

Mucins of the gastrointestinal tract: their role in pathogenesis and as diagnostic markers

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Introduction

The literature on mucin histochemistry of the gastrointestinal tract is voluminous and complex, yet the subject has not realized its potential, either in terms of explaining disease mechanisms or informing clinical practice. This paper will attempt to highlight the underlying principles that will hopefully support the development of understanding and effective approaches diagnosis and management in the coming years.

Classic mucin histochemistry

Epithelial mucin is a dual molecule, part carbohydrate and part protein. The protein backbone is densely shrouded in oligosaccharide chains (Fig. 1) and has, until recently, been inaccessible to the investigative tools of mucin histochemistry. Classic mucin histochemistry has been employed for many decades to study the most peripheral (and therefore most reactive) sugars on the oligosaccharide chains. These include the neutral sugars fucose (Fuc), galactose (Gal) and N-acetyl galactosamine (GalNAc) and the acid sugar sialic acid. Additional acidification may occur through the addition of sulfate to these sugars.

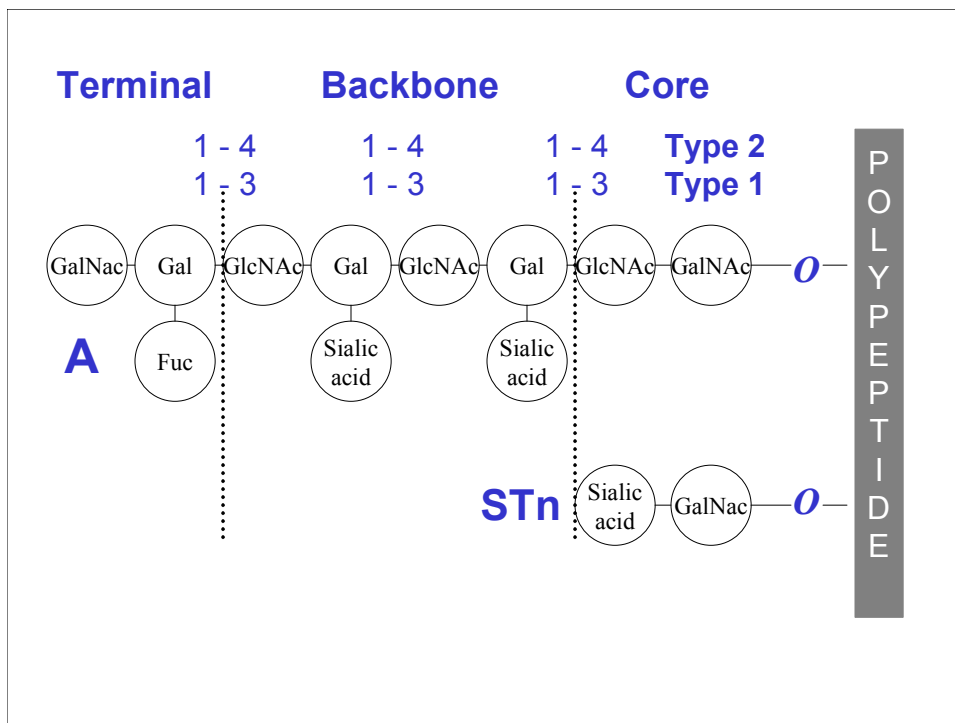


Fig.1: Example of a long oligosaccharide chain expressing terminal blood group substance A. The short disaccharide structure sialosyl Tn is shown below. These chains are linked covalently via GalNAc to serine or threonine in the apomucin (polypeptide).

Diastase periodic acid Schiff (dPAS) is the archetypal broad-spectrum mucin histochemical technique, reacting with both neutral and acidic mucin (though not O-acetyl sialic acid (see below)). Diastase PAS is useful for diagnosing poorly differentiated adenocarcinoma and signet ring cell carcinoma. The pattern of mucin staining is also useful for distinguishing cancer from non-malignant lesions including adenoma. Diastase PAS positive material in adenocarcinoma is generally distributed in lumens, along the glycocalyx, within intracytoplasmic lumina, but is not otherwise found intracellularly (signet-ring cell carcinoma is an exception). In non-malignant situations (including adenoma) mucin is largely located intracellularly. A distinction between acid (purple) and neutral mucin (magenta) can be made by combined staining with Alcian blue and dPAS (AB/dPAS). Acid mucins can be further distinguished into sulfomucin (brown) and sialomucin (blue) by the combined stain high iron diamine/AB (HID/AB). These techniques have proved useful in the diagnosis of intestinal metaplasia of gastric mucosa and Barrett's esophagus and the distinction between complete and incomplete forms of intestinal metaplasia.^{1, 2} The link between incomplete intestinal metaplasia and neoplasia is likely to be indirect. Incomplete intestinal metaplasia in both stomach and esophagus may be related to reflux, and particularly bile reflux, whereas complete intestinal metaplasia (which is mainly limited to the stomach) serves as a marker of infection by *H. pylori* in isolation.³⁻⁵ Therefore, the association between incomplete intestinal metaplasia and cancer may be explained by the shared pathogenic role of bile in both incomplete intestinal metaplasia and neoplasia.

Structure and synthesis of mucin

The biochemical structures in the terminal domains of oligosaccharide chains are blood group substances (BGS) (Fig. 2). Two major classes of BGS are represented in gastrointestinal mucin: types 1 and 2. These are determined by the type of chemical linkage between the carbon atoms of Gal and N-acetyl glucosamine (GlcNAc) (Fig.1). Type 1 BGS (1 –3 linkage) dominate in secretory (goblet or mucous cell) mucin and include Le^a, Le^b and Sialyl Le^a. Type 2 BGS (1 – 4 linkage), for example Le^x (CD15) and Le^y, are expressed along the apical membranes of crypt base columnar cells in the normal colorectum and are carried by the transmembrane apomucin MUC1.

The synthesis and expression of BGS are under genetic control. The stepwise addition of fucose and sialic acid to the precursor structure is regulated by highly specific fucosyl- and sialyltransferases (Fig. 2).⁶ The alterations in BGS expression that are observed in disease states are explained by the patterns of expression and mutual competition between glycosyltransferases. However, carbohydrate and apomucin (protein) biosynthesis and expression are co-regulated.⁷ Therefore carbohydrate expression is to a large extent determined by and dependent upon the expression of MUC genes (see below).

Lectin and immunohistochemistry can be used to study the expression of BGS. Lectins bind to specific sugars. For example, UEA-1 binds to fucose (though only in the configuration of Le^y of H substance type 2), DBA binds to GalNAc and PNA binds to

Gal-GalNAc and Gal-GlcNAc. Monoclonal antibodies bind to larger structures, for example the entire blood group substance, and are generally more specific than lectins.

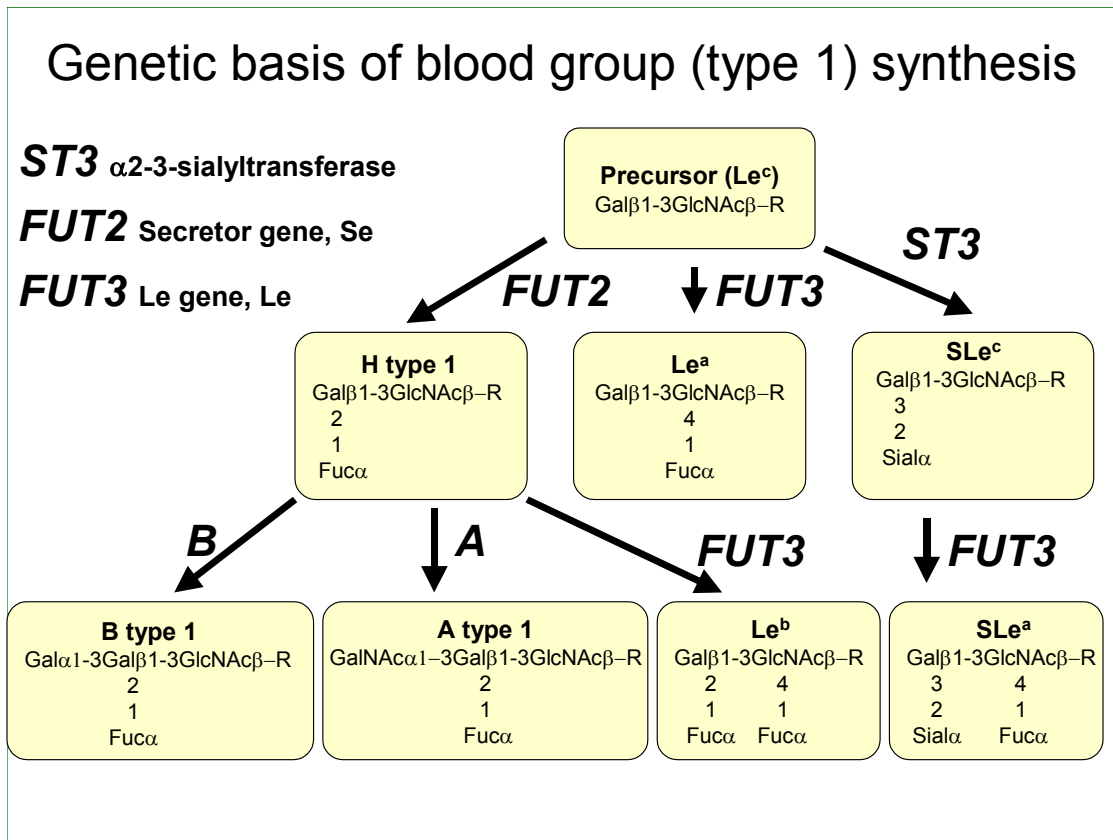


Fig. 2: Stepwise synthesis of type 1 blood group substances.

Sialic acid – a pivotal sugar

The structure of sialic acid is modified by the addition of sulfate and O-acetyl groups, these additions being particularly evident in colonic mucin. The variable structure of sialic acid has major effects on sensitivity to enzymatic digestion and reactivities with both PAS and monoclonal antibodies. The presence of O-acetyl groups (particularly at C4) renders sialic acid resistant to neuraminidase digestion, unreactive with PAS and unreactive with many moabs to structures containing sialic acid (e.g. STn and SLe^x).⁸ Absence of O-acetyl groups may be constitutional in subjects with a null O-acetyl transferase genotype (OAT-/OAT-). Goblet cell mucin will then express the 'tumor associated' structures STn and SLe^x. This occurs in about 9% of caucasians and a higher proportion of Asians.⁹ The same affect may be achieved in the laboratory by removing O-acetyl groups with KOH (saponification). The switch from O to N-acetyl sialic acid is acquired in multiple colorectal lesions: hyperplastic polyps, serrated adenomas, villous adenomas, serrated hyperplasia in ulcerative colitis and cancers producing secretory mucin.¹⁰⁻¹² The change is seen in adenomas, but only as a focal or subclonal phenomenon and is associated with high-grade dysplasia.¹³ The biochemical switch is

well demonstrated with the mild PAS technique, which is specific for N-acetyl sialic acid. Because N-acetyl sialic acid is sensitive to neuraminidase, the preceding lesions also show 'tumor associated' lectin binding patterns, for example neuraminidase PNA or neuraminidase DBA positivity.

Therefore, a single, simple change to the structure of sialic acid underlies numerous seemingly unrelated observations described in the literature. The data also show that many 'tumor-associated' changes are not tumor-associated at all. The only change is to the structure of sialic acid. The question is: why does this change occur and how does it fit with the accepted views on the histogenesis of colorectal neoplasia. The most plausible explanation is that there are two broad groups of colorectal neoplasm. In one there is metaplasia or transdifferentiation to a mucinous phenotype in which both small intestinal and gastric phenotypes are found. In this model, the sialic acid change is a component of the switch to a small intestinal phenotype. In the second, neoplasia is accompanied by loss of secretory mucins and there is retention of the normal crypt base, columnar cell phenotype.

MUC genes and lineage-based classifications

In precancerous and cancerous lesions of the gastrointestinal tract, patterns of differentiation may be characterized by the domination of a particular lineage, for example mucous/goblet cell versus absorptive/columnar cell. It is now possible to employ monoclonal antibodies to apomucins encoded by the MUC gene family and thereby achieve lineage-based classifications for gastrointestinal neoplasms (Table 1). For example, mucinous carcinomas may now be defined as cancers in which the majority of cells express such apomucins as MUC2 and/or MU5AC (Table 1). Non-mucinous neoplasms express transmembrane mucins such as MUC1.¹⁴ In the normal colon MUC2 shows co-localization with type 1 BGS and with STn. By contrast, MUC1 co-localises with type 2 BGS (e.g. Le^x and Le^y).^{14, 15} The same expression patterns are recapitulated in mucinous and non-mucinous cancers respectively. Many of the complex alterations in mucin structure described in the literature can therefore be reduced to two basic patterns determined by cell lineage.

The preceding approach to the classification of neoplasia is not only simple and scientifically correct, but has clinical significance. In colorectal cancer, the MUC1/MUC2 phenotypes occur mainly in the combinations: MUC2+/MUC1-, MUC2+/MUC1+ and MUC2-/MUC1+. The frequency of lymph node metastasis in these groups (a few MUC2-/MUC1- are included in the third group) was 10%, 45% and 60% respectively.¹⁵ Whilst aggression has been related to MUC1 expression in colorectal cancer, it may be that MUC1 is merely serving as a marker of a pathways implicating columnar cell differentiation that are fundamentally different from pathways implicating mucous cell differentiation (cells that show little or no expression of MUC1). It so happens that MUC2 up-regulation in colorectal lesions is accompanied by upregulation of gastric mucin MUC5AC. The MU2+/MUC5AC+ phenotype occurs in hyperplastic polyps, serrated adenoma and villous adenomas.¹⁶ The same phenotype dominates in sporadic MSI-H cancers.¹⁷ It is now becoming clear that sporadic MSI-H cancers arise

in serrated polyps. Whilst most MUC2-/MUC1+ colorectal cancers would be expected to arise in conventional adenomas, the same phenotype has been associated with cancers arising in dome epithelium.¹⁸ These observations greatly reinforce the scientific rationale for lineage-based classifications.

Mucin	Type	Location	Locus
MUC1	TM	G, I	1q21-24
MUC2	Sec	I	11p15.5
MUC4	TM	I	3q29
MUC5AC	Sec	G (f), I (tr)	11p15.5
MUC5AB	Sec	E, I (tr)	11p15.5
MUC6	Sec	G (mnc/gl)	11p15.5

TM *transmembrane*, Sec *secretory*, G *gastric*, I *intestine*, E *esophagus*, f *foveolar*, t *trace*, mnc *mucous neck cells*, gl *glands*

Table 1: MUC genes expressed in the gastrointestinal tract

In gastric cancer, MUC (and CD10) expression has been used to group cancers into: (1) gastric, (2) complete IM and (3) incomplete IM phenotypes.^{19, 20} These studies show that most gastric cancers show an incomplete IM or a gastric phenotype, regardless of whether or not the cancers are classified as ‘intestinal’ or ‘diffuse’. Such studies highlight the limitations of classical mucin histochemistry and the Laurén classification and invite re-appraisal of the role of incomplete intestinal metaplasia as a precancerous lesion.

The mixed gastrointestinal mucinous phenotype (MGMP)

Multiple gastrointestinal lesions express a mixture of gastric (MUC5AC, MUC6) and intestinal (MUC2) mucins. These include hyperplastic polyps and serrated adenomas of the colorectum, serrated hyperplasia in ulcerative colitis, incomplete intestinal metaplasia of gastric mucosa and Barrett’s esophagus.^{16, 21-23} The occurrence of the MGMP in the preceding lesions could be mere coincidence. Alternatively, these seemingly disparate lesions may be related through by a common underlying mechanism. For example, alteration of a master gene switch controlling differentiation pathways could occur in the above lesions. Epigenetic changes such as DNA methylation have been described in many of the preceding lesions whereas mutations are infrequent. Further

research into this area should yield novel and important molecular insights into the earliest changes in gastrointestinal neoplasia.

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