HHS Public Access

Author manuscript

Chembiochem. Author manuscript; available in PMC 2015 September 22.

Published in final edited form as:

Chembiochem. 2014 September 22; 15(14): 2106–2112. doi:10.1002/cbic.201402134.

Lactose-Functionalized Dendrimers Arbitrate the Interaction of Galectin-3/MUC1 Mediated Cancer Cellular Aggregation

Anna K. Michel[a], **Pratima Nangia-Makker**[b], **Avraham Raz**[b], and **Mary J. Cloninger**[a]

Mary J. Cloninger: mcloninger@chemistry.montana.edu

[a]Department of Chemistry and Biochemistry, Montana State University, 103 Chemistry and Biochemistry, Bozeman, MT 59717 (USA)

[b]The Departments of Oncology and Pathology, School of Medicine, Wayne State University, 110 East Warren Avenue, Detroit, Michigan 48201 (USA)

Abstract

By using lactose-functionalized poly(amidoamine) dendrimers as a tunable multivalent platform, we studied cancer cell aggregation in three different cell lines (A549, DU-145, and HT-1080) with galectin-3. We found that small lactose-functionalized G(2)-dendrimer **1** inhibited galectin-3-induced aggregation of the cancer cells. In contrast, dendrimer **4** (a larger, generation 6 dendrimer with 100 carbohydrate end groups) caused cancer cells to aggregate through a galectin-3 pathway. This study indicates that inhibition of cellular aggregation occurred because **1** provided competitive binding sites for galectin-3 (compared to its putative cancer cell ligand, TF-antigen on MUC1). Dendrimer **4**, in contrast, provided an excess of ligands for galectin-3 binding; this caused crosslinking and aggregation of cells to be increased.

Keywords

aggregation; cancer; galectin-3; lactosefunctionalized dendrimers; mucin 1

Introduction

Found inside and on the surface of most healthy cells, galectin-3 is involved in regulating cell growth, differentiation, adhesion, and death.[1] Galectin-3 is a chimeric lectin, with a carbohydrate recognition domain (CRD) specific for β-galactosides and a collagen-like tail capable of forming pentamers and other oligomers at high concentrations or when interacting with multivalent frameworks.[2–8] Galectin-3 is overexpressed in many forms of cancer,[9, 10] and there is a correlation between the amount of circulating galectin-3 and the aggressiveness of the cancer.[11, 12] Extracellular galectin-3 is involved in tumor formation and metastasis.[13–16] It has been shown that a natural ligand of cancer-associated galectin-3 is Mucin 1 (MUC1), a large transmembrane protein that is overexpressed and heavily glycosylated on cancer cells.[11, 17]

Correspondence to: Mary J. Cloninger, mcloninger@chemistry.montana.edu.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cbic.201402134.

MUC1 is found on the apical face of healthy epithelial cells (Figure 1A), but on cancer cells it is aberrantly glycosylated and expressed across the entire surface (Figure 1B).[11] Truncated glycosylation exposes the Thomsen–Friedenreich antigen (TF antigen), a serine- or threonine-linked disaccharide (Galβ1-3GalNAc-α-*O*-Ser/Thr) that is thought to be a natural ligand for galectin-3.[18] Circulating galectin-3 interacts with the TF antigen on MUC1, thereby polarizing the transmembrane mucin on the cell surface, exposing adhesion molecules, and allowing increased homotypic (tumor formation) and heterotypic (metastasis) cellular adhesions to occur (Figure 1C).[11, 17]

As galectin-3 can have a profound impact on the cell-surface display of proteins such as MUC1 (thereby influencing cellular functions), we report here a multivalent system to bind galectin-3 in the presence of cells. Multivalent display of galectin-3 has been shown to be nucleated by the binding of glycans to the CRD binding site, and protein–protein interactions are enabled by the carbohydrate binding event.[19] It has also been shown that galectin-3 shows a higher affinity for multivalent networks of β-galactosides than for monomeric carbohydrates.[20, 21] The increased affinity of galectin-3 for lactose-functionalized poly(amidoamine) (PAMAM) dendrimers as compared to lactose monomers has been specifically demonstrated.[22]

Multimerization of galectin-3 often leads to its activity in cancer and inflammation processes.[23, 24] In bacterial interactions, galectin-3 can bind directly to pathogens by serving as a crosslinker for microorganisms or as a cellular docking site;[25, 26] it can also act as a scaffold for the presentation of ligands such as lipopolysaccharides into an aggregate that stimulates cellular responses.[24] In cancer, although the effects of multivalency in some systems are not known, galectin-3 has been shown to regulate the expression of proteins such as p21 (a regulator of the cell cycle) by stabilizing the p21 protein.[27] In galectin binding studies with neuroblastoma cells, high affinity and selective binding of galectins was only observed when both the glycan ligand and the display of the ligand on the cell surface were optimized, thus strongly indicating that multivalent display of galectin-3 is of critical importance.[28] Polyvalent galectin-3 inhibitors have been shown to increase the susceptibility of diffuse large B-cell lymphoma (DLBCL) to chemotherapeutic agents.[29] Overall, current studies indicate that the propensity of galectin-3 to multimerize is essential for many biological functions in both healthy and cancerous systems.

Here, our focus was to use the natural affinity that galectin-3 has for lactose-functionalized dendrimers in order to survey galectin-3-mediated homotypic aggregation in three different cancer cell lines, thereby gaining insight into the mechanism by which cancer cellular aggregation occurs. A correlation between cancer cells aggregation in vitro and their metastatic potential in vivo has been demonstrated,[30, 31] thus suggesting the real value of a therapeutic agent to inhibit galectin-3-mediated cellular aggregation. By using PAMAM dendrimers, we were able to fine-tune the number of lactose groups on the multivalent framework, thus allowing more control over the outcome of the interaction. As described below, the aggregation can be either inhibited or enhanced depending on the number of lactose groups on the dendrimer.

Results and Discussion

Lactose-functionalized glycodendrimers

Previous work has established that galectin-3 has much stronger binding interactions with polymeric glycoclusters of its β-galactoside ligands than with the respective monomeric glycosides.[20–22] Here, we exploited the preference of galectin-3 for multivalent clusters of β-galactosides in experiments with our tunable multivalent lactose-functionalized dendrimers. By using glycodendrimers with displays of 15 to 100 lactose end groups attached to the dendrimer framework, we monitored the effects of multivalency on the homotypic aggregation of cancer cells. The lactose-functionalized PAMAM dendrimers that were used for these studies are shown in Scheme 1. The number of lactose end groups represents 95, 63, 75, and 47% functionalization (**1**, **2**, **3**, and **4**, respectively) of the dendrimer starting material (determined by MALDI-TOF MS), with reduced functionalization for larger dendrimers due to steric hindrance.[32]

Cellular aggregation assays with A549 cells and lactose-functionalized dendrimers 1–4

To test the effects of the different generations of lactose-functionalized dendrimers **1**–**4** on cellular aggregation, assays using the glycodendrimers were conducted on A549 lung carcinoma cells, with and without the addition of galectin-3. Addition of galectin-3 to the A549 cells induced cellular aggregation. When generation 2 and generation 3 glycodendrimers (**1** and **2**) were incubated with galectin-3 and A549 cells, the formation of (galectin-3 induced) cellular aggregates was inhibited. Incubation of the cells with **1** or **2** in the absence of galectin-3, however, had no impact on cellular aggregation (Figure 2A and B). When generation 4 and generation 6 glycodendrimers (**3** and **4**) were used, there was no apparent inhibition of galectin-3-induced aggregation. In the absence of additional galectin-3, however, glycodendrimer-induced aggregation was observed (Figure 2C and D). The smallest and largest lactose-functionalized dendrimers (**1** and **4**) caused the most pronounced effects. More subtle results were obtained when **2** or **3** was used. Example microscope images of control A549 cells, A549 cells for which galectin-3-mediated aggregation was inhibited by **1**, and aggregates of A549 cells that were formed upon addition of galectin-3 are shown in Figure 2E, F, and G, respectively. Assays with Trypan Blue indicated that the dendrimers are non-toxic (no effect on cellular viability; Figure S1 in the Supporting Information).

Cellular aggregation assays with DU-145 and HT-1080 cells and lactose-functionalized dendrimers 1–4

To test the generality of the results for A549 cells in the homotypic aggregation assay, two additional cell lines, DU-145 and HT-1080, were tested. These were chosen because of their expression levels of galectin-3; DU-145 cells express more galectin-3 than A549 cells; HT-1080 cells express less. The results corroborated those for A549 cells, although slight variations were observed for these different cell lines. Dendrimer **1** was able to inhibit galectin-3-induced aggregation for HT-1080 and DU-145 cell lines (Figure 3A). Dendrimer **2**, however, only induced aggregation of DU-145 cells (not HT-1080; Figure 3B). A possible explanation is that the results are impacted by the inherent amount of endogenous galectin-3 in each cell line. We measured the amount of native galectin-3 in each cell line by

Western blotting, and found that DU-145 cells had more total native galectin-3 than the other cell lines; HT-1080 had the lowest amount of native galectin-3 (Figure S2). Although only extracellular galectin-3 is thought to be involved in homotypic aggregation processes,[11] it can be inferred that an increase in total cellular galectin-3 results in an increase in cell-surface galectin-3. With less galectin-3 available for interaction with **2**, little effect on cellular aggregation was observed for HT-1080. As DU-145 and A549 have more galectin-3, the effect of **2** with these cell lines was more pronounced. These assays were also performed in the absence of additional galectin-3, and no change in aggregation was observed in any of the cell lines for generation 2 and 3 glycodendrimers (**1** and **2**; Figure S3A and B).

As with A549 cells, the lactose-functionalized generation 4 and 6 dendrimers (**3** and **4**) did not discernibly inhibit aggregation of DU-145 and HT-1080 cells when an aggregation-inducing amount of galectin-3 was added (Figure S3C and D). However, when no galectin-3 was added, a statistically significant increase in aggregation was observed when **4** was incubated with DU-145 cells (Figure 4B). Again, the lack of native galectin-3 in HT-1080 apparently prohibited aggregation.

Cellular aggregation assays with fixed cells

When assays with **1**–**4** were run without adding galectin-3, it was observed that **4** (the lactose-functionalized G(6)-dendrimer) caused cellular aggregation. To determine whether reorganization of cell-surface MUC1 by galectin-3 was responsible for the observed aggregation in the presence of **4**, cellular aggregation assays were performed with fixed A549 cells (Figure S4). When cells are fixed with paraformaldehyde the cell surface is not dynamic, and extracellular protein is removed in the washing procedure. Thus, galectin-3 cannot influence the localization of MUC1 at the cell surface.[11] When **4** was added with or without galectin-3 to fixed cells, no aggregates formed. This indicates that polarizable MUC1 on the cell surface and galectin-3 are required for cellular aggregation to be induced by **4**.

Fluorescence microscopy to determine the effect of the glycodendrimer on the cell-surface localization of galectin-3

Homotypic aggregation assays were performed as above for **1** and **4**, then cells were incubated with fluorescently labelled anti-galectin-3 antibody, so that the distribution of galectin-3 around the outside of the cells could be viewed (Figure 5). Dendrimers **1** and **4** were studied because they elicited the greatest responses in the aggregation assays. When untreated cells were incubated with the antibody, a faint green fluorescence was visible over the entire cell surface, thus indicating that galectin-3 was distributed across the cell surface (Figure 5A). When cells were treated with just galectin-3 and then with the antibody, the fluorescence was much more punctate, thus indicating that the galectin-3 (when in higher concentrations) was localized around the cells (Figure 5B).

Given the fixation data, these fluorescence images indicate that galectin-3 reorganizes the cell surface to increase the aggregation potential of the cells, most likely by interacting with MUC1 and exposing adhesion molecules on the cell surface (Figure 5B). When cells were

incubated with **1** but without additional galectin-3, the cells looked similar to untreated cells (the galectin-3 pattern was unchanged; Figure 5C). When incubated with **4**, the phenotype looked similar to that for galectin-3-induced aggregates (Figure 5D). When the cells were incubated in the presence of **1** and galectin-3, the cells looked similar to the untreated control (Figure 5E). Finally, when cells were incubated with **4** and galectin-3, large aggregates of galectin-3 were observed at the cell interfaces (Figure 5F). Indeed, a larger network of cells was observed when galectin-3 and **4** were added than for the galectin-3 control. The galectin-3 pattern also looked more punctate when both galectin-3 and **4** were present, thus suggesting aggregation of galectin-3 into large multivalent aggregates.

These results can be explained given that naturally occurring galectin-3 interacts with MUC1 at the cell surface, and this occurs in a distributed fashion (Figure 6). Although binding of galectin-3 to other cell-surface ligands is of course possible, MUC1 is the most significant target known.[11] When additional galectin-3 is introduced to the cells (or when galectin-3 is over-expressed by cells), more galectin-3 is present at the cell–cell interfaces, and cellular aggregation results (Figure 6B). This interaction is disrupted by the introduction of a small glycodendrimer, **1** (Figure 6C), because **1** diverts galectin-3 from the clusters on the cell surface, thus re-establishing the diffuse pattern of galectin-3 (as in Figure 6A). When a large glycodendrimer, **4**, is added in the presence of high concentrations of galectin-3, the aggregation seen in Figure 6B is augmented by multivalent crosslinking of the cells through formation of galectin/glycodendrimer matrices (Figure 6D).

These findings indicate that glycodendrimers effectively mediate cellular aggregation properties by intercepting and modifying the galectin-3/MUC1 pathway. These results establish glycodendrimers as tools to better understand multivalent processes that play important roles in cancer cellular processes. Glycodendrimers that target the galectin-3/MUC1 binding system might also yield important insights for the development of targeting agents, prodrug delivery systems, or diagnostics and imaging systems.

Conclusion

By using lactose-functionalized dendrimers as a tunable multivalent system, the level of cellular aggregation in three different cancer cell lines was modulated. The smaller dendrimer (**1**, a generation 2 dendrimer with 15 lactose end groups) inhibited galectin-3-induced aggregation for all three tested cancer cell lines. In contrast, dendrimer **4** (a larger, generation 6 dendrimer with an average of 100 lactose end groups) caused cancer cells to aggregate through a galectin-3 pathway. The results of the homotypic cellular aggregation assays indicated that inhibition by **1** was accomplished by providing competitive binding sites for galectin-3 (to divert binding from its putative cancer cell ligand, TF antigen on MUC1). Dendrimer **4**, in contrast, provided so many sites for galectin-3 binding that the cross linking of the cells increased, thus enhancing aggregation.

Experimental Section

Dendrimer synthesis

Lactose-functionalized dendrimers were synthesized as previously described.[32]

Galectin-3

Galectin-3 was produced by the protocol developed by the Raz lab.[32, 33] The concentration of galectin-3 was verified by a bicinchoninic acid (BCA) protein assay.[34]

Cell lines and cultures

Cell lines were chosen for robustness, extent of characterization, and galectin-3 expression level.[32–37] Human cell lines A549 (lung carcinoma), DU-145 (prostate cancer), and HT-1080 (fibrosarcoma) were purchased from ATCC (Manassas, VA) and cultured as recommended by ATCC. Cells that had been passaged less than 12 times were used for these experiments. Cells were dissociated from growth dishes by using trypsin (0.25 %)/EDTA (0.02%) (SAFC Biosciences/Sigma–Aldrich).

Homotypic aggregation assay

Cells were counted and suspended (16×10^6 cells per mL) in serum-free medium (SFM, F-12K (Gibco) for A549 and Eagle's Minimum Essential Medium (Gibco) for DU-145 and HT-1080 cell lines). Dendrimers were dissolved (2 mg mL^{-1}) in ultrapure water. Galectin-3 was used at 0.5 mg mL^{-1} in PBS. Galectin-3 (13 μL, 16.7 μM, 217 ρmol) and dendrimer solution (0, 10, 20, 30, or 40 μL) were mixed with SFM (total 53 μL) and vortexed. Cell suspension (15 μL, 240000 cells) was added to each reaction mixture, gently mixed, and incubated at 37°C for 1 h with gentle rotation. Although this order of addition was used for all assays, changing the order of addition did not impact the results. Three aliquots (10 μL) of each reaction mixture were viewed on a BC-364 inverted microscope (Jenco, Portland, OR). Four images were randomly chosen from each aliquot (selected primary data from assays performed on A549 cells in Figures S5–S7). The percentages of free and aggregated cells (aggregation defined as four or more cells clumped together) were determined by using the image analysis software Pixcavator (http://inperc.com/image_analysis.htm). To determine cell viability after the assay was completed, an aliquot (13 μL) of cells was incubated with Trypan Blue (13 μL), and the live/dead ratio was determined under 10× magnification in a hemocytometer (Bright-Line).

Fixed cell homotypic aggregation assay

Assays performed on fixed cells were performed as described above, but with cells fixed in paraformaldehyde (4% in PBS, pH 8) and washed three times with SFM.

SDS-PAGE and Western blots

Each cell line was grown to confluency, and cells were released by trypsin (0.25 %)/EDTA (0.02 %). Cells were washed twice with ice cold PBS buffer then incubated with NP40 cell lysis buffer and protease inhibitor cocktail (BD Bioscience) for 30 min on ice, and vortexed vigorously for 1 min every 5 min. The lysed cells were centrifuged (10 000 *g*, 20 min), and the total protein concentration in the supernatant for each cell line was determined by a BCA assay. Cell lysate (16 μg for each cell line: A549, HT-1080, and DU-145) with an appropriate amount of SDS sample buffer (3×) was loaded onto a 20% SDS polyacrylamide gel. Galectin-3 (10 mg) and MUC1 (3 mg; Sino Biological, Beijing, China) were also loaded. Gels were run at 200 V for 20 min, then at 100 V until complete, then transferred to

nitrocellulose membranes (1 h, 100 V). Membranes were then blocked in BSA (4% in Tris-Tween buffered saline (TTBS)) for 1 h at room temperature, incubated overnight in BSA (1% in TTBS) at 4°C with antihuman galectin-3–biotin (1:3000; eBioscience, San Diego, CA), then incubated for 1 h at room temperature in BSA (1% in TTBS) with strepaviden-HRP (1:1500; BD Biosciences). Between incubations, the membrane was washed with TTBS (3 × 5 min). The membrane was developed in 1-Step TMB Blotting Substrate (Thermo Scientific), scanned, and analyzed.

Microscopy

Homotypic aggregation assays were performed as described above. A sample (45 μL) of each reaction was added to complete medium (200 μL, F-12K (Gibco) supplemented with 10% fetal bovine serum for A549 and Eagle's Minimum Essential Medium (Gibco) supplemented with 10% and 5% fetal bovine serum for DU-145 and HT-1080 cell lines, respectively). Alexa Fluor 488-labeled galectin-3 antibody (1:25 dilution; R&D Systems, Minneapolis, MN) was added to each mixture and plated in an 8-chamber Nunc Lab-Tek II Chamber Coverglass System (Thermo Scientific) and incubated at 37°C overnight. The following day, cells were washed with SFM and fixed in paraformaldehyde (4%) for 20 min at room temperature. A coverslip was then placed on the microslide by using ProLong Gold Mountant with DAPI (Invitrogen/Life Technologies) and allowed to cure overnight at room temperature shielded from the light. Slides were then analyzed in an Eclipse TE200-U microscope (Nikon) with 40× oil immersion objective and in an LSM 510 inverted confocal microscope (Ziess).

Statistics

Statistical analysis used the unpaired two-tailed student's t-test. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Data are mean ± SD, $n \geq 3$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by NIGMS 62444.

References

1. Ochieng J, Furtak V, Lukyanov P. Glycoconjugate J. 2002; 19:527–535.
2. Sçrme P, Arnoux P, Kahl-Knutsson B, Leffler H, Rini JM, Nilsson UJ. J. Am. Chem. Soc. 2005; 127:1737–1743. [PubMed: 15701008]
3. Cooper DNW. Biochim. Biophys. Acta Gen. Subj. 2002; 1572:209–231.
4. Dumic J, Dabelic S, Flçgel M. Biochim. Biophys. Acta Gen. Subj. 2006; 1760:616–635.
5. Ahmad N, Gabius H-J, André S, Kaltner H, Sabesan S, Roy R, Liu B, Macaluso F, Brewer CF. J. Biol. Chem. 2004; 279:10841–10847. [PubMed: 14672941]
6. Dam TK, Gabius H-J, André S, Kaltner H, Lensch M, Brewer CF. Biochemistry. 2005; 44:12564–12571. [PubMed: 16156668]

7. Kübler D, Hung C-W, Dam TK, Kopitz J, Andr_ S, Kaltner H, Lohr M, Manning JC, He L, Wang H, Middelberg A, Brewer CF, Reed J, Lehmann W-D, Gabius H-J. Biochim. Biophys. Acta Gen. Subj. 2008; 1780:716–722.
8. Hsu D, Kuwabara I, Liu F. Transfusion Med. Hemother. 2005; 32:83–96.
9. Califice S, Castronovo V, Van Den Brûle F. Int. J. Oncol. 2004; 25:983–992. [PubMed: 15375548]
10. Newlaczyl AU, Yu L-G. Cancer Lett. 2011; 313:123–128. [PubMed: 21974805]
11. Zhao Q, Guo X, Nash GB, Stone PC, Hilkens J, Rhodes JM, Yu L-G. Cancer Res. 2009; 69:6799–6806. [PubMed: 19690136]
12. Chen C, Duckworth CA, Zhao Q, Pritchard DM, Rhodes JM, Yu L-G. Clin. Cancer Res. 2013; 19:1693–1704. [PubMed: 23401226]
13. Guzman-Aranguez A, Argüeso P. Ocular Surface. 2010; 8:8–17. [PubMed: 20105403]
14. Rapoport EM, André S, Kurmyshkina OV, Pochechueva TV, Severov VV, Pazynina GV, Gabius H-J, Bovin NV. Glycobiology. 2008; 18:315–324. [PubMed: 18256179]
15. Rapoport EM, Pochechueva TV, Kurmyshkina OV, Pazynina GV, Severov VV, Gordeeva EA, Belyanchikov IM, André S, Gabius H-J, Bovin NV. Biochemistry Moscow. 2010; 75:310–319. [PubMed: 20370609]
16. Song X, Xia B, Stowell SR, Lasanajak Y, Smith DF, Cummings RD. Chem. Biol. 2009; 16:36–47. [PubMed: 19171304]
17. Zhao Q, Barclay M, Hilkens J, Guo X, Barrow H, Rhodes JM, Yu L-G. Mol. Cancer. 2010; 9:154. [PubMed: 20565834]
18. Yu L-G, Andrews N, Zhao Q, McKean D, Williams JF, Connor LJ, Gerasimenko OV, Hilkens J, Hirabayashi J, Kasai K, Rhodes JM. J. Biol. Chem. 2007; 282:773–781. [PubMed: 17090543]
19. Lepur A, Salomonsson E, Nilsson UJ, Leffler H. J. Biol. Chem. 2012; 287:21751–21756. [PubMed: 22549776]
20. Vrasidas I, André S, Valentini P, Bçck C, Lensch M, Kaltner H, Liskamp RMJ, Gabius H-J, Pieters RJ. Org. Biomol. Chem. 2003; 1:803–810. [PubMed: 12929363]
21. Chabre YM, Roy R. Adv. Carbohydr. Chem. Biochem. 2010; 63:165–393. [PubMed: 20381707]
22. André S, Ortega PJC, Perez MA, Roy R, Gabius H-J. Glycobiology. 1999; 9:1253–1261. [PubMed: 10536041]
23. Nieminen J, Kuno A, Hirabayashi J, Sato S. J. Biol. Chem. 2007; 282:1374–1383. [PubMed: 17082191]
24. Fermino ML, Polli CD, Toledo KA, Liu F-T, Hsu DK, Roque- Barreira MC, Pereira-da-Silva G, Bernardes ES, Halbwachs-Mecarelli L. PloS One. 2011; 6:e26004. [PubMed: 22031821]
25. Kavanaugh D, Kane M, Joshi L, Hickey RM. Appl. Environ. Microbiol. 2013; 79:3507–3510. [PubMed: 23524672]
26. Quattroni P, Li Y, Lucchesi D, Lucas S, Hood DW, Herrmann M, Gabius H-J, Tang CM, Exley RM. Cell. Microbiol. 2012; 14:1657–1675. [PubMed: 22827322]
27. Wang Y, Balan V, Kho D, Hogan V, Nangia-Makker P, Raz A. Oncogene. 2013; 32:5058–5065. [PubMed: 23160381]
28. Kopitz J, Bergmann M, Gabius H-J. Iubmb Life. 2010; 62:624–628. [PubMed: 20665623]
29. Clark MC, Pang M, Hsu DK, Liu F-T, de Vos S, Gascoyne RD, Said J, Baum LG. Blood. 2012; 120:4635–4644. [PubMed: 23065155]
30. Otsuji E, Park Y-S, Tashiro K, Kojima N, Toyokuni T, Hakomori S-I. Int. J. Oncol. 1995; 6:319–327. [PubMed: 21556540]
31. Mo J, Sun B, Zhao X, Gu Q, Dong X, Liu Z, Ma Y, Zhao N, Liu Y, Chi J, Sun R. Melanoma Res. 2013; 23:254–263. [PubMed: 23752306]
32. Goodman C, Wolfenden M, Nangia-Makker P, Raz A, Cloninger M. Beil. J. Org. Chem. 2014; 10:1570–1577.
33. Nangia-Makker P, Balan V, Raz A. Methods Mol. Biol. 2012; 878:251–266. [PubMed: 22674139]
34. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC. Anal. Biochem. 1985; 150:76–85. [PubMed: 3843705]

35. Giard DJ, Aaronson SA, Todaro GJ, Arnstein P, Kersey JH, Dosik H, Parks WP. J. Natl. Cancer Inst. 1973; 51:1417–1423. [PubMed: 4357758]

36. Rasheed S, Nelson-Rees WA, Toth EM, Arnstein P, Gardner MB. Cancer. 1974; 33:1027–1033. [PubMed: 4132053]

37. Lieber M, Smith B, Szakal A, Nelsonrees W, Todaro G. Int. J. Cancer. 1976; 17:62–70. [PubMed: 175022]

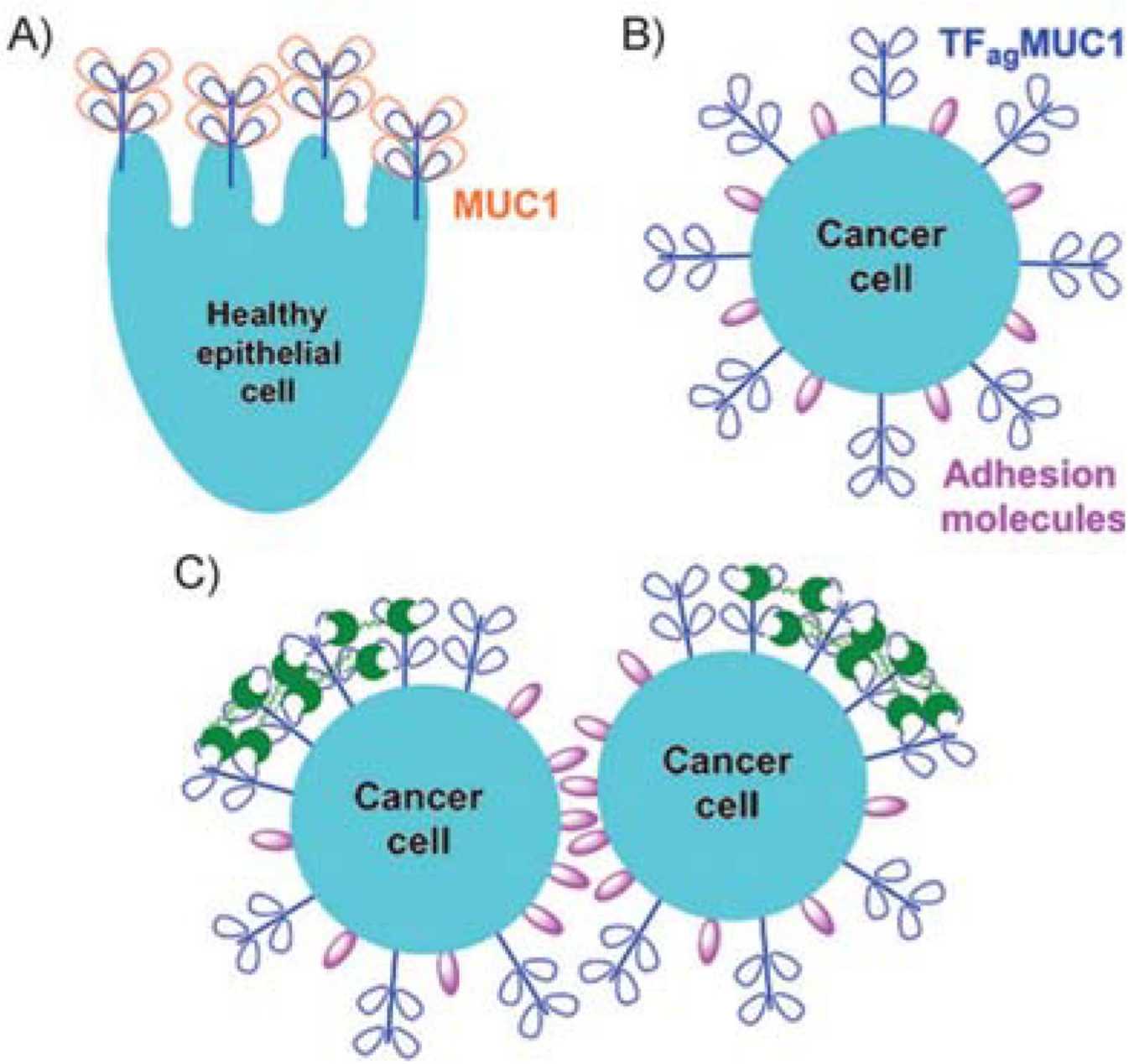


Figure 1.

Interaction of galectin-3 with MUC1 at the cell surface. A) A healthy epithelial cell with a normal distribution of MUC1. B) The surface of a cancer cell with over-expressed and aberrantly glycosylated MUC1. C) When circulating galectin-3 (green) is introduced to the cancer cell surface, its interaction with cancer-associated MUC1 causes adhesion molecules to be exposed, thus inducing aggregation (adapted from Yu et al.[17]).

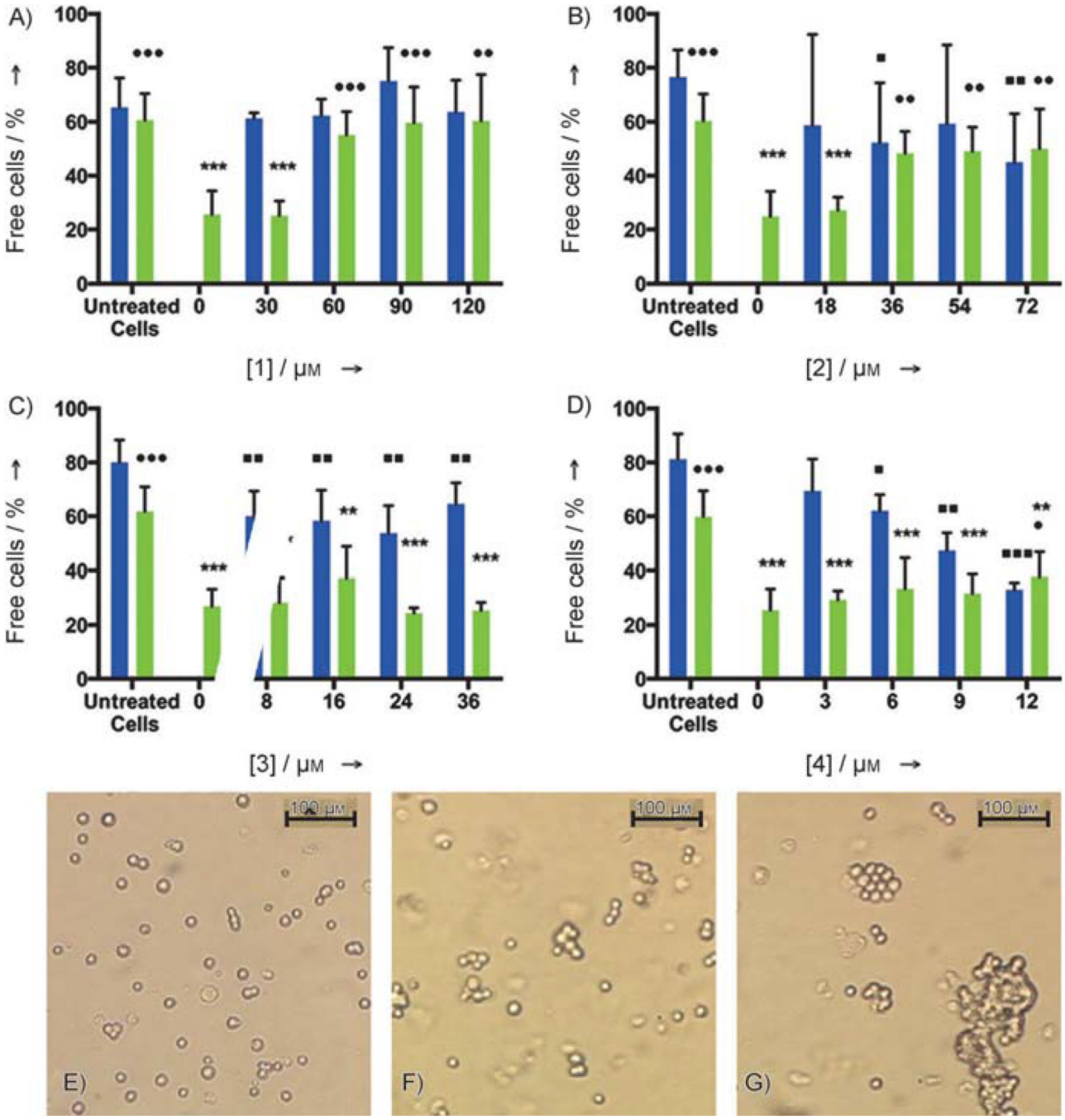


Figure 2.

Effect of lactose-functionalized dendrimers on untreated (blue bars) and galectin-3-induced (green bars) homotypic aggregates of A549 cells. In the assays without added galectin-3, ■ represents a statistical significance with respect to untreated cells. In the assays with added galectin-3, * identifies statistical significance with respect untreated cells, ● represents statistical significance with respect to the galectin-3 standard. One symbol represents $p < 0.05$, two symbols represent $p < 0.01$, and three symbols represent $p < 0.001$. No dendrimer was added to "Untreated Cells" or to the "0" entries on the *x*-axis. A) Compound **1** inhibits

galectin-3 aggregation but has no effect on cells without added galectin-3. B) and C) Dendrimers **2** and **3** have little effect on cellular aggregation. D) Aggregation results from the incubation of untreated cells with **4**. E) Micrograph of free cells. F) Micrograph of cells in the presence of galectin-3 and 60 μM **1**. G) Micrographs of galectin-3-induced cellular aggregates.

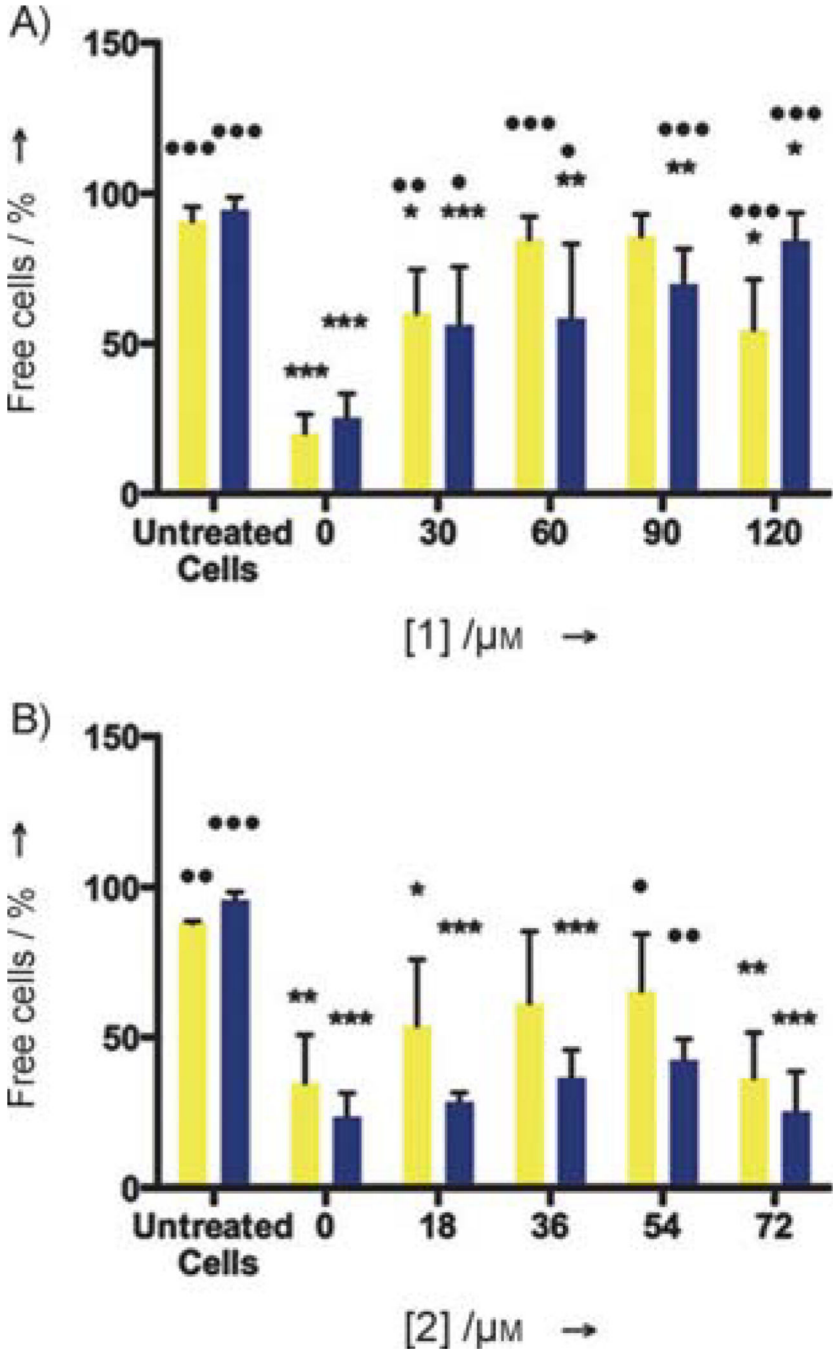


Figure 3.

Effect of lactose-functionalized dendrimers on galectin-3-induced homotypic aggregates of DU-145 (yellow bars) and HT-1080 cells (blue bars): * statistical significance with respect untreated cells, ● statistical significance with respect to the galectin-3 standard. One symbol represents $p < 0.05$, two symbols represent $p < 0.01$, and three symbols represent $p < 0.001$. No dendrimer was added to the "Untreated Cells" or to the "0" entries on the *x*-axis. A) Dendrimer **1** inhibits aggregation in both cell lines. B) Dendrimer **2** inhibits aggregation slightly by DU-145 cells, but has little effect on HT-1080 cells.

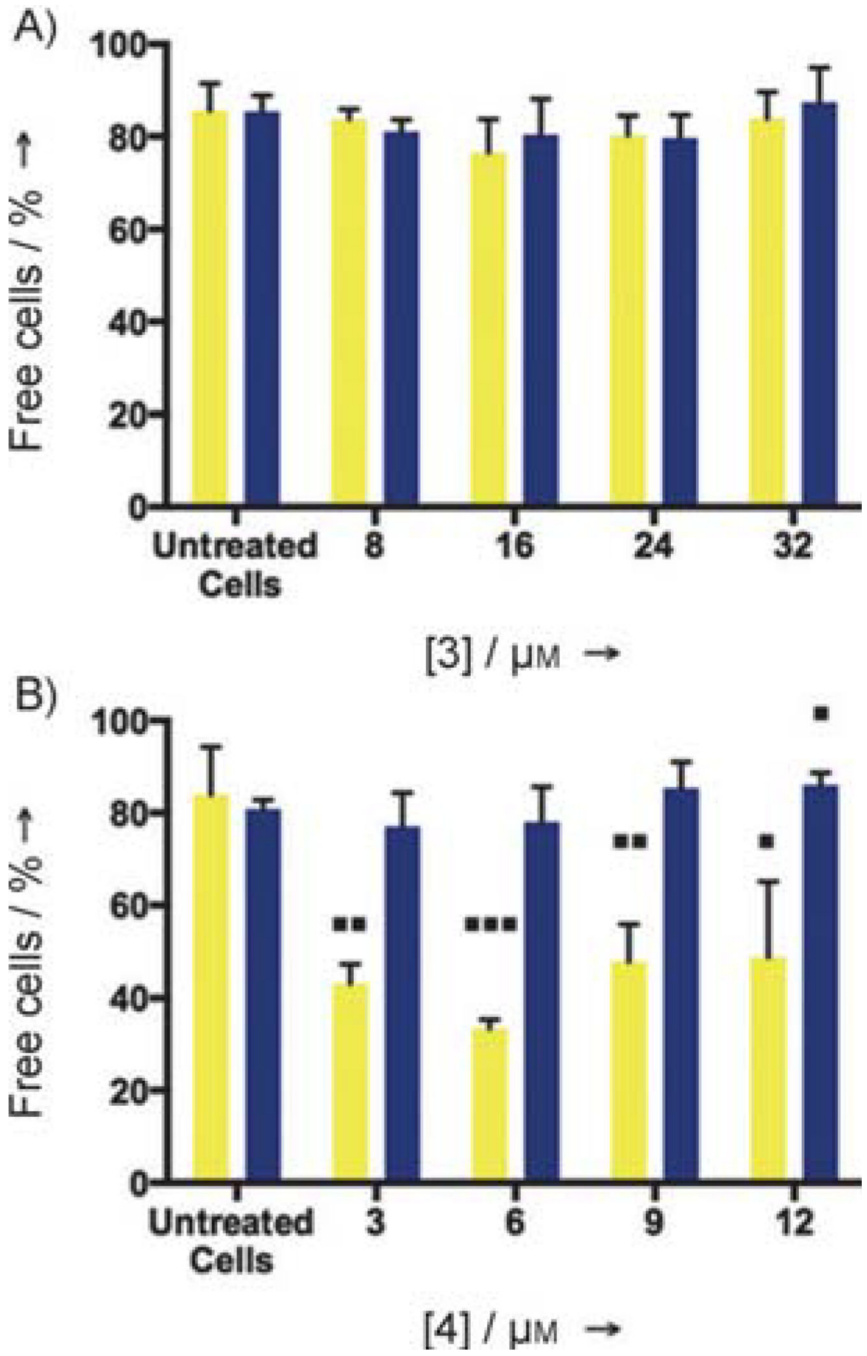


Figure 4.

Effect of lactose-functionalized dendrimers on DU-145 (yellow bars) and HT-1080 cells (blue bars) with no added galectin-3: ■ statistical significance with respect to untreated cells. One symbol represents $p < 0.05$, two symbols represent $p < 0.01$, and three symbols represent $p < 0.001$. A) Dendrimer **3** has no effect in either cell line on untreated cells. B) Dendrimer **4** causes aggregation in DU-145 cells, but has no effect on untreated HT-1080 cells.

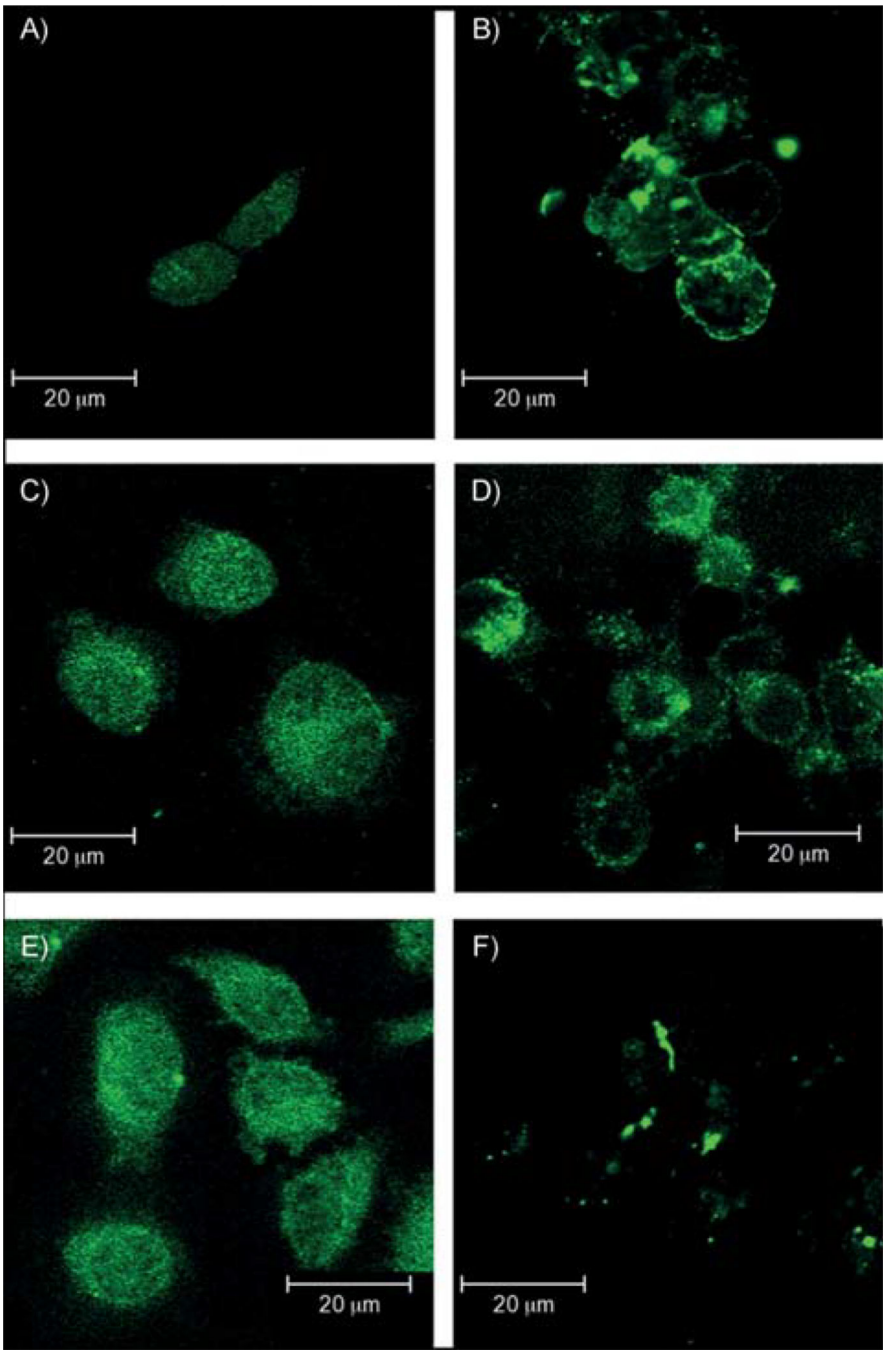


Figure 5.

Micrographs of A549 cells in the absence or presence of galectin-3 and lactose-functionalized dendrimers probed with fluorescent anti-galectin-3 antibody. A) Untreated cells. B) Cells incubated with galectin-3. C) Cells incubated with 120 μM **1**. D) Cells incubated with 12 μM **4**. E) Cells incubated with galectin-3 and 120 μM **1**. F) Cells incubated with galectin-3 and 12 μM **4**.

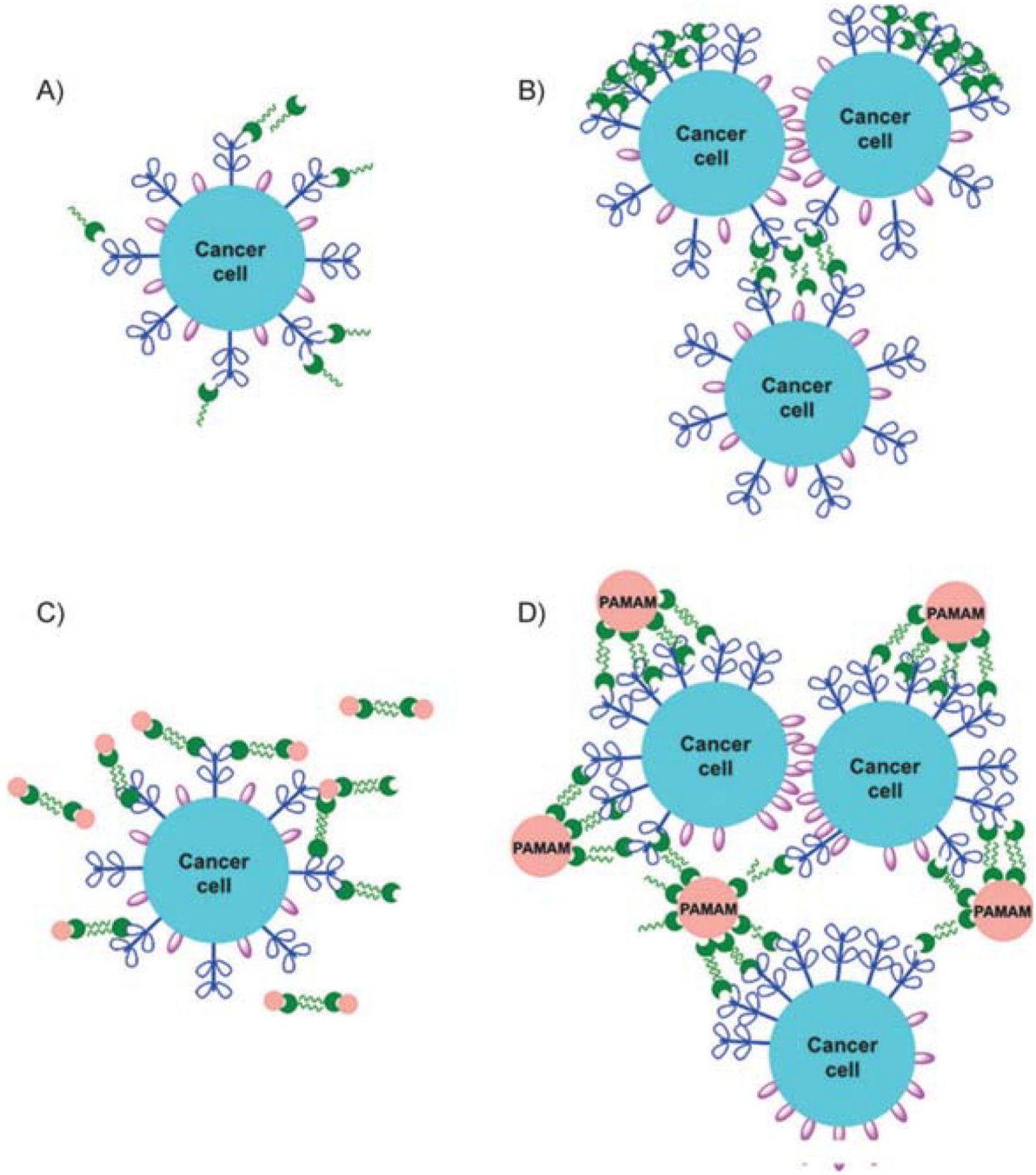
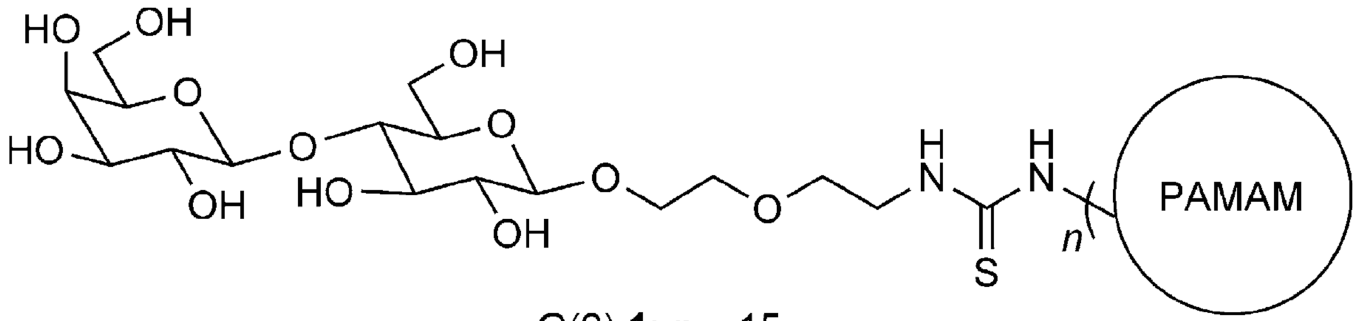


Figure 6.

Cancer cell-surface environment in the absence and presence of glycodendrimer. A) Untreated cell. B) Cells incubated with galectin-3. C) Cell incubated with **1** and galectin-3. D) Cells incubated with **4**.



G(2) **1**: *n* = 15
G(3) **2**: *n* = 20
G(4) **3**: *n* = 45
G(6) **4**: *n* = 100

Scheme 1.
Lactose-functionalized dendrimers.