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MINIREVIEW

Altered glycosylation in inflammatory bowel disease: A possible role in cancer development

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Ulcerative colitis and Crohn's disease (together known as Inflammatory Bowel Disease or IBD) are both associated with increased risk for colorectal cancer. Although it is conventional to emphasise differences between IBD-associated and sporadic colon cancer, such as a lower rate of Adenomatosis Polyposis Coli mutations and earlier p53 mutations, IBD-associated cancer has a similar dysplasia-cancer sequence to sporadic colon cancer, similar frequencies of major chromosomal abnormalities and of microsatellite instability and similar glycosylation changes. This suggests that IBD-associated colon cancer and sporadic colon cancer might have similar pathogenic mechanisms. Because the normal colon is arguably in a continual state of low-grade inflammation in response to its microbial flora, it is reasonable to suggest that both IBD-associated and sporadic colon cancer may be the consequence of bacteria-induced inflammation. We have speculated that the glycosylation changes might result in recruitment to the mucosa of bacterial and dietary lectins that might otherwise pass harmlessly though the gut lumen. These could then lead to increased inflammation and/or proliferation and thence to ulceration or cancer. The glycosylation changes include increased expression of onco-fetal carbohydrates, such as the galactose-terminated Thomsen-Friedenreich antigen (Galβ1,3GalNAcα-), increased sialylation of terminal structures and reduced sulphation. These changes cannot readily be explained by alterations in glycosyltransferase activity but similar changes can be induced *in vitro* by alkalinisation of the Golgi lumen. Consequences of these changes may be relevant not only for cell-surface glycoconjugates but also for intracellular glycoconjugates.

Keywords: **O-glycosylation, oncofetal antigens, inflammatory bowel disease, colon cancer, Golgi pH**

Inflammatory bowel disease-associated colon cancer

In both ulcerative colitis and Crohn's disease (together known as inflammatory bowel disease) there are strong associations between duration and extent of mucosal inflammation and colorectal cancer risk. Each condition has a prevalence of between one and five per thousand people in Western countries. Their clinical features overlap but ulcerative colitis affects only the colon and rectum whereas Crohn's disease may affect any part of the gastro-intestinal tract. Both diseases are commonly presumed to result from an altered host response to the normal intestinal bacterial flora [1]. In approximately one quarter of cases of Crohn's disease, particularly those with small intestinal involvement, there is an alteration in a gene called NOD2 [2,3] which encodes a protein that is selectively expressed in macrophages. Its function is not well understood but it is reasonable to assume that it may alter the macrophage response to bacteria. It is now recognised that the cancer risk is equivalent in both ulcerative colitis and Crohn's disease when there is a similar extent and duration of colonic disease and amounts to an approximately 5-fold overall relative risk in colorectal cancer compared with the age-matched general population [4]. In Crohn's disease, there is a convincing association between inflammation and cancer at various sites. Patients with small bowel Crohn's disease have an increased risk for small bowel cancer which is otherwise extremely rare [5] and cancers can occur at the site of inflammation in the anal canal, in fistula tracts and in surgical scars. In keeping with the development of cancer as a consequence of inflammatory bowel disease rather than as a co-inherited phenomenon, a recent study of over 30,000 cases of inflammatory bowel disease in Sweden, has shown no significantly increased risk of colorectal cancer in first-degree relatives of inflammatory bowel disease patients [6]. The presence of a

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family history of colon cancer does however increase this risk still further. Inflammatory bowel disease-associated cancer thus serves as an excellent model of inflammation-associated cancer and may also provide many important clues to understanding the pathogenesis of sporadic colorectal cancer.

Abberant mucosal glycosylation

Similar mucosal glycosylation alterations occur in colon cancer, adenomatous and metaplastic polyps and in pre-cancerous conditions of the colon such as ulcerative colitis and Crohn's disease [7] affecting intracellular, cell-surface and secreted glycoconjugates. Probably the commonest change is the shortening of the *O*-linked oligosaccharide side chains of glycoproteins [8,9]. Our own work has provided clear evidence of increased expression of the core 1 structure in *O*-linked oligosaccharides, the oncofetal Thomsen Friedenreich (TF) carbohydrate antigen (galactoseβ1,3N-acetylgalactosamine) α-linked to serine or threonine of the protein core) by mucins extracted from colon cancer and ulcerative colitis mucosal samples [9] (see Figure 1). Others have shown increased expression of short *O*-linked oncofetal antigens such as sialyl Tn (sialylα2,6N-acetylgalactosamine α-linked to serine or threonine of the protein core) [10,11]. In previous studies, we and others have also demonstrated that mucosal samples from the colons of patients with ulcerative colitis incorporate less sulphate into their

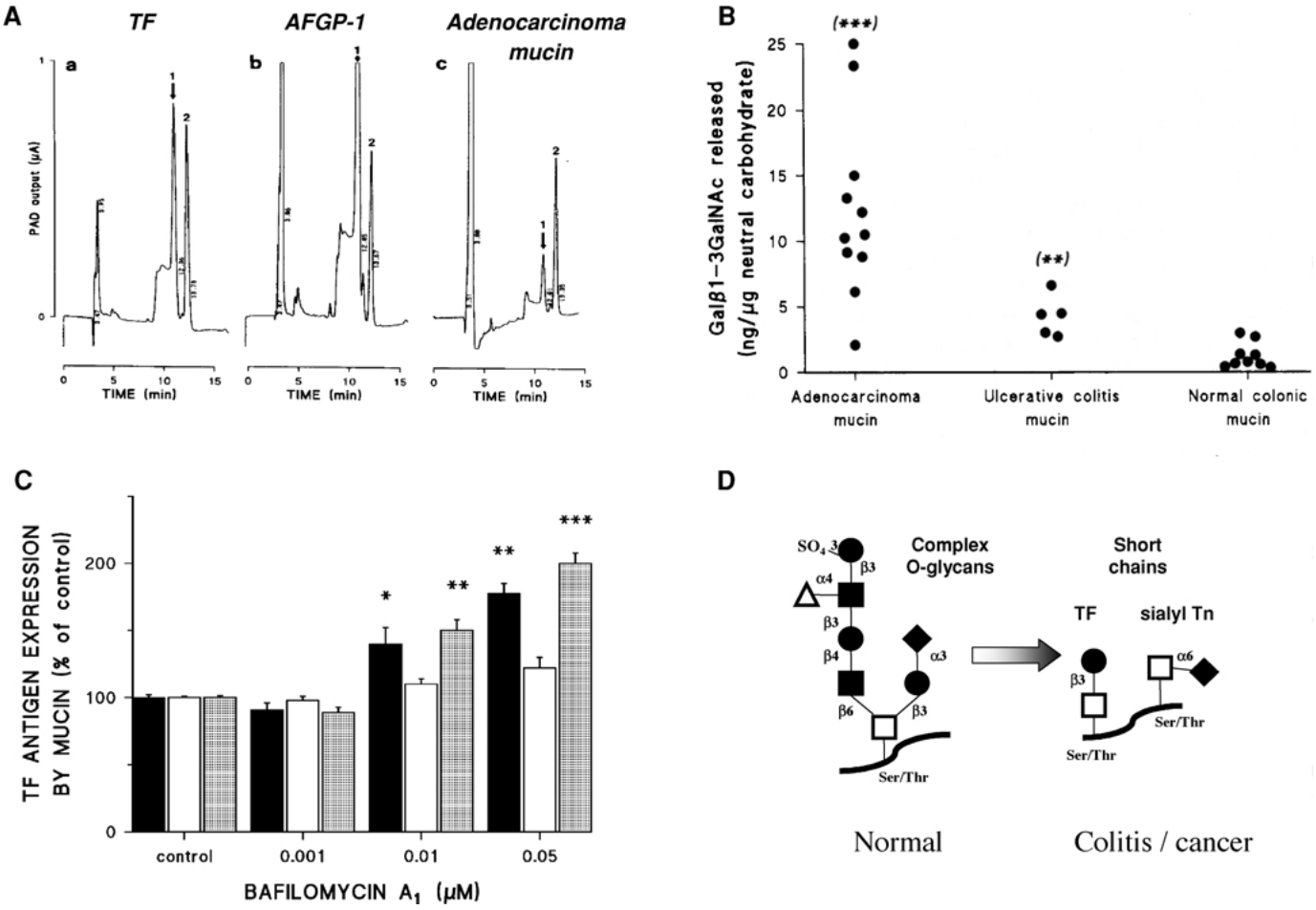

Figure 1. Panel A: HPAEC separation of (a) 0.5 μg TF antigen Galβ1-3GalNAcα- (1, arrows) and Galβ1-3GalNAcα- liberated by *O*-glycanase treatment of (b) 100 μg antifreeze glycopeptide AFGP-I and (c) colonic adenocarcinoma mucin. All samples contained 320 ng melibiose (2) as internal standard. From reference [9], reprinted with permission; Panel B: TF antigen liberated by *O*-glycanase from purified mucin was significantly higher in colonic adenocarcinoma ($n = 11$) and colitic ($n = 5$) mucin samples when compared with adjacent histologically normal tissue ($n = 9$). $^{***}P < 0.0001$ and $^{**}P = 0.0018$, respectively; ANOVA. From reference [9], reprinted with permission; Panel C: Bafilomycin A_1 increases expression of oncofetal TF antigen by mucin from LS174T colonocytes (as assessed by binding of PNA per 250 cpm ^{14}C-threonine labelled cellular [■], secreted [□] and total mucins [▒], $n = 16$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ indicate significant differences from control; (ANOVA). Reprinted with permission from reference [37]; Panel D: Aberrant mucosal glycosylation seen in colitis, colitis-associated cancer and colon cancer is characterised by increased expression of truncated O-glycans particularly oncofetal carbohydrate antigens such as TF (core 1) and sialyl Tn.

mucus when cultured *in vitro* than non-inflammatory bowel disease controls [12,13], supporting earlier histochemical evidence of reduced mucosal sulphation [14]. Although having similar glycosylation changes, South Asians with colitis, unlike their European counterparts, do not have reduced sulphomucin [15] and it has been speculated that this might be related to their apparent low risk for colitis-associated colon cancer. Other glycosylation changes include increased expression of ABO blood groups and increased sialylation of peripheral blood group structures resulting, for example, in di-sialyl and tri-sialyl Lewis antigen variants, particularly those with Galβ1,4GlcNAc linkage (Lewis^x and Lewis^y) [16]. Increased sialylation of mucins has also been documented by our own group in inflammatory-bowel disease colonic mucosal explants [17]. Some of these changes, notably sialyl Tn expression, have been shown to be markers of high risk for cancer development in inflammatory bowel disease mucosa [10,11] but glycosylation changes can often be found in the absence of dysplasia and seem to predate cytological malignant change in inflammatory bowel disease.

A similar colitis-cancer association exists in the Cotton-top tamarin, a New World monkey that is susceptible to a disease that is indistinguishable from human ulcerative colitis. The monkeys universally develop colitis in captivity and many go on to develop colon cancer subsequently [18]. Colon cancer has not been reported in the monkeys prior to the development of colitis. A selective mucin sub-class depletion has been reported in this animal similar to the changes seen in human ulcerative colitis [19]. This subclass is defined by its ion-exchange profile. Our opinion is that this subclass probably accounts for most of the pure mucin [20] but even if this just indicates mucin depletion it is a very interesting finding. As in human colitis, increased expression of oncofetal TF antigen (as demonstrated by increased peanut lectin binding), is seen on colonic mucosal glycoconjugates of Cotton-top tamarins with chronic colitis [21,22] with a marked degree of expression significantly associated with those animals that developed colorectal carcinoma [22]. Lectin histochemistry has also demonstrated interesting differences between mucins in those New World monkeys who are susceptible to colitis and colon cancer and those which are colitis susceptible but cancer resistant [23]. One of the key differences observed, is the presence of fucosylated *Ulex europaeus* (UEA-1) lectin-positive cellular glycans in the cancer-prone tamarins.

Mechanisms of altered glycosylation

It is not known whether the glycosylation abnormalities in colon cancer, colitis and colitis-associated cancer are determined by alterations in the relative activities of the relevant Golgi glycosyltransferases, altered substrate availability or changes in the amino-acid sequence of the glycoprotein.

The pattern of glycosylation of cell-surface glycoproteins can be altered as a consequence of altered splicing. We showed that cell surface expression of the TF oncofetal carbohydrate antigen on colon cancer cells can occur specifically on high molecular weight splice variants of the adhesion molecule CD44 whereas the standard CD44 from both normal and colon cancer tissues does not express TF [24]. Several studies have suggested a link between CD44 splicing, altered cell surface glycosylation and tumour cell behaviour. Transfection of a poorly tumorigenic rat colon cancer cell-line with human H blood group antigen-forming α(1-2) fucosyltransferase cDNA resulted in cell-surface expression of H antigens selectively borne on CD44v6 and conferred increased mobility and tumorigenicity to the transfected cells [25]. The expression of oncofetal carbohydrate antigens on CD44 splice variants provides a link between cancer-associated changes in glycosylation and CD44 splicing, both of which correlate with increased metastatic potential. The fact that high-molecular-weight CD44 variants are also found in colitis [26], may explain some of the glycosylation changes in that condition. The association between CD44 splicing and glycosylation suggests that the nature of *O*-glycosylation may be determined, at least in part, by the amino-acid sequence of the protein (CD44) undergoing glycosylation. It does not however explain the simultaneous change in glycosylation of secreted mucins that is commonly seen in disease states.

The reduction in mucosal sulphation seen in inflammatory bowel disease and colon cancer could itself explain some of the other changes because the TF antigen has been shown to be concealed by *O*-sulphate esters in the normal colon [27]. Moreover, a colonic mucin sulphotransferase, which undergoes progressively reduced expression from adenoma to cancer, has as its preferred acceptor the TF antigen [28]. However, in a meticulous study, Brockhausen and colleagues have shown that, although there are changes in expression of the relevant glycosyl-, sialyl-, and sulpho-transferases in colon cancer, these changes correlate relatively poorly with the changes in carbohydrate expression [29]. They speculated that other explanations, including altered arrangement of transferases within the Golgi, might be responsible.

The relative positions in the Golgi of the different glycosyl-, sialyl- and sulpho-transferases are critically important in determining *O*-glycan structure [30,31] and these positions can be affected by intra-Golgi pH [32]. We have speculated that the changes in *O*-glycosylation seen in colitis, colitis-associated cancer and in sporadic colon cancer might all result from reduced Golgi acidification, possibly mediated by inflammatory cytokines generated as a result of bacterial-mucosal interaction [33]. Intra-Golgi pH is maintained by the activity of an electrogenic vacuolar ATP-dependent proton pump (V-ATPase) [34,35] which can be blocked by the macrolide bafilomycin A_1 [36]. We have recently shown that increasing the intra-Golgi pH of goblet cell-differentiated colonocytes following treatment with bafilomycin A_1, mimics the decreased mucin sulphation and increased oncofetal TF antigen expression seen in colon cancer, colitis and colitis-associated cancer [37] (see Figure 1). Work by Axelsson and colleagues [38] confirms that the altered glycosylation that results from altered intra-Golgi pH

is mediated by altered position of glycosyltransferases within the Golgi. Intra-Golgi pH has been little studied in cancer but there is some evidence of defective Golgi acidification in cancer cells [39]. Interestingly, the contrasting glycosylation changes seen in cystic fibrosis (undersialylation of plasma membrane glycoconjugates) are shown to be due to hyper-acidification of the *trans*-Golgi as a result of the dysfunctional CFTR (cystic fibrosis transmembrane conductance regulator) gene product [40].

We have recently found that some of the inflammatory bowel disease and cancer-associated glycosylation changes can be induced in cultured goblet cell-differentiated colon cancer cell lines by the pro-inflammatory cytokine, tumor necrosis factor alpha (TNF-α) [41,42]. This includes abberant mucin synthesis and expression, increased oncofetal TF expression and reduction in mucosal sulphation. Others have shown increased cell-surface expression of the cancer-related carbohydrate antigens sialyl Lewis^x, in response to TNF-α [43], and Lewis^y, in response to interferon-alpha and interferon-gamma [44]. TNF-α has recently been demonstrated to alter glycosyl- and sulfotransferases of the human bronchial mucosa responsible for the biosynthesis of Lewis x and of its sialylated and sulfated forms on mucins [45]. However, further studies are needed to determine whether pro-inflammatory cytokines mediate aberrant glycosylation changes in colitis and colitis-associated cancer by altered glycosyltransferase expression or through alteration of intra-Golgi pH. In addition, studies show that CD44 expression and splicing in colonocytes may also be modified by cytokines particularly IL-4 and IL-13, IFN-γ and TNF-α [46–48] so this may represent an alternative mechanism for inflammation-induced alterations in cell surface glycosylation.

Is colonic mucosal inflammation the driving force for increased risk of cancer?

Inflammatory bowel disease is associated with increased mucosal production of pro-inflammatory cytokines by infiltrating mononuclear cells, a process that is mediated by NFκB (nuclear factor kappa B) [49]. NF-κB regulates the promoters of a variety of genes whose products are critical for inflammatory processes, including pro-inflammatory cytokines, such as tumour necrosis factor alpha (TNF-α), IL-1β, IL-6 and IL-8 [50] and cyclo-oxygenase 2 (COX2) [51]. There are several differences in the cytokine response between ulcerative colitis and Crohn's disease yet both conditions have similar cancer risks.

The cytokine profile in Crohn's disease is generally thought to typify a T helper1-mediated, cell-mediated response with increased levels particularly of IL-2 and IL-12, interferon gamma (IFN-γ) and TNFα, whereas ulcerative colitis is more usually associated with a cytokine profile typical of a T helper 2, antibody-mediated, response with elevated IL-4, 5,6 and 10 [1]. Interleukin 10 knockout mice show increased production of pro-inflammatory cytokines and develop colitis-associated adenocarcinoma [52] that is histologically similar (well differentiated but with irregular glandular structure) to the adenocarcinomas seen in the other mouse colitis models, G$\alpha_{1\text{-}2}$ and dominant negative N-cadherin [53]. Inflammation-associated stroma has also been shown to promote the conversion of colonic adenoma cells to adenocarcinoma cells in nude mice [54].

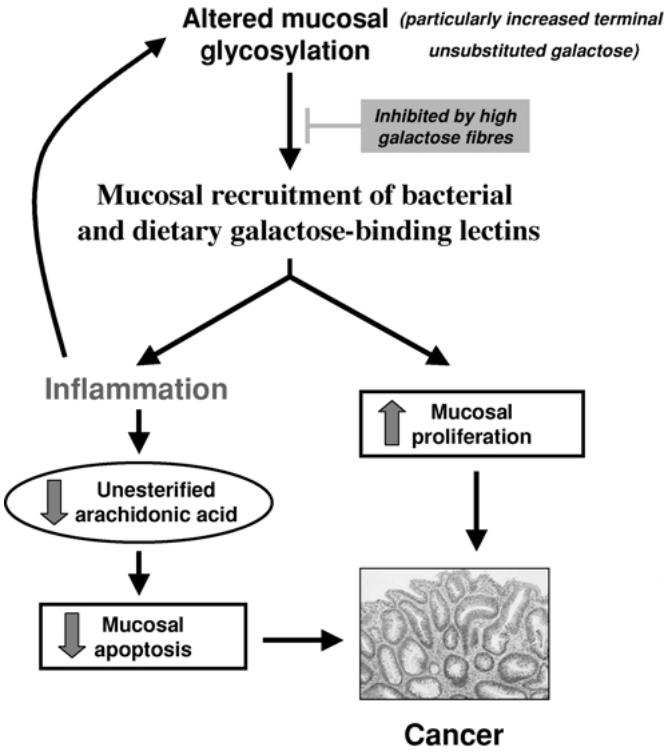

Figure 2. A hypothesis for altered mucosal glycosylation as a cause of colonic inflammation and cancer. Adapted from reference [33].

Inflammation in all these conditions including ulcerative colitis and Crohn's disease leads to increased expression of COX2 and/or lipoxygenase both of which cause depletion of intracellular unesterified arachidonic acid, a molecule with pro-apoptotic activity. Mesalazine (5-aminosalicylic acid, 5-ASA), a drug which is routinely used to prevent relapse of inflammatory bowel disease, inhibits lipoxygenase and has been shown markedly to reduce the cancer risk in inflammatory bowel disease patients [55].

If inflammation is indeed a common feature of IBD and sporadic colon cancer then this makes it even more likely that the similar glycosylation changes seen in these conditions could represent an important part of their pathogenesis rather than just a curious epiphenomenon (see Figure 2).

Consequences of altered mucosal glycosylation with relevance to cancer

Until recently, very little was known about the functional significance of changes in epithelial carbohydrate expression but it is now clear that they play a major role in determining the

proliferative, invasive and metastatic properties of tumour cells. Individuals who express increased amounts of TF antigen in their rectal mucosae have a 40% increase in rectal mitotic index after 7 days of daily ingestion of peanuts, which contain a mitogenic TF-binding lectin [56]. This is proof of concept of the hypothesis that alteration in mucosal cell surface glycosylation may lead to functionally important changes as a consequence of mucosal recruitment of intraluminal lectins, which may be of dietary or microbial origin. Since increased expression of terminal unsubstituted galactose is a common glycosylation change in colonic malignancy and pre-malignancy, it is possible that dietary fibres that are rich in galactose (usually of vegetable origin since cereal fibres are largely devoid of galactose) may protect against colon cancer by competitive inhibition of intraluminal galactose-binding lectins [57]. The interaction of disease-related cell surface glycoconjugates with intraluminal lectins could be quantitatively as important as interaction with growth factors in determining the rate of epithelial proliferation and may have other important consequences for mucosal epithelial function. A case-control study of diet and colorectal cancer [58] has provided evidence to support the protective effects of high-galactose fibres which may explain some of the discrepancies between the epidemiological evidence for a protective effect of vegetables and the disappointing results of intervention studies with dietary fibre. Circulating TF antibodies, which are present in all humans after weaning, probably as a reaction to intestinal bacterial carbohydrate antigens, have also been shown to have the potential to interact with TF expressed on cancer cell membranes [59].

Conversely, a TF-binding lectin in edible mushrooms causes inhibition of proliferation without cytotoxicity [60], as a result of internalization and inhibition of nuclear localization sequence (NLS)-dependent nuclear protein import [61], showing the potential importance of intracellular as well as cell surface glycosylation. This combination of anti-proliferative effect and lack of cytotoxicity is unusual and implied that the lectin might prove a useful tool for exploring the functional implications of glycosylation (and disease-related changes in glycosylation) of cellular glycoproteins. We have recently shown that one of the major intracellular ligands for the mushroom lectin is a TF-expressing, N-terminal truncated cytoplasmic form of a stress protein, Orp150 (Hypoxia responsive protein), which in its non-truncated form is localised mainly in the endoplasmic reticulum (ER) as a result of an ER signal peptide at its N terminus. We have shown that the truncated cytoplasmic Orp150 is itself essential to NLS-dependent nuclear protein import [62].

The increased expression of oncofetal carbohydrate antigens by cell surface glycoproteins also has the potential to result in alteration of the mucosal-associated flora, for example by recruiting bacteria with lectins specific for TF or sialyl Tn. It is intriguing that the pathogenic amoeba, *Entamoeba histolytica* possesses a TF-binding lectin that is essential for its pathogenicity. This should imply that patients with inflammatory bowel disease who have increased mucosal TF expression should be particularly prone to develop amoebic dysentery in endemic areas. Recent studies have demonstrated the presence of increased mucosa-associated bacteria [63] including a new class of "adhesive and invasive" *E coli* in ileal biopsies from patients with Crohn's disease [64,65] and separate studies have shown an intriguing increase in apparently intra-epithelial *E coli* in histologically normal mucosa distant from colon cancer [66]. This is compatible with the hypothesis that not only IBD-associated colon cancer but even sporadic colon cancer could be the result of colonic bacterial-mucosa interactions in a way analagous to *Helicobacter pylori* and gastric cancer. Although there are differences between the molecular abnormalities found in colitis-associated dysplasia (a relatively increased frequency of p53 mutations and reduced frequency of APC mutations) and those in sporadic adenomas, the natural history and molecular biology of colitis-associated cancer and sporadic colon cancer are otherwise remarkably similar. This is compatible with the hypothesis that sporadic colon cancer could occur secondarily to the low-grade inflammation that arguably exists in the normal human colon [33].

Concluding remarks

There seems little doubt that the increased risk of cancer in the inflammatory bowel diseases ulcerative colitis and Crohn's disease is due to the effects of inflammation, probably mediated at least in part by their effects on cytokines and hence on prostaglandin metabolism, apoptosis and glycosylation. The possibility remains that some of the glycosylation changes might be inherited rather than acquired. The similarities between the molecular mechanisms of colitis-associated cancer and sporadic cancer seem to outweigh the differences and make it reasonable to speculate that even "sporadic" colon cancer might be largely secondary to inflammation. Whether the glycosylation changes are an essential part of this process or just an innocent bystander remains to be firmly established but there is increasing evidence that the cell surface and intracellular glycosylation changes seen in colonic disease are likely to be functionally important, particularly in the relationship between the colon's mucosa and its luminal contents.

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References

1 Shanahan F, Inflammatory bowel disease: Immunodiagnostics, immunotherapeutics, and ecotherapeutics, *Gastroenterology* **120**, 622–35 (2001).

2 Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr RH, Achkar JP, Brant SR, Bayless TM, Kirschner BS, Hanauer SB, Nunez G, Cho JH, A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease, *Nature* **411**, 603–6 (2001).

3 Hugot JP, Chamaillard M, Zouali H, Lesage S, Cezard JP, Belaiche J, Almer S, Tysk C, O'Morain CA, Gassull M, Binder V, Finkel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel JF, Sahbatou M, Thomas G, Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease, *Nature* **411**, 599–603 (2001).

4 Ekbom A, Helmick C, Zack M, Adami HO, Increased risk of large-bowel cancer in Crohn's disease with colonic involvement, *Lancet* **336**, 357–9 (1990).

5 Collier PE, Turowski P, Diamond DL, Small intestinal adenocarcinoma complicating regional ileitis, *Cancer* **55**, 516–21 (1985).

6 Askling J, Dickman PW, Karlen P, Brostrom O, Lapidus A, Lofberg R, Ekbom A, Colorectal cancer rates among first-degree relatives of patients with inflammatory bowel disease: A population-based cohort study, *Lancet* **357**, 262–6 (2001).

7 Rhodes JM, Yu LG, Glycosylation and disease, In *Encyclopaedia of Life Sciences*, http://www.els.net. (Nature Publishing Group, London, 2000.)

8 Boland CR, Deshmukh GD, The carbohydrate composition of mucin in colonic cancer, *Gastroenterology* **98**, 1170–7 (1990).

9 Campbell BJ, Hounsell E, Finnie IA, Rhodes JM, Direct demonstration of increased expression of Thomsen-Friedenreich antigen (Galβ1-3GalNAc) by mucus in colon cancer and inflammatory bowel disease, *J Clin Invest* **95**, 571–6 (1995).

10 Karlen P, Young E, Brostrom O, Lofberg R, Tribukait B, Ost K, Bodian C, Itzkowitz S, Sialyl-Tn antigen as a marker of colon cancer risk in ulcerative colitis: Relation to dyplasia and DNA aneuploidy, *Gastroenterology* **115**, 1395–404 (1998).

11 Itzkowitz SH, Marshall A, Kornbluth A, Harpaz N, McHugh JB, Ahnen D, Sachar DB, Sialosyl-Tn antigen: Initial report of a new marker of malignant progression in long-standing ulcerative colitis, *Gastroenterology* **109**, 490–7 (1995).

12 Raouf AH, Tsai HH, Parker N, Hoffman J, Walker RJ, Rhodes JM, Sulphation of colonic and rectal mucin in inflammatory bowel disease: Reduced sulphation of rectal mucus in ulcerative colitis, *Clin Sci* **83**, 623–6 (1992).

13 Corfield AP, Myerscough N, Bradfield N, Corfield Cdo A, Gough M, Clamp JR, Durdey P, Warren BF, Bartolo DC, King KR, Williams JM, Colonic mucins in ulcerative colitis: Evidence for loss of sulfation, *Glycoconj J* **13**, 809–22 (1996).

14 Filipe MI, Mucins in the human gastrointestinal epithelium: A review, *Invest Cell Pathol* **2**, 195–216 (1979).

15 Probert CS, Warren BF, Perry T, Mackay EH, Mayberry JF, Corfield AP, South Asian and European colitics show characteristic differences in colonic mucus glycoprotein type and turnover, *Gut* **36**, 696–702 (1995).

16 Kim YS, Yuan M, Itzkowitz SH, Sun Q, Kaizu T, Palekar A, Trump BF, Hakamori S, Expression of Le Y and extended Le Y blood group antigens in human malignant, premalignant and non-malignant colonic tissues, *Cancer Res* **46**, 598–609 (1986).

17 Parker N, Tsai HH, Ryder SD, Raouf AH, Rhodes JM, Increased rate of sialylation of colonic mucin by cultured ulcerative colitis mucosal explants, *Digestion* **56**, 52–6 (1995).

18 Chalifoux LV, Bronson RT, Colonic adenocarcinoma associated with chronic colitis in cotton top marmosets, *Saguinus oedipus. Gastroenterology* **80**, 942–6 (1981).

19 Podolsky DK, Madara JL, King N, Sehgal P, Moore R, Winter HS, Colonic mucin composition in primates. Selective alterations associated with spontaneous colitis in the cotton-top tamarin, *Gastroenterology* **88**, 20–5 (1985).

20 Raouf AH, Parker N, Iddon D, Ryder S, Langdon-Brown B, Milton JD, Walker R, Rhodes JM, Ion-exchange chromatography of purified colonic mucus glycoproteins in inflammatory bowel disease: Absence of a selective subclass defect, *Gut* **32**, 1139–46 (1991).

21 Moore R, King N, Alroy J, Differences in cellular glycoconjugates of quiescent, inflamed, and neoplastic colonic epithelium in colitis and cancer-prone tamarins, *Am J Pathol* **131**, 484–9 (1988).

22 Boland CR, Clapp NK, Glycoconjugates in the colons of new world monkeys with spontaneous colitis. Association between inflammation and neoplasia, *Gastroenterology* **92**, 625–34 (1987).

23 Moore R, King N, Alroy J, Characterization of colonic cellular glycoconjugates in colitis and cancer-prone tamarins versus colitis and cancer-resistant primates, *Am J Pathol* **131**, 477–83 (1988).

24 Singh R, Campbell BJ, Yu L-G, Fernig DG, Milton JD, Goodlad RA, FitzGerald AJ, Rhodes JM, Cell surface-expressed Thomsen-Friedenreich antigen in colon cancer is predominantly carried on high molecular weight splice variants of CD44, *Glycobiology* **11**, 587–92 (2001).

25 Goupille C, Hallouin F, Meflah K, Le Pendu J, Increase of rat colon carcinoma cells tumorigenicity by α(1-2) fucosyltransferase gene transfection, *Glycobiology* **7**, 221–9 (1997).

26 Camacho FI, Munoz C, Sanchez-Verde L, Saez AI, Alcantara M, Rodriguez R, CD44v6 expression in inflammatory bowel disease is associated with activity detected by endoscopy and pathological features, *Histopathology* **35**, 144–9 (1999).

27 Martinez-Menarguez JA, Ballesta J, Aviles M, Madrid JF, Castells MT, Influence of sulphate groups in the binding of peanut agglutinin. Histochemical demonstration with light- and electron-microscopy, *Histochem J* **24**, 207–16 (1992).

28 Kuhns W, Jain RK, Matta KL, Paulsen H, Baker MA, Geyer R, Brockhausen I, Characterization of a novel mucin sulphotransferase activity synthesizing sulphated *O*-glycan core 1,3-sulphate-Gal beta 1-3GalNAc alpha-R, *Glycobiology* **5**, 689–97 (1995).

29 Yang JM, Byrd JC, Siddiki BB, Chung YS, Okuno M, Sowa M, Kim YS, Matta KL, Brockhausen I, Alterations of *O*-glycan biosynthesis in human colon cancer tissues, *Glycobiology* **4**, 873–84 (1994).

30 Rabouille C, Hui N, Hunte F, Kieckbusch R, Berger EG, Warren G, Nilsson T, Mapping the distribution of Golgi enzymes involved in the construction of complex oligosaccharides, *J Cell Sci* **108**, 1617–27 (1995).

31 Rottger S, White J, Wandall HH, Olivo J-C, Stark A, Bennett EP, Whitehouse C, Berger EG, Clausen H, Nilsson T, Localization of three human polypeptide GalNAc-transferases in HeLa cells suggests initiation of *O*-linked glycosylation throughout the Golgi apparatus, *J Cell Sci* **111**, 45–60 (1998).

32 Zhang Y, Doranz B, Yankaskas JR, Engelhardt JF, Genotypic analysis of respiratory mucous sulphation defects in cystic fibrosis, *J Clin Invest* **96**, 2997–3004 (1995).

33 Rhodes JM, Campbell BJ, Inflammation and colorectal cancer: Inflammatory bowel disease-associated cancer and sporadic cancer compared. *Trends Mol Med* **8**, 10–6 (2002).

34 Glickman J, Croen K, Kelly S, Al-Awqati Q, Golgi membranes contain an electrogenic H^+ pump in parallel to a chloride conductance, *J Cell Biol* **97**, 1303–8 (1983).

35 Wu MM, Grabe M, Adams S, Tsien RY, Moore HP, Machen TE, Mechanisms of pH regulation in the regulated secretory pathway, *J Biol Chem* **276**, 33027–35 (2001).

36 Bowman EJ, Siebers A, Altendorf K, Bafilomycins: A class of inhibitors of membrane ATPases from microorganisms, animal cells, and plant cells, *Proc Natl Acad Sci USA* **85**, 7972–6 (1988).

37 Campbell BJ, Rowe G, Leiper K, Rhodes JM, Increasing the intra-Golgi pH of cultured LS174T goblet-differentiatied cells mimics the decreased mucin sulphation and increased Thomsen-Friedenreich antigen (Galβ1-3GalNAcα-) expression seen in colon cancer, *Glycobiology* **11**, 385–93 (2001).

38 Axelsson MAB, Karlsson NG, Steel DM, Ouwendiijk J, Nilsson T, Hansson GC, Neutralization of pH in the Golgi apparatus causes redistribution of glycosyltransferases and changes in the *O*-glycosylation of mucins, *Glycobiology* **11**, 633–44 (2001).

39 Schindler M, Grabski S, Hoff E, Simon SM, Defective pH regulation of acidic compartments in human breast cancer cells (MCF-7) is normalized in adriamycin-resistant cells (MCF-7adr), *Biochemistry* **35**, 2811–7 (1996).

40 Poschet JF, Boucher JC, Tatterson L, Skidmore J, Van Dyke RW, Deretic V, Molecular basis for defective glycosylation and *Pseudomonas* pathogenesis in cystic fibrosis lung, *Proc Natl Acad Sci* **98**, 13972–7 (2001).

41 Campbell BJ, Oxley C, Singh R, Yu L-G, Rhodes JM, TNF-alpha decreases the sulphation of mucins and CD44 in human colonic epithelial cells: An effect which may explain the low mucosal sulphation seen in inflammatory bowel disease, *Gastroenterology* **118**, A3836 (abstract) (2000).

42 Campbell BJ, Krishna Y, Rhodes JM, TNF-α causes reduced mucin synthesis and increased oncofetal carbohydrate expression by HT29-MTX cells, *Gastroenterology* **122**, T1044 (abstract) (2002).

43 Kuninaka S, Yano T, Yokoyama H, Fukuyama Y, Terazaki Y, Uehara T, Kanematsu T, Asoh H, Ichinose Y, Direct influences of pro-inflammatory cytokines (IL-β, TNF-α, IL-6) on the proliferation and cell-surface antigen expression of cancer cells, *Cytokine* **12**, 8–11 (2000).

44 Flieger D, Hoff AS, Sauerbruch T, Schmidt-Wolf IGH, Influence of cytokines, monoclonal antibodies and chemotherapeutic drugs on epithelial cell adhesion molecule (EpCAM) and Lewis^y antigen expression, *Clin Exp Immunol* **123**, 9–14 (2001).

45 Delmotte P, Degroote S, Lafitte JJ, Lamblin G, Perini JM, Roussel P, Tumor necrosis factor alpha increases the expression of glycosyltransferases and sulfotransferases responsible for the biosynthesis of sialylated and/or sulfated Lewis x epitopes in the human bronchial mucosa, *J Biol Chem* **277**, 424–31 (2002).

46 Trejdosiewicz LK, Morton R, Yang Y, Banks RE, Selby PJ, Southgate J, Interleukin 4 and 13 upregulate expression of CD44 in human colonic epithelial cell-lines, *Cytokine* **10**, 756–65 (1998).

47 Rosenberg WMC, MacDonald D, CD44 regulation and function in ulcerative colitis, *Gut* (suppl I) **48**, A272 (abstract) (2001).

48 Wimmenauer S, Steiert A, Wolff-Vorbeck G, Xing B, Baier PK, Ruckauer KD, Kirste G, von Kleist S, Influence of cytokines on the expression of fas ligand and CD44 splice variants in colon carcinoma cells, *Tumour Biol* **20**, 294–303 (1999).

49 Jobin C, Sartor RB, The IκB/NF-κB system: A key determinant of mucosal inflammation and protection, *Am J Physiol* **278**, C451–62 (2000).

50 Neurath MF, Fuss I, Schurmann G, Pettersson S, Arnold K, Muller-Lobeck H, Strober W, Herfarth C, Buschenfelde KH, Cytokine gene transcription by NF-κB family members in patients with inflammatory bowel disease, *Ann NY Acad Sci* **859**, 149–59 (1998).

51 Agoff SN, Brentnall TA, Crispin DA, Taylor SL, Raaka S, Haggitt RC, Reed MW, Afonina IA, Rabinovitch PS, Stevens AC, Feng Z, Bronner MP, The role of cyclooxygenase 2 in ulcerative colitis-associated neoplasia, *Am J Pathol* **157**, 737–45 (2000).

52 Berg DJ, Davidson N, Kuhn R, Muller W, Menon S, Holland G, Thompson-Snipes L, Leach MW, Rennick D, Enterocolitis and colon cancer in interleukin 10-deficient mice are associated with aberrant cytokine production and CD4(+) TH1-like responses, *J Clin Invest* **98**, 1010–20 (1996).

53 Rennick DM, Fort MM, Lessons from genetically engineered animal models. XII. IL-10 deficient [IL-10(−/−)] mice and intestinal inflammation, *Am J Physiol* **278**, G829–33 (2000).

54 Okada F, Kawaguchi T, Habelhah H, Kobayashi T, Tazawa H, Takeichi N, Kitagawa T, Hosokawa M, Conversion of human colonic adenoma cells to adenocarcinoma cells through inflammation in nude mice, *Lab Invest* **80**, 1617–28 (2000).

55 Eaden J, Abrams K, Ekbom A, Jackson E, Mayberry J, Colorectal cancer prevention in ulcerative colitis: A case-control study, *Aliment Pharmacol Ther* **14**, 145–53 (2000).

56 Ryder SD, Jacyna MR, Levi AJ, Rizzi PM, Rhodes JM, Eating peanuts increases rectal proliferation in individuals with mucosal expression of peanut lectin receptor, *Gastroenterology* **114**, 44–9 (1998).

57 Rhodes JM, Unifying hypothesis for inflammatory bowel disease and related colon cancer: Sticking the pieces together with sugar, *Lancet* **347**, 40–4 (1996).

58 Evans RC, Fear S, Ashby D, Hackett A, Williams E, van der Vliet M, Dunstan FDJ, Rhodes JM, Diet and colorectal cancer: An investigation of the lectin/galactose hypothesis, *Gastroenterology* **122**, 1784–92 (2002).

59 Yu LG, Jansson B, Fernig DG, Milton JD, Smith JA, Gerasimenko OV, Jones M, Rhodes JM, Stimulation of proliferation in human colon cancer cells by human monoclonal antibodies against the TF antigen (Galactose β 1-3 N-Acetyl-galactosamine), *Int J Cancer* **73**, 424–31 (1997).

60 Yu LG, Fernig DG, Smith JA, Milton JD, Rhodes JM, Reversible inhibition of proliferation of epithelial cell lines by *Agaricus bisporus* (edible mushroom) lectin, *Cancer Res* **53**, 4627–32 (1993).

61 Yu LG, Fernig DG, White MRH, Spiller DG, Appleton P, Evans RC, Grierson I, Smith JA, Davies H, Gerasimenko OV, Petersen O, Milton JD, Rhodes JM, Edible mushroom (*Agaricus bisporus*) lectin, which reversibly inhibits epithelial cell proliferation, blocks NLS-dependent nuclear protein import, *J Biol Chem* **274**, 4890–9 (1999).

62 Yu LG, Andrews N, Weldon M, Gerasimenko OV, Campbell BJ, Singh R, Grierson I, Petersen OH, Rhodes JM, An N-terminal truncated form of Orp150 is a cytoplasmic ligand for the anti-proliferative mushroom Agaricus bisporus lectin and is required for NLS-dependent nuclear protein import, *J Biol Chem* **277**, 24538–45 (2002).

63 Swidsinski A, Ladhoff A, Pernthaler A, Swidsinski S, Loening-Baucke V, Ortner M, Weber J, Hoffmann U, Schreiber S, Dietel M, Lochs H, Mucosal flora in inflammatory bowel disease, *Gastroenterology* **122**, 44–54 (2002).
64 Darfeuille-Michaud A, Neut C, Barnich N, Lederman E, Di Martino P, Desreumaux P, Gambiez L, Joly B, Cortot A, Colombel JF, Presence of adherent Escherichia coli strains in ileal mucosa of patients with Crohn's disease, *Gastroenterology* **115**, 1405–13 (1998).
65 Boudeau J, Glasser AL, Masseret E, Joly B, Darfeuille-Michaud A, Invasive ability of an Escherichia coli strain isolated from the ileal mucosa of a patient with Crohn's disease, *Infect Immun* **67**, 4499–509 (1999).
66 Swidsinski A, Khilkin M, Kerjaschki D, Schreiber S, Ortner M, Weber J, Lochs H, Association between intraepithelial Escherichia coli and colorectal cancer, *Gastroenterology* **115**, 281–6 (1998).

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