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Research paper

Structure and anticancer activity of native and modified polysaccharides from brown alga *Dictyota dichotoma*

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ABSTRACT

The laminaran DdL and fucoidan DdF were obtained from the brown alga *Dictyota dichotoma*. DdF was a sulfated (28.9%) and acetylated heteropolysaccharide containing fucose, galactose, mannose and glucose (57.9, 20.4, 12.4 and 9.2 mol%, respectively). DdL was a 1,3;1,6-β-D-glucan with the main chain built from 1,3-linked glucose residues and single glucose residue in branches at C6 (one branch on three glucose residues of the main chain). Sulfated (43.7%) laminaran DdLs was obtained from DdL by sulfation. It was determined that sulfates occur at C2, C4 and C6 of glucose residues. The anticancer effect of DdF, DdL, and DdLs (200 μg/mL) was studied *in vitro* on colon cancer cells HCT-116, HT-29, and DLD-1. The effect of polysaccharides (40 μg/mL) on colony formation of DLD-1 cancer cells after irradiation (4 Gy) was investigated first. All polysaccharides showed a synergistic effect with X-ray irradiation against cancer cells, decreasing the amount and size of cancer cells colonies.

1. Introduction

Fucoidans are the sulfated algal polysaccharides, which exhibit a wide spectrum of biological activitity, including immunomodulatory, anticoagulant, anticancer, radioprotective and antiviral properties (Ale, Mikkelsen, & Meyer, 2011; Kusaykin et al., 2008). Additionally, fucoidans are non-toxic to organisms and have no side effects. Substances possessing these properties can be used in combined cancer therapy, which is comprised of surgical, radiation and medicinal treatment. There are only a few studies devoted to the radioprotective activity of fucoidans (Byon et al., 2008; Lee et al., 2008; Lee, Bae, Cho, & Rhee, 2009; Qiong et al., 2011), although these polysaccharides are surely prospective non-toxic radioprotectors.

The brown algae *Dictyota dichotoma* belongs to the family Dictyotaceae (order Dictyotales). The algae from this order – *Canistrocarpus cervicornis* (Camara et al., 2011), *Dictyopteris delicatula* (Magalhaes et al., 2011), *D. plagiogramma* (Percival, Rahman, & Weigel, 1981), *D. polypodioides* (Sokolova, Ermakova, Awada, Zvyagintseva, & Kanaan, 2011), *Dictyota dichotoma* (Abdel-Fattah, Hussein, & Fouad, 1978; Hussein, Fouad, & Abdel-Fattah, 1979; Rabanal, Ponce, Navarro, Gomez, & Stortz, 2014), *D. menstrualis* (Albuquerque et al., 2004), *Lobophora variegata* (Medeiros et al., 2008), *Padina gymnospora* (Silva et al., 2005), *P. pavonica* (Hussein, Abdel-Aziz, & Salem, 1980; Men'shova et al., 2012), *P. tetrastromatica* (Karmakar et al., 2009; Rao, Sastry, & Rao, 1984), *Spatoglossum schroederi* (Leite et al., 1998; Rocha et al., 2005), *Stoechospermum marginatum* (Adhikari et al., 2006) – produce predominantly heterogeneous fucoidans. Polysaccharides from *D. dichotoma* have been obtained and investigated by some research groups (Abdel-Fattah, Hussein, & Fouad, 1978; Hussein, Fouad, & Abdel-Fattah, 1979; Rabanal, Ponce, Navarro, Gomez, & Stortz, 2014). It was shown that obtained fucoidans are sulfated heteropolysaccharides. The system of fucoidans (more than 60 fractions), containing fucose, galactose, mannose, xylose, glucose, rhamnose, arabinose and uronic acid residues, was obtained and investigated in study (Rabanal, Ponce, Navarro, Gomez, & Stortz, 2014).

Although fucoidans from this alga have been characterized, there are no data on the isolation and structural characterisation of other algal polysaccharides – laminarans in the literature sources. The laminarans (1,3;1,6-β-D-glucans) are also of interest due to their biological effects and non-toxicity. Earlier, we established that chemical and enzymatic modification of laminarans can improve the biological activity of derivatives in comparison with native polysaccharides (Elyakova et al., 2007; Malyarenko et al., 2017; Menshova et al., 2014; Zvyagintseva, Elyakova, & Isakov, 1995;).

Thus, the aim of this work was to investigate the structural

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characteristics of polysaccharides from *Dictyota dichotoma*, to modify the native laminaran, and to study the anticancer and radioprotective activities of fucoidan, laminaran and its sulfated derivative.

2. Experimental

2.1. Materials

Organic solvents, inorganic acids and salts, sodium hydroxide and trifluoroacetic acid (TFA) were commercial products (Laverna-Lab, Moscow, Russia). Standards (mannose, rhamnose, glucose, galactose, xylose and dextrans) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Sorbents for chromatography were Polychrome-1 (Reakhim, Moscow, Russia), DEAE-cellulose Sigma–Aldrich (St. Louis, MO, USA), Macro-Prep DEAE (Bio-Rad Laboratories, Hercules, CA, USA) and Amberlite CG-120 (Serva Electrophoresis GmbH, Heidelberg, Germany).

Basal Medium Eagle (BME), McCoy's 5A Modified Medium (McCoy's 5A), RPMI-1640 Medium, trypsin, fetal bovine serum (FBS) and agar were purchased from Gibco/Life Technologies (Carlsbad, CA, USA). MTS reagent – 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide was purchased from Promega Corporation (Madison, WI, USA). Phosphate buffered saline (PBS), L-glutamine and penicillin–-streptomycin solution (10000 U/mL, 10 μg/mL) were from Sigma–Aldrich (St. Louis, MO, USA).

Human colorectal adenocarcinoma HCT-116 (ATCC® no. CCL-247™), HT-29 (ATCC® no. HTB-38™), DLD-1 (ATCC® no. CCL-221™) cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA).

A sample of the algae *Dictyota dichotoma* (Dd) was collected from Peter the Great Bay in August 2014, Sea of Japan (Russia). Fresh algal biomass (100 g) was powdered and pre-treated with 70% aqueous ethanol (w/v = 1:10) at room temperature for 10 days. Defatted alga was air-dried.

2.2. Instruments

NMR spectra were obtained on an Avance DPX-500 NMR spectrometer (Bruker BioSpin Corporation, Billerica, MA, USA) resonating at 75.5 MHz at 35 and 60 °C. The sample concentration was 15 mg of polysaccharide/mL of D_2O for 1D and 2D experiments.

2.3. General methods

2.3.1. Analytical procedures

Total carbohydrates were quantified by the phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Monosaccharide composition was determined by HPLC with an ISA-07/S2504 column (0.4 × 25 cm, Shimadzu, Kyoto, Japan), a bicinchoninate assay and a C-R2 AX integrating system (Shimadzu, Kyoto, Japan) after hydrolysis with 2 M trifluoroacetic acid (6 h, 100 °C). Monosaccharides (rhamnose, ribose, mannose, fucose, galactose, xylose, and glucose) were used as standards for HPLC. The protein and polyphenol contents were determined using the Bradford method (Bradford, 1976) and a modification of the Folin–Ciocalteau method (Singleton & Rossi, 1965), respectively. The amount of sulfate groups was determined by using the $BaCl_2$ gelatin method (Dodgson, 1961).

2.3.2. Molecular weight determination

Samples of polysaccharides were analyzed using a high-performance liquid chromatography instrument (Agilent 1100 Series, Germany) equipped with a refractive index detector and a series-connected gel-filtration column (TSK gel G4000 SW and TSK gel G2000 SW, Tosoh Co., Tokyo, Japan). Elution was performed with a 0.05 M aqueous solution of Na_2SO_4 at 50 °C with a flow rate of 0.5 mL/min. The molecular weights of polysaccharides were estimated using dextrans of molecular weights 5, 6, 10, 40, 70, 100, 200 and 450–650 kDa as reference standards.

2.3.3. Polysaccharide extraction

Samples of defatted, dried and powdered algal fronds (100 g) were extracted twice with 0.1 M HCl (2 L) for 2 h at 60 °C. The extracts were combined, centrifuged, dialyzed, concentrated on a rotary evaporator (2.5 h, 45 °C) and lyophilized to obtain the polysaccharide fraction DdP (8.4 g).

2.3.4. Anion-exchange chromatography of polysaccharides on DEAE-cellulose

A solution of polysaccharide DdP in 50 mL of 0.1 M HCl was applied to a DEAE-cellulose column (Cl^- form, 21 × 3 cm) equilibrated with 0.1 M HCl. The laminaran-containing fraction was eluted with water, neutralized and concentrated on rotary evaporator (40 min, 45 °C). Then, the column was successively washed with 1 and 2 M NaCl solutions until the disappearance in the eluate of a positive reaction for carbohydrates by the phenol-sulfuric acid method (Dubois et al., 1956) in each case. The fraction containing fucoidan was eluted with 2 M NaCl, then concentrated on rotary an evaporator (40 min, 45 °C), dialyzed and lyophilized with a yield of 1.1 g.

2.3.5. Hydrophobic chromatography of laminaran on Polychrome-1

The solution of laminaran was applied to a Polychrome-1 column (15 × 4.5 cm). The column was subsequently washed with water and 5% aqueous ethanol. Then, the pure laminaran (L) DdL was eluted with 15% aqueous ethanol until the disappearance in the eluent of a positive reaction for carbohydrates by the phenol-sulfuric acid method (Dubois et al., 1956). The eluate was concentrated on a rotary evaporator (40 min, 45 °C) and lyophilized. The yield of the laminaran fraction DdL was 1.3 g.

2.3.6. Removal of polyphenols from the fucoidan fraction

A sample of the fucoidan fraction was dissolved in water (100 mL), to which was added 30% aqueous H_2O_2 (20 mL), and then 10% aqueous NH_3 until to the solution reached pH 8.5. The resulting mixture was kept at room temperature for 17 h in the dark. Then, the solution was centrifuged, and the supernatant was dialyzed, concentrated on a rotary evaporator (40 min, 45 °C) and lyophilized to obtain the pure fucoidan fraction DdF with a yield of 739 mg.

2.3.7. Anion-exchange chromatography of fucoidan on Macro-Prep DEAE

A solution of fucoidan in 0.1 M NaCl (0.5 g in 10 mL) was applied to a Macro-Prep DEAE (Bio-Rad, USA) column (Cl^- form, 2.5 × 9 cm) equilibrated with 0.1 M NaCl. Then the column was successively eluted with a linear gradient of NaCl (from 0.1 to 2 M). The eluate was analyzed by the phenol-sulfuric acid method (Dubois et al., 1956). The obtained fraction of fucoidan DdF was concentrated on a rotary evaporator (40 min, 45 °C), dialyzed and lyophilized with a yield of 415 mg.

2.3.8. Sulfation of laminaran

Pyridine (30 mL) in a bottle was cooled in an ice bath, and chlorosulfonic acid (10 mL) was slowly dropped into the pyridine solution and incubated for 1 h to form a mixture. The laminaran DdL (1 g) was dissolved in 100 mL of dimethylformamide, and the resulting solution was added to the mixture. Then, the bottle with reagents was kept in a hot water bath at 75 °C for 1.5 h. Subsequently, the solution was cooled, resolved in water (250 mL) and neutralized with NaOH. The obtained sulfated (s) laminaran DdLs was precipitated by 96% ethanol and centrifuged. The precipitate was dissolved in water, dialyzed and lyophilized with a yield of 377 mg.

2.4. Biological assays

2.4.1. Cell culture

Human colorectal adenocarcinoma cells HCT-116 and HT-29 were cultured in McCoy's 5A medium; DLD-1 cells were maintained in RPMI-1640 medium. Culture media were supplemented with 10% FBS and penicillin–streptomycin solution. The cell cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO_2.

2.4.2. Soft agar assay

Cells (8.0×10^3) were seeded in 6-well plate and treated with the polysaccharides (laminarans, their sulfated derivative and fucoidans) (40 or 200 μg/mL) in 1 mL of 0.3% BME agar containing 10% FBS, 2 mM L-glutamine and 25 μg/mL gentamicin. The cultures were maintained at 37 °C in a 5% CO_2 incubator for 14 days, and the cell colonies were scored using a microscope Motic AE 20 (Xiamen, China) and the Motic Image Plus computer program.

2.4.3. Radiation exposure

Irradiation was deliver at room temperature using single doses from the X-ray system XPERT 80 (KUB Technologies, Inc, Milford, CT, USA). The applied doses were from 4 to 8 or 4 Gy for the colony formation assay. The absorbed dose was measured using an X-ray radiation clinical dosimeter DRK-1 (Axelbant LLK, Moscow, Russia).

2.4.4. Cell irradiation

Cells were cultured in a sterile environment and kept in an incubator at 5% CO_2 and 37 °C to promote growth. Cell stocks were subcultured every 3–4 days by rinsing the cells in phosphate buffered saline (PBS), adding trypsin to detach the cells from the tissue culture flask, and transferring 10–20% of the harvested cells to a new flask containing fresh growth media.

Cells (5.0×10^5) were seeded in 60 mm dishes and exposed to radiation at a dose rate from 4 to 8 Gy at 24 h after plating. Immediately after irradiation, cells were returned to the incubator for recovery. Three hour late cells were harvested and used for the soft agar assay to establish the effect of radiation on colony formation of DLD-1 cells.

Cells (5.0×10^5) were plated in 60 mm dishes and incubated for 24 h. After the incubation, cells were exposed either in the presence or absence of 40 μg/mL polysaccharides (native and sulfated laminarans and fucoidan) for an additional 24 h before irradiation at a dose of 4 Gy. Immediately after irradiation, cells were returned to the incubator for recovery. Three hours later, cells were harvested and used for the soft agar assay to establish the effect of polysaccharides and radiation on colony formation of DLD-1 cells.

3. Results and discussion

3.1. Purification and structural characteristics of polysaccharides from brown alga D. dichotoma

Water-soluble polysaccharides were isolated from the brown alga *Dictyota dichotoma* collected from Peter the Great Bay, Sea of Japan, Far East of Russia. We used anion-exchange chromatography on DEAE-cellulose for separation of the laminaran and fucoidan fractions. The laminaran fraction was eluted by water, the fraction contained mannuronic acid and low-sulfated heterogeneous fucoidan – by 1 M NaCl, and high-sulfated pure fucoidan – by 2 M NaCl. Then, the laminaran was further purified by hydrophobic chromatography on Polychrome-1 to obtain the fraction DdL (1.3% of defatted algae weight).

Analysis of polyphenol content showed that the fucoidan fraction eluted by 2 M NaCl from a DEAE-cellulose column contained a significant amount of polyphenols (3.2% of sample weight). We removed polyphenols from the fucoidan fraction according to known method (Urvantseva et al., 2004). Earlier, this method was applied successfully to fucoidan from the brown algae *Fucus evanescens*. Fucoidan was subsequently purified by anion-exchange chromatography on Macro-Prep DEAE. The obtained fucoidan fraction DdF was 0.4% of defatted algae weight.

Analysis of the monosaccharide composition of polysaccharide fractions revealed that DdL is pure glucan, and DdF is a heteropolysaccharide containing fucose, galactose, mannose and glucose (57.9, 20.4, 12.4 and 9.2 mol%, respectively). Based on HPLC, the molecular weight interval was 5–6 kDa for laminaran and 250–280 kDa for fucoidan fractions.

The sulfate content of DdF was 28.9% of the total sample weight. Earlier fucoidans from the brown algae *D. dichotoma* were investigated by other groups (Abdel-Fattah et al., 1978; Hussein et al., 1979; Rabanal et al., 2014). It was shown that this alga produce sulfated heteropolysaccharides, containing fucose, galactose, mannose, xylose and glucuronic acid. Our results are in good agreement with published data.

The fucoidan DdF has a very complex and difficult to elucidate ^{13}C NMR spectrum. This spectrum contains intensive signals at 16.2–16.5 and 61–62 ppm corresponding to C6 of α-L-fucose and unsulfated galactose, mannose or glucose, respectively. Signals corresponding to acetyl groups and uronic acids are at 21–22 and 175–176 ppm.

3.2. The structure of native and sulfated laminaran

Laminarans are water-soluble polysaccharides of brown algae, consisting of 1,3- and 1,6-linked b-D-glucose residues. Laminarans from different species of algae are known to vary due to the ratio of 1,3:1,6 bonds and variants of including of these bonds in the molecule of β-D-glucan (Zvyagintseva, Shirokova, & Elyakova, 1994). Laminarans usually have a molecular weight of 4–5 kDa and contain a main chain from 1,3-linked b-D-glucose residues with a small number (≤10%) of branches at C6 as single b-D-glucose residues.

The structure of laminaran DdL was investigated by 1D (^1H, ^{13}C) and 2D (COSY, HSQC, HMBC) NMR spectroscopy. The ^1H NMR spectrum of DdL contains group of two doublets at 4.76 and 4.78 ppm (residues A and A'), and one doublet at 4.53 ppm (residue B) in anomeric region. A full analysis of 2D spectra COSY and HSQC (Fig. 1A) allowed to the determination of glucose proton signals and C/H correlation. The chemical shifts of this laminaran are shown in Table 1.

The HSQC spectrum showed that glycosidic bonds are in position 3 of residue A and positions 3 and 6 of residue A' (downfield location of H3/C3 resonances of A and A' at 3.79/86.0 and 3.79/85.5 ppm, respectively, and H6/C6 resonances of A' at 4.22, 3.88/69.9 ppm). The analysis of HMBC spectrum (Fig. 1B) confirmed of these results. Correlation peaks H1(A)/C3(A') and H1(A')/C3(A) at 4.78/85.5 and 4.76/86.0 corresponded to the presence of 1,3-bonds between A and A' residues. Cross-peak H1(B)/C6(A') at 4.53/69.9 showed that glucose residue B was linked to C6 of residues A'.

The ratio of bonds 1,3:1,6 in the laminaran DdL was estimated by comparing the intensity of anomeric proton signals in ^1H NMR spectrum (group of two doublets belonged to residues A and A' and the doublet of residue B) and amounted to 3:1 (Kim et al., 2000).

Thus, our investigation showed that laminaran DdL is a regular polysaccharide, which contain a main chain build from 1,3-linked glucopyranose residues (A and A') with the branches of single glucose (B) at C6 (ratio of bonds 1,3:1,6 = 3:1). The structure of DdL is presented in Fig. 2.

In this investigation, regular laminaran DdL with a determined structure was sulfated (s) to obtain the laminaran derivative DdLs with yield of 38% of native polysaccharide weight. The degree of sulfation of sulfated laminaran was 43.7%. The structure of DdLs was investigated by ^{13}C NMR spectroscopy. The downfield location of signals C2, C4 and C6 at 80–80.5, 75.8–76.2 and 68.8 ppm corresponded that DdLs has sulfate groups at position 2, 4 and 6. The molecular weight range of the sulfated laminaran fraction was 9–12 kDa.

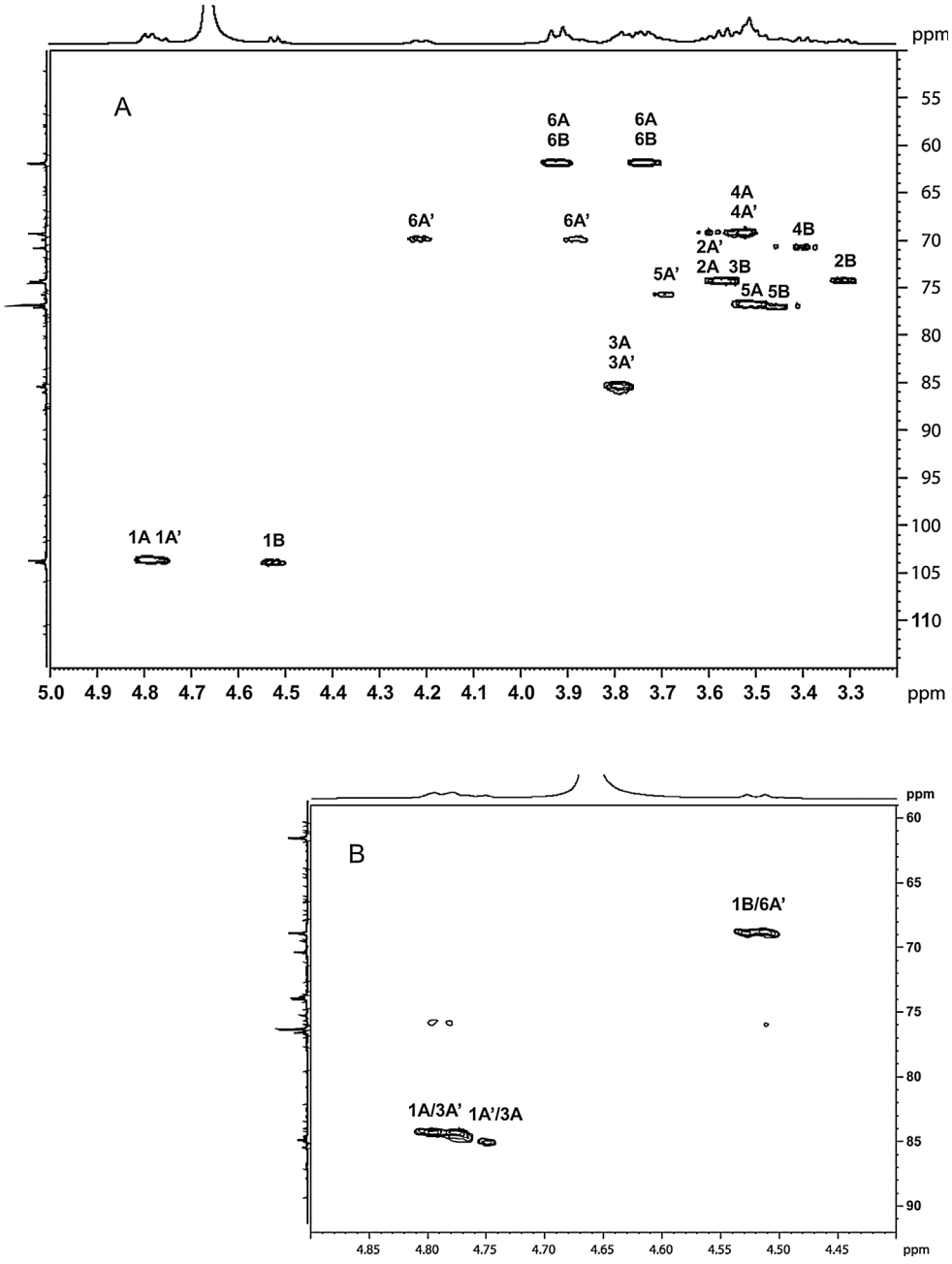

Fig. 1. HSQC (A) and HMBC (B) NMR spectra of laminaran DdL.

Table 1
NMR data for the laminaran DdL.

Fragment of structure	C1	C2	C3	C4	C5	C6
A → 3)-β-D-Glc*p*-(1 → 3)-	103.5	74.3	86.0	69.4	76.8	61.8
B β-D-Glc*p*-(1 → 6)-	104.0	74.3	74.4	70.9	77.1	61.8
A' → 3,6)-β-D-Glc*p*-(1 → 3)-	103.5	74.3	85.5	69.4	75.9	69.9
	H1	H2	H3	H4	H5	H6
A → 3)-β-D-Glc*p*-(1 → 3)-	4.78	3.57	3.79	3.53	3.51	3.93; 3.74
B β-D-Glc*p*-(1 → 6)-	4.53	3.31	3.55	3.40	3.46	3.93; 3.74
A' → 3,6)-β-D-Glc*p*-(1 → 3)-	4.76	3.57	3.79	3.53	3.70	4.22; 3.88

3.3. Biological activity of polysaccharides

We determined the effect of the polysaccharides DdL, DdLs and DdF (at a concentration of 200 μg/mL) on colony formation of human colon cancer cells HCT-116, HT-29, and DLD-1 using the soft agar clonogenic assay (Fig. 3).

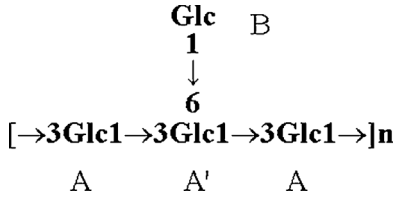

Fig. 2. Structure of the laminaran DdL.

The results revealed that all investigated polysaccharides showed slight inhibition of colony formation of HCT-116 and HT-29 cells: less than 10% compared with non-treated cells (control) for DdL and DdLs for both cell lines; and 6% and 26% for HCT-116 and HT-29 cells, respectively, for DdF. The highest inhibitory activity of these polysaccharides was observed for DLD-1 cells.

The percentage of inhibition was approximately 50% for DdF, and sulfated laminaran DdLs also possessed a powerful inhibitory activity:

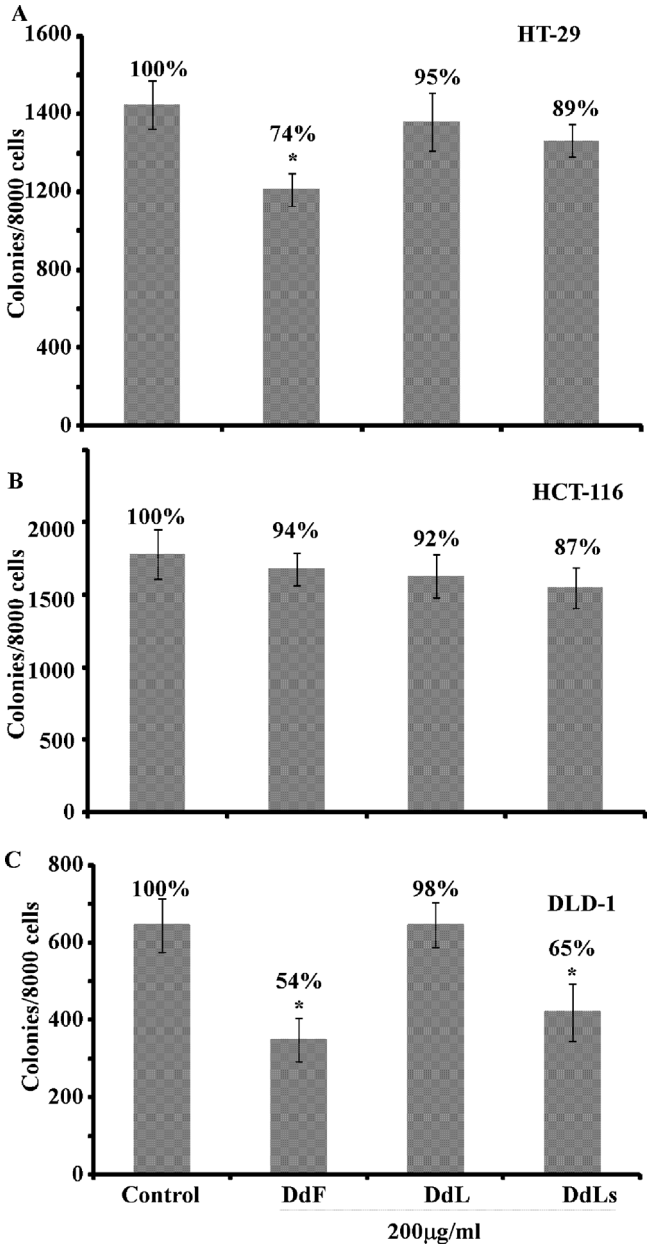

Fig. 3. Inhibitory effect of native (DdF, DdL) and modified (DdLs) polysaccharides from *D. dichotoma* against colony formation of human colon cancer cells HCT-116, HT-29, and DLD-1.

DdLs prevented the formation and growth of colonies of these human cancer cells at 35%.

The brown algae *Saccharina cichorioides* produced polysaccharides, the structure of which was well characterized. The fucoidan from *S. cichorioides* was fucan with a main chain of 1,3-linked α-L-fucose residues and a small number of 1,4-linked fucose residues. A non-significant number of single α-L-fucose residues were in the branches at the C2 position. Sulfate groups occupied positions 2 and 4 of the fucopyranose residues (Anastyuk et al., 2010; Anastyuk et al., 2017; Zvyagintseva et al., 2003). The laminaran from *S. cichorioides* was glucan with a main chain of 1,3-linked glucose residues. Single glucose residues were found on the branches at the C6 position (ratio of bonds 1,3:1,6 = 9:1) (Zvyagintseva et al., 1994).

The effect of fucoidan and laminaran from the brown alga *S. cichoriodes* was previously demonstrated by our group. The fucoidan was showed to inhibit colony formation of human colon cancer DLD-1 cells by 75% (Vishchuk, Ermakova, & Zvyagintseva, 2013). The laminaran from *S. cichorioides* and its sulfated derivative suppressed colony formation of colon cancer HCT-116 cells by 16 and 32%, respectively, compared with non-treated cells (Malyarenko et al., 2017).

The fucoidans from brown algae differ widely in their structural characteristics, and their anticancer activity can depend on their molecular weight, monosaccharide composition, branching and content of sulfated and acetyl groups. The specific relationship between fucoidan structure and anticancer effect are not determined. For example, galactofucans from *Sargassum duplicatum*, *Sargassum feldmannii*, Padina *boryana* and *Dictyota divaricata* with different content of galactose, sulfate and acetyl groups have widely variable anticancer activity *in vitro* (Shevchenko et al., 2017). While structural simplification of fucoidan from *Saccharina gurjanovae* by of lowering its molecular weight, desulfation at C2 and removal of galactose residues did not decrease its anticancer activity (Shevchenko et al., 2015). Fucoidans from the brown algae *S. cichoriodes* and *D. dichotoma* were very different in their structure, and their biological effects were also different.

The published data about the effect of laminarans on colony formation of cancer cells is very limited. It is known that laminaran from brown seaweed *Laminaria digitata* (commercial preparation from Sigma–Aldrich) was found to inhibit cell growth of colorectal cancer cells HT-29 by 40% with lower cytotoxicity on IEC-6 intestinal epithelial cells (Park, Kim, Kim, & Nam, 2012; Park, Kim, Kim, & Nam, 2013). Another group of authors showed that the same laminaran suppressed proliferation of colorectal cancer cells LOVO by 38.8%. Moreover, they obtained sulfated laminaran and determined its antiproliferative activity in LOVO cells. It was found that after sulfated modification the growth inhibitory activity of sulfated laminaran was enhanced, and the inhibition rate was 86% at the same dose (Ji et al., 2012; Ji, Ji, & Meng, 2013).

In studies (Ermakova et al., 2013; Menshova et al., 2014) the anticancer effect of laminaran fractions from *Eisenia bicyclis* was investigated. The laminaran from this alga had an unusual structure and was a complex, branched high-molecular-weight 1,3;1,6-β-D-glucan with a significant amount of 1,6-linked galactose residues (ratio of bonds 1,3:1,6 = 3:2). It was shown that decreasing the molecular weight of native laminaran to a determined limit and increasing the content of 1,6-linked glucose residues increased the anticancer effect.

Recently, the *in vitro* anticancer activity of the laminarans isolated from the brown seaweeds *S. cichorioides*, *S. japonica* and *F. evanescens* and their chemically sulfated derivatives was demonstrated through evaluation of their efficacy in inhibiting the proliferation, colony formation and migration of human colorectal adenocarcinoma, malignant melanoma and breast adenocarcinoma cells (Malyarenko et al., 2017). The results revealed that the sulfation of laminarans increased their anticancer effect. In the present work, we showed that inhibiting activity of sulfated laminarans from *Dictyota dichotoma* was the highest compared with that of native laminaran. We also proposed that it was related to the degree of sulfation.

Next, we studied the effect of the investigated polysaccharides on colony formation cancer cells after irradiation. DLD-1 cells were chosen because the effect of the investigated polysaccharides on colony formation of this cell line was highest. We developed conditions for irradiation of DLD-1 cells. The number of colonies was reduced by 8, 18 and 23% compared with the control at radiation doses of 4, 6 and 8 Gy, respectively (Fig. 4A). We choose a dose of 4 Gy to study the effect of the investigated polysaccharides on colony formation cancer cells after irradiation. First we determined the effect of the polysaccharides DdL, DdLs and DdF at a concentration of 40 μg/mL on colony formation of DLD-1 human cancer cells using the soft agar clonogenic assay (Fig. 4B). DdL, DdLs and DdF at 40 μg/mL did not inhibit this process (data not shown). However,it was shown that the investigated polysaccharides dramatically decreased the number and size of cancer cells colonies irradiated with 4 Gy. Fucoidan decreased the number and size of DLD-1 cells colonies at 7 and 14% compared with cells irradiated with 4 Gy, respectively. DdL decreased the number and size of DLD-1

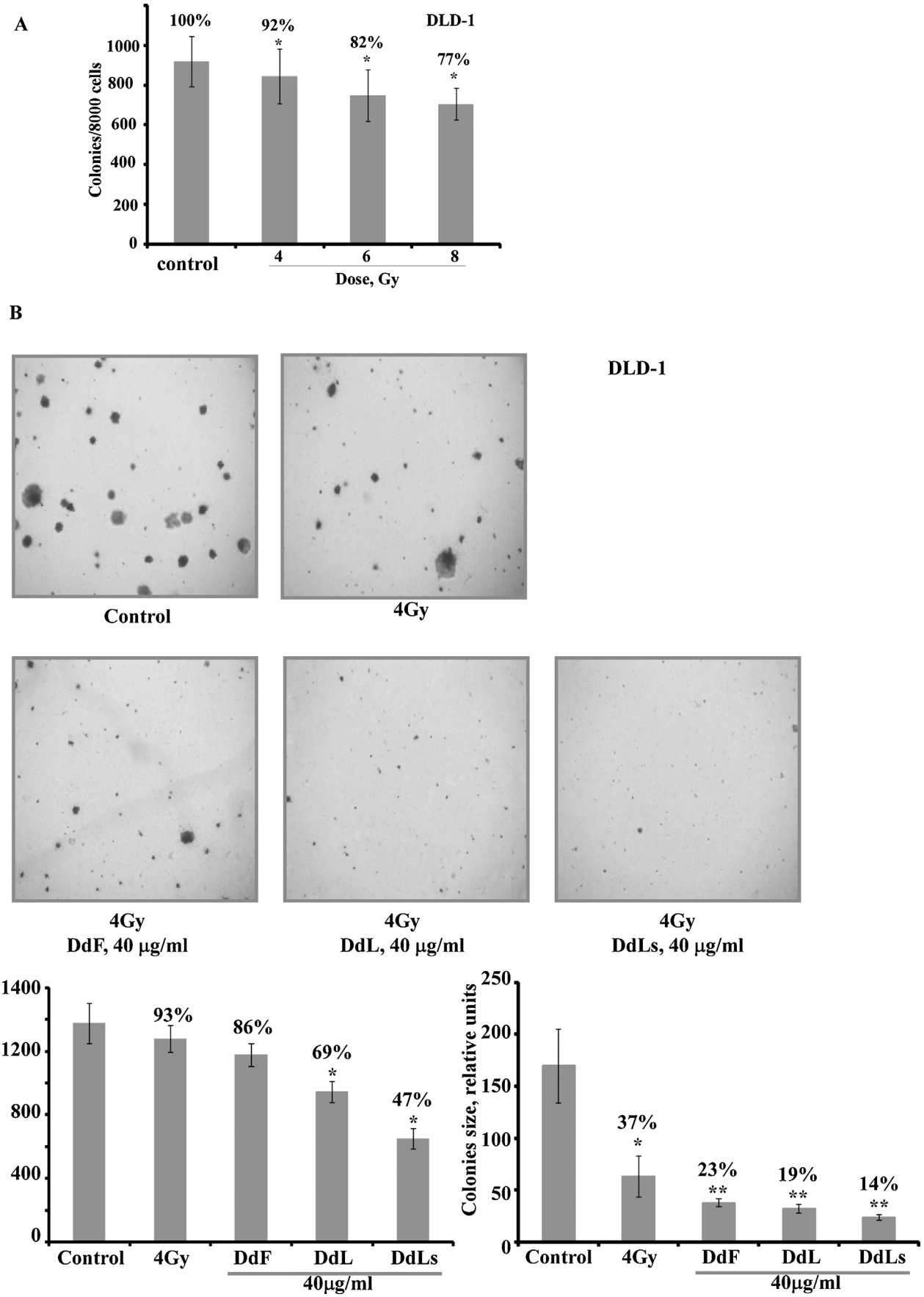

Fig. 4. Effect of radiation and native (DdF, DdL) and modified (DdLs) polysaccharides from *D. dichotoma* against colony formation of human colon cancer cells DLD-1. (A) Effect of different doses of radiation on colony formation of DLD-1cells. (B) Synergistic effect of DdF, DdL and DdLs with radiation (4 Gy) against amount and size of colony formation of DLD-1cells.

cells colonies by 24 and 18%, respectively, whereas DdLs resulted in corresponding decreases of – at 46 and 23%, respectively. This finding is very important, because during irradiation of the human body, normal cells are also exposed to irradiation. Polysaccharide can increase the effect of irradiation on cancer cells and safeguard the survival of normal cells. It was demonstrated for the first time that fucoidan, laminaran and sulfated derived of laminaran inhibited not only the number of irradiated cell colonies but also their size.

4. Conclusion

In the results of this investigation, the highly purified laminaran DdL and fucoidan DdF were obtained from the brown alga *Dictyota dichotoma*. Fucoidan was determined to be a sulfated (28.9%) and acetylated heteropolysaccharide containing fucose, galactose, mannose and glucose residues (57.9, 20.4, 12.4 and 9.2 mol%, respectively). Laminaran was 1,3;1,6-β-D-glucan with a 1,3:1,6 = 3:1 bond ratio. The structure of DdL was investigated by 2D spectroscopy NMR; laminaran contained predominantly repeating structural units of tetrasaccharide with three 1,3-linked β-D-glucopyranose residues and branch of single glucose at C6.

The structure of DdL was modified by sulfation to obtain the sulfated (43.7%) laminaran derivative DdLs. Sulfate groups were identified at C2, C4 and C6 of glucose residues.

The anticancer effect of three obtained polysaccharides DdF, DdL and DdLs was studied *in vitro* at a concentration of 200 μg/mL on human colon cancer cells HCT-116, HT-29 and DLD-1. The native laminaran had little anticancer effect on all cell lines. Sulfated polysaccharides showed slight inhibition of colony formation of HCT-116 and HT-29 cells and powerful inhibition of DLD-1 cells (46 and 35% for DdF and DdLs, respectively).

We firstly investigated the effect of DdF, DdL and DdLs (40 μg/mL) against cells of DLD-1 cancer cells after irradiation. No investigated polysaccharides showed anticancer activity at this concentration, but they decreased the amount (7, 24 and 46% for DdF, DdL, and DdLs, respectively) and size (14, 18 and 23% for DdF, DdL, and DdLs, respectively) of colony of cancer cells irradiated with 4 Gy.

We have demonstrated the *in vitro* anticancer activity of polysaccharides from the brown alga *Dictyota dichotoma* through evaluation of their inhibitory activity of colony formation of human colorectal adenocarcinoma cells. The current study showed that fucoidan, laminaran and sulfated laminaran showed a synergistic effect with X-ray radiation against cancer cells. Thus, the application of the investigated polysaccharides as additional therapy during radiotherapy can provide a possible strong effect with a low dose of radiation.

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