The human intestinal cell lines Caco-2 and LS174T as models to study cell-type specific mucin expression

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Mucin expression was studied during proliferation and differentiation of the enterocyte-like Caco-2 and goblet cell-like LS174T cell lines. Caco-2 cells express mRNAs of MUC1, MUC3, MUC4 and MUC5A/C whereas MUC2 and MUC6 mRNAs are virtually absent. Furthermore, MUC3 mRNA is expressed in a differentiation dependent manner, as is the case for enterocytes. Concomitantly MUC3 protein precursor (~550 kDa) was detected in Caco-2 cells. In LS174T cells mucin mRNAs of MUC1, MUC2 and MUC6 are constitutively expressed at high levels, whereas MUC3, MUC4 and MUC5A/C mRNAs are present at low levels. At the protein level LS174T cells express the goblet cell specific mucin protein precursors MUC2, MUC5A/C and MUC6 with apparent molecular masses of about 600 kDa, 470/500 kDa and 400 kDa respectively. MUC3 protein is not detectable. Furthermore, human gallbladder mucin protein (~470 kDa precursor), of which the gene has not yet been identified, is expressed in LS174T cells. In addition, synthesis and secretion of the goblet cell specific mature MUC2, MUC5A/C and human gallbladder mucin was demonstrated in LS174T cells. It is concluded that Caco-2 and LS174T cell lines provide excellent in vitro models to elucidate the cell-type specific mechanisms responsible for mucin expression.

Keywords: mucin-type glycoproteins, MUC1–6 mRNA biosynthesis, protein biosynthesis, Caco-2, LS174T, human intestine

Abbreviations: SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis; DMEM, Dulbecco's modified Eagle's medium; EMEM, Eagle's minimum essential medium; Endo-H, endo-β-N-acetylgalactosaminidase H, HGBM, human gallbladder mucin; dpc, days past confluence; PBS, phosphate buffered saline.

Introduction

The gastrointestinal epithelium and other mucosa are covered by mucus. The mucus glycoproteins (mucins) are the most important structural components of mucus [1]. Mucins play an important role in cytoprotection. Alterations in intestinal mucins, especially in glycosylation patterns and expression levels, are thought to be associated with diseases such as inflammatory bowel disease and carcinoma [2–4]. Mucins are large glycoproteins and are either secretory or membrane-bound (reviewed in [5, 6]). The central part of the polypeptide backbone of epithelial mucins consists of many tandemly repeated amino acid sequences. These tandem repeats are rich in serine and threonine residues and may vary in number between individuals, but are invariably highly O-glycosylated. The C- and N-termini protrude from the heavily O-glycosylated central part, do not contain tandem repeats, and, for secretory mucins, contain many cysteine residues forming inter- and intramolecular disulfide bonds. Usually the termini have some N-glycosylation [6].

Due to diverse O-glycosylation, each mucin gene product eventually forms a heterogeneous set of molecules. Several different human epithelial mucin genes have been identified, named MUC1–8 [5]. MUC1, of which the gene has been fully sequenced, codes for a membrane-bound mucin [7–9]; MUC2 and MUC7 cDNAs have been completely sequenced and code for secretory mucins [10–12]. Of MUC3, 4, 5A/C, 5B, 6 and 8 only partial cDNA sequences are known [13–19]. Whether the latter cDNAs encode secretory mucins has

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to await their full sequence determination and cell biological measurement of the secretory status of each of these mucins.

Mucin expression is tissue- as well as cell-type specific and varies according to differentiation. For instance in the small intestine, MUC2 and MUC3 mRNA are prominently expressed: MUC2 is confined to goblet cells while MUC3 is largely confined to enterocytes, although some MUC3 expression is detectable in goblet cells [20]. In the human large intestine MUC2 is the prominent mucin [21] and is goblet cell specific, whereas in the stomach, which also produces a copious mucin layer, it is hardly detectable [2, 22]. In addition to MUC2 and MUC3, several other mucins, namely MUC1, MUC4, MUC5A/C, MUC5B and MUC6, have been reported to be expressed to some extent in the intestine [5]. Of the other known mucins MUC8 is not expressed in the small intestine as detected by Northern blot analysis and MUC7 expression seems restricted to the salivary glands [12, 17]. Besides the mucins of which the genes have been identified (MUC1–8) intestinal expression of at least one other mucin has been reported [23]. This mucin, human gallbladder mucin (HGBM), was originally identified in the human gallbladder, where it is prominently expressed and secreted [24]. In summary, MUC1–6 and HGBM are expressed in the intestine of which MUC2 and MUC3 predominate in the small intestine and MUC2 predominates in the large intestine.

In the intestine, besides the tissue- and cell-type specific expression, the differentiation status of small intestinal epithelial cells correlates closely with their position along the crypt-villus axis. In the small intestine MUC2 mRNA expression is detected along the whole crypt-villus axis; whereas MUC3 mRNA expression shows a gradient along the crypt-villus axis, with high expression on the villus tips [20]. In contrast, MUC1 expression is turned off early in differentiation when crypt cells differentiate and migrate up the villi [25]. Therefore, the expression of each mucin in the intestine seems to be regulated specifically during differentiation, but how this expression is regulated is still unknown.

Cell lines constitute good in vitro models to investigate the tissue- and cell-type specific mucin expression during proliferation and differentiation. In addition, these relatively homogeneous populations of cells allows one to investigate the regulation of cell-type specific mucin gene expression. To be able to understand cell-type specific mucin expression in the intestine we have characterized the mucin expression of two different human colon adenocarcinoma cell lines, LS174T and Caco-2. LS174T cells contain mucous granules, produce significant amounts of secretory mucin and have similarity to goblet cells [26, 27], while Caco-2 is a well differentiated enterocyte-like cell line developing a brush border [28–30]. After confluency Caco-2 differentiates into enterocyte-like cells, expressing i.e. the small intestinal glycohydrolases lactase and sucrase-isomaltase in an in vitro differentiation dependent fashion [30, 31]. The aim of this study was to characterize these two cell line models, for enterocytes and goblet cells, with respect to mucin biosynthesis during in vitro proliferation and differentiation. Mucin expression in LS174T and Caco-2 cells was therefore investigated at mRNA and protein level at various stages of cyto-differentiation.

Materials and methods

Chemicals were obtained from the following manufacturers: Amersham International, Amersham, Bucks., UK; Gibco/Life Technologies, Breda, The Netherlands; New England Biolabs, Beverly, MA, USA; Merck, Darmstadt, Germany; Sigma Chemical Co., St Louis, MO, USA; BioRad, Richmond, CA, USA; Pharmacia, Uppsala, Sweden; BDH, Poole, Great Britain and Boehringer Mannheim, Mannheim, Germany; ICN, Costa Mesa, CA, USA.

Cell culture

The LS174T cell line was obtained from American Type Culture Collection. The Caco-2 cell line was donated by Dr W.J. Van’t Hoff, University of Utrecht. LS174T and Caco-2 were cultured in 4 cm² tissue culture wells and 25 cm² tissue culture flasks respectively (Costar, MA, USA) in Dulbecco’s Modified Eagle’s Medium (DMEM, Gibco) with 4.5 g l⁻¹ glucose, 3.7 g l⁻¹ NaHCO₃, supplemented with 0.1 mM non-essential amino acids (Gibco), 50 U ml⁻¹ penicillin (Sigma), 50 μg ml⁻¹ streptomycin (Sigma). In addition the medium was supplemented with 20% and 10% fetal calf serum (Gibco) for LS174T and Caco-2 cells respectively. Cells were cultured in 5% CO₂ at 95% relative humidity and 37 °C. Medium was replaced daily for LS174T and once every 2–3 days for Caco-2. Cells were routinely treated with trypsin at near-confluent densities and split 1:2 (LS174T) or 1:4 (Caco-2). For experiments, LS174T and Caco-2 cultures were used between passages 112–122 and 95–102 respectively. Medium of post-confluent LS174T cells was centrifuged at 800 rpm to collect all living cells growing in suspension; these were reintroduced into fresh medium. Furthermore, for RNA experiments cells from one single passage of LS174T or Caco-2 were used and cells were seeded at identical densities in a set of wells or flasks, respectively, in order to isolate RNA at various days past confluency (dpc).

RNA blotting and quantification

MUC1 (450 bp), MUC2 (900 bp) and MUC3 (400 bp) cDNA sequences, cloned in the Eco RI site of pBluescript (Stratagene), have been described previously [7–10, 13]. All cDNA probes, except MUC5A/C contain tandemly repeated sequences. A MUC4 (48 bp) sequence was