR spectrum of the copolymer β-CD-CL has both characteristic peaks of β-CD and ε-CL. According to the results, it can be preliminarily judged that β-CD and ε-CL are successfully connected. After β-CD-CL was loaded with scutellarein, the above-mentioned characteristic peak remained unchanged, and the C=C peak of the benzene ring in scutellarein appeared at 1592.4 cm^{-1}. After coupling the cRGD cyclic peptide, the stretching vibration peak C=O of the amide bond is 1659.4 cm^{-1}, and the bending vibration absorption peak of the primary amine NH bond is 1551.9 cm^{-1}. This result indicated that the scutellarein load and the cRGD couple joint are successful (Fig. 3).

Gel permeation chromatography detection of β-CD-CL

The polydispersity of molecular weight is one of the basic characteristics of polymers, and the properties of polymers are closely related to their molecular weight and molecular weight distribution. The molecular weight of β-CD-CL copolymer is 10396 detected by Gel permeation chromatography, which is consistent with the molecular weight of the polymer (Fig. 4).

Physicochemical characteristics of β-CD-CL-Scu-cRGD

Transmission electron microscopy (TEM) was used to investigate the particle size of the β-CD-CL-Scu-cRGD. The PDI of β-CD-CL-Scu was 0.254, and Zeta Potential was − 19.3 mV. While the particle size of the β-CD-CL-Scu was 127.1 nm (Table 1). As showing in (Fig. 5A), the images revealed that the particles present a uniformly dispersed spherical shape with an obvious core–shell structure. According to the image of TEM the mean diameter of β-CD-CL-Scu-cRGD was obtained to be 140.2 nm. β-CD-CL-Scu-cRGD drug-loaded targeting microspheres Pdi was 0.21, and Zeta Potential was − 11.3 mV (Table 1). High-performance liquid chromatography was used to detect encapsulation efficiency and drug loading of β-CD-CL-Scu-cRGD. HPLC detects Scu to draw the standard curve as $Y = 2E^{-0.8X} - 0.00661$ ($R^2 = 0.995$). The cumulative release efficiency of Scu was shown in (Fig. 5B). The encapsulation efficiency (EE %) was 33.50% and the drug loading(DL%) was 20.22% (Table 1). To preliminarily evaluate the validity

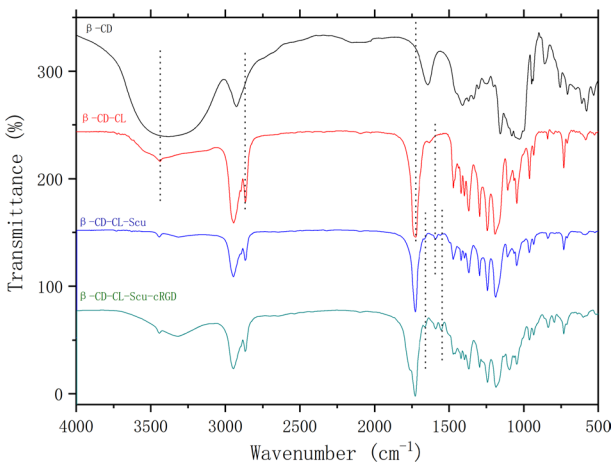
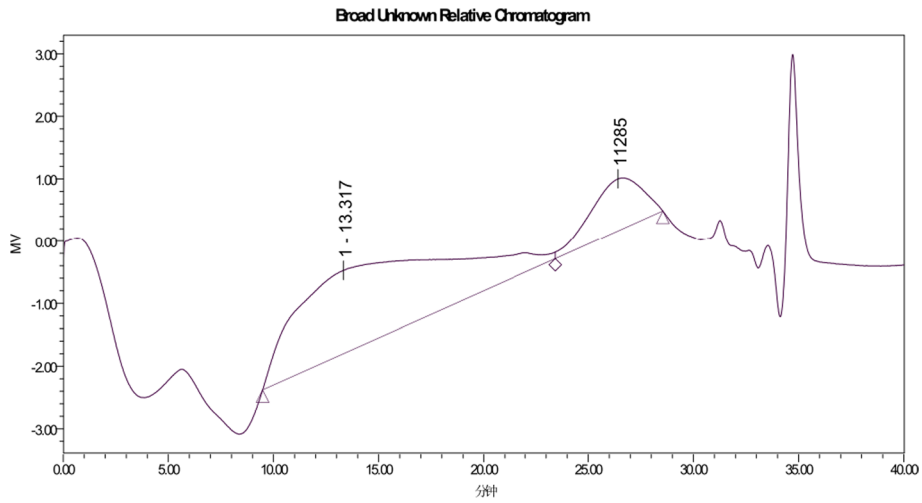


Fig. 3 Infrared spectrum analysis results of β-CD, β-CD-CL, β-CD-CL-Scu, and β-CD-CL-Scu-cRGD



Broad Unknown Relative Peak Table

	Distribution Name	Mn (Daltons)	Mw (Daltons)	MP (Daltons)	Mz (Daltons)	Mz+1 (Daltons)	Polydispersity	Mz/Mw	Mz+1/Mw
1		10396	14846	11285	20745	27084	1.428081	1.397314	1.824269

Fig. 4 β-CD-CL gel permeation chromatography test results

Table 1 Physicochemical characteristics of nanoparticle

Sample	Particle size (nm)	PDI	Zeta potentials (mV)	EE%	DL%
β-CD-CL-Scu-cRGD	140.2	0.21	− 11.3	33.50%	20.22%
β-CD-CL-Scu	127.1	0.254	− 19.3	–	–

of nanoparticles, the stability of β-CD-CL- Scu-cRGD in neutral conditions was studied. β-CD-CL-Scu-cRGD displayed excellent biostability in neutral conditions solution for 7 days (Fig. 5C). To verify the aggregation of nanoparticles in a solution containing fetal bovine serum, β-CD-CL-Scu-cRGD displayed excellent biostability in MCCOY'5A with 10% FBS for 7 days (Fig. 5D).

β-CD-CL-Scu-cRGD inhibited proliferation and promoted apoptosis in colon cancer cells

The result of flow cytometry showed that the targeted nanoparticle group (β-CD-CL-Scu-cRGD) showed strong fluorescence, indicating that the nanoparticles were bound and swallowed by LoVo cells after being stained by IR780. Non-target nanoparticle group (β-CD-CL-Scu), only weaker red fluorescence appeared with the cells, and the fluorescence intensity was significantly lower than that of the targeted group (Fig. 6A). To investigate the effect of β-CD-CL-Scu-cRGD on malignant biological behavior of colon cancer cells, MTT, colony formation and flow cytometry were applied to detect proliferation and apoptosis. MTT assay showed that the β-CD-CL-cRGD empty group had no cytotoxicity in LoVo cells. The IC_{50} of β-CD-CL-Scu-cRGD is (35.31 ± 1.32), the IC_{50} of Scu is (43.70 ± 3.21), and the IC_{50} of β-CD-CL-Scu (40.10 ± 2.41) (Fig. 6B). As shown in Fig. 6C, the β-CD-CL-Scu-cRGD can significantly inhibit the proliferation of

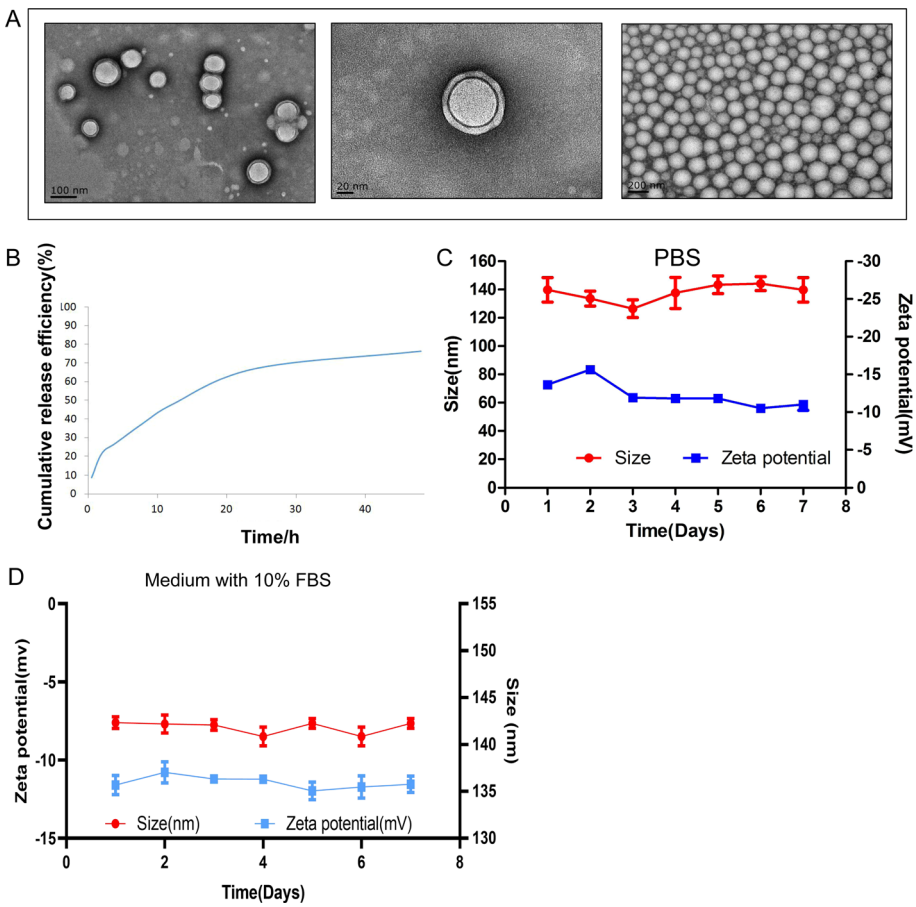


Fig. 5 Physicochemical characteristics and of nanoparticle. **A** The image of β-CD-CL-Scu-cRGD detected by TEM. **B** The Cumulative release curve of Scu. **C** 7-day stability test results of the complex under neutral conditions (PBS). **D** 7-day stability test results of the RPMI1640 with 10% FBS

colon cancer cells. As shown in Fig. 6D, Compared with 24 h, the cell viability of β-CD-CL-Scu-cRGD, Scu, and β-CD-CL-Scu at 48 h decreased significantly, while blank and β-CD-CL-cRGD were not significantly changed. Clonal formation assay is regarded as the gold standard for detecting tumor cell proliferation. The present study also revealed that β-CD-CL-Scu-cRGD can significantly decrease the clone efficiency of colon cancer cells (Fig. 6E, F). The flow cytometry results revealed that the apoptotic rate of colon cancer cells was significantly increased following β-CD-CL-Scu-cRGD treatment (Fig. 6G, H). These data indicated that the β-CD-CL-Scu-cRGD has a good anti-tumor effect in vitro.

β-CD-CL-Scu-cRGD inhibits the growth of LoVo in vivo

Based on our results demonstrating that β-CD-CL-Scu-cRGD inhibited proliferation and promoted apoptosis in vitro, we wished to determine whether this embellish compound attenuates the development of colon cancer in vivo. Colon cancer graft was induced by injecting LoVo cells into Balb/c nude mice. At 30 days following the injection of β-CD-CL-Scu-cRGD and Scu cells, the tumor-bearing mice were sacrificed by cervical dislocation and the solid tumors were removed and arranged with their volume measured and

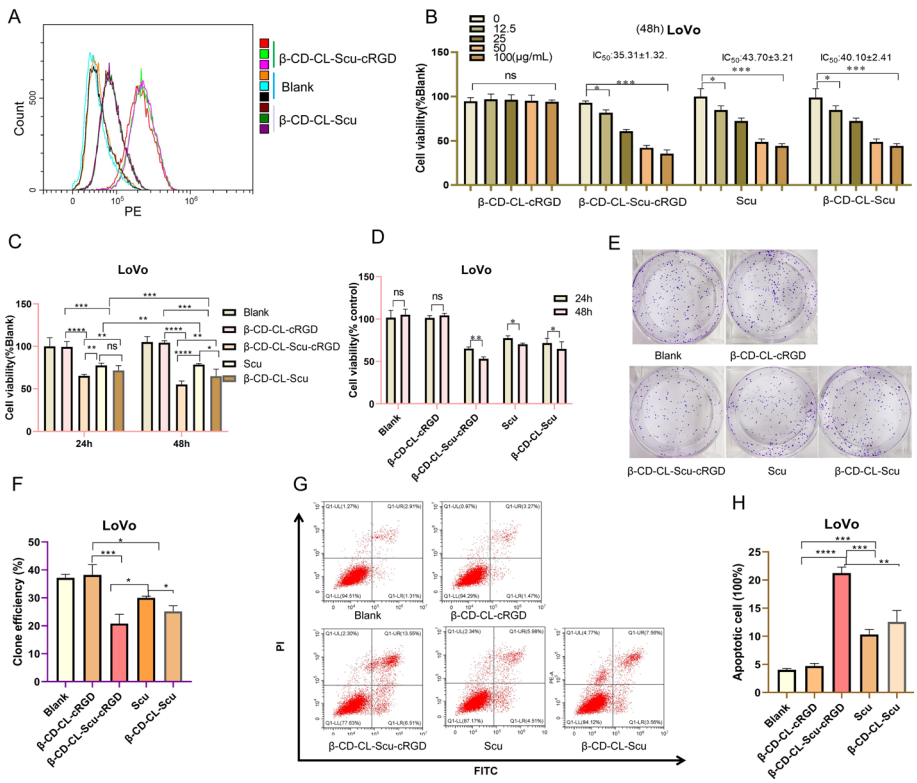


Fig. 6 Effects of β-CD-CL-Scu-cRGD on proliferation and apoptosis of colon cancer cells (LoVo). LoVo cells were treated with Scu (40 μg/mL) or β-CD-CL-Scu-cRGD (40 μg/mL). **A** The fluorescence intensity of nanoparticles in LoVo cells by flow cytometry. **B** IC_{50} of β-CD-CL-Scu-cRGD, β-CD-CL-cRGD and Scu to LoVo cells. **C** Cell proliferation was determined by MTT assay. **D** Cell viability of 24 h and 48 h. **E** Images of the cell colony assay showing stained colonies resulting from different treatments. **F** Statistics of cell colony assay. **G** Images of the cell apoptosis by flow cytometry. **H** Statistics of cell apoptosis. *p < 0.05, **p < 0.001, ***p and ****p < 0.0001

analyzed (Fig. 7A, B). As shown in Fig. 7C, all mice showed varying degrees of weight loss during the experiment, with the most pronounced decrease in the β-CD-CL-Scu-cRGD-treated group. The HE results showed that there was obvious mitosis in the control group, and the cells were in a state of obvious value-added. Necrotic tumor cells appeared in the Scu treatment group, while apoptotic tumor cells appeared in the β-CD-CL-Scu-cRGD treatment group (Fig. 7D). The results of immunohistochemistry showed that the expression levels of Ki67 and $\alpha_v\beta3$ in the control group were the highest, while the expression levels in the β-CD-CL-Scu-cRGD treatment group were the lowest. The levels of Ki67 and αvβ3 in the β-CD-CL-Scu group were greater than those in the Scu group but smaller than those of β-CD-CL-Scu-cRGD (Fig. 7D, E). These results indicate that the proliferation ability of LoVo cells is the weakest in β-CD-CL-Scu-cRGD, and β-CD-CL-Scu-cRGD can target the expression of $\alpha_v\beta3$.

Discussion

Natural products have always been the most productive source for drug development, especially as anticancer agents. Previous studies have demonstrated that scutellarein has anticancer properties (Thomford et al. 2018; Koehn and Carter 2005; Gao

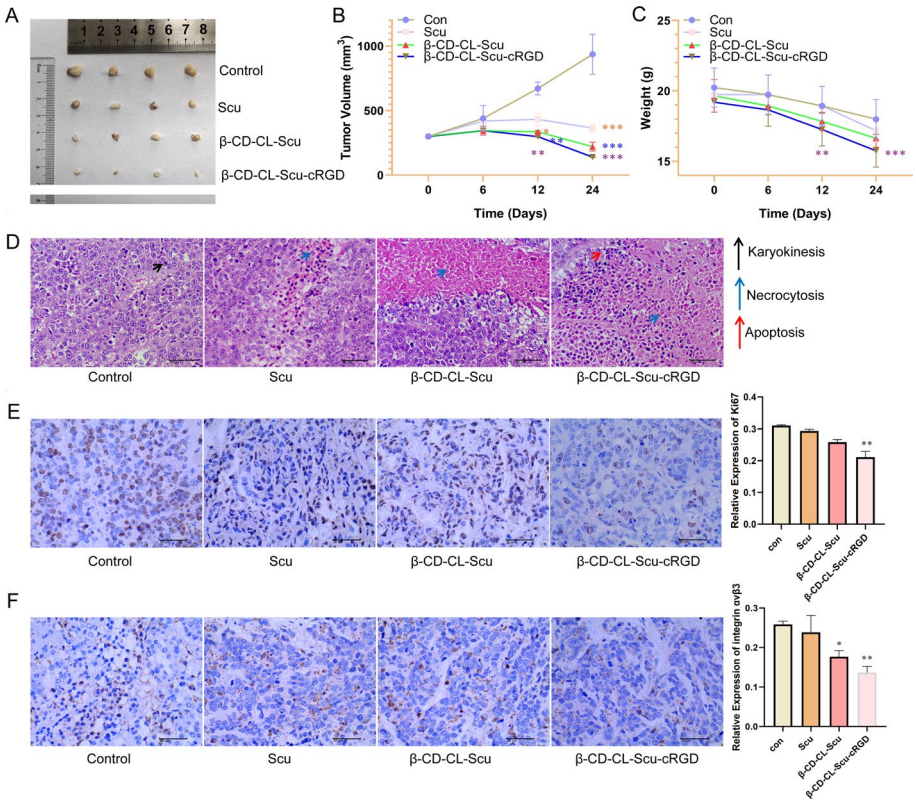


Fig. 7 β-CD-CL-Scu-cRGD inhibits cell proliferation and viability of LoVo. **A** Image of transplanted tumor. **B** Statistics of transplanted tumor volume. **C** Weight change of transplanted tumor mice. **D** HE staining of transplanted tumor. **E** Ki67 expression in transplanted tumor. **F** Integrin $\alpha_v\beta3$ expression in transplanted tumor. Karyokinesis: chromatin aggregation forming chromosome splitting in two. Necrocytosis: cell swelling and rupture, nuclear lysis, and eosinophilic staining of cytoplasm. Apoptosis: chromatin condensation, cell shrinkage, and fragmentation. Asterisk compared with con group, *p < 0.05, **p < 0.001, ***p < 0.0001

et al. 2008). However, the water solubility and bioavailability of scutellarein are low (Fu et al. 2011; Shi et al. 2015). Based on these observations, the present study was designed to investigate the ability of scutellarein loaded in nanoparticles to attenuate the development of human colon cancer by using in vitro model. Our results revealed that β-CD-CL-Scu-cRGD has a better potential to inhibit tumor formation of colon cancer cells than scutellarein.

In the past two decades, nanoparticle-based drug delivery systems have been developed to treat various diseases, including colon cancer (Pandey et al. 2016; Madni et al. 2017). Many types of nanoparticles, including polymer-based, lipid-based, viral, inorganic, and drug-conjugated, have been designed to target and destroy cancer cells (Pang et al. 2007; Alan 2008). β-CD-CL was appropriately designed for the delivery materials, and anti-tumor properties of scutellarein were significantly promoted when loaded in nanoparticles. In this study, the average diameter of β-CD-CL-Scu-cRGD was 140.2 nm, the zeta potential was − 11.3 mV, the drug encapsulation rate was 33.5%, and the drug load was 20.22%. It indicated that encapsulating scutellarein is effective. The physical and chemical properties of β-CD-CL-Scu-cRGD are a permeable, stable, and inexpensive component of scutellarein targeted delivery to cancer

cells. Compared with free Scutellarein, β-CD-CL-Scu-cRGD has a stronger cytotoxic effect on LoVo cells.

The RGD sequence is a highly conserved amino acid sequence shared by a variety of extracellular matrix components, which is a recognition site for the combination of integrins and extracellular matrix ligands or basement membrane components (Geertje et al. 2014). Integrin receptors are expressed on the surface of colon cancer (Zhou et al. 2011). Previous studies (Du et al. 2015) have shown that cRGD containing arginine-glycine-aspartate acid can be selectively taken up by cancer cells through receptor-mediated endocytosis. Nanoparticles carrying cRGD participate in the proliferation and apoptosis of LoVo cells. Therefore, the carbodiimide technique was used to form a stable peptide bond between the amino group of the cRGD side chain and the carboxyl group of β-CD-CL in this experiment. The cRGD was conjugated onto the surface of the nanoparticle to obtain β-CD-CL-Scu-cRGD. The connection method is relatively stable and is less affected by the environment inside and outside the body, and obtained nanoparticles have higher biological activity.

Previous studies have shown that Ki67 expression is closely related to tumor differentiation, invasion, and metastasis (Yerushalmi et al. 2010; Bubendorf et al. 2015; Dowsett et al. 2007). $\alpha_v\beta3$ can affect cell growth, migration, and adhesion during angiogenesis (Garanger et al. 2007). Integrin $\alpha_v\beta3$ is an ideal tumor treatment target, and its ligand RGD peptide can specifically bind to drug molecules and transport them to the targeted tumor site. Therefore, the application of the RGD sequence is not only anti-angiogenesis but also drug targeting (Li et al. 2007). The results of this experiment showed that β-CD-CL-Scu-cRGD significantly inhibited the growth of colon cancer transplantation tumors and suppressed the expression of Ki67 and αvβ3, all of which showed that β-CD-CL-Scu-cRGD could target and inhibit the growth of colon cancer.

Conclusion

The present study is the first to investigate the effect of a novel nanoparticle-loaded scutellarein on LoVo. Our findings revealed that β-CD-CL-Scu-cRGD more efficiently controls the viability, proliferation of LoVo than Scutellarein through targeting integrin receptors. Our data suggested that nano-niosome could be used as an ideal carrier to deliver scutellarein for possible therapeutic approaches to LoVo. However, these findings should be interpreted with caution. Further in vitro and in vivo investigations are warranted to confirm the anti-tumor effect of β-CD-CL-Scu-cRGD through targeting integrin receptors. Meanwhile, the lower encapsulation efficiency is mainly due to the poor solubility of scutellarein (chloroform, acetone, methanol, acetonitrile have been tried), compared to curcumin, paclitaxel, etc.

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Author's contributions
JW and TL finished the experiment. CY, SZ, XY and JL contributed to the acquisition of reagents and materials. YL supervised this project and assisted in the peer review response and the writing of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

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Competing interests
The authors declare that they have no competing interests.

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