Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 26 (3-4)
Sofia ● 2019

Profile of Cell-Surface Glycopeptides and Role of Guerin 51 kDa gCBP for Tumor Cell Adhesion

*J. Stoyloff**

Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

* Corresponding author e-mail: info.jstoyloff@gmail.com

We found mainly tri-antennary and small amount of tetra-antennary glycopeptides with terminal galactose at the cell surface of Guerin 'heavy' cell's subpopulation (GH). On the other hand, Guerin 'light' cells (GL) contained oligomannoside and hybrid type oligosaccharides with terminal galactose and glucose. Next we used isolated cell-surface glycopeptides from GH cells in solid-phase inhibition assay. We found drastic reduction of adhered GH cells on laminin-coated plates in the presence of tri- and tetra-antennary cell-surface glycopeptides. We also saw drastic reduction of adhesion of GH cells on fibronectin-coated plates in presence of tri- and tetra-antennary cell-surface glycopeptides. Macromolecular ligand asialofetuin (AsFet) was also potent at inhibiting GH adhesion to fibronectin-coated plates. On collagen-coated plates most potent inhibition was observed by tri-antennary cell-surface glycopeptides, followed by tetra-antennary glycopeptides and asialofetuin.

Key words: galectin, cancer, adhesion

Introduction

Galectins participate in carbohydrate-mediated adhesion of tumor cells to extracellular matrix (ECM), as well as in homo- and heterotypic adhesions of tumor cells. Glycoproteins of the matrix are N-glycosylated and potential ligands for galectins. These ligands include laminin, fibronectin and collagen IV [9].

Secreted galactose-binding proteins could participate in cell-to-extracellular matrix (ECM) adhesion through bridging carbohydrate moieties of cell-surface glycoproteins and ECM glycoproteins. For example, galectin-1 is secreted outside colon cancer (Colo201) cells where it helps adhesion and spreading of these cells to fibronectin-, laminin-, and collagen-coated plates [6].

Review of the literature shows two models of oligomerization of lectins. In the ligand-induced oligomerization model, galectins bind cell-surface glycoproteins, followed by oligomerization. The other model of oligomerization postulates that oligomerization takes place before binding of ligands. Cell-surface N-linked

glycans are potential ligands for galectins with implications for oligomerization and adhesion. Galectin-3 binds to cell surface glycoproteins, including branched N-glycans [3]. Another report shows that Gal-3 binds specifically to beta-galactoside residues of cell surface glycoproteins [4].

The aim of the present study was to characterize adhesion of subpopulations of Guerin tumor cells to solid-phase immobilized glycoproteins of ECM. We also investigated the composition of cell-surface glycoproteins of Guerin tumor cells (GTC), in order to reveal the role of branching in adhesion. Finally, we present data of ligand-induced oligomerization of 51 kDa gCBP and implications of this oligomerization for adhesion of GTC.

Materials and Methods

Separation of Guerin tumor cells (GTC) subpopulations

Guerin tumor cells were separated according to their buoyant densities as described in [13].

Metabolic labeling of cell-surface glycoproteins with radioactive sugars

Monolayer of GH cells were detached by trypsinization, resuspended in complete RPMI medium containing 1 μCi/ml D-[14C] glucosamine (45 to 60 mCi/mmol), and grown for two days. Then Guerin tumor cells were labeled in a solution containing 0.01 mCi/ml [3H] mannose.

Isolation of cell-surface glycopeptides from GTC

Guerin tumor cells were washed with 0.15M NaCl/0.01M sodium bicarbonate, pH 7.5 and then incubated in the same buffer containing 0.05% tripsin (type III, Sigma) for 20 min at 37°C. The reaction was stopped with 0.003% soy trypsin inhibitor and the supernatant was used for lectin affinity chromatography.

Lectin affinity chromatography

Affinity chromatography was performed as described in [5].

Isolation of secreted 51 kDa gCBP from Guerin tumor cells

GTC in cell culture medium were incubated for 3 h at 37°C with radioactive ^{35}S methionine (500 μCi). Cell were washed and incubated in cell culture medium for 24 h [12] to allow maximum secretion of 51 kDa gCBP. Secreted 51 kDa gCBP was then isolated by gel filtration.

Inhibition assay

Inhibition of adhesion of GTC to solid-phase immobilized glycoproteins of ECM was done as described by Horiguchi [6]. Laminin, fibronectin or collagen (5 μg/ml in 0.1M carbonate buffer, pH 9.6) were added to microtiter plates and incubated for 3 h at 37°C. After washing with PBS, a solution of ^{35}S 51 kDa gCBP was added and incubated for 2 h in the presence of tri- and tetra-antennary cell-surface glycopeptides.

Results

We found mainly tri-antennary and small amount of tetra-antennary glycopeptides with terminal galactose at the cell surface of Guerin heavy (GH) cells (**Fig. 1**). Guerin light (GL) cells contained predominantly oligomannoside and hybrid type oligosaccharides with terminal galactose and glucose.

Cell-surface glycopeptides from GH cells are ligands of 51 kDa gCBP, as observed in inhibitory experiments shown in Figs. 2, 3 and 4. Approximately 9 × 10^5 GH cells/well adhere to laminin-coated plates. However, we see a drastic reduction of adhered cells in the presence of tri- and tetra-antennary cell-surface glycopeptides (**Fig. 2**).

Adhesion of GH cells to fibronectin-coated plates is shown in **Fig. 3**. Here we can also see a drastic reduction of adhesion in the presence of tri- and tetra-antennary cell-surface glycopeptides. Macromolecular ligand asialofetuin (AsFet) is also a potent inhibitor of GH adhesion to fibronectin-coated plates. It is evident that the most potent inhibition is by tetra-antennary cell-surface glycopeptides, followed by tri-antennary glycopeptides and asialofetuin.

Finally we investigated the inhibition of adhesion of GH cells on collagen IV-coated plates. Here most potent inhibition was observed by tri-antennary cell-surface glycopeptides, followed by tetra-antennary glycopeptides and asialofetuin (**Fig. 4**).

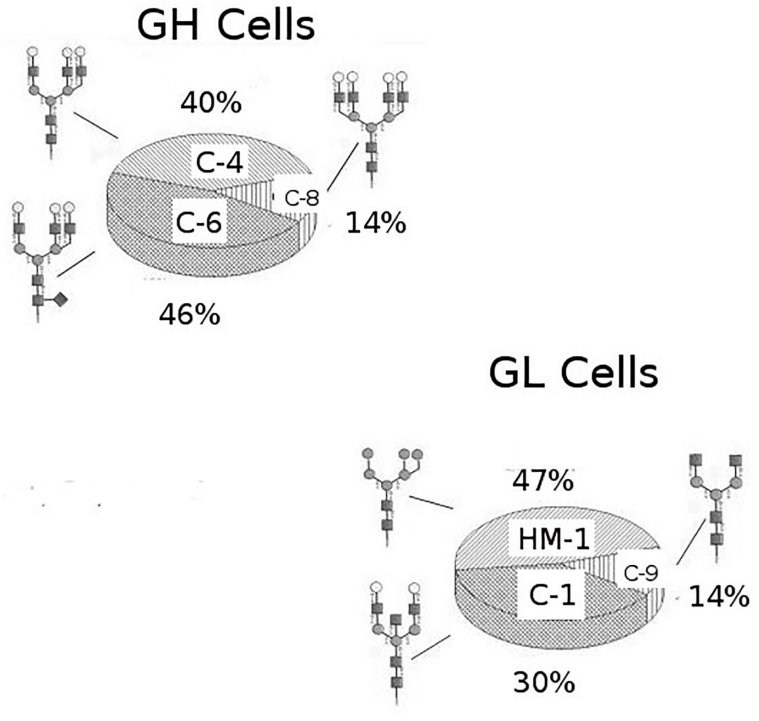

Fig. 1. Glycoprofile of cell surface glycoproteins of Guerin tumor cell's subpopulations

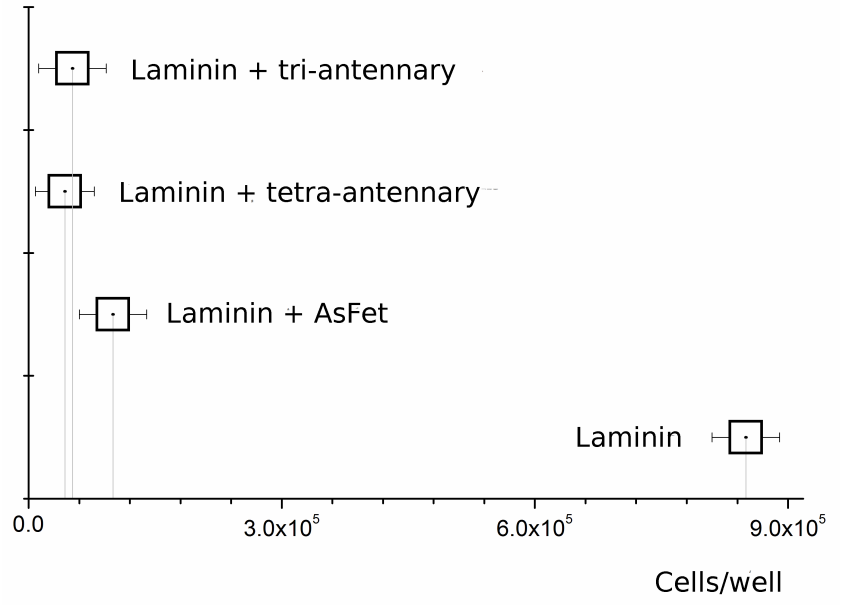

Fig. 2. Adhesion of Guerin "heavy" (GH) subpopulation to laminin. **Laminin** – adhesion of GTC to laminin, **Laminin + AsFet** – inhibition of adhesion with AsialoFetuin, **Laminin + tri-antennary** – inhibition of adhesion with tri-antennary cell-surface glycopeptides, **Laminin + tetra-antennary** – inhibition of adhesion with tetra-antennary cell-surface glycopeptides

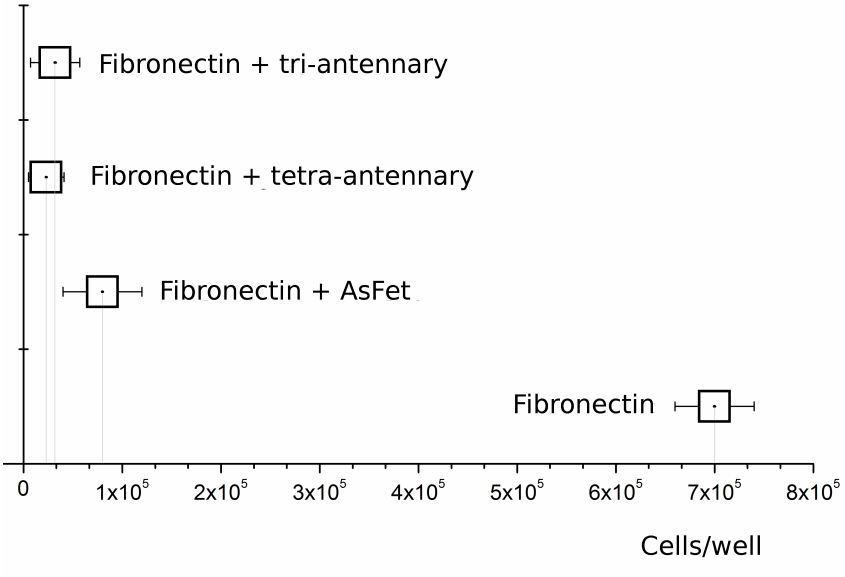

Fig. 3. Adhesion of Guerin "heavy" (GH) subpopulation to fibronectin. **Fibronectin** – adhesion of GTC to laminin, **Fibronectin + AsFet** – inhibition of adhesion with AsialoFetuin, **Fibronectin + tri-antennary glycopeptides** – inhibition of adhesion with tri-antennary cell-surface glycopeptides, **Fibronectin + tetra-antennary glycopeptides –** inhibition of adhesion with tetra-antennary cell-surface glycopeptides.

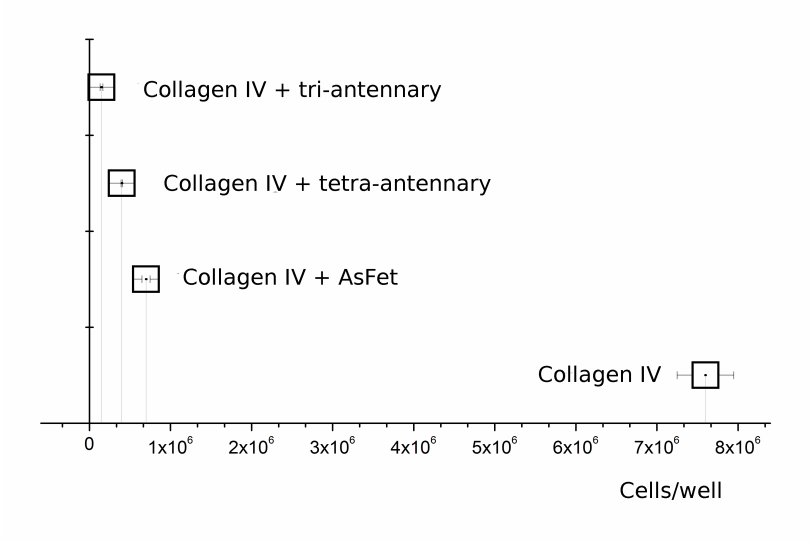

Fig. 4. Adhesion of Guerin "heavy" (GH) subpopulation to collagen IV. **Collagen IV** - adhesion of GTC to laminin, **Collagen IV + AsFet** - inhibition of adhesion with AsialoFetuin, **Collagen IV + tri-antennary glycopeptides** - inhibition of adhesion with tri-antennary cell-surface glycopeptides, **Collagen IV + tetra-antennary glycopeptides -** inhibition of adhesion with tetra-antennary cell-surface glycopeptides.

Comparing the inhibition of adhesion on laminin, fibronectin and collagen IV-coated plates, we found that the most potent inhibition in the case of laminin and fibronectin was brought about by tetra-antennary glycopeptides, whereas for collagen IV the most potent inhibition was caused by tri-antennary cell-surface glycopeptides.

Discussion

Analysis of membrane glycoproteins of 3T3 cells shows a decrease in bi-antennary glycopeptides, with a simultaneous increase in tri- or tetra-antennary glycopeptides [10]. Membrane subfractions of rat pheochromocytoma (PC12) cells consist of large tri- and tetra-antennary complex oligosaccharides accounting for 82 to 97% of the membrane glycoproteins [7]. The increase in antennae number of glycoproteins leads to increased binding to galectins [9]. Glycan branching of glycoproteins also correlates with the metastatic potential of oral squamous carcinoma [14]. Increased branching leads to increased binding to galectins, as showed by Andre et al. [1].

In situ oligomerization of galectins is shown by Nieminen et al. [8] in binding of galectin-3 to solid-phase immobilized asialofetuin. *In situ* oligomerization of galectin-3 on cell surface leads to cluster formation of receptors, as shown by FRET technique [8]. Oligomerization is a unique feature of secreted galectin-3, leading to formation of ordered galectin-glycan lattices on the cell surface. Balan et al. [6] show that galectin-3 is monomer in solution but in the presence of a ligand, galectin-3

polymerizes up to pentamers. Oligomerization of galectin-3, after ligand binding, occurs on cell surfaces within the physiological concentrations of the lectin. It has thus been proposed that oligomerization of the N-terminal domains of galectin-3 molecules, after ligand binding by the C-terminal domain, is responsible for adhesion of tumor cells to solid-phase immobilized glycoproteins [8]. Recombinant galectin-3, with missing N-domain, does not mediate adhesion between neutrophils and endothelial cells [11], pointing to the role of N-domain in oligomerization.

Conclusion

Our data show mainly tri-antennary and small amount of tetra-antennary oligosaccharides at the cell surface of Guerin heavy cells.

Guerin tumor cells secrete 51 kDa gCBP, which is involved in adhesion of GTC to plates coated with laminin, fibronectin and collagen IV. Inhibition experiments with cell-surface glycopeptides, isolated from Guerin cells, point to the role of these glycopetides as ligands for 51 kDa gCBP. We can assume that the secreted protein binds to laminin (fibronectin, collagen IV) and oligomerizes *in situ***.** According to the 'ligand-induced' model, receptors oligomerize only in the presence of their ligands. The low MW ligand galactose was able to induce oligomerization of 51 kDa gCBP. We found also that multivalent ligands were able to induce di- and trimers, but not tetramers of 51 kDa gCBP. It can be concluded that adhesion of GTC is mediated by 51 kDa gCBP, which form ligand-induced oligomers.

References

1. **André, S., S. Kojima, G. Gundel, R. Russwurm, X. Schratt.** Branching mode in complex-type tri-antennary N-glycans as regulatory element of their ligand properties. – *Biochim. Biophys. Acta*, **176**(5), 2006, 768-782.
2. **Balan, V., P. Nangia-Makker, D. Kho, Y. Wang, A. Raz.** Tyrosine-phosphorylated galectin-3 protein is resistant to prostate-specific antigen (PSA) cleavage. – *J. Biol. Chem.*, **287**(8), 2012, 5192-5198.
3. **Boscher, C, Y. Z. Zheng , R. Lakshminarayan, L. Johannes, J. W. Dennis.** Galectin-3 protein regulates mobility of N-cadherin and GM1 ganglioside at cell-cell junctions of mammary carcinoma cells. – *J. Biol. Chem.*, **287**(39), 2012, 32940-32952.
4. **Chiu, C., S. Strugnell, O. Griffith, S. Jones, A. Gown.** Diagnostic utility of galectin-3 in thyroid cancer. – *Am. J. Pathol.*, **176**(5), 2010, 2067-2081.
5. **Cummings, R., S. Kornfeld.** Fractionation of asparagine-linked oligosaccharides by serial lectin-Agarose affinity chromatography. A rapid, sensitive, and specific technique. – *J. Biol. Chem.*, **257**(19), 1982, 11235-11240.
6. **Horiguchi, N., K. Arirnota, A. Mizutani, Y. Endo-Ichikawa, H. Nakada.** Galectin-1 induces cell adhesion to the extracellular matrix and apoptosis of nonadherent human colon cancer Colo20J cells. – *Biochem. (Tokyo)*, **134**(6), 2003, 869-874.
7. **Margolis, R., S. Salton, U. Margolis.** Complex carbohydrates of cultured PC12 pheochromocytoma cells. Effects of nerve growth factor and comparison with neonatal and mature rat brain. – *J. Biol. Chem.*, **258**(7), 1983, 4110-4117.
8. **Nieminen, J., A. Kuno, J. Hirabayashi, S. Sato.** Visualization of galectin-3 oligomerization on the surface of neutrophils and endothelial cells using fluorescence resonance energy transfer. – *J. Biol. Chem.*, **282**(2), 2007, 1374-1383.
9. **Ozeki, Y., T. Matsui, Y. Yamamoto, K. Titan**i. Tissue fibronectin is an endogenous ligand for galectin. – *Glycobiology*, **5**, 1995, 255-261.
10. **Santer, U., F. Gilbert, M. Glick.** Change in glycosylation of membrane glycoproteins after transfection of NIH 3T3 with human tumor DNA. – *Cancer Res.*, **44(9)**, 1984, 3730-3735.

11. **Sato, S., N. Ouellet, I. Pelletier, M. Simard, A. Rancourt, M. G. Bergeron.** Role of galectin-3 as an adhesion molecule for neutrophil extravasation during streptococcal pneumonia. – *J. Immunol.*, **168**(4), 2002, 1813-1822.
12. **Stoyloff, J., S. Ivanov.** Isolation and characterization of galactose-specific carbohydrate-binding protein from Guerin tumor cells. – *Brain Res. Bull.*, **83**(1-2), 2010, 65-75.
13. **Stoyloff, J., S. Ivanov.** Guerin tumour cells have different isoprofile and cellular distribution of 51 kDa galactose-binding protein and different activity of the thermostable Regan enzyme. – *Comp. Rend. Acad. Bulg. Sci.*, **65**(3), 2012, 353-358.
14. **Tanda, N., S. Mori, M. Nose, T. Saito, S. Song.** Expression of Phaseolus vulgaris leukoagglutinin-binding oligosaccharides in oral squamous cell carcinoma: possible association with the metastatic potential. – *Pathol. Int.*, **46**(9), 1996, 639-645.