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Research Article

Some Biological Effects of Galactose-Specific Lectins from Prostate Tissue with Different Pathologies in Tumor Cells Model *In vitro*

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- Cells survival viability
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Abstract

Introduction: The biological effects of cytoplasmic galactose-specific lectins fraction isolated from unaffected, as well as post-operational pathological (benign hyperplasia, BPH, high-grade intraepithelial neoplasia, HGPIN, tumor precursor, atypical adenomatous hyperplasia, AAH, and prostate adenocarcinoma, PC) prostate tissues were studied on tumor cell lines (MEC1 lymphocytes cell line and Human prostate PC3-cell line).

Methods and Results: Cytosolic galactose-specific lectin fractions from prostate tissue with different diagnosis were purified by affinity chromatography. The lectin effects were studied on cell viability by MTT reduction method and on apoptosis by flow cytometry method. All testified lectin fractions, except from AAH tissue, markedly reduced also the cell viability in MEC1 and PC3 cell lines and fraction isolated from HGPIN and PC tissues in time dependent manner increased apoptosis in MEC1 line lymphocytes.

Conclusions: Cytosolic galactose-specific lectins fractions depend on the form of glandular tissue disease and also on the type of cell (lymphocytes, PC3, used in the research). Our findings suggest that lectins isolated from HGPIN and PC tissues can have defensive anti-tumor properties in tumors cell - MEC1 line lymphocytes and prostate PC3 cell line.

ABBREVIATIONS

AAH: Atypical Adenomatous Hyperplasia; BPH: Benign Prostate Hyperplasia; HGPIN: High-Grade Intraepithelial Neoplasia; PC: prostate adenocarcinoma; Post-op: Post Operation

INTRODUCTION

It has been recognized that the structure of cell surface glycans can change under different physiological and pathological conditions. In cancer biology, aberrant glycosylation changes, resulting in expression of altered carbohydrate determinants on many glycoproteins and glycolipids has been known as one of the most important changes related to tumor malignancy [1]. Some of these significant interactions related to carbohydrates are mediated through binding to specific proteins interacting with carbohydrate determinants. Specific proteins selectively binding [2,3].

Prostate cancer is the second leading cause of cancer death in males after lung cancer [4]. In prostate cancer galectins have been implicated in many cellular processes such as proliferation, apoptosis, migration and invasion [5,6] and are considered as potential prognostic markers and potential therapeutic targets for this type of pathology [7].

The prostate tissue could have different grades of pathologies which in turn could lead to the formation of prostate adenocarcinoma (PC) [8]. These forms are as follows: benign hyperplasia (BPH), high-grade intraepithelial neoplasia (HGPIN,

tumour precursor) [9], atypical adenomatous hyperplasia (AAH) and PC [8]. Each of them is characterized with specific histology and molecular signature [5].

In our previous study, we have shown that **a**ffinity purified cytosolic galactose-specific lectins fractions from normal and pathological prostate tissue samples are characterized with different protein composition and they expressed different effects on cell viability and apoptosis of healthy blood lymphocytes in vitro [10].

The present study is focused on cytoplasm galactose-specific lectins effects on cell viability, and apoptosis in the pathological cells on the example of MEC1 cell line lymphocytes and Human prostate cancer PC3 cell line. MEC1 cell line was established from EBV-seropositive patient's Chronic Lymphocytic Leukemia cells and iswidely used as a model for CLL pathogenesis. We have expanded the source of galactose-specific lectins and carried out experiments on lectins purified from prostatic tissues with the following diagnosis: BPH, HGPIN, AAH, PC as well as from unaffected tissue (norm).

MATERIALS AND METHODS

Editorial Policies and Ethical Consideration

The ethical approval of the study as long as the guidelines followed to carry out the study was also obtained from Georgian National Center for disease control and public health (Institutional Review Board Letter 2021-069). Approval for the use of the tissues was obtained from the local ethical committee at A. Tsulukidze Urology National Modern Center, Pathomorphology Laboratory at the 1st Clinical Hospital and Clinic of SJC "Modern Medical Technologies", and the patients' written consent. However, through dissemination process, we made sure that the patients' data protection system is firmly implemented and that no leakage of personal data or the results of the diagnosis, prognosis etc. into public domain takes place. This was insured through the implementation of modern guidelines of ethics and data protection as planned in this application. The study design was approved by the internal review board of the institution. The scientific research was carried out at Ivane Javakhishvili Tbilisi State University, Faculty of Exact and Natural Sciences, Department of Biology, in tight coordination of chairs of Biochemistry and Immunology/Microbiology.

Patient's characteristics and samples

Prostate post-operational tissue samples were obtained from: A. Tsulukidze Urology National Modern Center, Pathomorphology Laboratory at the 1st Clinical Hospital and Clinic of SJC "Modern Medical Technologies". Human prostate tissue samples were obtained from patients undergoing open trans-abdominal prostatectomies for benign prostate hyperplasia and transurethral resection of the prostate (TURP). The numbers of testified tissues were: 1. Unaffected (N, normal) tissue isolated after cytoprostatectomies, n=7 (age 47-68 years); 2. benign prostatic hyperplasia (BPH) with LGPIN diagnosis, n=35 (age 55-70 years); 3. with HGPIN diagnosis n=17 (age 50-68 years); 4. with AAH diagnosis n=22 (age 50-75 years) and 5. PC diagnosis n=11 (age 54-67 years). All patients were otherwise healthy. After removal of tissue specimens' pathological conditions were confirmed by histopathology. The diagnoses were drawn according to the histopathology diagnostic criteria [11], by examining of needle biopsies (before the operation) and paraffin-embedded slices (after an operation). The cells in slices with AAH diagnosis had clear cytoplasm, lack malignant nuclear features, prominent nucleoli occur in 21-25% of cases. The nuclear size and anisonucleosis are the main features of cells in slices with LGPIN diagnosis.

Prostate epithelial and stroma were not separated from each other, and the whole tissue was used for lectin purification.

Reagents

Affinity chromatography sorbent-agarose with immobilized the NAcGal (Cat.N.A2787), WST1-reagent (4-[3-(4-Iodophenyl)-2-(4-nitro-phenyl)-2H-5-tetrazolio]-1,3-benzene sulfonate) (Cat.N.5015944001), Histopaque1077, Propidium iodide (PI,P4864), RNaseA (89836) were purchased from Sigma-Aldrich, Dorset, UK), RPMI-1640, Fetal bovine serum, Protein Assay Kit (Sigma, USA) were purchased from Sigma Chemical, CO.,USA. Commercial lectins (PNA (*Arachis hypogaea* (peanut agglutinin), SNA (*Sambucus nigra*,), HPA (*Helix pomatia*), PHA *(Phaseolus vulgaris*-phytohemagglutinin), SBA (soybean agglutinin), Con A (*Canavaliaensiformis,*jak-bean) and WGA (*Triticum vulgaris,* wheat germ agglutinin) were purchased from Sigma-Aldrich.

Prostate galactose-specific lectins isolation procedure

Galactose-specific lectins were isolated from the cytosolic fractions (S_3-fraction) of human prostate post-operational tissue with different diagnoses. Prostate tissues were homogenized in 0.32 M sucrose and centrifuged at 1000× g for 10 min. The supernatant (S1) was collected and centrifuged at 17,000×g for 55min, resulting in the S2 supernatant and the P2 pellet (crude mitochondrial fraction). S2 was centrifuged at 100,000×g for 1 to generate the P3 pellet (microsomal fraction) and S3 supernatant (cytosolic fraction).

For lectin purification these cytosolic fractions were saturated by ammonium sulphate (to 80% saturation), the mixtures allowed to stand overnight at 4°C. The precipitates were collected by centrifugation at 12,000 *g* for 20 min, re-dissolved in a minimal volume of phosphate buffer saline (PBS), pH 7.4, dialysed overnight against the PBS at 4°C, and then centrifuged at 12,000 *g* for 15 min. Obtained supernatants were examined for hemagglutination activity and sugar specificity, and were used for the purification of galactose-specific lectins by affinity chromatography (agarose with immobilized the NAcGal ("Sigma-Aldrich", Cat.N.A2787). The galactose-specific lectins fractions were eluted with 0.2 M galactose, 0.2M lactose and 0.05 M glycine-HCl buffer (pH 3.0) at 0.5 ml/min flow rate and immediately neutralised. Lectins fractions were dialysed and concentrated by the filter (AmiconUltra-15 Centrifugal Filter10 kDa, UFC901008). The yield of galactose-specific lectin fraction from 1 g of post-operation tissue was approximately 70-100 mkg of protein.

Peripheral Blood Mononuclear Cells (PBMCs) isolation procedure

PBMCs were isolated from the blood of healthy (35-50 year) men. PBMCs were isolated in Histopaque1077 gradient (density:

1.076–1.078 g/mL) (Sigma-Aldrich, Dorset, UK), and cell concentration was adjusted as required in RPMI-1640 medium supplemented with 10% fetal bovine serum (both Sigma-Aldrich) [12]. Cells number was counted in a hemocytometer and the concentration of cells adjusted to the required number for each experiment.

Cell Culture

PC-3 cells line (Sigma-Aldrich) were maintained in the RPMI 1640 containing 10% FBS, 100 units/ml penicillin, 100 µg/ml streptomycin, L-glutamine and sodium pyruvate. Cells were incubated in humidified atmosphere (37°C, 5% CO_2).

MEC1-cells line (donated by the University of Westminster, UK) [13] were cultured in RPMI 1640 supplemented with 10% heat-inactivated FBS,100 units/ml penicillin, and 100 µg/ml streptomycin in humidified incubator at 37°C and 5% CO_2. Cells grew in suspension, forming small and large clumps with round shape. The morphology of growing cells was evaluated on cytocentrifuge smears stained with May-Grunewald Giemsa (MGG). We counted the number of cells per 24 hours by hemocytometer (Sigma).

Cell viability experiments

The effect of lectins on the cell survival was studied in model experiments by MTT reduction method [13] using the WST1-reagent(4-[3-(4-Iodophenyl)-2-(4-nitro-phenyl)-2H-5-tetrazolio]-1,3-benzenesulfonate) ("Sigma Aldrich"5015944001). The experiments were carried out on cancer cells - leukemiaMEC1-linelymphocytes and prostate cancer PC3-line cells culture). The concentration of cytosolic galectins comprised 5 µg/10x10^6 lymphocytes/well (200 µl incubation area) and 10x10^5 PC3 cells/well. Cell suspensions were incubated with purified lectins fractions from different sources in 96-well tissue culture plates. Microtiter plates were kept for 1 h at 37°C, after which the medium was removed, and the culture washed with PBS. To each microtiter well the FBS-RPMI10% was added, followed by the 10µl WST-1. Plates were incubated in an incubator at 37^0C for 30 min. Results have been read twice: immediately after WST-1 addition and second time 30 min (incubation time) later. The absorbance was measured by ELISA reader Multiscan GO Microplate Reader (Thermo Scientific, USA) at 430 nm (WST-1) and with 630 nm as a reference read-out. A blank with WST-1 alone was measured and subtracted from all values.

Apoptotic Cell Cycle Analysis (Apoptosis assay)

The effects of cytosol galactose-specific lectins fractions isolated from PC and HGPIN diagnosed tissue cells were also studied on lymphocytes apoptosis by flow cytometry method. Lectin concentration comprised 5 µg/10x10^6cells (200 µl incubation area), the incubation time was 1 h, and cells were washed with PBS. Cell cycle analysis was carried out at 0 sec, 24 h, 48 h, 72 h. The apoptotic cells numbers were counted (only viable cells have been assessed). DNA content of propidium iodide (PI,P4864) stained cells was measured by flow cytometry and separated into phases of the cell cycle based on the PI fluorescence [14,15]. PI intercalates into double-stranded nucleic acids, binds to DNA stoichiometrically and its fluorescence is enhanced upon binding. The enzyme RNaseA was used to degrade double-stranded RNA. Cell concentration was adjusted to 1x10^6 cells/ml for each sample, centrifuged at 600g for 5 min, washed in PBS, the pellets were well vortexed, fixed in ice-cold 70% ethanol added drop-by-drop to the vortexes cells and maintained overnight at 4°C. Cells were then stained with a fluorochrome solution containing 20 µg/ml PI in PBS, 50 µg/ml RNase A ("Sigma-Aldrich", 89836) for 30 min at 37°C and analyzed by FACScan flow cytometer (Becton & Dickinson). The DNA histogram showed the cell cycle distribution of the non-apoptotic cells. The apoptotic cells should be observed as a distinct sub-G1 peak of the hypo diploid DNA.

Sugar-specificity control experiments

In hemagglutination assays, cell viability and apoptosis experiments, control samples contained 0.1M galactose in a final concentration.

Statistical Analysis

The data of cell viability experiments were analyzed by one-way analysis of variances (ANOVA) with factor – lectin source (BPH, HGPIN, AAH and PC). The data of apoptosis were analyzed by two-way ANOVA with factors: time of incubation and lectin source (HGPIN, and PC). Planned comparisons were carried out with student t-test. All tests were two-tailed unless otherwise stated, and P value less than or equal to 0.05 was taken as significant. All significant results are reported.

RESULTS

The effect of galactose-specific lectins fractions from prostate tissue on leukemic MEC1-line lymphocytes viability in model experiments

One-Way ANOVA revealed that the effect of the factor of lectin source on the leukemia MEC1-line lymphocytes viability was significant (F5, 29=18.19, P<0.001) (Figure 1). Cytosolic lectin fractions from all sources except AAH diagnosis significantly reduced the viability of leukemia MEC1-line lymphocytes

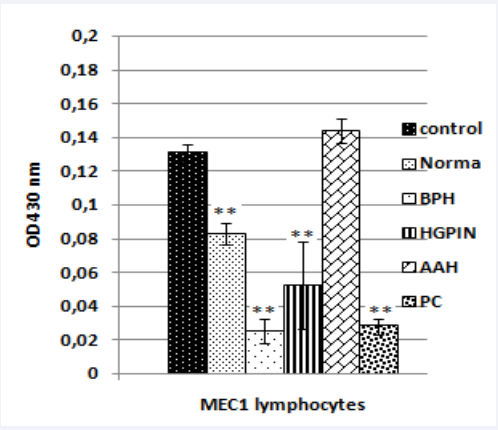

Figure 1 The effect of cytoplasm galactose-specific lectins fractions isolated from normal and diseased prostate tissue on leukemia MEC1-line lymphocytes viability. The data are mean ± SEM. **- p<0.001 as compared to controls. Other comparison results are provided in the manuscript.

(Normal cytosolic lectins vs Control cells T=6.53, P≤ 0.001; BPH vs. Control cellsT= 12.48 P≤ 0.0001; BPH vs. normal cytosolic lectins T= 6.32 P≤ 0.001; BPH vs. AAH T= 14.56P≤ 0.001; HPGIN vs Control cells T=2.96 P= 0.018; HPGIN vs AAH T = 3.83 P = 0.005; PC lectins vs Control cells T = 17.10 P≤ 0.001 PC lectins vs normal cytosolic lectins T= 7.64 P≤ 0.001; PC lectins vs AAH lectins T=19.22 P≤ 0.001, for all comparisons DF=8). No other differences were significant.

Thus, the cytosolic galactose-binding lectin fractions from prostatic tissues with different pathologies have different effects on cell viability depending on the lectin's fractions source (prostatic tissues diagnoses).

The effect of commercially available plant lectins on lymphocytes viability

Considering that lectins effect is exerted in relation of lectins with the specific carbohydrate determinant of glycoconjugates, the effect was compared with the effect of some commercially available plant lectines ("Sigma-Aldrich") with different carbohydrate specific lectins.

The lectins– PNA (*Arachis hypogaea* (peanut agglutinin),β-galactose-), SNA (*Sambucus nigra*, sialic acid-, α-NAcNANA(2-)6) Gal/NAcGal), HPA (*Helix pomatia,* βD-galactose), PHA *(Phaseolus vulgaris*-phytohemagglutinin, bi- and tri-antenal N-glycan-), SBA (soybean agglutinin,NAcGal), Con A (*Canavaliaensiformis,*jak-bean, mannose-, D-manopiranose-, D-glucopiranoside) and WGA (*Triticum vulgaris,* wheat germ agglutinin, NAcGlu-) were also used (Figure 2). The lectins effect on MEC1 cell line was compared with effect on normal lymphocytes (healthy blood).

Among the galactose-specific lectins the PNA and HPA (but not SBA lectin) significantly reduced the cell viability in both, normal [F(16,46)=16,171] and MEC1-line lymphocytes [F(10,21)=49,8, (p < 0.001)]. No effect was revealed in MEC1 line lymphocytes viability by SNA and Con A lectins. Con A decreased only normal lymphocytes surviving and WGA reduced in both, normal and MEC1–line lymphocytes viability (Figure 2).

The effect of galactose-specific lectins fractions from prostate tissue on prostate PC3-cells viability

One-way ANOVA revealed that the effect of the factor–lectin source is significant on the viability of the PC-3 cells (F5,29 =9.83, P≤ 0.001). In general, all lectins decreased the PC-3 cell viability. The strongest effect is observed for lectins from BPH tissue (BPH vs Control cells T=24.23 P≤ 0.001 DF = 8; BPH vs lectins from normal tissue T = 2.71 P = 0.027 P<0.05 DF = 8; BPH vs HGPIN T = 8.35 P≤ 0.001 DF = 8 and BPH vs AAH T= 10.31 P≤ 0.0001 DF = 8). All other lectins from prostatic tissue with different diagnosis significantly decrease the cell viability of PC-3 cells as compared to control conditions (lectins from normal tissue vs Control cells T= 2.86 P = 0.021 DF = 8; lectins from HGPIN diagnosis as compared to control cells T= 4.46 P≤ 0.001 DF = 8; lectins from AAH diagnosis as compared to control cells T= 9.93 P≤ 0.001 DF = 8 and lectins from PC cells as compared to control cells T= 4.00 P≤ 0.001 P 0.004 DF = 8) (Figure 3).

Among the commercial lectins with different carbohydrate-specificity the SBA (NAcGal), PNA (β-galactose), PHA (N-glycan)

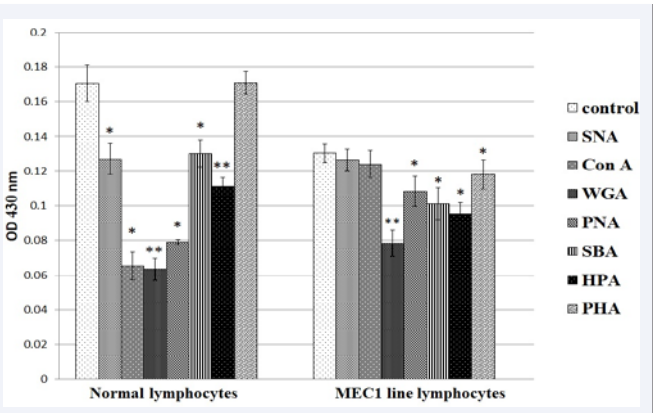

Figure 2 Influence of commercial different carbohydrate-specific lectins on viability of normal blood and leukemia MEC1-line lymphocytes. Note: The data are mean ± SEM. *p < 0.05, **p < 0.001.

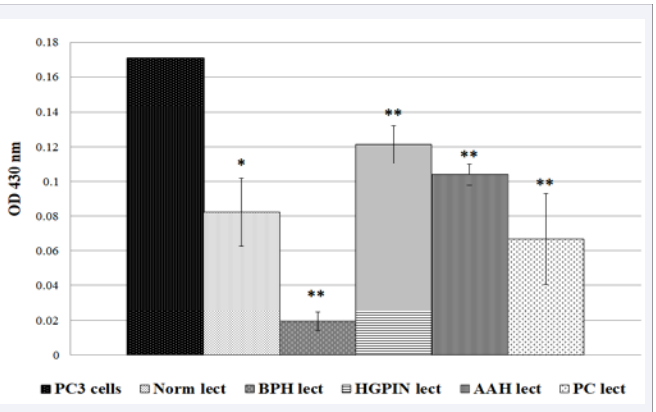

Figure 3 The effect of cytoplasm lectins fraction on prostate cancer PC3-line cells viability. Note: The data are mean ± healthy and SEM. *- p<0,05, **- p<0.001 as compared to controls. Other comparison results are provided in the manuscript.

and ConA (mannose) significantly decreased the PC3 cells viability (p<0,001, [F(10,21)=49,8], no effect had SNA (sialic acid-specific lectin) (Figure 4) as in MEC1 lymphocytes viability (Figure 2).

The effect of galactose-specific lectins fraction on cell apoptosis

Based on previous studied of the effect of galactose-specific lectins from prostate tissue with different pathology on the normal blood lymphocytes [10], were able to study the role of cytosolic galactose-specific lectins fraction on apoptosis from PC and HGPIN tissue lectins. The effect of lectins fraction on the cell cycle was studied in *vitro* in MEC1 line lymphocytes.

Two way ANOVA revealed that the effects of factors lectins source and time were significant (F1,23=2221.61 P≤ 0.001 and F3,23=247.81, P≤ 0.001) and their interaction is also significant (F3,23=247.19, P≤ 0.001).

In control cells significant decrease is observed at 48h as compared 0h (T= 3.36 P = 0.028 DF = 4) and 72h time points (T = 6.72 P = 0.003 DF = 4) (Figure 5).

Cytosolic galactose specific lectin fraction from prostatic tissue with HPGIN diagnosis significantly decreases the apoptosis of leukemic MEC-1 line lymphocytes at 48h (48h vs 0 h T = 7,63 P= 0.002 DF = 4; 48h vs 24h T= 10.01 P= 0.001 DF = 4) followed by sharp increase at 72 h (72h vs 48h T= 27.36 P≤ 0.001 DF = 4).

Incubation of leukemia MEC1-line lymphocytes with cytosolic galactose binding lectins from prostatic tissue with PC diagnosis leads to gradual increase of apoptosis and starting from 24h the levels are significantly higher as compared to 0h value (24hvs0h T= 24.39 P≤ 0.001; 48h vs 0h T = 30.75P≤ 0.001; 72h vs 0h T = 45.05 P≤ 0.001 for all comparisons DF= 4). The highest rate of apoptosis is observed at 72h significantly exceeding the corresponding values at 24h and 48h (T= 11.81 P≤ 0.001 DF = 4; T= 14.88 P≤ 0.001 DF = 4 respectively) (Figure 5).

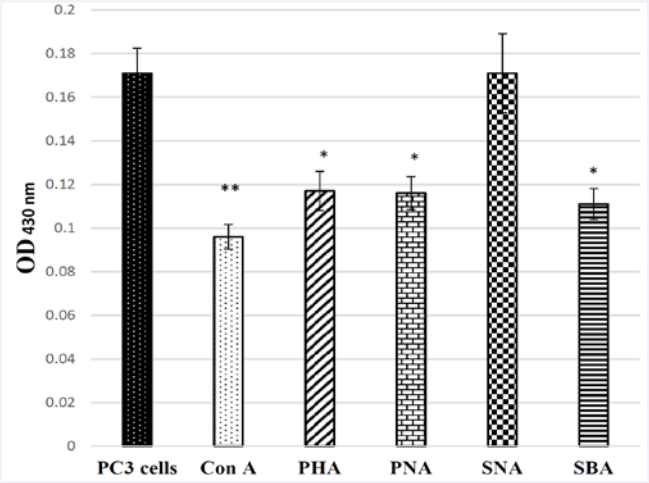

Figure 4 Commercial lectins effect on viability of PC3-cells. Con A – mannose-spec., PHA – N-glycan-sp., PNA- β-galactose-sp., SNA- sialic acid-spec.,SBA – NAcGal-spec. lectins.Note: The data are mean ± SEM. *$p < 0.05$, **$p < 0.001$.

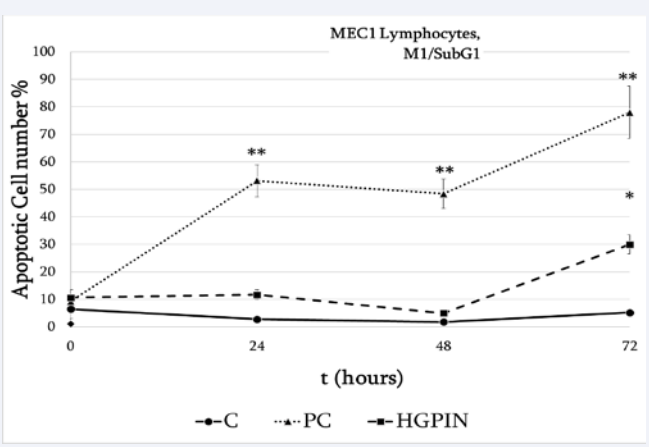

Figure 5 Influence of cytosolic galactose-specific lectins from tissue with the different diagnosis on peripheral blood leukemic MEC1-line lymphocytes apoptosis by flow cytometry technique.Sub-G1 (apoptotic) cells number - %. The data are mean ± SEM. **$p < 0.001$, as compared to initial time point. Other comparison results are provided in the manuscript.

DISCUSSION

In the presented study discussed the effects of cytoplasmic galectins fractions isolated from normal and diseased human prostate tissue with different diagnosis (BPH, HGPIN, AAH and PC) on cell viability and apoptosis in tumor cell cultures (leukemia MEC1-line lymphocytes and prostate PC3).

Unlike normal healthy lymphocytes [10], a different effect is manifested in the case of tumor cells. The effect also varies depending on the cell type. The same lectin affects differently on leukemia MEC1-line lymphocytes and PC3 cell viability. All lectins from normal as well as from diseased prostate tissue except from AAH diagnosis are decreasing viability of leukemic MEC1-line lymphocytes, and all forms of lectin fractions decreased the PC3 cells viability.

In all cases of experiments, the effects of cytosolic galectin fractions on the cell's viability are conditioned by a specific binding with cells membrane surface glycoconjugates. Lectins effects are mediated by carbohydrate-binding domains since the addition of galactose in the incubation medium completely abolished these effects. So, the change of lectin biological function is depending on the glycoconjugate's carbohydrate structure. Glycosylation is one of the most common co- or posttranslational modifications. Malignant transformation is associated with abnormal glycosylation, changes the carbohydrate structure of the membrane and becomes the primary cause of metastasis [6,16]. The differences in glycosylation of proteins within the diseased prostate tissue, such as BPH and prostatic carcinoma comparing with normal tissues [16], were revealed. The carcinoma tissue intensifies the expression of proteins with the galactose residues [6,16,17].

Presumably the β-galactose-/N-acetyl-galactosamine/ lactose-containing glycoconutates are exposed on the normal and leukemic MEC1-line lymphocytes and PC-3-line cells membrane surface. The use of the exogenous (plant) lectins with different carbohydrate-specificity revealed also exhibited of mannose-/N-acetylglucosamine-containing glycoconjugates too (Figure 2,4). However, the β-galactose-specific lectins (as from prostate tissue as testified plant galactose-specific lectins) act differently on the viability of different types of cells. This effect is due to the fact that lectins influence on the cell viability and apoptosis through different pathways and some being more effective than others in specific cell lines [18].

The galactose-specific lectins expression varies according to prostate state pathologies [6,17]. The composition of cytosolic galactose-speciic lectins in isolated fractions (norm, BPH, HGPIN, AAH, PC) is different [10] and may contribute to various steps in tumor progression. Despite this galectins from PC and HGPIN (tumor precursor) diagnosed prostate tissue are increasing apoptosis as in normal lymphocytes [14], as in leukemic MEC1-line lymphocytes. We suggest that galectins from PC and HGPIN in both cases involved a similar mechanism of apoptosis.

CONCLUSIONS

There was studied the biological properties of cytosolic galactose-specific lectins, isolated from unaffected (norm) tissue (after cytoprostatectomies) and diseased prostate tissue with

different diagnoses (BPH/LGPIN, HGPIN, AAH and PC) in vitro system.

We conclude that the effect of cytosolic galactose-specific lectins fractions 1) depends on the form of glandular tissue disease and also on the type of cell (lymphocytes, PC3, used in the research), 2) is likely to depend significantly on the cell surface glycoconjugate's nature and structure and 3) the effect of cytosolic galactose-specific lectins fractions isolated from HGPIN and PC tissues can have defensive and anti-tumor properties in tumors cell – MEC1 line lymphocytes and prostate PC3 cell line.

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