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Structural analysis of a novel sulfated galacto-fuco-xylo-glucurono-mannan from *Sargassum fusiforme* and its anti-lung cancer activity

Weihua Jin [a], Hong Tang [a], Jinmei Zhang [a], Bin Wei [b], Jiadong Sun [c,d], Wenjing Zhang [e], Fuming Zhang [f], Hong Wang [b], Robert J. Linhardt [f,g,*], Weihong Zhong [a,**]

[a] College of Biotechnology and Bioengineering, Zhejiang University of Technology, Hangzhou 310014, China
[b] Collaborative Innovation Center of Yangtze River Delta Region Green Pharmaceuticals & College of Pharmaceutical Sciences, Zhejiang University of Technology, Hangzhou 310014, China
[c] Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI 02881, USA
[d] Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institutes of Health, Bethesda, MD 20878, USA
[e] Department of Endocrinology, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou 310016, China
[f] Department of Chemical and Biological Engineering, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY 12180, USA
[g] Department of Biological Science, Departments of Chemistry and Chemical Biology and Biomedical Engineering, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY 12180, USA

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ABSTRACT

Polysaccharide (HFSGF) was purified from *Sargassum fusiforme*. Autohydrolysis and gel column chromatography were performed to fractionate HFSGF into three components (HFSGF-S, HFSGF-L and HFSGF-H). Compositional analysis, mass spectrometry and nuclear magnetic resonance spectroscopy were used to elucidate the structural features of HFSGF. HFSGF-S was a mixture of sulfated galacto-fuco-oligomers, from the branches terminal ends; in HFSGF-L, the branches of HFSGF, was a sulfated galactofucan, containing a backbone of 1,3-linked α-L-fucan sulfated at C2/4 and/or C4 and interspersed with galactose (Gal); and in HFSGF-H, the backbone of HFSGF, was composed of alternating 1,2-linked α-D-mannose (Man) and 1,4-linked β-D-glucuronic acid (GlcA), branched with sulfated galactofucan or sulfated fucan, 1,3-linked α-L-fucan sulfated at C2/4 and/or C4 and partly interspersed with Gal. Some fucose (Fuc) residues were also partially branched with xylose (Xyl). The anti-lung cancer activities of HFSGF-L and HFSGF-H against human lung cancer A549 cells *in vitro* and A549 xenograft tumor growth *in vivo* were determined. HFSGF-H had higher activity *in vitro* (IC_{50} ~12 mg/mL for 24 h) and *in vivo* (tumor inhibition ~51%.) than HFSGF-L, indicating that HFSGF-H might be a leading compound for a potential new therapeutics for the treatment of lung cancer.

1. Introduction

Lung cancer is responsible for many deaths with approximately 631,000 people expected to die in China in 2015 according to the National Cancer Institute of China. In 2019, the number of Americans expected to die from lung and bronchus cancer is 142,670, nearly three-times the 51,020 deaths expected from colorectal cancer, the second most common cause of cancer death [1]. Lung cancer consists of two major types, small cell lung cancer and non-small cell lung cancer that accounts for about 80% of all types of lung cancer [2]. Therefore, there is an urgent need to discover compounds for the prevention and treatment of lung cancer.

Sargassum fusiforme grows in the temperate seaside areas of the northwest Pacific Ocean, including China, Japan and Korea [3]. It is considered a longevity food in Japan. It is also used as a Chinese herbal medicine and has been documented in "Shen Nong's Herbal and Compendium of Materia Medica". In addition, *Sargassum fusiforme* is used to treat cancer, as documented in "Wai Tai—Cui Shi Hai Zao Shan". Nowadays, many active compounds are derived from *Saragassum fusiforme*, including polysaccharides [3–7], phlorotannins [8], phytoserols [9], fucosterol [10], and other compounds [11–14]. Polysaccharides from *Sargassum fusiforme* possesses a variety of biological activities, including inhibition of HIV-1 infection [15,16], immunomodulatory activity [17–19], improvement of intestinal function [20,21], macrophage activation [22], neuroprotective activity [23], prevention of osteoarthritis [24], anti-aging [25], ameliorating learning memory deficiencies [26], anti-inflammatory and anti-diabetic effects [27,28], prevention of skin damage [29] and anti-tumor activity [7,30–36].

* Correspondence to: R.J. Linhardt, Department of Chemical and Biological Engineering, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY 12180, USA.
** Corresponding author.
E-mail addresses: linhar@rpi.edu (R.J. Linhardt), whzhong@zjut.edu.cn (W. Zhong).

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Table 1
Chemical compositions of the polysaccharides studied.

Samples	Yields (%)	Fuc (%)	UA (%)	SO_4 (%)	Total Sugar (%)	Mw (kDa)	Monosaccharides (molar ratio)				
							Man	GlcA	Gal	Xyl	Fuc
HFSGF	27.89	42.85	4.12	26.08	63.03	162.8	0.04	0.03	0.30	0.03	1
HFSGF-H	42.50	26.55	9.56	24.89	66.76	111.5	0.12	0.13	0.45	0.09	1
HFSGF-L	28.75	54.91	–	33.12	63.66	12.8	–	–	0.19	–	1

Crude polysaccharides from *Sargassum fusiforme* usually contain fucose (Fuc), galactose (Gal), mannose (Man), rhamnose (Rha), xylose (Xyl), glucose (Glc), and glucuronic acid (GlcA), accompanied with sulfate substitution. Li et al. [3], Wang et al. [37] and Cong et al. [35] reported the structural features of heteropolysaccharides and Hu et al. [26,38] showed two unusual backbones: 1): →4)-α-GalAp-(1 → 4)-α-Hexp-(1 → 4)-α-GalAp-(1 → 4)-α-Fucp-(1 → 4)-α-GalAp-(1 → and 2): →3)-β-L-Fucp-(1 → 3,4)-β-L-Fucp-(1 → 3,4)-β-L-Fucp-(1 → and connected in an alternating fashion with →3,4)-α-D-GlcAp-(1→, →4)-β-D-Xylp-(1→, →4)-α-D-Galp-(1→, →3,6)–α-D-Manp-(1→, where Hex is hexose and p is pyranose. Jin et al. [39] reported the structure features of sulfated galactofucans.

2. Materials and methods

2.1. Preparation of sulfated galactofucan (HFSGF) from Sargassum fusiforme

Crude polysaccharide (HF) was prepared according to the modification of a previous study [23]. HF (5 g) was then purified by anion exchange chromatography on a DEAE-Bio Gel Agarose FF gel (6 cm × 40 cm) eluted with water (5 L) (HFL), 0.5 M (5 L) (HFH) and 2 M NaCl (5 L) (HFSGF). The polysaccharides were then dialyzed, concentrated and precipitated with ethanol.

2.2. Depolymerization of HFSGF by autohydrolysis

Autohydrolysis was performed according to a modified method [39]. Briefly, HFSGF (0.8 g) was converted to the H^+-form using a cation exchange column and left for 72 h at room temperature. The mixture was neutralized with 5% NH_4OH solution in water. The solution was concentrated and precipitated by ethanol. Finally, after two fractionations the supernatant was named HFSGF-S and the precipitate was named HFSGF-C. HFSGF-S was desalted on a Sephadex G-10 column (4.5 × 40 cm) while HFSGF-C was fractionated on a Bio-Gel P-10 column (2.6 × 100 cm) eluted with 0.5 M NH_4HCO_3 into two fractions, HFSGF-H and HFSGF-L, and desalted on a Sephadex G-10 column (4.5 × 40 cm).

2.3. Compositional analysis

The molar ratio of monosaccharides and Fuc contents were determined as described by Zhang et al. [40]. The sulfated contents were determined by a modified method of Dodgson and Price [41]. Uronic acid (UA) concentration was determined by a modified carbazole method [42]. The molecular weights of the polysaccharides were evaluated by GPC-HPLC on TSK G3000 PWxl column (7 μm 7.8 × 300 mm) with elution in 0.05 M Na_2SO_4 at a flow rate of 0.5 mL min^{-1} at 40 °C with refractive index detection. Ten different molecular weight dextrans, purchased from the National Institute for the Control of Pharmaceutical and Biological Products (China), were used as weight standards. The total yields were calculated from the equation: product weight / raw material weight × 1000.

2.4. Mass spectral analysis of oligosaccharides

Electrospray ionization (ESI) – mass spectrometry (MS) and ESI chemically induced dissociation (CID) - MS/MS were performed on a LTQ ORBITRAR XL (Thermo Scientific). The samples were dissolved in CH_3CN-H_2O (1:1, *v*/v). The solution was centrifuged, and the supernatant was analyzed. Mass spectra were collected in the negative-ion mode at a flow rate of 5 μL min^{-1}. The capillary voltage was set to −3000 V, and the cone voltage was set at −50 V. The source temperature was 80 °C, and the desolvation temperature was 150 °C. The

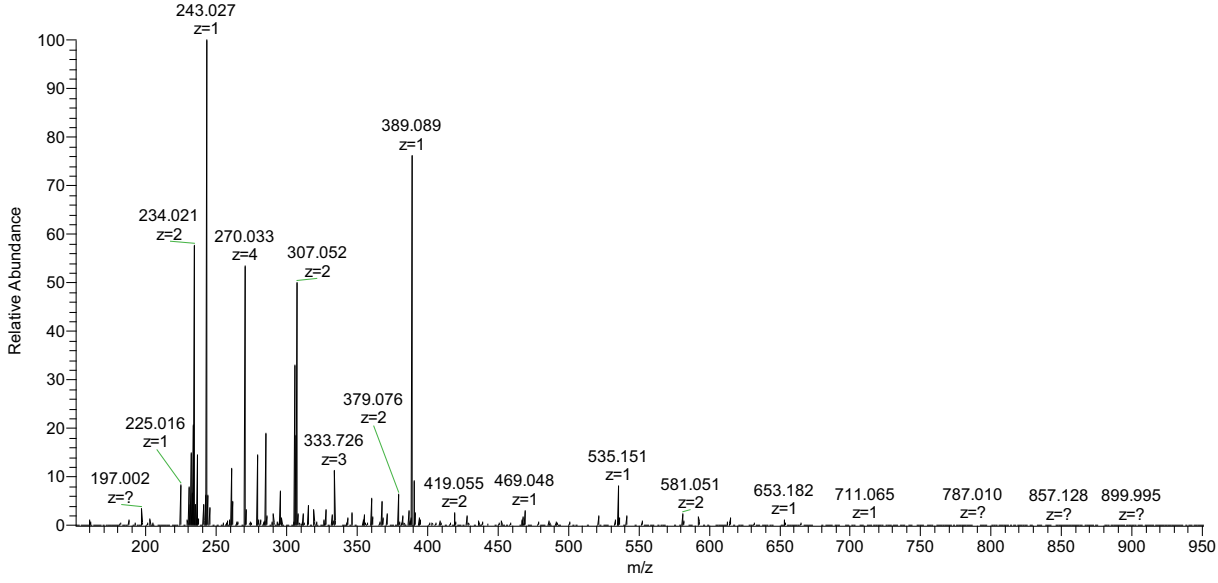

Fig. 1. Negative-ion mode ESI-MS spectrum of HFSGF-S.

collisional energy was optimized between 10 and 50 eV. All spectra were analyzed using Xcalibur.

2.5. Nuclear magnetic resonance (NMR) spectroscopy

Polysaccharides (50 mg) were co-evaporated with deuterium oxide (99.9%) twice before dissolving in deuterium oxide (99.9%) containing 0.1 μL deuterated acetone. NMR and two-dimensional spectra were recorded at a Bruker AVANCE III 600 MHz at 25 °C. The chemical shifts were adjusted to the internal standard (deuterated acetone, 2.05 and 29.92 ppm, respectively).

2.6. Anti-tumor activity

Anti-tumor activities of polysaccharides against human lung cancer A549 cells were determined. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT) assay was used to measure cell viability. Briefly, cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum and penicillin-streptomycin (100 units/mL) in an atmosphere of 5% CO_2 at 37 °C. The cells (100 μL) were then seeded in a 96-well plate at a density of 1×10^4 cells/well for 24 h. Subsequently, the cells were divided into the following three groups: (1) blank group, which only contained medium for 24 h and 48 h; (2) control group in which cells were added for 24 h and 48 h; and (3) experimental groups in which cells and polysaccharides at different concentrations (2, 4, 6, 8, 10 and 12 mg/mL) were cultivated in medium for 24 h and 48 h. Then, 10 μL of MTT (5 mg/mL) was added to each well. After 4 h of incubation, the supernatants were removed. And dimethyl sulfoxide (DMSO) (100 μL) was added. Next, the absorbance was measured at 570 nm, and the inhibition rate was determined using the following equation: Cell Inhibition rate (%) = (Ac − A1) / (Ac − A0) × 100, where A0 was the absorbance of the blank, A1 was the absorbance in the presence of samples, and Ac was the absorbance of the control.

2.7. Xenograft tumor model

Four-week-old male BALB/c-nu nude mice were purchased from Shanghai Sipu-Bikai Experimental Animal Co., LTD (Shanghai, China). All mice studies were approved by the Animal Ethics Committee of Zhejiang University of Technology Animal Center in accordance with the animal care and use guidelines. Tumors were established by giving subcutaneous injection of 2.5×10^6 (0.2 mL) A549 cells into the right flank of mice. When the tumor volume was approximately 100 mm^3, the mice were divided into the following three groups: (1) control group in which mice were administrated *i.p.* with normal saline; (2) experimental group (HFSGF-H) in which mice were administrated *i.p.* with HFSGF-H doses at 100 mg/kg (0.2 mL) every day; and (3) experimental group (HFSGF-L) in which mice were administrated *i.p.* with HFSGF-L doses at 100 mg/kg (0.2 mL) every day. Body weight and tumor volumes were measured every two days with a balance and with a vernier caliper, respectively. The tumor volume was calculated with the formula: $V = 1/2 \times \text{length} \times \text{width}^2$ and the relative tumor volume (RTV) was calculated with the formula: Vn/V0, where Vn is the tumor volume at the "n" day after administration and V0 is the tumor volume before administration. After three weeks of treatment, the mice were euthanized to excise tumors, heart, liver, spleen, lungs, kidney and brain. Cisplatin at 1.0 mg/kg every day was used as a treatment in the same way as a positive control. Tumor inhibition was calculated according to the following formula: [(mean tumor weight of control group - mean tumor weight of treated group) / mean tumor weight of control group] × 100. The organ coefficients for nude mice were calculated as follows: organ weight × 1000 / body weight.

3. Results and discussion

3.1. Chemical compositions of HFSGF, HFSGF-H and HFSGF-L

Polysaccharide (HFSGF) was obtained by anion exchange chromatography on a DEAE-Bio Gel Agarose FF gel (6 cm × 40 cm) with elution by 2 M NaCl. Autohydrolysis reaction is frequently used in the structural determination of polysaccharides [39,43–45]. Autohydrolysis reaction was performed to obtain the structures of HFSGF. Two fractions, HFSGF-S and HFSGF-C, were obtained. MS was performed to analyze HFSGF-S. HFSGF-C was fractionated on a Bio-Gel P-10 Gel column into two fractions, HFSGF-H and HFSGF-L. The chemical compositions of HFSGF and its derivatives are shown in Table 1. The molar ratio of monosaccharides of HFSGF was 0.04: 0.03: 0.30: 0.03: 1, Man: GlcA: Gal: Xyl: Fuc, indicating that it was sulfated galactofucan, accompanied with slightly glucuronomannan or branched with Xyl as suggested by previous studies [3,35,37,46]. After autohydrolysis HFSGF-C was converted into two fractions, one of high molecular weight (HFSGF-H) and one of low molecular weight (HFSGF-L). HFSGF-H had the same kind of monosaccharides as HFSGF and HFSGF-L containing only Gal and Fuc, suggesting that HFSGF-L was a relatively pure sulfated galactofucan and HFSGF-H might be sulfated galacto-fuco-xylo-glucuronomannan.

3.2. Structural analysis of HFSGF and its derivatives

The yield of HFSGF-S was 8.8%. And the molar ratio of monosaccharides of HFSGF-S was 0.20:1.00 (Gal: Fuc). MS was performed to analyze HFSGF-S (Fig. 1). HFSGF-S contained mono-sulfated fuco-oligomers (*m/z* 243.027 (−1), 389.089 (−1) and 535.151 (−1)), di-sulfated fuco-oligomers (234.021 (−2) and 307.052 (−2)), tri-sulfated fuco-oligomers (333.054 (−3)), di-, tri-, tetra- and penta-sulfated galacto-fuco-oligomer (315.050 (−2), 285.039 (−3), 270.033 (−4) and 261.230 (−5)). The proposed compositions of the ions are summarized in Table 2. Based on previous studies [39,47], the major components of HFSGF-S were suggested to be the branches or terminal ends of the backbone of HFSGF. Thus, we concluded that HFSGF might have branches or terminal ends, mainly with mono- and di- sulfated fuco-oligomers and tetra-sulfated galacto-fuco-oligomer, slightly with tri-sulfated fuco-oligomers and

Table 2
Proposed compositions of the ions of HFSGF-S.

m/z	Charge	Composition	m/z	Charge	Composition
234.021	2	$[Fuc_2(SO_3H)_2\text{-}2H]^{2-}$	307.052	2	$[Fuc_3(SO_3H)_2\text{-}2H]^{2-}$
243.027	1	$[Fuc(SO_3H)\text{-}H]^{-}$	315.050	2	$[GalFuc_2(SO_3H)_2\text{-}2H]^{2-}$
261.230	5	$[GalFuc_5(SO_3H)_5\text{-}5H]^{5-}$	333.054	3	$[Fuc_4(SO_3H)_5\text{-}3H]^{3-}$
270.033	4	$[GalFuc_4(SO_3H)_4\text{-}4H]^{4-}$	389.089	1	$[Fuc_2(SO_3H)\text{-}H]^{-}$
285.039	3	$[GalFuc_3(SO_3H)_3\text{-}3H]^{3-}$	535.151	1	$[Fuc_3(SO_3H)\text{-}H]^{-}$

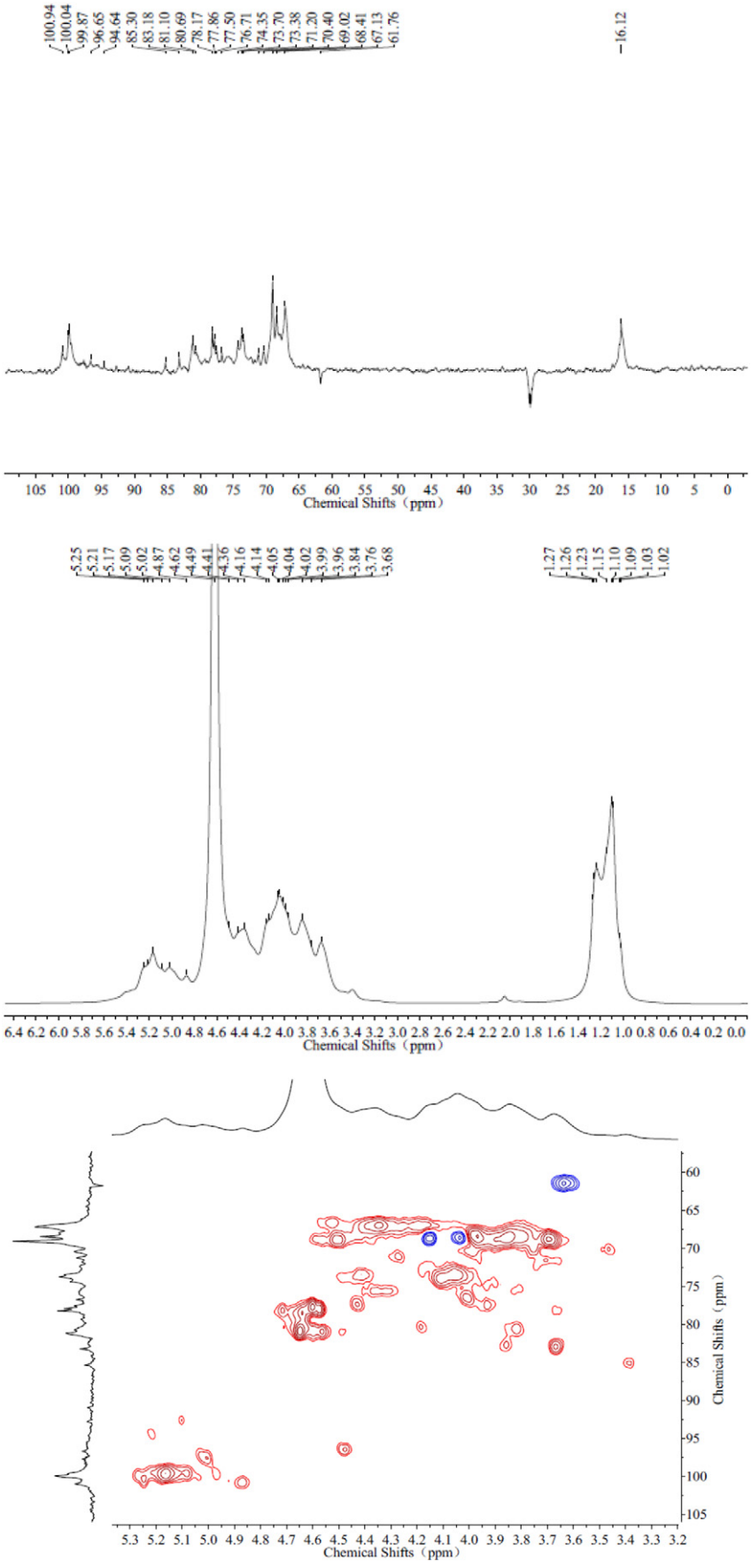

Fig. 2. The DEPTQ spectrum (upper panel), ^{1}H NMR spectrum (Middle) and HSQC spectrum (lower panel) of HFSGF-L.

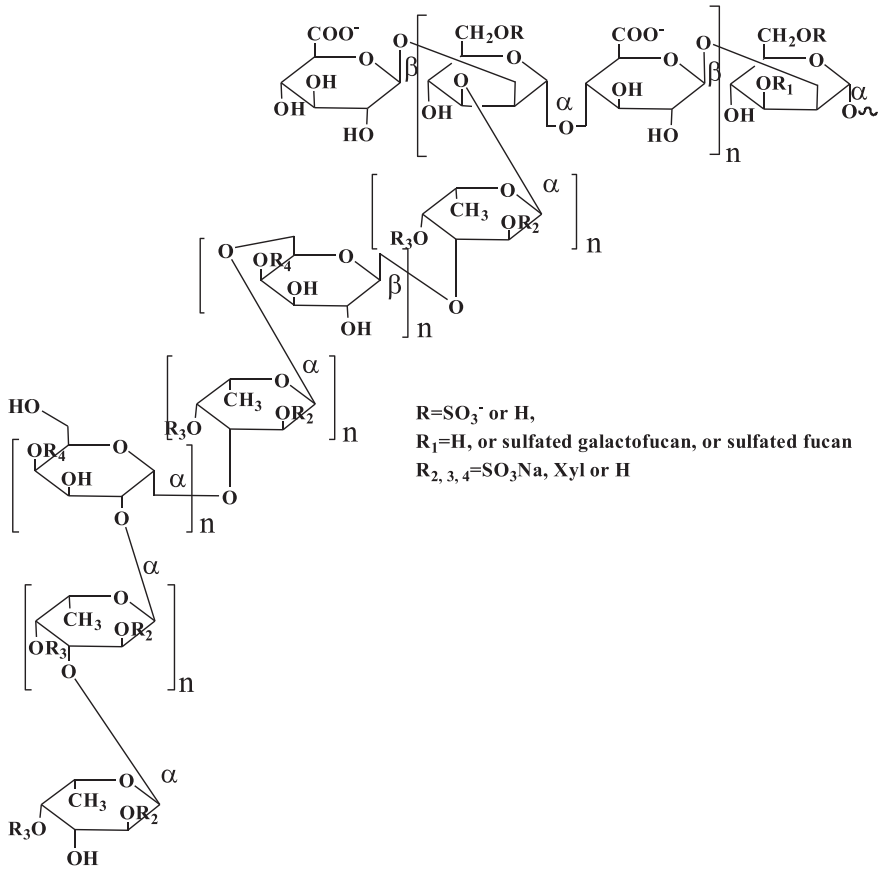

Fig. 3. The proposed structure scheme of HFSGF-H.

sulfated galacto-fuco-oligomers. The degree of polymerization (DP) of branches was no more than six.

HFSGF-C was fractionated into HFSGF-H and HFSGF-L on a Bio-Gel P-10 Gel column. The chemical analysis of HFSGF-H showed that it contained 26.6% Fuc, 24.9% sulfate content and 66.8% total sugar, while HFSGF-L had 54.9% Fuc, 33.1% sulfate content and 63.7% total sugar. The molecular weights of HFSGF-H and HFSGF-L were 111.5 kDa and 12.8 kDa (Fig. S1), respectively. The molar ratios of HFSGF-H and HFSGF-L were 0.12: 0.13: 0.45: 0.09: 1, Man: GlcA: Gal: Xyl: Fuc and 0.19:1, Gal: Fuc, respectively. From the above discussion, it was proposed that the unstable sulfate, the oligomers (HFSGF-S) and the low molecular weight polysaccharide (HFSGF-L) were split from HFSGF, leaving HFSGF-H. Thus, we concluded that HFSGF-H was the backbone of HFSGF while HFSGF-S and HFSGF-L could be the branches or the terminal ends of HFSGF.

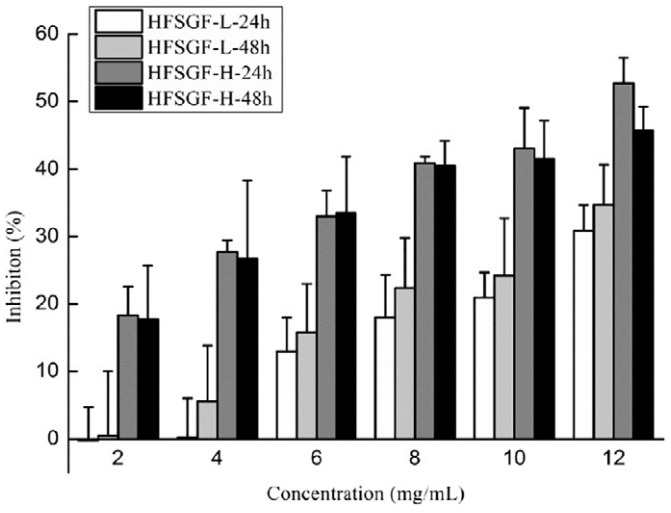

Fig. 4. Concentration-and time-dependent cytotoxic effects of HFSGF-H and HFSGF-L on A549 cells. Cells were cultured in 96-well plate and treated with different doses of HFSGF-H and HFSGF-L (2–12 mg/mL) for 24 and 48 h. The cell viability was analyzed by MTT assay. Data are presented as means ± SD of three independent experiments ($n = 3$).

The molar ratio in HFSGF-L of sulfate to (Gal+Fuc) was approximately 0.85 while the molar ratio of sulfate to Fuc was approximately 1.03, indicating that sulfate was mainly on Fuc. DEPTQ, ^{1}H NMR and two-dimensional NMR of HFSGF-L was performed to elucidate the structural feature, and the results are shown in Fig. 2. The spectra of HFSGF-L showed resonances with chemical shifts 99.87 (C-1)/5.02–5.17 (H-1), 68.41 (C-2)/3.96–4.04 (H-2), 73.38–76.71 (C-3)/4.05–4.16 (H-3), 81.10 (C-4)/4.47–4.62 (H-4), 67.13 (C-5)/4.36 (H-5) and 16.12(C-6)/1.10–1.27 (H-6) ppm that are characteristic of a 1,3-linked α-L-fucopyranose sulfated at C4 and weaker resonances with chemical shifts, 100.04 (C-1)/5.25 (H-1), 96.65 (C-1)/4.49 (H-1) and 94.64 (C-1)/5.21 (H-1), were assigned to 1,2-linked α-D-galactopyranose, 1,6-linked β-D-galactopyranose and 1,3-linked α-L-fucopyranose sulfated at C2 and C4, respectively. Therefore, we concluded that HFSGF-L contained a backbone of alternating 1,2-linked α-D-galactopyranose $(\alpha\text{-D-Gal})_n$ and 1,3-linked α-L-fucopyranose residues $(\alpha\text{-L-Fuc})_n$, sulfated mainly at C2 and C4 and/or C4 of fucopyranose and terminated by 1,6-linked β-D-galactopyranose at the reducing end.

Owing to the high molecular weight of HFSGF-H, the NMR spectra were complicated and unclear. HFSGF-H was degraded with dilute sulfuric acid [48] and glucuronomannan-oligomers were obtained. According to the previous studies [49,50], glucuronomannan was more stable than sulfated galactofucan under acidic conditions. Thus, we concluded

that the HFSGF-H backbone was glucuronomannan. The sulfated galactofucan might be branched at the C3 of Man residue based on previous studies [3]. Thus, we propose the structure of HFSGF-H in Fig. 3.

3.3. HFSGF-H and HFSGF-L inhibited the proliferation of A549 cells

A549 cells were exposed to increasing concentrations of HFSGF-H and HFSGF-L for 24 and 48 h, and cell viability was measured by MTT assay to evaluate the proliferation inhibition by HFSGF-H and HFSGF-L. HFSGF-H and HFSGF-L inhibited the growth of A549 cells in a dose-dependent manner (Fig. 4). HFSFG-L showed low inhibition activity (<10%) at the concentrations of <4 mg/mL. The inhibition rates of HFSGF-L reached about 30% after incubation for 24 h and 48 h at the concentration of 12 mg/mL, indicating that HFSGF-L showed lower cytotoxicity. Compared to HFSGF-L, HFSGF-F showed stronger inhibition. However, the inhibition rates of HFSGF-H were approximately 50% at the concentration of 12 mg/mL. The inhibition of HFSGF-H after incubation for 24 h and 48 h were similar.

3.4. HFSGF-H and HFSGF-L attenuated A549 xenograft tumor growth in vivo

We evaluated its medicinal effects in BALB/c-nu mice to determine whether HFSGF-H and HFSGF-L could suppress the growth of lung tumor *in vivo*. Cisplatin was used as positive control for the *in vivo* assay (Fig. S2). After 21 days treatment, the relative tumor volume (RTV) data in Fig. 5A suggested HFSGF-H and HFSGF-L significantly attenuated A549 xenograft tumors compared with the negative control group. The tumor inhibition rates, of HFSGF-L, HFSGF-H and cisplatin were 22.7%, 50.61% and 53.26%, respectively, suggest that HFSGF-H behaves similarly to cisplatin (Fig. 5B, C, S2). There were no apparent differences in the body weights among the HFSGF-H and HFSGF-L groups, indicating that HFSGF-H and HFSGF-L were potent and well tolerated medicines (Fig. 5D). Table 3 shows the organ coefficients of the nude mice. Compared to the control group, the organ coefficient of spleen in HFSGF-H group increased compared with the control group. In addition, the organ coefficient of kidney in HFSGF-H group increased significantly.

Polysaccharides from *Sargassum fusiforme* are a family of sulfated heteropolysaccharides, mainly consisting of Fuc, Gal, Man, Xyl, Glc and GlcA. It has been reported [32] that the tumor inhibition rates on nasopharyngeal carcinoma (NPC) of crude polysaccharide (SFPS) from *Sargassum fusiforme* at the concentrations of 50 mg/kg, 100 mg/kg and 200 mg/kg are 18%, 28% and 43%, respectively. In addition, SFPS can inhibit human hepatocellular carcinoma HepG2 cells at the concentrations of 100 mg/kg, 200 mg/kg and 400 mg/kg with the inhibition rates 19%, 31% and 45%, respectively [7]. Moreover,

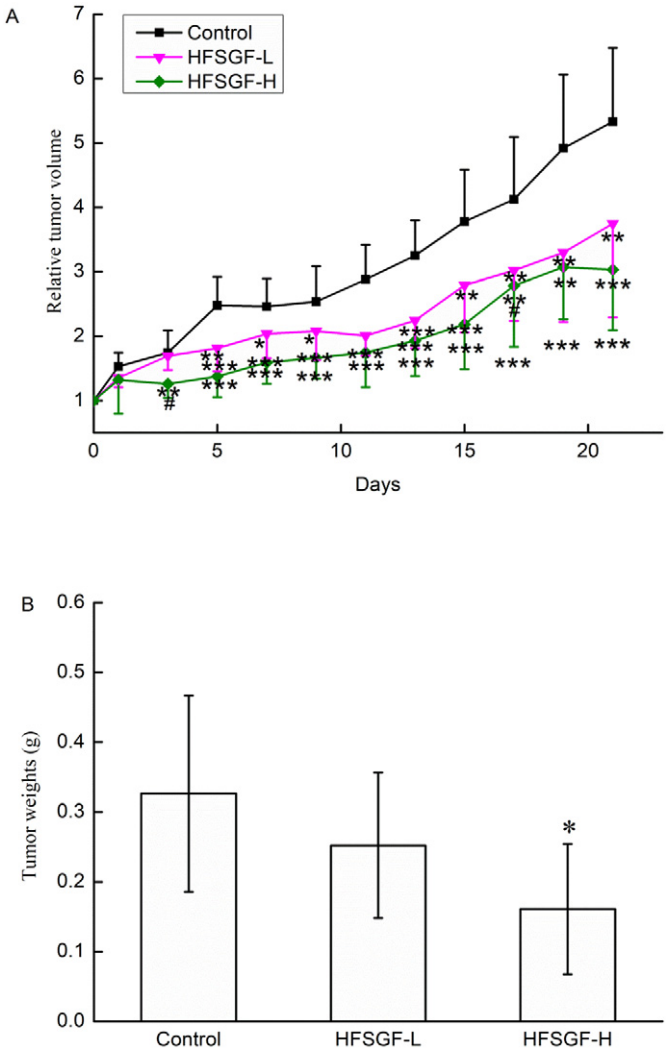

Fig. 5. Attenuation of A549 cell xenograft tumor growth in BALB/c-nude mice by HFSGF-H and HFSGF-L. (A) The relative tumor volume (RTV) of each group. $^{**}p < 0.01$, $^{***}p < 0.001$ *versus* Control. (B) Tumor weight of each group. $^{*}p < 0.05$ *versus* Control. (C) Representative images of the A549 xenograft tumors from each group at day 21. (D) The effects on the body weights of treated mice. $^{*}p < 0.05$ *versus* Control.

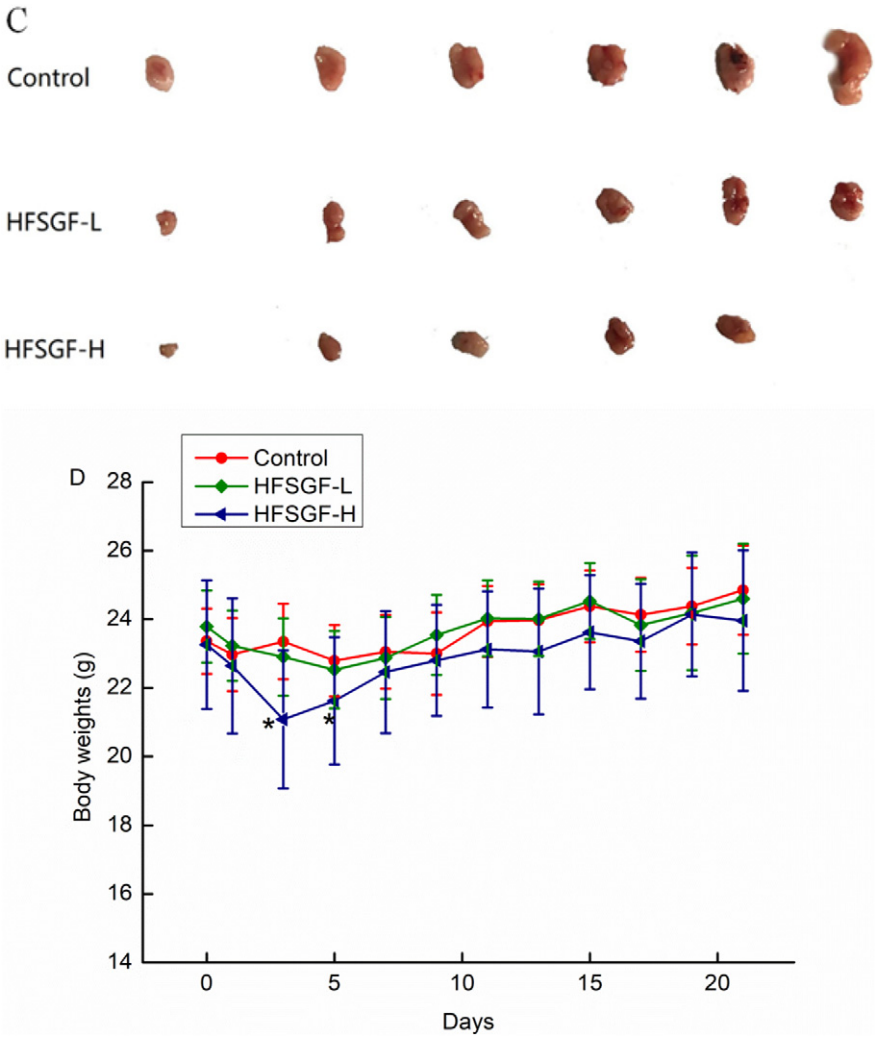

Fig. 5 (*continued*).

SFPS also can inhibit the proliferation of SPC-A-1 cells at the concentrations of 20 mg/kg and 40 mg/kg [36]. All these polysaccharides were crude polysaccharides. Therefore, isolation and purification of the polysaccharides were performed to determine their active constituents. Anion exchange chromatography was performed tofractionate polysaccharides into laminaran, sulfated heteropolysaccharides and sulfated galactofucan [51]. In the present study, a novel polysaccharide (HFSGF) was purified and characterized as sulfated galacto-fuco-xylo-glucuronomannan. After autohydrolysis, HFSGF was fractionated into three fractions, HFSGF-S was sulfated galacto-fuco-oligomers, derived from the branches or terminal ends; HFSGF-L, the branches of HFSGF, was a sulfated galactofucan, containing a backbone of 1,3-linked α-L-fucan sulfated mainly at C4 and interspersed with galactose (the linkages might be 1,2-linked and 1,6-linked); and HFSGF-H had the backbone of glucuronomannan, composed of alternating 1,2-linked α-D-Man and 1,4-linked β-D-GlcA, the branches of sulfated galactofucan or sulfated fucan that was 1,3-linked α-L-fucan sulfated mainly at C4 and partly interspersed with Gal. In addition, some Fuc residues might be partially branched with Xyl [3,35,37,46]. And the anti-lung cancer activities of HFSGF-H and HFSGF-L were determined *in vitro* and *in vivo*. HFSGF-H had higher activity in cells experiments than HFSGF-L, confirming by the results on A549 xenograft tumor model. Particularly interesting was that HFSGF-H behaved similarly to cisplatin. The organ coefficients of spleen in HFSGF-H group increased, compared to the control group, suggesting that HFSGF-H might have immunomodulatory activities [7,30–32].

Table 3
Organ coefficients of nude mice.

Organ	Control	HFSGF-L	HFSGF-H
Heart	6.2 ± 0.7	6.0 ± 0.8	5.9 ± 0.9
Liver	66.3 ± 3.6	64.9 ± 4.2	70.9 ± 4.5
Spleen	4.9 ± 0.8	4.7 ± 0.7	6.2 ± 0.4*
Lungs	6.7 ± 1.2	6.8 ± 1.5	6.4 ± 0.7
Kidney	17.7 ± 1.5	18.6 ± 1.4	19.3 ± 0.8*
Brain	17.4 ± 1.0	17.5 ± 1.9	17.5 ± 1.4

* $p < 0.05$ *versus* Control.

4. Conclusion

A novel polysaccharide (HFSGF) was purified from *Sargassum fusiforme*. After autohydrolysis and gel column chromatography,

HFSGF was fractionated into three fractions, HFSGF-S, HFSGF-L and HFSGF. It was interesting to note that HFSGF-S was a mixture of sulfated galacto-fuco-oligomers. HFSGF-L, as a sulfated galactofucan, contained a backbone of 1,3-linked α-L-fucan sulfated mainly at C4 and interspersed with galactose (the linkages might be 1,2-linked and 1,6-linked). HFSGF-H, as a sulfated galacto-fuco-xylo-glucuronomannan, contained a backbone of alternating 1,2-linked α-D-Man and 1,4-linked β-D-GlcA, branched mainly with sulfated galactofucan or sulfated fucan. HFSGH-H showed higher anti-lung cancer activity *in vitro* and *in vivo* than HFSGF-L. Therefore, it was suggested that HFSGF-H might be a leading compound for the potential new therapeutics for the treatment of lung cancer.

CRediT authorship contribution statement

Weihua Jin: Conceptualization, Methodology, Data curation, Formal analysis, Funding acquisition, Resources, Writing - original draft, Project administration. **Hong Tang:** Conceptualization, Methodology, Investigation, Formal analysis. **Jinmei Zhang:** Conceptualization, Methodology, Visualization. **Bin Wei:** Methodology, Writing - review & editing. **Jiadong Sun:** Methodology, Writing - review & editing. **Wenjing Zhang:** Methodology, Writing - original draft, Writing - review & editing. **Fuming Zhang:** Methodology, Writing - review & editing. **Hong Wang:** Writing - review & editing, Funding acquisition. **Robert J. Linhardt:** Writing - review & editing. **Weihong Zhong:** Writing - review & editing, Supervision.

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Declaration of competing interest

The authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2020.01.275.

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