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Biochemistry

Anticancerogenic Activity of the Mannose-Specific Lectin Isolated from Rhizomes of the Georgian Endemic Mountain Plant *Polygonatum obtusifolium* (C.Koch) Miscz. ex Gross

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ABSTRACT. In the present work, we report the effect of a novel mannose-specific lectin (SABA-1) on in vitro cultures of normal and cancer cells. The cytotoxic effect was estimated by means of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test. The extinction index was determined with Biotek EL 312 counter at the wavelength of 570 nm. The anticancerogenic and control experiments were conducted in triplicate for each concentration of SABA-1. The studies were performed on five types of cells, derived directly from the parent tissue human skin, lung, ovarian and breast cancer and on normal mouse fibroblasts. Exposure to SABA-1 resulted in remarkable concentration-dependent inhibition of growth of all tested cancer cells. According to the obtained results, the 10, 50, 100 μg/ml concentrations of SABA-1, which are lethal for malignant tumor cells, it does not affect normal fibroblast cells. Maximum cytotoxic effect of SABA-1 against cancer cells of skin, lung, ovarian and breast cancer is revealed at 100 μg/ml concentration and it attains 72, 60, 63 and 68%. Incubation of SABA-1 with α-methyl-mannopyranoside, which caused screening of its sugar-binding centres, fully inhibits cytotoxic activity of the mentioned lectin against the cells of the primary in vitro cultures of all tested tumour cells. The obtained results show that cytotoxic effect of SABA-1 on cancer cells should be conditioned by its specific interaction with α-methyl-mannopyranoside containing membrane receptors, located on surfaces of cancer cells and the transmission of signal, inducing apoptosis-self destruction of cells.

Key words: *Polygonatum obtusifolium* mannose-specific lectin, anticancerogenic activity.

Genus *Polygonatum* Mill. unites important medicinal plants, which are often used for the treatment and prevention of many human diseases [1]. *Polygonatum obtusifolium* (C. Koch) Miscz. ex Grossh (King Solomon's-seal, Solomon's seal), which belongs to the family Convallariaceae[2].

A variety of biologically active compounds were identified from these plants, which include lignans, flavonoids, coumarins, phenolics, fatty acids, steroids, triterpenes, tannins and alkaloids [3]. This structurally diverse set of compounds was found to possess a wide range of therapeutic activities, in particular, analgesic, antidiabetic, antituberculosis, antiviral, cytotoxic, antiaging, antiinflammatory, antidiar-

rhoeal, anti-microbial, anti-oxidant, anti-malarial properties, insecticidal activities and was found to be effective against respiratory diseases and osteoporosis.

However, so far little is known about the biologically active proteins of this plant family, although a number of researchers published data that the carbohydrate binding proteins isolated from *Polygonatum odoratum* had a remarkable effect against human immunodeficiency virus [HIV] and possessed antiproliferative activity against cancer cells in the cell culture studies [4].

In this study, we characterized some significant biological properties of SABA-1, in terms of its cytotoxic activities against cultured primary cells of human cancer.

Materials and Methods

The underground parts, the rhizomes of *Polygonatum obtusifolium*, which are extensively used in folk medicine due to its essential medicinal properties, served as the object of this study. Polygonatum mannose-specific lectin (SABA-1) was isolated as described previously [5].

The cytotoxic effect of the SABA-1 on the human malignant tumours was studied *in vitro* on the short-term primary cultures derived directly from the parent tissue of human skin, lung, ovarian and breast cancer. The tumour tissue was taken from the untreated patients subjected to the surgery. For the separation of cells the tissue was treated mechanically and then disaggregated with enzymes. The suspension of the separated cells was prepared on RPMI 1640 area, to which fetal bovine serum (10%) and gentamicin (50μg/ml) were added. Cells were seeded into 96-well microplates (cancer cells = 5×10^4 cells/well). For the determination of the cell viability we used 0.2% solution of the trypan blue, a vital dye stipulated in the project. In the experiments without SABA-1 the cell viability was in the range of 90-95%. All manipulations were carried out in sterile environments. The cells were cultivated on the microtiter plates with 96 wells in conditions of three different concentration of SABA-1 (10, 50, 100 μg/ml). In the control wells nutrient medium of standard quantity was placed. The anticancerogenic and control experiments were conducted in triplicate for each concentration of SABA-1. The incubation was carried out at 37°C during 48 hours in the conditions of humidity and 5% of CO_2. The cytotoxic effect was estimated by means of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test [6] after 3-hour-incubation. For extraction the solution consisting of SDS (10%), isobutanol (50%) and HCI (0.01 N) was used. The extinction index was determined with Biotek EL 312 counter at the wavelength of 570 nm.

Hemagglutination activity was determined visually in 96-well immunological microtiter U-plates, using a

Table. Effect of SABA-1 on growth inhibition of 2 days old *in vitro* primary cell cultures derived from human skin, lung, ovarian and breast cancer

Cancer Types	SABA-1 g/ml	0	10	50	100	SABA-1+ α-methyl-mannopyranoside (400 mM)
Skin cancer	AV	0.326	0.283	0.166	0.091	0.320
	NC(%)	100	87	51	28	100
	GI (%)	0	13	49	72	0
Ovarian cancer	AV	0.318	0.218	0.176	0.130	0.315
	NC(%)	100	67	54	40	100
	GI (%)	0	33	46	60	0
Lung cancer	AV	0.328	0.277	0.179	0.121	0.329
	NC(%)	100	85	55	37	100
	GI (%)	0	15	45	63	0
Breast cancer	AV	0.322	0.261	0.192	0.104	0.321
	NC(%)	100	80	59	32	100
	GI (%)	0	20	41	68	0

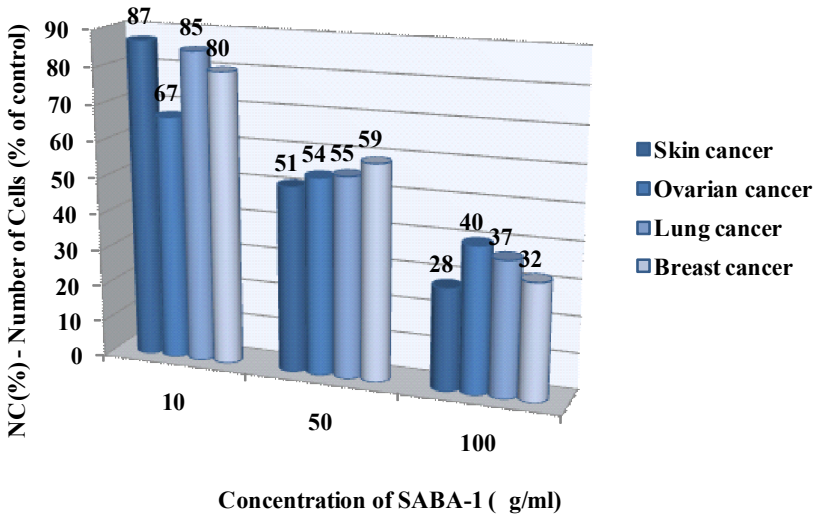

Fig. 1. Cytotoxic effect of SABA-1 on 2 days old primary cell cultures derived from human skin, lung, ovarian and breast cancer

AV - Average Value (average value of triplicate measurements of the extinction index)
NC(%) - Number of Cells (% of control)
GI (%) - Growth Inhibition%

hemagglutination test on rabbit trypsin-treated erythrocytes according to the method of Takatsy [7].

Inhibition of the cytotoxic effect of SABA-1 with α-methyl-mannopyranoside

Initial solution of the SABA-1 (1 mg/ml) was added by equal quantities – 400 mM α-methyl-mannopyranoside and was incubated on the shaker in the thermostat at 37°C temperature for 3 hours. After the period of incubation, the free molecules of α-methyl-mannopyranoside, unbound with SABA-1, were removed by means of dialysis carried out in dialysis packs during the night. For sterilization the dialyzate was filtered through the filters with 0.22 mμ pore diameter (Millipore) and were tested on hemagglutination activity towards the trypsin treated rabbit erythrocytes, and, then on cytotoxicity towards the cancer cells in short-term suspension cultures *in vitro*.

Protein concentration was measured by the method of Lowry, et al. [8].

All experiments were performed in triplicate. Statistical analysis was performed using Student's t-test and p-values < 0.05 considered significant.

Results and Discussion

In order to reveal the nonspecific cytotoxic action of SABA-1, its influence on the short-term cell culture of normal human fibroblasts was studied. According to the obtained results, the 10, 50, 100 μg/ml concentrations of SABA-1, which are lethal for malignant tumor cells, it does not affectnormal fibroblast cells.

Data presented in the Table and the Figure evidence that the same concentrations of SABA-1 had well expressed cytotoxic effects on primary short-term cell cultures, derived directly from the parent tissues of Human skin, lung, ovarian and breast cancer. The results clearly show that there is a direct correlation between those two indices, particularly simultaneously with growing of the concentration of SABA-1, its cytotoxic effect increases sharply.

As seen from the Table and the Figure maximum cytotoxic effect of SABA-1 against cancer cells of skin, lung, ovarian and breast cancer is revealed at 100 μg/ml concentration and it attains 72, 60, 63 and 68%.

In the next series of experiments with the aim of revealing molecular mechanisms of cytotoxic action of

SABA-1 on cancer cells the effect of α-methyl-mannopyranoside on cytotoxic activity of SABA-1 was studied. In works, published earlier we showed that sugar-binding centres of SABA-1 reveal high specificity to α-methyl-mannopyranoside. Data presented in the Table show that incubation of SABA-1 with α-methyl-mannopyranoside, which caused screening of its sugar-binding centres, fully inhibits its cytotoxic activity of the mentioned lectin against the cells of the primary *in vitro* cultures of all tested tumour cells.

The obtained results show that cytotoxic effect of SABA-1 on cancer cells should be conditioned by its specific interaction with α-methyl-mannopyranoside containing membrane receptors, located on surfaces of cancer cells and the transmission of signal, inducing apoptosis - self destruction of cells.

ბიოქიმია

მთის მცენარის, საქართველოს ენდემის *Polygonatum obtusifolium* (C. Koch) Miscz. ex Grossh. ფესურადან იზოლირებული მანოზა-სპეციფიკური ლექტინის ანტიკანცეროგენული აქტივობა

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მედიკო-ბიოლოგიური სამეცნიერო-კვლევითი ცენტრი „ალექსის“, შპს, თბილისი
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წინამდებარე ნაშრომში წარმოდგენილია საქართველოს ენდემური მცენარის *Polygonatum obtusifolium* (C. Koch) Miscz. ex Grossh. ფესურადან იზოლირებული ახალი, მანოზა-სპეციფიკური ლექტინის (SABA-1) ციტოტოქსიკური ეფექტი ნორმალური და კიბოს უჯრედების *in vitro* კულტურებზე. ციტოტოქსიკური ეფექტი შეფასდა MTT ტესტის მეთოდით. ექსპერიმენტები ჩატარდა ხუთი ტიპის უჯრედებზე: ადამიანის კანის, ფილტვის, საკვერცხის, სარძევე ჯირკვლის ქსოვილებიდან მიღებულ კიბოს და თაგვის ნორმალურ ფიბრობლასტურ უჯრედთა კულტურებზე. უჯრედების ექსპოზიციამ SABA-1 მიმართ აჩვენა ლექტინის კონცენტრაციაზე დამოკიდებული ციტოტოქსიკური აქტივობა ტესტირებული კიბოს უჯრედების მიმართ და ნეიტრალობა ნორმალური ფიბრობლასტების მიმართ. SABA-1-ის ინკუბაცია α-მეთილ-მანოპირანოზიდთან სრულად თრგუნავდა SABA-1-ით ინდუცირებულ ციტოტოქსიკურ აქტივობას. განხილულია SABA-1-ის კიბოს უჯრედებზე ციტოტოქსიკური მოქმედების მექანიზმი.

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