DMBT1 expression and glycosylation during the adenoma–carcinoma sequence in colorectal cancer

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Abstract
The gene DMBT1 (deleted in malignant brain tumour-1) has been proposed to play a role in brain and epithelial cancer, but shows unusual features for a classical tumour-suppressor gene. On the one hand, DMBT1 has been linked to mucosal protection, whereas, on the other, it potentially plays a role in epithelial differentiation. Thus its function in a particular tissue is of mechanistic importance for its role in cancer. Because the former function requires secretion to the lumen and the latter function may depend on its presence in the extracellular matrix, we decided to investigate DMBT1 expression, location and its mode of secretion during malignant transformation in colorectal cancer. Using human colorectal PC/AA cell lines and tissue sections from individual patients, we have examined the expression of DMBT1 and its glycosylation in the adenoma–carcinoma sequence leading to the adenocarcinoma phenotype.

Introduction
Changes in the glycosylation of a variety of glycoproteins, including mucins and tumour-suppressor-gene products, feature among the many studies designed to investigate the mechanisms of malignant transformation in CRC (colorectal cancer) at the molecular level [1–3].

The nature and mechanism leading to these defects is poorly understood. Secreted and membrane-bound glycoproteins are produced continuously to create a mucosal surface micro-environment, which functions as part of the innate protective programme. Tumour cells modulate this programme during proliferation and the development of invasive phenotypes, and also in order to attain the ability to metastasize. This implies that each stage of malignant transformation will be associated with a different glycosylation profile and that the identification of antigenic structures among the different oligosaccharides present in the glycoproteins may be used for tumour staging.

Among the glycoproteins that are synthesized and secreted by intestinal epithelial cells are the mucins and a variety of protective proteins, including tumour suppressor proteins. DMBT1 (deleted in malignant brain tumour-1) is a potential tumour-suppressor gene with tissue-specific cellular and subcellular expression and which has been correlated with its action in tumour development in brain and epithelial tumours [4]. Little is known regarding the glycosylation of this molecule and whether it correlates with its cellular location and protective function.

The availability of cultured human colorectal cell lines established as a model for the adenoma–carcinoma sequence [5] provides the means to examine the role of selected glycoproteins that make a significant contribution to the structure of the mucosal protective barrier and its battery of protective proteins. These cell lines have been used to examine the properties of DMBT1 in CRC.

Glycosylation and mucins in CRC
The surface epithelium of the intestinal tract is coated with a protective layer of mucus. This mucus constitutes a physical protective barrier between the tissue and the aggressive external environment, including the enteric microflora. Mucins are the major components in mucus and confer its rheological properties such as elasticity, adherence and viscosity. They are high-molecular-mass molecules and possess a large variety of glycans.

A number of studies have shown altered glycosylation of mucins in CRC [2,3,6]. These defects have been linked with cellular growth, differentiation, transformation, adhesion and invasive potential.

Expression of MUC2 and MUC3, the genes encoding the major mucins found in the normal intestinal tract, is strongly diminished in colorectal adenocarcinomas, with concomitant induction of MUC5AC. In order to determine the importance of mucins in the progression to CRC, knockout mice for Muc2 were generated. This work showed that Muc2 can regulate the formation of malignant tumours in the small intestine and colon of these mice, and therefore functions as a tumour suppressor [7].

Modifications of glycosylation are characteristic of CRC cells, and a variety of cell-surface glycan antigens have been identified. Colon cancer has been characterized by an increase

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in the expression of T [Galβ1 → 3-GalNAc-(N-acetylgalactosamine)], α (GalNAcα1 → Ser/Thr), sialyl-Tn, sialyl-Lea (Lewis x) and sialyl-Lea (Lewis a) antigens. It has been shown that cells expressing these epitopes are resistant to natural killer and cytotoxic T-cells and also metastasize more readily. Expression of sialyl-Tn and sialyl-Lea antigens are associated with a poorer survival rate of patients with colon cancer, suggesting an important role for these glycan structures in the biology of CRC [2,3,6]. Based on these results, a variety of screening markers for malignant transformation in CRC and other cancers have been developed, including SSEA (stage-specific embryonic antigen) and CA19.9. However, not one of these alone has been sufficiently sensitive for the detection of early cancer. They have been used to confirm diagnosis and detect recurrence.

Recently, we have been able to identify a glycan repertoire for each stage of the human gastrointestinal tract using NMR and MS methods [8,9].

DMBT1: molecular properties and relationship to CRC

DMBT1 is a secreted glycoprotein, which is believed to mediate mucosal homeostasis and it has been proposed as a candidate tumour-suppressor gene and implicated in the development of epithelial cancers through its down-regulation [4]. The significance of the gene and its product was initially recognized with regard to its loss in brain tumours. A role for the protein in the development of malignant disease was suggested by its chromosomal location (10q25.3-26.1), which frequently shows losses of heterozygosity in a range of tumours. The high homology of its SRCR (scavenger receptor cysteine-rich) domains and SIDs (SRCR interspersed domains), and their occurrence as multiple tandem repeats, also represents a genomic structure that may be susceptible to instability. Accordingly, it is believed that DMBT1 plays a role in epithelial cancer development, including CRC [10].

Cloning of the protein demonstrated a number of forms. The N-terminus contains up to 13 SRCR domains separated by short SIDs rich in serine and threonine. To the C-terminal end of the SRCR domains and SIDs, one zona pellucida and two CUB (complement protein subcomponents C1r/C1s, urchin embryonic growth factor and bone morphogenetic protein 1) domains are located, and a putative transmembrane domain has also been identified [4].

DMBT1 and glycosylation

DMBT1 is a glycoprotein, with major glycosylation accounting for up to 30% of its dry mass. It contains 14 N-linked oligosaccharide amino acid triplet sites, and the SIDs have many serine and threonine locations for O-glycosylation. Very little information concerning the nature of DMBT1 glycosylation is available. A study of the O-glycosylation of DMBT1 forms found in human tears has been reported [11], but no information is available for the gastrointestinal forms of DMBT1, and the potential roles for glycosylation have not been reported or examined with regard to tissue origin, normal function or pathological significance. Our present work is designed to address the question of DMBT1 glycosylation against the background of known defects, that have been identified in well-characterized, cultured human CRC cell lines and tissues from patients with tumours at different stages of the adenoma–carcinoma sequence.

Protective functions at the mucosal surface and in colorectal neoplasia: a role for DMBT1

We have examined the role of secreted and membrane-associated mucins of the MUC gene family in gastrointestinal disease and, in particular, in the process of malignant transformation [6]. Neoplastic transformation in the colorectum has been characterized in terms of the adenoma–carcinoma sequence [12], and a cultured cell model (PC/AA) has been described and shown to represent stages in the progression to cancer through the adenoma–carcinoma sequence [5]. These include pre-malignant and malignant stages originating from a single colonic tubular adenoma with mild dysplasia and show altered O-glycosylation through the progression to adenocarcinoma and mucinous carcinoma phenotypes [13,14]. We have used this model to examine the significance of MUC gene expression and the glycosylation patterns that are associated with progression to adenocarcinoma and mucinous carcinoma phenotypes [13,14]. Independent support for a role of MUC genes in the development of intestinal cancers has recently come from study of a Muc2-knockout mouse [7]. This work has shown that loss of the major secretory mucin gene (Muc2) leads to the appearance of adenocarcinomas in the intestine. Our own work has shown that this gene is down-regulated in the development of the adenocarcinoma phenotype [13,14]. A role for MUC2 glycosylation in neoplastic transformation is implicated by these studies, but has not yet been examined in any detail.

DMBT1 has mucin-like properties and may share some biological functions with these molecules. The interrelationship of DMBT1 to the mucosal barrier is not clear. A normal functional role for the molecule has not yet been demonstrated, and the significance of the abundant glycosylation remains to be characterized and examined for functional importance.

Preliminary evidence for glycosylated DMBT1 in colorectal cells during malignant transformation

Cells from the PC/AA cultured cell model of the adenoma–carcinoma sequence were grown as described previously [13,14]. The cell layer from three cell lines PC/AA (non-tumorigenic), AA/C1 (non-tumorigenic) and SB10C (tumorigenic in nude mice) were extracted with 6 M GdmCl (guanidinium chloride) and subjected to isopycnic density gradient centrifugation in 4 M GdmCl/CsCl. Reactivity with the anti-DMBT1h12 serum [10] was seen over a broad density range (1.32–1.42 g/ml) (Figure 1), which overlapped with the gel-forming mucins (1.35–1.55 g/ml). These results indicate
that DMBT1 is expressed throughout the progression to malignancy in this model of CRC transformation. In addition, DMBT1 remains O-glycosylated, in contrast with the mucins ([13]; and C. Robbe and A. Corfield, unpublished work), implicating a selective pattern of molecular glycosylation in these cell lines.

Further support for the pattern of DMBT1 expression was obtained from a series of patients with adenomas of increasing malignant potential (tubular, tubulovillous and villous adenomas), where increased DMBT1 immunoreactivity was detected relative to normal controls. We have demonstrated previously the loss of MUC2 and appearance of MUC5AC in such adenomas [15]. These results are contrary to reports of down-regulated DMBT1 mRNA levels in CRC (reviewed in [4]).

Conclusions and perspective
The role of DMBT1 in normal colorectal mucosa and the nature of cancer-related changes remain to be clarified. The initial claims of down-regulation in CRC are not supported in the initial studies presented in this review using the PC/AA cultured cell culture model of the adenoma–carcinoma sequence and in individual adenoma patients. However, it should be emphasized that these are the initial studies in the colorectal system, and that the use of the cultured cells appears to be a valuable asset in future work. Closer analysis of the molecular properties of DMBT1 and, in particular, its glycosylation patterns during the adenoma–carcinoma sequence in the colorectum, is currently in progress.

References

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