Review Article *Spatula DD. 2015; 5(1):15-19*

Carbohydrate-mediated drug targeting-A review

Karbonhidrat aracılı ilaç hedeflemesi-Bir derleme

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ABSTRACT
Conventional drugs used in cancer therapy have extended the survival time of patients, however mortality has not significantly decreased. It is evident that there is a need to develop a new, more effective and less toxic compounds in cancer treatment. A promising new approach is based on the deciphering of biological information encoded in β-galactose-containing glycoconjugates and to find out the biological role(s) of carbohydrate binding proteins/galectins, which able to convert encoded information into biological and pathological reactions. Galectins can alter cellular interactions and have important roles in cell cycle, apoptosis and metastases. This review aimed to provide an overview on the potential advantages concerning understanding the mechanism of action of galectin-3 (gal-3) as a therapeutic agent for cancer. Galectin-3 has been found to be upregulated in many types of cancer including colon, gastric, renal and non-small cell lung cancer. Results of several studies indicating that gal-3 plays a dynamic role in the tumorogenesis and metastatic process, and showing that blockers/inhibitors of gal-3 could have important therapeutic potential in cancer.
Keywords: Galectin-3; Cancer; Drug; Carbohydrates.

ÖZET
Kanser tedavisinde kullanılan konvesiyonel ilaçlar hastaların ömrünü uzatmakla birlikte ölüm oranları anlamlı düzeylerde azaltılabilmiş değildir. Günümüzde kanser tedavisinde kullanılmak üzere toksisitesi azaltılmış ve etkinliği artırılmış yeni ilaçların geliştirilmesinin gerekliliği ortadadır. Glikokonjugatların yapısında şifrelenmiş olan biyolojik bilgiyi çözmeye yönelik çalışmalar ile bu bilginin deşifre edilip biyolojik ve patalojik olaylarda kullanılmasına aracılık eden karbonhidrat bağlayan proteinlerin/galektinlerin üzerine yapılan çalışmalar ümit vermektedir. Galektinler hücreler arası etkileşimde, hücre siklusunda, apoptozisde ve metastazda rol oynamaktadırlar. Bu derlemenin amacı galektin-3'ün kanser tedavisinde hedef olarak seçilmesinin avantajının ne olabileceği ve bunun mekanizmasının anlaşılmasına yönelik genel bir bilginin verilmesi amaçlanmıştır. Kolon, mide, böbrek ve küçük hücreleri olmayan akciğer kanserlerinde galektin-3 sentezinin uyarıldığı görülmüştür. Çok sayıda çalışmadan elde edilen bulgular galektin-3'ün tümörogeneziste ve metastazda rol oynadığını göstermiştir. Kanser tedavisinde galektin-3 blokörlerinin/inhibitörlerinin kullanılma potansiyelinin yüksek olduğu görülmüştür.
Anahtar kelimeler: Galektin-3; Kanser; İlaç; Karbonhidratlar.

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Received March 23, 2015 ; **Accepted** March 27, 2015
DOI 10.5455/spatula.20150330105811
Published online in ScopeMed (www.scopemed.org).
Spatula DD..

INTRODUCTION

Based on the widely held belief that carbohydrates are dull compounds and that they serve only as structural or protective materials (e.g., cellulose in plants and chitin in insects) and as an energy source (glycogen in animals), but lack any biological specificity, a long time proteins and carbohydrates were considered separate classes of natural products. However, there is expanding evidence that carbohydrates act as recognition determinants in different physiological and pathological processes [1-11]. Following the realization that carbohydrates endowed with a huge potential for encoding biological information, researches conceived that carbohydrates can play an important role in different physiological and pathological processes [12,13]. Biological information encoded in the carbohydrate structures are deciphered through interactions with complementary sites on carbohydrate-binding proteins/lectins [14].

Oligosaccharides present in human tissues are often linked to lipids or proteins. The resultant glycoconjugates are found within the cells, both in the cytoplasm and subcellular organelles, and in the cell membranes as well as in extracellular space [15].

Oligosaccharides are to be encountered in the macromolecules, such as structural proteins, transport proteins, enzymes, hormones, immunoglobulins and cell adhesion molecules [15]. The biological role of these carbohydrate structures may include the modulation of the functional activity of a protein masking of such epitopes and the provision of ligands for specific binding sites that mediate cell-cell or cell-matrix interactions.

Alterations in the expression and localisation of O- and N-glycan chains of glycoproteins were detected in tumour cells. Branching and sialylation of N-glycans in cancerous tissues is proven, while O-glycans are often truncated and sialylated [16-18]. The new branches come into existence in tumour cells provides attachment places for additional N-acetyllactosamine (LacNAc) chains, sialic acid residues and other carbohydrate structures that may be in action in the cell adhesion or other biological processes and in the protection of the cancer cell surface, promoting survival in the blood stream and invasion of tissues [18,19]. Abnormal glycosylation patterns in colon cancer tissues are revealed. Likewise, changed carbohydrate activities in human colorectal polyps are reported.

After realization that lectins have a gift to decipher the encoded biological information in the carbohydrate structures and they play numerous biologicals roles such as in cell growth and differentiation, in interactions of cells with their environment, and also in a variety of pathological processes, studies on lectins increased promptly. An expanding body of studies is focusing on biochemical properties and physiological roles of galectins.

Galectins are carbohydrate binding proteins with 15 different members. According to the structure of the carbohydrate recognition domain (CRD) galectins are divided into 3 subgroups: proto-type, tandem-repeat type and chimera-type. Galectin-3 is the only galectin in the chimera-type. Galectins can be expressed by almost all cell types, however different cells usually express different type of galectins [20,21]. They are synthesized on cytoplasmic ribosomes and don't cary any signal peptide to be targeted for classical ER/Golgi secretion pathway [21]. Galectins can be found virtually in all organelles, outside the cell, at the cell surface or in the extracellular matrix (ECM) [20]. Depend on the biological function of cells they can have ability to secrete galectins, strictly controlled during development, transformation or in health or disease. The localization of galectins can affect the role of these proteins. Actually intracellular or extracellular localization of galectins may have opposing effects. For example, while intracellular gal-3 protects cells from apoptosis extracellular gal-3, however induces cell death.

Studies on galectins provided that galectins are involved in a wide variety of cellular processes that include pre mRNA splicing, cell growth regulation, cell adhesion, embryogenesis, inflammation, immune function, apoptosis, angiogenesis and tumor metastasis. Studies focused on the role of gal-3 in different malignancies revealed that expression of gal-3 in the head, neck, gastric or anaplastic large cell lymphoma tumors, thyroid and central nervous system (CNS) tumors is up-regulated. Contrary to these statement expression of gal-3 is down-regulated in carcinomas of the uterus, breast and ovary. Based on the NWGR motif primarily associated with the C-terminus of gal-3, this protein exhibits similarities with the anti-apoptotic protein Bcl-2. This anti-apoptotic activity is abolished with a single amino acid substitution, such as glycine 182 to alanine [22,23].

GALECTIN-3 AS A MODULATOR OF CELLULAR PROSESSES

Galectin-3 is a carbohydrate binding protein with a molecular weight about 29-35 kD showing binding specifity for galactose residues, particularly prefers to bind LacNAc structures. Galectin-3 is a unique chimera-type member of galectin family containing three different structural domains such as an NH_2-terminal domain which contains a serine phosphorylation site, a collagen-like sequence and a COOH-terminal domain containing a single CRD with Asp-Trp-Gly-Arg amino acid motif (NWGR) responsible for the anti-apoptotic action of gal-3 [24-30]. Galectin-3 participates in numerous biological processes like cell adhesion, cell activation, cell growth and differentiation, cell cycle, and apoptosis [31–34]. Like other galectins, gal-3 is present both inside and outside cells [35–37] and interacts with other intracellular and extracellular ligands. Main localization site of gal-3 is cytosol, however it can also passes membranes reaching the nucleus, mitochondria and the extracellular environment. Galectin-3 localized in the extracellular space has innumerous binding sites, mostly polylactosamine-rich molecules in the ECM or on the cell surface. Through the binding to multivalent carbohydrates gal-3 can form pentamers that modulate intracellular signaling cascade [34]. Extracellular gal-3 acts like an adhesion molecule by cross-linking neighboring cells or cells and ECM components. Due to these interactions with polylactosamine-rich molecules gal-3 plays a key role in the extracellular modulation of

tumor progression [31]. Overexpression of gal-3 in various human solid tumors is well known fact.

Protein glycosylation is to be seen almost in the all healthy tissues and it so widespread that nearly half of all known proteins in eukaryotes are being glycosylated. Oligosaccharide residues in proteins secreted or expressed on the cell surface or within the cell affect protein conformation, localization as well as its role playing in biological processes. Alterations in the carbohydrate residues of proteins may have relevant effects of the cell behavior and cell activity. Even a tiny aberration in glycosylation of proteins may have huge harmful effects on the physiological and pathological events, such as cell growth, migration, cell adhesion, endocytosis and tumor development and metastasis [31].

Galectin-3 present inside of the cells is mostly a monomeric soluble protein [31]. Interaction of gal-3 with specific carbohydrate residues of glycoproteins or glycolipids leads binding of the other gal-3 monomers via N-terminal domains to this complex establishing a pentameric structures. This multivalent complex consist of five gal-3 and their carbohydrate structures makes galectin–glycan clusters termed lattices cross-linked with carbohydrate-containing glycoproteins or glycolipids can modulate cell function [31].

In tumor progression, due to the alterations in some cancer cells they gain the ability to invade surrounding tissues and through blood or lymphatic vessels they can penetrate basement membranes and endothelial walls, son they can colonize in distant organs. This complex and dynamic process accounts for the majority of cancer-related deaths [38,39]. Based on the changes in proteins related in cell–cell and cell–matrix adhesions invasion and metastasis processes of cancer cells are quite complex event. Extracellular gal-3 seems to play a crucial role in these processes [31,38]. Several studies proved that in migration and invasion of cancer cells gal-3 expression is up-regulated. Up-regulation of gal-3 expression is detected especially in lung cancer, breast cancer, melanoma [31], gastric cancer [40], sarcoma [41], and in leukemia [31]. Interactions of adhesive molecules with ECM is crucial for cell migration [42]. To this end, it is proven that gal-3 interacts with ECM localized specific glycoproteins such as fibronectin, collagen IV, elastin, laminin, and hensin [31]. Growing and multiplying of tumor cells need continuous delivery of nutrients and oxygen. Delivery of these substances is requiring neovascularization or angiogenesis. Establishing new capillaries is a quite complex process demands activation of endothelial cells and migration of them. In this neovascularization process for the activation and migration of the endothelial cells gal-3 plays an important role. Galectin-3 increases motility of endothelial cells and to promote angiogenesis by interacting with integrins and other cell surface molecules.

It is a well-known fact that diminishing of intercellular adhesions pav the way for tumor cells to invasion and metastasis. Binding of gal-3 to N-cadherin destabilizes the cell–cell junctions, which might favor cell migration process. Interaction with cancer-associated transmembrane mucin protein (MUC1) and gal-3 increases tumor cell aggregation and survival. Studies performed showed that in a galectin-null sarcoma cell line adhesion of cancerous cells to laminin decreased remarkably.

GALECTIN-3 INHIBITION AS A TOOL TO PREVENT TUMOR GROWTH AND METASTASIS

It is proven that gal-3 is a oncogenic protein and plays crucial role on the regulation of cell growth, cell adhesion, cell proliferation, angiogenesis, and apoptosis. Intracellular gal-3 promotes malignant cell transformation by stimulating cell growth and inhibiting apoptosis [31]. Some studies reported that inhibition of gal-3 expression in certain human carcinoma cell lines resulted in the loss of the malignant cell phenotype and slower tumor growth. Galectin-3 promotes cell cycle progression by downregulating the expression of cyclin E and cyclin A and upregulating the expression of cell cycle inhibitors p21 and of cyclin D. Galectin-3 has a role in tumor progression by regulating apoptosis. It is shown that increased expression of intracellular gal-3 in certain cancerous cells protected these cells from apoptosis. Galectin-3 maintains mitochondrial integrity and block release of pro-apoptotic factors, thereby preventing apoptotic cell death. Using anticancer drugs translocates gal-3 either from the cytosol or the nucleus to the mitochondria and blocks changes in the mitochondrial membrane potential. Inducing of translocation of gal-3 to mitochondria prevents apoptosis.

Studies on the role of gal-3 in tumor growth and metastasis gave us a clue that tumor growth or dissemination of cancer cells throughout the body could be prevented by targeting gal-3. Galectin-3 blockers that specifically inhibit this lectin are needed to be design and set as drugs. Numerous approaches could be taken to target gal-3 for therapeutic intention. Carbohydrate residues specific for gal-3 could be taken as target molecules using peptidomimetics to block LacNAc structures or galactose residues. Once they are blocked by certain drugs, it is no more possible for gal-3 to act as a

mediator in cancer progression. In addition to the binding sites for gal-3, this carbohydrate binding protein itself could be taken as target molecule. Carbohydrate based compounds contains binding sites for gal-3 could be taken into consideration as a gal-3 blockers. For example, intravenous injection of modified citrus pectin (MCP), gained by degradation of highly branched citrus pectin, into mice widely blocked melanoma cells metastasis to the lung. Furthermore, it is proven that MCP inhibits binding of melanoma cells to laminin and oral intake of MCP in mice inhibits tumor growth, angiogenesis and metastasis [42-46]. Furthermore, the thiodigalactoside diester Td131_1 has been revealed to be very specific molecule inhibits gal-3 effectively [47-49].

CONCLUSION

The roles of gal-3 in human cancer are well known. Targeting the actions of galectins in the tissues of cancer patients may help to prevent cancer progression and attempts to cure cancer patient by targeting of gal-3 represent a promising therapeutic strategy for preventing cancer progression. Numerous inhibitors to target gal-3 can be isolated from natural products but also they can be synthesized chemically. Clinical outcomes of anti-Bcl-2 drugs in cancer therapy the best results for patients with cancer may be achieved by using gal-3 inhibitors such as MCP, thiodigalactoside diester Td131_1, with other anticancer drugs.

REFERENCES

1. Sharon N. Glycoproteins now and then: A personal account. Acta Anat. 1998; 161: 7-17.
2. Bourrilon R, Aubery M. Cell surface glycoproteins in embryonic development. Int Rev Cytol. 1989; 116: 257-338.
3. Dennis RP. A review of biological significance of carbohydrates on glycoproteins and methods for their analysis. Eds.: Alavi A, Axford JS. (eds) In: Glycoimmunology; 1995; 1-10.
4. Gabius HJ, Kayser K, Gabius S. Protein-Zucker-Erkennung: Grundlagen und medizinische Anwendungen am Beispiel der Tumorlektinologie. Naturwissenschaften. 1995; 82: 533-43.
5. Geyer H, Geyer R. Strategies for glycoconjugate analysis. Acta Anat. 1998; 161: 18-35.
6. Mann PL. Membrane oligosaccharides: structure and function during differentiation. Int Rev Cytol. 1988; 112: 67-96.
7. Muramatsu T. Developmentally regulated expression of cell surface carbohydrates during mouse embryogenesis. Cell Biochem. 1998; 36: 1-14.
8. Perillo NL, Madeline EM, Baum LG. Galectins: versatile modulators of cell adhesion, cell proliferation, and cell death. J Mol Med. 1998; 76: 402-412.
9. Schmidt RR. Neu Methoden zur Glycosid- und Oligosaccharidsynthese-gibt es Alternativen zur Koenigs-Knorr- Methode? Angew. Chem. 1986; 98: 213-236.
10. Sharon N, Liss H. Carbohydrates in cell recognition. Sci. Am. 1993; 268: 82-89.
11. Zanetta JP, Badeache A, Maschke S, Marschal P, Kuchler S. Carbohydrates and soluble lectins in the regulation of cell adhesion and proliferation. Histol Histopathol. 1994; 9: 385-412.
12. Bevilacua M, Nelson RM. Selectins. J Clin Invest. 1993; 91:379-387.
13. Hakomori S, Igarashi Y. Functional role of glycospingolipids in cell recognition and signaling. J Biochem. 1995; 118:1091-1101.
14. Sharon N, Liss H. Lectins-proteins with a sweet tooth: functions in cell recognition. Essays Biochem. 1995; 30: 59-75.
15. Varki A. Biological roles of oligosaccharides: all of the theories are correct. Glycobiology. 1993; 3: 97-130.
16. Cheresh DA, Reisfeld RA, Varki A. O-Acetylation of disialoganglioside GD3 by human melanoma cells creates an unique antigenic determinant. Science. 1984; 225: 844-846.
17. Kageshita T, Hirai S, Kimura T. Association between sialyl Lewisa expression and tumor progression in melanoma. Cancer Res. 1995; 55: 1748-1751.
18. Seyrek, K, Seyrek-İntaş, K, Keskin, A, Kargın Kıral, F, Musal, B, Toplu, N. Biochemical and Histochemical Detection of the Sialic Acids in Mammary Tumours of Bitches. Rev. Med. Vet-Toulouse. 2005; 156(5): 258-263.
19. Brockhausen I. Clinical aspects of glycoprotein biosynthesis. Crit Rev Clin Lab Sci. 1993; 30: 165-151.
20. Liu FT, Patterson RJ, Wang JL. Intracellular functions of galectins. Biochim Biophys Acta. 2002; 1572(2-3): 263-273.
21. Lepur A. Fuctional properties of galectin-3 Doctoral thesis. Lund University, Deparment of Laboratory Medicine, Section of Microbiology, Imminology and Glycobiology. 2012.
22. Yang RY, Hsu DK, Liu FT. Expression of galectin-3 modulates T-cell growth and apoptosis. Proc Natl Acad Sci USA. 1996; 25: 6737–42.
23. Akahani S, Nangia-Makker P, Inohara H, Kim HR, Raz A. Galectin-3: a novel anti-apoptotic molecule with a functional BH1 (NWGR) domain of Bcl-2 family. Cancer Res. 1997; 57: 5272–76.
24. Lobsanov YD. et al. Crystallization and preliminary X-ray diffraction analysis of the human dimeric S-Lac lectin (L-14-II). J Mol Biol. 1993; 233, 553-555.
25. Lobsanov YD. et al. X-ray crystal structure of the human dimeric S-Lac lectin, L-14-II, in complex with lactose at 2.9-A resolution. J Biol Chem. 1993; 268: 27034-27038.
26. Seetharaman J. et al. X-ray crystal structure of the human galectin-3 carbohydrate recognition domain at 2.1-A resolution. J Biol Chem. 1998; 273: 13047-13052.
27. Birdsall B. et al. NMR solution studies of hamster galectin-3 and electron microscopic visualization of surface-adsorbed complexes: evidence for interactions between the N and C-terminal domains. Biochemistry. 2001; 40: 4859-4866.
28. Liao DI. et al. Structure of S-lectin, a developmentally regulated vertebrate beta galactoside-binding protein. Proc Natl Acad Sci U S A. 1994; 91: 1428-1432.
29. Mehul B, Hughes RC. Plasma membrane targeting, vesicular budding and release of galectin-3 from the cytoplasm of mammalian cells during secretion. J Cell Sci. 1997; 110: 1169-1178.
30. Almkvist J, Karlsson A. Galectins as inflammatory mediators. Glycoconj J. 2004; 19: 575-581.
31. Fortuna-Costa A, Gomes AM, Kozlowski EO. et al. Extracellular galectin-3 in tumor progression and metastasis. Front Oncol. 2014; 4: 1-9.
32. DiLella S, Sundblad V, Cerliani J, Guardia C, Estrin D, Vasta G. et al. When galectins recognize glycans: from biochemistry to physiology and back again. Biochemistry. 2011; 50(37): 7842–7857.
33. Liu FT, Rabinovich G. Galectins as modulators of tumour progression. Nat Rev Cancer. 2005; 5(1): 29–41.

34. Newlaczyl A, Yu LG. Galectin-3–a jack-of-all-trades in cancer. Cancer Lett. 2011; 313(2):123–8.
35. Hsu D, Zuberi R, Liu F. Biochemical and biophysical characterization of human recombinant IgE-binding protein, an S-type animal lectin. J BiolChem. 1992; 267(20): 14167–14174.
36. Dumic J, Dabelic S, Flögel M. Galectin-3: an open-ended story. Biochim Biophys Acta. 2006; 1760 (4): 616–635.
37. Takenaka Y, Inohara H, Yoshii T, Oshima K, Nakahara S, Akahani S. et al. Malignant transformation of thyroid follicular cells by galectin-3. Cancer Lett. 2003; 195 (1): 111–119.
38. Elad-Sfadia G, Haklai R, Balan E, Kloog Y. Galectin-3 augments K-Ras activation and triggers a Ras signal that attenuates ERK but not phosphoinositide 3-kinase activity. J Biol Chem 2004; 279 (33): 34922–34930.
39. Hanahan D, Weinberg R. Hallmarks of cancer: the next generation. Cell. 2011; 144 (5): 646–674.
40. Talmadge J, Fidler I. AACR centennial series: thebiology of cancer metastasis: historical perspective. Cancer Res. 2010; 70 (14): 5649-5669.
41. Kim SJ, Shin JY, Lee KD, Bae YK, Choi IJ, Park S. et al. Galectin-3 facilitates cell motility in gastric cancer by up-regulating protease-activated receptor-1(PAR-1) and matrix metalloproteinase-1 (MMP-1). PLoS One. 2011; 6 (9): e25103.
42. Melo F, Butera D, Junqueira M, Hsu D, da Silva AM, Liu FT. et al. The promigratory activity of the matricellular protein galectin- 3 depends on the activation of PI-3 kinase. PLoS One. 2011; 6 (12):e29313.
43. Friedl P, Alexander S. Cancer invasion and the microenvironment: plasticity and reciprocity. Cell. 2011; 147 (5): 992–1009.
44. Georgiadis V. et al. Lack of galectin-1 results in defects in myoblast fusion and muscle regeneration. Dev Dyn. 2007; 236: 1014-1024.
45. Nangia-Makker P, Hogan V, Honjo Y, Baccarini S, Tait L. et al. Inhibition of human cancer cell growth and metastasis in nude mice by oral intake of modified citrus pectin. J Natl Cancer Inst. 2002; 94: 1854–1862.
46. Inohara H, Raz A. Effects of natural complex carbohydrate (citrus pectin) on murine melanoma cell properties related to galectin-3 functions. Glycoconjugate Journal. 1994; 11: 527–532.
47. Pienta KJ, Naik H, Akhtar A, Yamazaki K, Replogle TS. et al. Inhibition of spontaneous metastasis in a rat prostate cancer model by oral administration of modified citrus pectin. J Natl Cancer Inst. 1995; 87: 348–353.
48. Yan J, Katz A. PectaSol-C modified citrus pectin induces apoptosis and inhibition of proliferation in human and mouse androgen-dependent and-independent prostate cancer cells. Integr Cancer Ther. 2010; 9: 197–203.
49. Harazono Y, Nakajima K, Raz A. Why anti-Bcl-2 clinical trails fail: a solution. Cancer Metastasis Rev. 2014; 33: 285-294