Lectin–epithelial interactions in the human colon

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Abstract

Similar changes in glycosylation occur in the colonic epithelium in inflammatory conditions such as ulcerative colitis and Crohn's disease and also in colon cancer and precancerous adenomatous polyps. They include reduced length of O-glycans, reduced sulfation, increased sialylation and increased expression of oncofetal carbohydrate antigens, such as sialyl-Tn (sialylα2-6GalNAc), and the TF antigen (Thomsen–Friedenreich antigen) Galβ1-3GalNAcα-Ser/Thr. The changes affect cell surface as well as secreted glycoproteins and mediate altered interactions between the epithelium and lectins of dietary, microbial or human origin. Different TF-binding lectins cause diverse effects on epithelial cells, reflecting subtle differences in binding specificities e.g. for sialylated TF; some of these interactions, such as with the TF-binding peanut lectin that resists digestion, may be biologically significant. Increased TF expression by cancer cells also allows interaction with the human galactose-binding lectin, galectin-3. This lectin has increased concentration in the sera of patients with metastatic cancer and binds TF on cancer cell surface MUC1 (mucin 1), causing clustering of MUC1 and revealing underlying adhesion molecules which promote adhesion to endothelium. This is likely to be an important mechanism in cancer metastasis and represents a valid therapeutic target. Tools are now available to allow fast and accurate elucidation of glycosylation changes in epithelial disease, characterization of their potential lectin ligands, whether dietary, microbial or human, and determination of the functional significance of their interactions. This should prove a very fruitful area for future research with relevance to infectious, inflammatory and cancerous diseases of the epithelia.

Glycosylation changes in colonic disease

Altered epithelial glycosylation is commonly present in colonic disease. Similar abnormalities are found in neoplasia (colorectal cancer, precancerous adenomatous polyps and hyperplastic polyps) and inflammation [1–5]. They affect particularly the O-linked mucin-type glycans initiated by GalNAc α-Ser/Thr, and changes include shortening of O-glycans, increased sialylation, reduced sulfation and increased expression of oncofetal glycans such as the TF antigen (Thomsen–Friedenreich antigen) (galactose β1-3 GalNAc α-Ser/Thr) and sialyl-Tn (sialyl 2-6 GalNAc α-Ser/Thr) [6–8]. These changes are not exclusive to the colon and similar changes have been shown in other malignant epithelia including breast and pancreas. The mechanisms for these glycosylation changes are probably complex. Some of the glycosylation changes, notably the increased expression of sialyl-Lewis[x] and di-sialyl-Lewis[x], are associated with increased activity of the relevant α3-sialyltransferase and α3-fucosyltransferase IV in colorectal cancer [9]. Colon cancer O-glycans more commonly have a core 1 (TF antigen, galactose β1-3 GalNAc α-Ser/Thr) structure, whereas normal colonic mucins have a predominance of core 3 glycans (initiated by GlcNAc β1-3 GalNAc α-Ser/Thr) [10]. The GlcNAc-transferase that is needed for synthesis of the core 3 structure typically has very low expression in colon cancer

[11]. Other glycosylation changes tend not to correlate so well with glycosyltransferase activity [12] and may more commonly result from Golgi disorganization. In normal cells, the GlcNAc-transferases responsible for initiation of core 1 and core 2 O-glycans are localized in the *cis*-Golgi, whereas in some cancers they have been found throughout all Golgi compartments [13]. Cell culture experiments have shown that prevention of the normal Golgi acidification by ammonium chloride treatment or by blockade of the Golgi proton pump with bafilomycin A$_1$ results in the Golgi apparatus becoming fragmented and disorganized and simulates the glycosylation changes found in cancer, including increased TF expression [14]. A similar Golgi fragmentation has also been shown to occur in human colon cancer tissue [15,16]. Other mechanisms could also be involved in disease-associated changes in glycosylation; unsialylated TF antigen, the peanut lectin [PNA (peanut agglutinin)] receptor, is particularly expressed in colon cancer cells by high-molecular-mass mass splice variants of the cell surface glycoprotein CD44 [17,18]. This undergoes alternative splicing in cancer and has itself been shown to be relevant to metastasis, with reduction of metastases shown in animal models as a result of treatment with antibodies against CD44. The lower molecular mass CD44 variants do not express the PNA receptor, even in the same cancerous cells, implying that the transferases relevant to TF expression may require a core protein amino-acid sequence that is expressed in only the high-molecular-mass splice variants. Further investigation here might help to clarify some of our present uncertainty concerning the amino-acid sequences around serine/threonine residues that

are required for O-glycosylation to occur. Some changes in cell surface glycosylation could also result from the action of intraluminal microbial enzymes. Bacterial sialidase (neuraminidase) treatment of tissue sections *in vitro* has for example been shown to reveal the oncofetal TF antigen in normal colonic epithelia [19].

Although we had speculated that some of the changes in colonic epithelial glycosylation seen in colon cancer and inflammatory bowel disease might be genetically determined [20], there is little evidence to support this. The glycosylation changes are similar in both inflammatory bowel disease and colon cancer and yet it is now known that there is no increased risk of colorectal cancer among first degree relatives of individuals with inflammatory bowel disease unless they themselves also have inflammatory bowel disease [21]. Glycosylation abnormalities are intriguingly found in the colonic epithelium of unaffected identical twins of individuals with inflammatory bowel disease, but the changes are confined to the surface epithelium and are associated with NF-κB (nuclear factor κB) activation occurring in the absence of microscopic inflammation [22]. The mechanism for this NF-κB activation is unknown, but it is reasonable to speculate that it might be the result of microbial–epithelial interaction, perhaps occurring as a consequence of an inherited defect in the function of the mucosal barrier.

Interaction with dietary lectins

The altered epithelial glycosylation seen in colonic disease affects both secreted and cellular glycoproteins and has the potential to allow recruitment to the mucosa of carbohydrate-binding proteins (lectins) that may not otherwise bind. These can be of dietary, microbial or human origin. There is also a potential for interaction between lectins and, as an example of this, dietary lectins may either increase [23] or inhibit recruitment to the mucosa of bacteria. Thus, in animal models, ingestion of snowdrop lectin [GNA (*Galanthus nivalis* agglutinin)] has been shown to be protective against *Salmonella* infection [24]. Many dietary lectins including those present in legumes are tightly globular proteins that resist enzymatic degradation in the intestine. Peanut lectin, for example, can be extracted from human faeces after peanut ingestion in a bioactive state, i.e. retaining haemagglutinating and pro-proliferative activity [25]. The importance of interactions between dietary lectins and the mammalian intestine has been relatively little investigated. Most emphasis has been placed on the toxic lectins such as PHA (phytohaemagglutinin) present in red kidney beans that causes a severe gastroenteritis if ingested without adequate prior heating, typically as a consequence of a chilli con carne that has been slow-cooked at low temperature [26]. We have assessed a range of dietary, and therefore non-toxic, lectins for their functional effects on human colon epithelial cells with a particular focus on lectins that bind the TF antigen. Peanut lectin has been shown to increase proliferation in colon cancer cell lines [27], in cultured human colonic mucosal biopsies [28,29] and *in vivo*, causing a 40% increase in rectal mucosal

Figure 1 | Inhibition of nuclear import of synthetic NLS peptide in colon epithelial cells by edible mushroom lectin (ABL)

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NLS-BSA-FLUOS

- ABL   + ABL
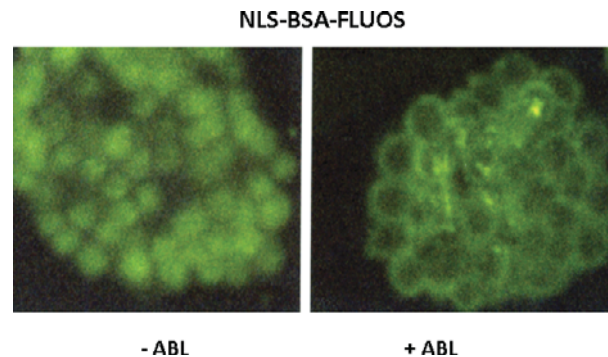
proliferation in individuals who ate a packet of peanuts per day for 5 days and who had increased TF expression in their colonic epithelia [25]. Conversely, the edible mushroom ABL (*Agaricus bisporus* lectin)/ABA (*A. bisporus* agglutinin), which binds not only TF but also sialylated TF, causes reversible inhibition of epithelial cell proliferation [30] as a consequence of internalization of the lectin and consequent inhibition of NLS (nuclear localization sequence)-dependent nuclear protein import (Figure 1) [31,32]. Another TF-binding dietary lectin, jacalin, present in jackfruit, also inhibits proliferation, but via a different mechanism: activation of PHAP1 (putative HLA class II-associated protein I) with consequent release and activation of a phosphatase, PP2A (protein phosphatase 2A), that dephosphorylates and hence deactivates ERK (extracellular-signal-regulated kinase) [33]. Investigation of the antiproliferative effect of jacalin thus led to clarification of an important cell regulatory mechanism by showing that PP2A becomes active after its release from complex with PHAP1 as a consequence of phosphorylation of PHAP1, itself induced by jacalin. It seems highly likely that these effects of dietary TF-binding lectins are mimicking effects of human galactose-binding lectins or galectins. These very different responses to lectins that have relatively similar binding specificities reflect the subtleties of lectin–carbohydrate interaction, with even slight differences in ligand binding resulting in different cell surface binding, different cellular localization (ABL is internalized, for example, whereas PNA is not) and completely different cellular responses [34].

Implications for cancer causation

We have investigated the possibility that ingestion of peanut lectin, by increasing colonic mucosal proliferation, might increase colon cancer risk and that food components that contain soluble fibre with a high galactose content, which might be expected to block interaction between dietary TF-specific lectins and the mucosa, could reduce colon cancer risk. A case control study used a telephone-interview food frequency questionnaire to compare pre-illness diet

Table 1 | Odds ratios for colorectal cancer (all sites) according to dietary intake

Association of dietary factors with risk for colorectal cancer: a case control study addressing the hypothesis that intake of foods high in lectins, such as legumes, might increase risk for colorectal cancer, whereas vegetable fibres [NSP (non-starch polysaccharide)] that are high in galactose might be protective. Univariate odds ratios (95% confidence intervals) are for colorectal cancer according to dietary intake. Values that were significant are shown in bold [35].

Variable	Quartile of intake			
	Lowest	Second	Third	Highest
Total NSP	1.0	1.09 (0.75–1.59)	0.97 (0.66–1.40)	1.10 (0.75–1.60)
Fruit and vegetable NSP	1.0	1.19 (0.80–1.78)	1.12 (0.75–1.66)	1.01 (0.67–1.52)
Cereal NSP	1.0	1.35 (0.92–1.98)	1.16 (0.78–1.73)	1.24 (0.84–1.83)
NSP galactose	1.0	1.00 (0.70–1.43)	0.75 (0.53–1.07)	**0.67 (0.47–0.95)**
Non-legume green vegetable	1.0	0.86 (0.58–1.29)	1.01 (0.68–1.50)	**0.54 (0.35–0.81)**
Legumes	1.0	**1.61 (1.08–2.39)**	1.18 (0.79–1.76)	1.37 (0.97–1.94)
Energy	1.0	1.40 (0.96–2.03)	**1.81 (1.23–2.65)**	**2.73 (1.81–4.12)**
Protein	1.0	1.35 (0.96–1.91)	1.20 (0.84–1.72)	**1.61 (1.11–2.33)**
Fat	1.0	**1.47 (1.01–2.12)**	**1.87 (1.28–2.74)**	**2.23 (1.53–3.25)**
Calcium	1.0	**1.52 (1.07–2.16)**	1.05 (0.74–1.50)	1.39 (0.97–1.97)
Red meat	1.0	0.96 (0.65–1.42)	1.03 (0.64–1.66)	**1.51 (1.06–2.15)**
Alcohol	1.0	1.0 (zero intake)	1.35 (0.99–1.85)	**1.70 (1.19–2.43)**
Exercise	1.0	1.0*	**0.39 (0.22–0.71)**	0.34 (0.19–0.61)
Peanuts†	**1.37 (1.01–1.85)**			
Broccoli†	**0.67 (0.45–1.00)**			
Aspirin†	**0.28 (0.18–0.43)**			

*Over 50% of the total sample values for exercise were similar, and separation of the lower two quartiles is not possible.

†Odds ratios are for regular consumers compared with non/irregular consumers.

in patients with colorectal cancer in comparison with controls who were matched for age and general practitioner. This showed a modest but significant increase in risk for colorectal cancer in regular peanut consumers and a significant protective effect of a high intake of galactose-rich soluble fibre (Table 1) [35]. An increased risk for rectal cancer had previously been shown among individuals with a high intake of peanut butter [36]. Peanuts are of course only one of many dietary sources of lectins and our study also showed an increased risk of colorectal cancer in association with a high dietary intake of legumes, a food source that is particularly rich in lectins. This is interesting as vegetarians, who often obtain much of their protein from legumes, tend not to have a significantly low risk for colorectal cancer despite their high intake of fruits and vegetables and avoidance of red meat [37]. This suggests that it might be more specifically non-legume vegetables that are protective against colorectal cancer.

Implications for interaction with pathogens

The potential for disease-related glycosylation changes to result in altered recruitment to the mucosa of microbes is also intriguing and very little explored. *Entamoeba histolytica*, the causative organism of amoebic dysentery, has a TF-binding lectin that is essential for its pathogenicity, and it seems plausible that inflammatory bowel conditions that lead to increased mucosal TF expression could greatly increase susceptibility to invasive amoebiasis. It is also a

reasonable hypothesis that sialidase-secreting bacteria in the colonic lumen could have a symbiotic relationship with *E. histolytica* and co-incubation of bacteria with *E. histolytica* can in some experimental circumstances be shown to increase pathogenicity [38], although bacteria can also reduce invasiveness of *E. histolytica* by releasing glycosidases and proteases that degrade the *Entamoeba* lectin that is essential for its adherence [39]. Alterations in the mucosa-associated microbiota have been reported both in inflammatory bowel disease and in colorectal cancer, particularly an increase in mucosa-associated *Escherichia coli* that have a characteristic adherent and invasive phenotype [40,41]. To date, we have only found one cancer *E. coli* isolate that has specificity for the cancer-associated TF glycan, but the ability of all the isolates to bind to the epithelium can be blocked *in vitro* by the presence of soluble fibres (non-starch polysaccharides), particularly those present in edible plantains [40]. We believe that inhibition of mucosal recruitment of bacteria could be an important mechanism by which dietary components might protect against colonic disease, including cancer, and further studies are needed to investigate this.

A potential role for galectin-3 interaction with epithelial glycans in cancer metastasis

There is a family of 15 human galactose-binding lectins, the galectins, and their functional interactions with epithelial cells are only beginning to be explored. In colonic disease, changes

Figure 2 | **Asymmetric localization of MUC1 on the cell surface of human colon epithelial cells in response to incubation with recombinant galectin-3 and consequential exposure of adhesion molecules allowing endothelial adhesion to HUVECs (human umbilical vein endothelial cells)**

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−galectin-3   +galectin-3   +galectin-3
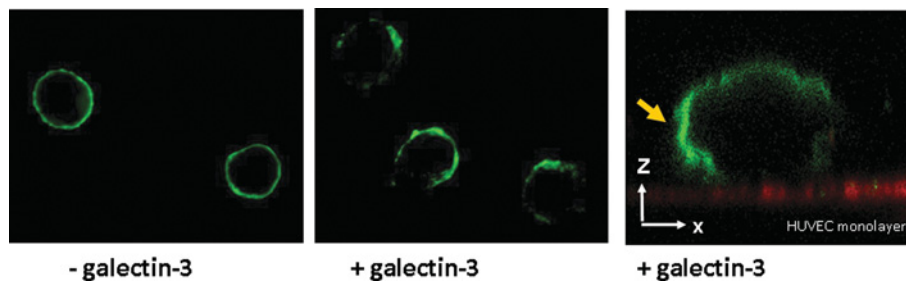
in galectin-3 expression and cellular distribution seem particularly striking. In epithelial cancers, including colon cancer, there is increased expression of galectin-3 combined with a shift from nuclear to cytoplasmic localization [42] and, particularly in patients with metastatic cancer, an increase in circulating concentrations [43]. Galectin-3 has been shown *in vitro* to increase adherence of cancer cells to endothelial cells via its interaction with TF expressed on the transmembrane mucin, MUC1 (mucin 1), and consequent clustering of MUC1 with revealment of underlying adhesion molecules that are otherwise concealed by the larger MUC1 molecule (Figure 2) [44]. Expression of MUC1-bearing TF glycans is in itself a feature of epithelial cancers that correlates with an increased risk for metastasis and has been studied as a potential target for anticancer vaccines [45]. Selectin–carbohydrate interactions are also involved in adhesion of epithelial cancer cells to endothelium. Expression of sialyl-Lewis[x] and other Lewis variants such as di-sialyl-Lewis[x] is often increased in colon cancer and correlates with poor prognosis. They are potential ligands for E-selectin and are also likely to be directly involved in cancer metastasis [46].

Much of this work has focused on interactions with the TF oncofetal carbohydrate antigen because of its potential for disease-related alterations in the mucosa-associated microbiota, but it is likely that there will also be many functionally important interactions between normal glycans on the healthy epithelium and lectins of dietary microbial and human origin. Many of these interactions may represent appropriate targets for novel therapies and this should be a very fertile field for research for years to come.

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