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FLOW CYTOMETRIC ANALYSIS OF NORMAL AND OSTEOARTHRITIC CHONDROCYTES WITH LECTINS

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ABSTRACT. Osteoarthritis (OA) is the most common of all joint diseases, but the molecular basis of its onset and progression is controversial. One characteristic of cartilage is that some chondrocytes reside in areas rich in ECM and it is well known that glycoproteins are abundant on the cell surface and in cartilage ECM. Our working hypothesize was that modification of glycoconjugates contribute to pathogenesis of chondrocyte and we have undertaken a comparative study of normal and OA chondrocytes analysis with fluorescently labeled lectins. Our results clearly indicate that the loss of sialic acid and exposure of the penultimate β-galactose residues of OA chondrocytes glycoconjugates cell surfaces renders cells more susceptible to the membrane damage, which may trigger apoptosis. This could further clarify the relevance of the glycophenotype for chondrocyte function or its significance, if any, as a marker for the development of new chondroprotective agents.

Keywords: chondrocytes, osteoartritic cartilage, flow cytometric analysis, lectins

INTRODUCTION

Articular cartilage is a specialized, avascular and aneural connective tissue that provides covering for the osseous components of diarthrodial joints. It serves as a load-bearing material, absorbs impact and is capable of sustaining shearing forces (Martel-Pelletier et al., 2008).

Structurally, the cartilage is formed by chondrocytes (cartilaginous cells) and the extracellular matrix. Chondrocytes are present in small number, number which represent about 1 % from the tissue, and are responsible for synthesis, remodeling and the turnover rate of the extracellular matrix macromolecules including type II collagen, aggregating proteoglycans and non-collagenous proteins (Muir, 1995). Chondrocytes are the only cell type present in the cartilaginous tissue and the cellular density vary depending the cartilage area and the individual age of the people (Vignon et al., 1976; Mitrovic et al., 1983). The extracellular matrix ensure the integrity of the tissue and gives it the biomechanical properties. It is formed by water (65-80 % from the cartilage mass), proteins (collagen fibres, glicoproteins and binding glicoproteins) and proteoglicans. The extracellular matrix, the cell surface and the serum contains glycoprotein glycans. These glycans act as an interface on the cell surface and modulate protein properties, including folding, secretion, targeting, and protease resistance (Kobata, 1992; Wong, 2005).

Covalent association of an oligosaccharide chain called glycans with protein or lipid generates structures called glicoconjugates by Jean Montreuil in 1984 (Montreuil, 1984). Glycoproteins are classified into two groups: glycans attached to an asparagine residue through a nitrogen atom (N-glycans) and those attached to a serine or threonine oxygen (O-glycans) (Kobata, 1992). Both types have been intensively studied, and many studies report structural and functional analyses of N-glycans (Wacker et al., 2002). Based on these data, a relationship between N-glycan alteration and disease has recently been emerged (Yamashita, 1993; Bratosin, 1995; Landberg et al., 1995; Reitter et al., 1998; Jaeken & Carchon, 2001; Xiping et al., 2003; Wang et al., 2005). Untill now, the N-glycan alteration in the articular cartilage has been very little studied, but a hypothese is that the sugars attached to proteins contribute to chondrocyte metabolism. This hypothesis appears based on the fact that glycoproteins are abundant on the cell surface and in cartilage ECM.

Several studies shows that glycan modifications of proteins contribute to the pathogenesis of some diseases (Goldring, 2000; Bluteau et al., 2001; Wang et al., 2005) like osteoarthritis, an age-related disorder generally affecting articular cartilage characterized by degradation and loss of articular cartilage, subchondral bone remodeling and inflammation of the synovial membrane. The alterations in osteoarthritic cartilage are numerous

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pigments were calculated a, b and carotenoids, for each species, the ecosystem types (Table 3) were calculated pigments a, b and carotenoids for each species in each ecosystem (Table 4). In all cases, standard deviations were compared for different averages of deposition of dust-free surfaces, with the influence of dust deposits.are numerous and involve morphologic and metabolic changes in chondrocytes, as well as biochemical and structural alterations in the extracellular matrix macromolecules (Martel-Pelletier et al., 2008; Silaghi et al., 2011).

The diverse carbohydrate structures present in the glycocalyx of cells can serve as targets for biorecognitive proteins such as lectins (Toegel et al., 2007). Lectins are proteins or glycoproteins of non-immune origin that recognize and binds specific to glucidic structures and wich aglutinates cells and/or precipitates glycoconjugates. Lectins were first discovered in plants but are also present in the most living organisms (bacteria, animals and humans) (Gavrovic-Jankulovic & Prodanovic, 2011). In the study of membrane glycoconjugates changes or in the study of membrane receptors, lectins are widely used. Lectins are used to characterise glycoconjugate distribution in tissues as they possess at least one noncatalytic domain which binds reversibly to specific mono- or oligosaccharides (Liener et al., 1986). Lectins have also been used in histological studies in order to examine cartilage development, matrix synthesis and structural changes encountered in osteoarthritis (Vertel et al., 1985; Schuenke et al., 1985; Farnum et al., 1988; Hoedt-Schmidt et al., 1989; Goetz et al., 1991; Zschaebitz et al., 1995).

The usefulness of lectins occurs also in detecting and studying carbohydrates and the ubiquitous occurrence of lectins in nature provide a major stimulus for their continuing evaluation as feasible excipients (Toegel et al., 2007).

Taking account of all of these data, we emitted the hypothese that modification of glycoconjugates contribute to pathogenesis of chondrocyte and we have undertaken a comparative study of normal and OA chondrocytes analysis with fluorescently labeled lectins: WGA-FITC (wheat germ agglutinin) from Triticum vulgare to detect GlcNAcβ1-4GlcNAcβ1-4GlcNAc and Neu5Ac (sialic acid), SNA-FITC from Sambucus nigra, for Neu5Acα2-6Gal(NAc)-R sialic acid, 2-6Gal, MAA-FITC from Mackia amurensis to detect α2,3- linked sialic acid and RCA from Ricinus communis to detect Galβ1-4GlcNAc β1-R.

MATERIALS AND METHODS

Dulbecco's modified Eagle's medium (DMEM) was obtained from Cambrex Bio Science (Verviers, Belgium), fetal calf serum, penicillin, streptomycin, amphotericin B and L-glutamine were from Gibco (Carlsbad, USA). Hyaluronidase, trypsin, collagenase from Clostridium histoliticum were from Sigma (St. Louis, USA). Maackia amurensis agglutinin (MAA); Ricinus communis agglutinin (RCA), Sambucus nigra agglutinin (SNA), Wheat germ agglutinin (WGA) were from EY Laboratories (San Mateo, CA, USA). The flow cytometer was a Becton-Dickinson FACScan apparatus (San Jose, CA, USA) with CellQuest Pro software for acquisition and analysis.

Isolation of chondrocytes

Normal and osteoarthritic articular chondrocytes were isolated as described elsewhere with a few modifications (Green, 1971; Kuettner et al., 1982) from patients with osteoarthritis undergoing arthroplasty under sterile techniques (CF 2 Hospital, Bucharest, Romania). All enzymatic solutions were prepared in Dulbecco's modified Eagle's medium (DMEM) supplemented with a mixture of antibiotics and antimycotics (penicillin 10 U/ml, streptomycin 10 mg/ml, amphotericin B 0.025 mg/ml), L-glutamine 0.002M and 10% of fetal calf serum. The fragments of cartilage were minced into small pieces and incubated with 0.1% of sheep teste hyaluronidase in DMEM medium for 20 min at 37°C. The pieces were washed with PBS (Phosphate Saline Buffer pH 7.4, osmolality 320-330 mosmol kg-1) and maintained in a trypsin solution (0.25 g/100 ml PBS buffer pH 7.4) for 60 min at 37°C. The articular cartilage pieces were washed again with PSB buffer and incubated at 37°C and 5% CO2 overnight in 0.2 % collagenase from Clostridium histolyticum in DMEM medium with 10% fetal calf serum. Cells were then centrifuged for 15 min at 3000 rpm, washed with PBS buffer and then centrifuged for 15 min at 3000 rpm.

Determination of the lectin binding capacity of chondrocytic cells

50 µl of a different dilution of the respective fluorescein-labelled lectin in PBS buffer was added to 50 µl cell-suspension (3×105 cells) and incubated for 15 min at 4°C. Afterwards, the cells were resuspended in 900 µl particle free PBS in order to provide a single cell suspension suitable for flow cytometric analysis. For estimation of autofluorescence, control samples consisting of unlabelled cells were included in all experiments. The binding for each lectin was studied at concentration ranging from 0 to 50 mM in order to determine the optimal lectin concentration.

Flow cytometric analysis

Flow cytometric analyses were performed on a FACScan cytometer (San Jose, CA, USA) using CellQuest Pro software for acquisition and analysis. Cells in suspension in isotonic PBS buffer pH 7.4 were gated for the light scatter channels on linear gains, and the fluorescence channels were set on a logarithmic scale with a minimum of 10,000 cells analyzed in each condition. The results were expressed as MFI value of the logarithmic fluorescence intensity.

RESULTS

As shows in the overlay histogram presented in Figure 1, regarding the binding capacity of the wheat germ agglutinin from Triticum vulgare (WGA-FITC), the osteoarthritic sample presented a smaller value of the

MFI (MFI = 49) comparing to the normal cartilage (MFI = 71), which indicate a poor expression for the GlcNAcβ1-4GlcNAcβ1-4GlcNAc and Neu5Ac at the OA sample comparing to normal.

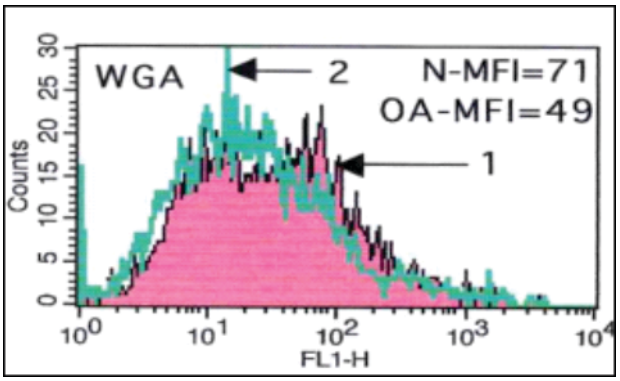

Fig.1 Analyses of membrane GlcNAcβ1-4GlcNAcβ1-4GlcNAc and Neu5Ac desialylation of normal (1) and osteoarthritic chondrocytes (2) using WGA-FITC lectin. Numbers represent fluorescence mean values (MFI). Abscissae: log scale green fluorescence intensity of WGA-FITC lectin (FL1). Ordinates: relative cell number. Number of counted cells: 10,000. Results presented are from one representative experiment of three performed.

In Figure 2 is presented the overlay histogram which express the binding capacity of the Sambucus nigra lectin (SNA-FITC) in order to determine the Neu5Acα2-6Gal(NAc)-R sialic acid desialylation. The osteoarthritic sample had a MFI = 49 value and the the normal cartilage had a MFI = 71 value, which also indicates a smaller capacity for binding the lectine of the osteoarthritic sample comparing to normal sample.

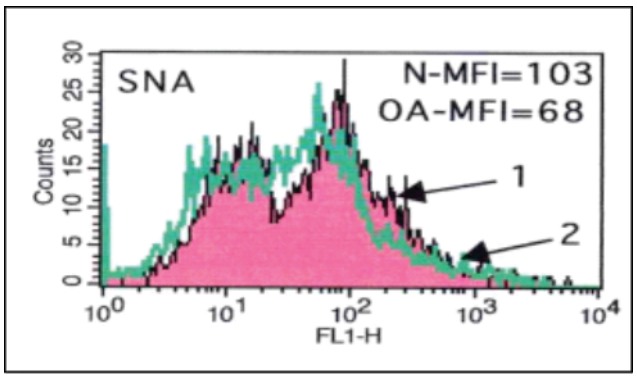

Fig. 2 Analyse of membrane Neu5Acα2-6Gal(NAc)-R sialic acid desialylation of normal (1) and osteoarthritic chondrocytes (2) using Sambucus nigra lectin (SNA-FITC). Numbers represent fluorescence mean values (MFI). Abscissae: log scale green fluorescence intensity of SNA-FITC lectin (FL1). Ordinates: relative cell number. Number of counted cells: 10,000. Results presented are from one representative experiment of three performed

In the same way, Mackia amurensis lectin (MAA-FITC) binding capacity (Figure 3) was smaller for the OA sample (MFI= 149) comparing to the normal sample (MFI=286).

Regarding the analyses made in order to determine the expression of Galβ1-4GlcNAc β1-R using Ricinus communis lectin (RCA-FITC), as is shows in Fig. 4, the MFI value indicates a higher quantity of Galβ1-4GlcNAc β1-R in OA chondrocytes compared to normal. The flow cytometric analyses reveal an MFI=633 for the normal sample and an MFI=530 for the osteoarthritic sample, confirming desialilation evidenced by sialic acid specific lectins.

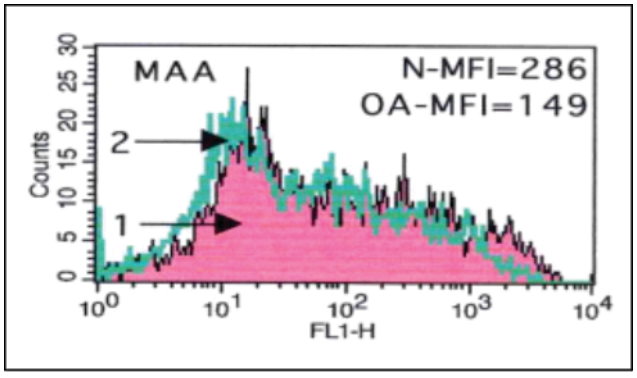

Fig. 3 Analyse of membrane α2,3- linked sialic acid desialylation of normal (1) and osteoarthritic chondrocytes (2) using Mackia amurensis lectin (MAA-FITC). Numbers represent fluorescence mean values (MFI). Abscissae: log scale green fluorescence intensity of MAA-FITC lectin (FL1). Ordinates: relative cell number. Number of counted cells: 10,000. Results presented are from one representative experiment of three performed.

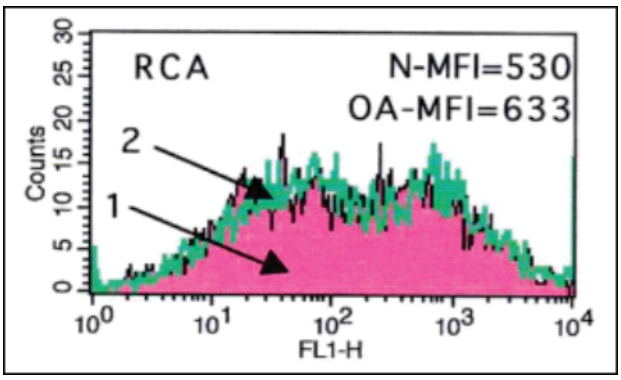

Fig. 4 Analyse of membrane Galβ1-4GlcNAc β1-R resulted after desialylation of normal (1) and osteoarthritic chondrocytes (2) using Ricinus communis lectin (RCA). Numbers represent fluorescence mean values (MFI). Abscissae: log scale green fluorescence intensity of RCA-FITC lectin (FL1). Ordinates: relative cell number. Number of counted cells: 10,000. Results presented are from one representative experiment of three performed

As shows in Figure 5, all the used lectins showed a desialilation of osteoarthritis chondrocytes compared to normal chondrocytes. From all of the lectines used, the WGA had the lowest binding capacity, with an MFI=71 for the normal and an MFI = 49 for the OA sample, compared with the MFI for the SNA lectin (103 for normal sample and 68 for the OA sample) and MAA lectin with an MFI = 286 for normal and 149 for the OA sample.

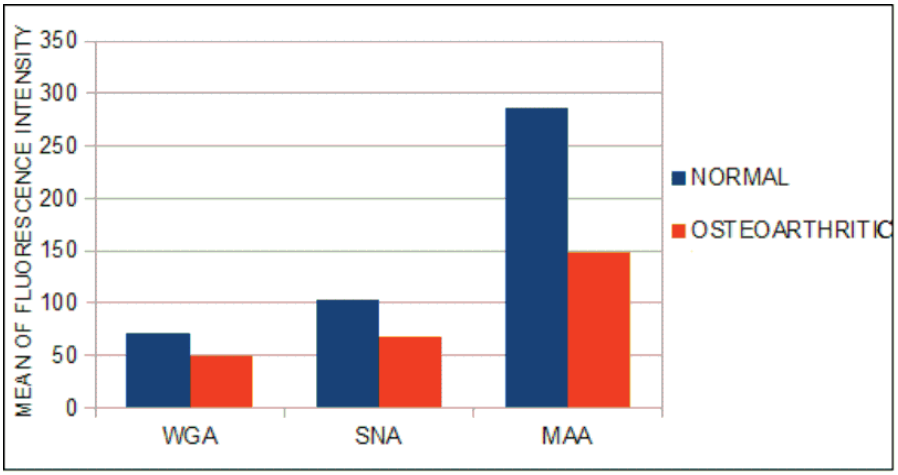

Fig. 5 Mean of fluorescence intensity of Wheat germ agglutinin (WGA), Sambucus nigra agglutinin(SNA) and Maackia amurensis agglutinin (MAA)

For the RCA binding capacity we obtained a higher binding capacity in the OA sample compared to control, as it shows in Figure 6, confirming the osteoarthritic chondrocytes desialilation followed by exposure of β-gal residues.

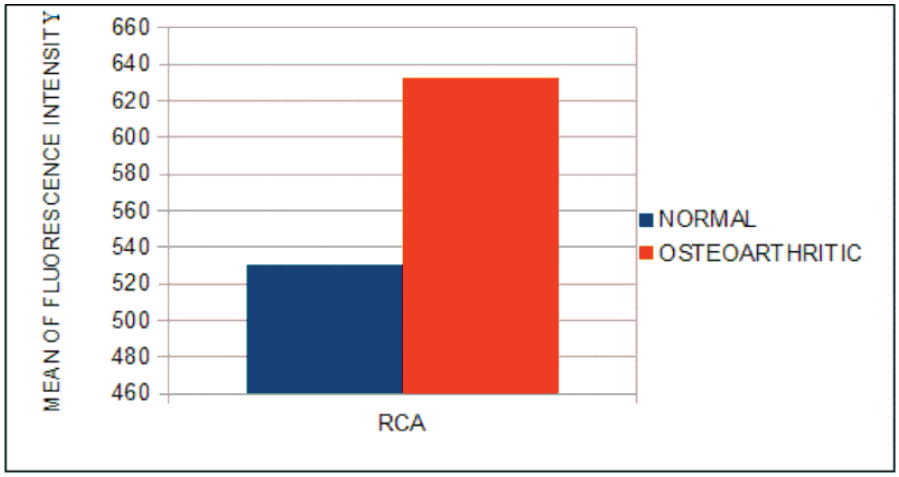

Fig. 6 Mean of fluorescence intensity of Ricinus communis agglutinin (RCA)

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DISCUSSIONS

Human articular cartilage is characterised by a sparse population of chondrocytes embedded in an abundant extracellular matrix (Toegel et al., 2007). The biochemical performance of cartilage depends on the biochemical and biophysical properties of extracellular matrix macromolecules and thus on the normal metabolic activity and homeostatic status of chondrocytes (Aigner et al., 2007).

The extracellular matrix (ECM) of cartilage ensure the integrity of the tissue, gives it the biomechanical properties and consist of water, proteins and numerous glycosylated proteins (Knudson & Knudson, 2001). Beside structural proteoglycans such as aggrecan and adhesive proteins such as chondroadherin, glycosylated cell surface receptors, including CD44 and integrins, play critical roles in the mediation of chondrocyte-matrix interactions (Knudson & Knudson, 2001; Dudhia, 2005; Nicoll et al., 2002). Protein glycosylation represents a common co-translational and posttranslational modification of proteins, conferring protection against proteolytic degradation or controlling protein folding and various factors can regulate cellular glycosylation and the expression of specific glycosyltransferases (Howard et al., 1993; Wagers et al., 1998; Brockhausen et al., 1998; Delmotte et al., 2002; Yang et al., 2004). Thus, changes in the cellular glycophenotype can potentially affect cell functions such as cell adhesion, cell surface receptor activity and the onset of apoptosis (Heyder et al., 2003; Yang et al., 2007). Sialic acids (Neu5Ac in humans) are negatively charged sugars typically found at the terminal positions of N- and O-linked oligosaccharides attached to cell surfaces or secreted glycoproteins. Despite the significance of glycoproteins for ECM assembly in cartilage tissue, however, little is known about the regulation of the chondrocyte glycophenotype. Recently, Toegel et al. founds that differences in the molecular phenotype between primary human chondrocytes and chondrocyte cell lines are reflected by specific cellular lectin-binding patterns (Toegel et al., 2007, 2009) and Yang and coworkers have shown that cytokine treatment of human and bovine chondrocytes results in specific glycosylation changes related to apoptosis and altered cell proliferation (Yang et al., 2007).

Recent studies have suggested a relationship between altered glycosylation and disease conditions showing that altered N glycans of serum immunoglobulin G molecules contribute to the pathophysiology of rheumatoid arthritis (Watson et al., 1999). Using animal model of osteoarthritis, there were studies that showed the altered N-glycosylation of cartilage tissue (Matsuhashi et al., 2008).

Previously we demonstrated using fluorescent marked lectins that the enzimatic desialilation is the first stage of erythrocyte apoptosis (Bratosin et al., 1995; Bilyy & Stoika 2003). Other studies also have shown that desialilation of nucleated eukaryotic cells is the first stage of cellular apoptosis phenomenon. Thus, in the same time, in the field of osteoartritic pathology we demonstrated the involvement of apoptosis in this disease (Takacs-Buia et al., 2008; Takacs-Buia et al., 2009; Bratosin et al., 2011). From this point, owing the potential implications of the chondrocyte glycophenotype in the pathophysiology of joint diseases such as osteoarthritis, we had the idea to investigate with lectins the degree of desialilation of the osteoarthritic chondrocytes. We investigated the sialylation processes in human and osteoarthritic chondrocytes to find if a desialilation process occurs in osteoarthritis. We compared the sialylation pattern of normal chondrocytes with osteoarthrithic chondrocytes, using the following lectines: WGA-FITC (wheat germ agglutinin) from Triticum vulgare to detect GlcNAcβ1-4GlcNAcβ1-4GlcNAc and Neu5Ac (sialic acid), SNA-FITC from Sambucus nigra, for Neu5Acα2-6Gal(NAc)-R sialic acid, 2-6Gal, MAA-FITC from Mackia amurensis to detect α 2,3- linked sialic acid and RCA from Ricinus communis to detect Galβ1-4GlcNAc β1-R.

The WGA-FITC (wheat germ agglutinin) from Triticum vulgare lectin, SNA-FITC from Sambucus nigra lectin and MAA-FITC from Mackia amurensis lectin shows lower MFI values of the OA samples compared with control, clearly indicate a loss of the sialic acid in the osteoarthrytic chondrocytes compared to control.

The results we obtained also indicate that the RCA lectin binding capacity was better at the OA sample which indicates an increased quantity of Galβ1-4GlcNAc β1-R to the OA sample compared with norma, which proves their externalisation after desialilation.

CONCLUSIONS

Taking all of the results obtained together, our results clearly indicate that the loss of sialic acid and exposure of the penultimate β-galactose residues of OA chondrocytes glycoconjugates cell surfaces renders cells more susceptible to the membrane damage, which may trigger apoptosis. Based on the presented results, future studies might aim to specify the glycoprofile of chondrocytes that is related to OA.

This could further clarify the relevance of the glycophenotype for chondrocyte function or its significance, if any, as a marker for the development of new chondroprotective agents.

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REFERENCES

Aigner T, Soder S, Gebhard PM, Mcalinden A and Haag J, Mechanisms of Disease: role of chondrocytes in the pathogenesis of osteoarthritis-structure, chaos and sesescence. Nature Clinical practice Rheumatology, 3, 391-399, 2007.

Bilyy RO, Stoika RS, Lectinocytochemical detection of apoptotic murine leukemia L1210 cells. Cytometry, 56A, 89-95, 2003.

Bluteau G, Conrozier T, Mathieu P, Vignon E, Herbage D, Mallein-Gerin F. Matrix metalloproteinase-1, -3, -13 and aggrecanase-1 and -2 are differentially expressed in experimental osteoarthritis. Biochim. Biophys. Acta, 1526, 147-158, 2001.

Bratosin D, Iordachel C, Rugina A, Gheorghe AM, Ciotec ACL, Efimov N, Sidoroff M, Cellular methods for in vitro engineering of human autologous cartilage. A review. Studia Universitas "Vasile Goldis" Life science series, 21, 175-181, 2011.

Bratosin D, Mazurier J, Debray H, Lecocq M, Boilly B, Alonso C, Moisei M, Motas C, Montreuil J, Flow cytofluorimetric analysis of young and senescent human erythrocytes probed with lectins. Evidence that sialic acids control their life span. Glycoconj. J., 12, 258-267, 1995.

Brockhausen I, Schutzbach J, Kuhns W, Glycoproteins and their relationship to human disease. Acta Anat, 161, 36–78, 1998.

Delmotte P, Degroote S, Lafitte JJ, Lamblin G, Perini JP, Roussel P, Tumor Necrosis Factor alpha increases the expression of glycosyltransferases and sulfotransferases responsible for the biosynthesis of sialylated and sulfated Lewis × epitopes in the human bronchial mucosa. J. Biol. Chem., 277, 424-431, 2002.

Dudhia J, Aggrecan, aging and assembly in articular cartilage. Cell. Mol. Life Sci., 62, 2241-2256, 2005.

Farnum CE, Wilsman NJ, Lectin-binding histochemistry of intracellular and extracellular glycoconjugates of the reserve cell zone of growth plate cartilage. J. Orthop. Res., 6, 166-179, 1988.

Gavrovic-Jankulovic M, Prodanovic R, Drug Delivery: Plant Lectins as Bioadhesive Drug Delivery Systems. Journal of Biomaterials and Nanobiotechnology, 2, 614-621, 2011.

Goetz W, Fischer G, Herken R, Lectin binding in the embryonal and early fetal human vertebral column, Anat. Embryol., 184, 345-353, 1991.

Goldring MB, The role of the chondrocyte in osteoarthritis. Arthritis Rheum., 43, 1916-1926, 2000.

Heyder P, Gaipl US, Beyer TD, Voll RE, Kern PM, Stach C, Kalden JR, Herrmann M, Early detection of apoptosis by staining of acid-treated apoptotic cells with FITC-labeled lectin from Narcissus pseudonarcissus. Cytometry, 55A, 86-93, 2003.

Hoedt-Schmidt S, Scheid A, Kalbhen DA, Histomorphological and lectinhistochemical confirmation of the antidegenerative effect of diclofenac in experimental osteoarthrosis. Arzneim.-Forsch., 39, 1212–1219, 1989.

Howard S, Anastassiades T, Differential effects of bone associated factors on newly synthesized anionic glycoconjugates by articular chondrocyte cultures from adult and immature bovines. J. Rheumatol., 20, 2083–2094, 1993.

Jaeken J, Carchon H, Congenital disorders of glycosylation: the rapidly growing tip of the iceberg. Curr. Opin. Neurol., 14, 811-815, 2001.

Knudson CB, Knudson W, Cartilage Proteoglycans. Semin. Cell. Dev. Biol., 12, 69-78, 2001.

Kobata A, Structure and function of the sugar chains of glycoprotein. Eur. J. Biochem., 209, 483-501, 1992.

Landberg E, Pahlsson P, Lundblad A, Arnetorp A, Jeppsson JO, Carbohydrate composition of serum transferrin isoforms from patients with high alcohol consumption. Biochem. Biophys. Res. Commun., 210, 267-274, 1995.

Liener IE, Sharon N, Goldstein IJ, The Lectins, Properties, Functions, and Applications in Biology and Medicine. Academic Press., Orlando, FA, 1986.

Martel-Pelletier J, Boileau C, Pelletier JP, Roughley JP, Cartilage in normal and osteoarthritis conditions. Best Practice & Research Clinical Rheumatology, 22, 351-384, 2008.

Matsuhashi T, Iwasaki N, Nakagawa H, Hato M, Kurogochi M, Majima T, Minami A, Nishimura SI, Alteration of Nglycans related to articular cartilage deterioration after anterior cruciate ligament transection in rabbits. Osteoarthr. Cartilage, 16, 772-778, 2008.

Mitrovic D, Quintero M, Starkovic A, Ryckeweart A, Cell density of adult human femural condylar articular surface in joints with normal and fibrillated surface. Lab Invest, 49, 309-316, 1983.

Montreuil J, Spatial conformation of glycans and glycoproteins. Biology of the Cell, 51, 115-131, 1984.

Muir H, The chondrocyte, architect of cartilage. Biomechanics, structure, function and molecular biology of cartilage matrix macromolecules. Bioessays, 17, 1039-1048, 1995.

Nicoll SB, Barak O, Csóka AB, Bhatnagar RS, Stern R, Hyaluronidases and CD44 undergo differential modulation during chondrogenesis. Biochem. Biophys. Res. Commun., 292, 819-825, 2002.

Reitter JN, Means RE, Desrosiers RC, A role for carbohydrates in immune evasion in AIDS. Nat. Med., 4, 679-684, 1998.

Schuenke M, Schumacher U, Tillmann B, Lectin-binding in normal and fibrillated articular cartilage of human patellae. Virchows Arch. A., 407, 221–231, 1985.

Silaghi C. N., Craciun A. M., Cristea V., Matrix Gla protein: the inhibitor of vascular and osteoarticular calcifications. Human & Veterinary Medicine 3(3), 178-190, 2011.

Takacs-Buia L, Bratosin D, Gheorghe AM, Iordachel C, Efimov N, Caloianu M, Montreuil J, Flow cytometric analysis of cell death and viability of osteartritic chondrocytes cultured in presence of apoptosis inhibitors for tissue engineering. Studia Universitas "Vasile Goldis", Life science series, 19, 7-13, 2009.

Takacs-Buia L, Iordachel C, Efimov N, Caloianu M, Montreuil J, Bratosin D, Pathogenesis of Osteoarthritis: Chondrocyte Replicative Senescence or Apoptosis?. Cytometry Part B, 74 B, 356-362, 2008.

Toegel S, Harrer N, Plattner VE, Unger FM, Viernstein H, Goldring MB, Gabor F, Wirth M, Lectin binding studies on C-28/I2 and T/C-28a2 chondrocytes provide a basis for new tissue engineering and drug delivery perspectives in cartilage research. Journal of Controlled Release, 117, 121-129, 2007.

Toegel S, Plattner V E, Wu S Q, Goldring MB, Chiari C, Kolb A, Unger FM, Nehrer S, Gabor F, Viernstein H, Wirth M, Lectin binding patterns reflect the phenotypic status of in vitro chondrocyte models. In Vitro Cell Dev Biol Anim., 45, 351-360, 2009.

Vertel BM, Morrell JJ, Barkman LL, Immunofluorescence studies on cartilage matrix synthesis, Exp. Cell Res. 158 423-432, 1985.

Vignon E, Arlot M, Patricot L, Vignon G, The cell density of human femoral head cartilage. Clin. Orthop., 121, 303-308, 1976.

Wacker M, Linton D, Hitchen PG, Nita-Lazar M, Haslam SM, North SJ Panico M, Morris RH, Dell A, Wren WB, Aebi M, N-linked glycosylation in Campylobacter jejuni and its functional transfer into E. Coli. Science, 298, 1790-1793, 2002.

Wagers AJ, Waters CM, Stoolman LM, Kansas GS, Interleukin 12 and interleukin 4 control T cell adhesion to endothelial selectins through opposite effects on alpha1, 3-fucosyltransferase VII gene expression. J. Exp. Med., 188, 2225-2231, 1998.

Wang X, Inoue S, Gu J, Miyoshi E, Noda K, Li W, Mizuno-Horikawa Y, Nakano M, Asahi M, Takahashi M, Uozumi N, Ihara S, Lee SH, Ikeda Y, Yamaguchi Y, Aze Y, Tomiyama Y, Fujii J, Suzuki K, Kondo A, Shapiro SD, Lopez-Otin C, Kuwaki T, Okabe M, Honke K, Taniguchi N, Dysregulation of TGF-b1 receptor activation leads to abnormal lung development and emphysema-like phenotype in core fucose-deficient mice. Proc Natl Acad Sci U S A, 102, 15791-15796, 2005.

Watson M, Rudd PM, Bland M, Dwek RA, Axford JS, Sugar printing rheumatic diseases: a potential method for disease differentiation using immunoglobulin G oligosaccharides. Arthritis Rheum., 42, 1682-1690, 1999.

Wong CH, Protein glycosylation: new challenges and opportunities. J. Org. Chem., 70, 4219-4225, 2005.

Xiping W, Julie MD, Shuyi W, Huxiong H, Kappes JC, Xiaoyun W, Salazar-Gonzalez JF, Salazar MG, Kilby JM, Saag MS, Komarova NL, Martin AN, Hahn B, Kwongk PD, Shaw GM, Antibody neutralization and escape by HIV-1. Nature, 422, 307-312, 2003.

Yamashita K, Ideo H, Ohkura T, Fukushima K, Yuasa I, Ohno K, Takeshita K, Sugar chains of serum transferrin from patients with carbohydrate deficient glycoprotein syndrome. Evidence of asparagine-N-linked oligosaccharide transfer deficiency., J. Bio. Chem., 268, 5783-5789, 1993.

Yang X, Lehotay M, Anastassiades T, Harrison M, Brockhausen I, The effect of tnfalpha on glycosylation pathways in bovine synoviocytes. Biochem. Cell. Biol., 82, 559-568, 2004.

Yang X, Yipa J, Anastassiades T, Harrison M, Brockhausen I, The action of tnfα and tgfβ include specific alterations of the glycosylation of bovine and human chondrocytes. Biochim. Biophys. Acta, 1773, 264-272, 2007.

Zschaebitz A, Krahn V, Gabius HJ, Weiser H, Khaw A, Biesalski HK, Stofft E, Glycoconjugate expression of chondrocytes and perichondrium during hyaline cartilage development in the rat. J. Anat., 187, 67-83, 1995.