The role of mucins in gastrointestinal barrier function during health and disease

Mucins and gastrointestinal barrier dysfunction: partners in crime in inflammation and cancer

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Summary

Mucins are the gatekeepers of the mucus barrier and are aberrantly expressed in various gastrointestinal pathologies, including pathogen infection, inflammation and uncontrolled growth and spread of abnormal cells. Although several studies have emphasized their role in gastrointestinal mucosal barrier dysfunction, they are often still considered as passive mediators of the mucosal barrier instead of regulators/modulators. In this review, we will cover the crosstalk between mucins and gastrointestinal barrier function during health and disease by focusing on the one hand on the bidirectional interaction between mucins and the gut microbiota and on the other hand on the molecular mechanisms involved in key cell signalling pathways such as inflammation, cell-cell interactions, cell differentiation, proliferation and survival. Additionally, we also highlight the potential use of mucins in the diagnosis, follow-up and treatment of gastrointestinal diseases such as chronic inflammatory diseases and cancer.

Search strategy and selection criteria

An extensive literature search was performed on PubMed with multiple combinations of the following search terms present in the title and/or abstract: “MUC*” AND “stomach”, “colon”, “intestinal”, “gastrointestinal”, “gastrointestinal tract”, “IBD”, “Crohn”, “ulcerative colitis”, “colorectal”, “dysplasia”, “cancer”, “adenocarcinoma”, “inflammation”, “barrier integrity”, “barrier dysfunction”, “microbiome”, “microbiota”, “cell-cell adhesion”, “cell interaction”, “cell junctions”, “cell differentiation”, “cell polarity”, “cell death”, “cell survival”, “cell proliferation”. Results were limited to research/review articles published from the year 1996. The following inclusion criteria were used: (1) studies in English and (2) only original research papers or meta-analyses. The final references were selected based on originality and relevance to the scope of this review. To limit the final number of references included in the manuscript, we referenced previously published review papers that had already summarized the findings of several included research papers whenever possible.

1. Introduction

The general architecture of the gastrointestinal (GI) tract features a thick mucus barrier, an epithelium and the inner lamina propria hosting innate and adaptive immune cells (Appendix 1). The mucus barrier separates the external luminal content from underlying host tissues and plays a pivotal role in the communication between the complex and dynamic gut microbiota and the mucosal immune system (Appendix 1).[1]

Mucins (MUC) are the gatekeepers of the mucus barrier and are characterized by domains rich in proline, threonine and serine that are heavily glycosylated (i.e. PTS domains).[2] They are functionally and structurally heterogeneous and are transcribed from large genes containing multiple exons which encode for various functionally unique domains, i.e. sperm protein enterokinase agrin (SEA), epidermal growth factor-like (EGF), Nidogen-like (NIDO), the von-Willebrand factor-like (vWF)C/D, immunoglobulin (Ig)-like, adhesion-associated domain in MUC4 and other proteins (AMOP), glycosyl hydrolases family 18 (GH18), and the cytoplasmic tail (CT) (Figure 1).[2] Mucins are expressed either as secretory or transmembrane glycoproteins. The secreted gel-forming mucins (MUC2, MUC5AC, MUC5B, MUC6; Figure 1) form the major constituents of the mucus layer and are produced by specialized mucus cells or goblet cells that are found throughout the GI epithelium. This review will focus on the gastric and colonic mucosa (Appendix 2) as the secreted mucus from both the stomach and the colon consists of two layers, an inner adherent layer that is sterile and difficult to dislodge and an outer loose layer that is home to microbes and able to interact with secreted mucins (Appendix 2).[1] Underneath the mucus layer, GI epithelial cells, particularly those that do not produce mucus, present a dense forest of highly diverse transmembrane mucins (MUC1, MUC4, MUC12, MUC13, MUC15, MUC17, MUC20, MUC21; Figure 1), which form the glycocalyx (Appendix 1).[3] Besides preventing infection by microorganisms that live in the gut, transmembrane mucins possess several EGF domains on their extracellular (C-terminal) tail and numerous phosphorylation sites on their intracellular (N-terminal) domain enabling them to participate in the intracellular signal

transduction and to play an important role in the homeostasis of mucosal epithelial cells (Figure 1 & Appendix 1).[2]

The GI epithelium is well organized to allow appropriate functioning and maintenance of the barrier. In particular, individual epithelial cells are mechanically tied to one another by intercellular junctions (i.e. tight junctions, adherens junctions, and desmosomes), which are essential to maintain cell–cell adhesion and for the regulation of paracellular permeability (Appendix 1).[4] Apart from linking neighbouring cells, tight junctions are also involved in regulating apical-basal cell polarity, which is established by the mutual interaction of three polarity complexes: i.e. the defective partitioning [PAR; PAR3 – PAR6 – aPKC], Crumbs [CRB3 – PALS1 – PATJ], and Scribble [SCRIB – DLG – LGL] complexes (Appendix 1).[4] The GI epithelium represents a defensive barrier against environmental and microbial attacks, carrying out several critical functions including antigen presentation, antimicrobial peptide production, mucin expression and maintaining a tight physical barrier. The latter is achieved via the regulation of well-coordinated processes, including proliferation, migration and differentiation of epithelial/stem cells as well as epithelial cell survival/controlled cell death.[2]

2. Mucins as mediators of gastrointestinal barrier dysfunction upon inflammation

Mucins are often considered passive mediators of the mucosal barrier, understating their importance in regulating epithelial barrier function. Aberrant mucin expression has been described in various GI pathologies that involve a dysfunctional gut barrier, such as IBD and colorectal cancer (CRC).[2] The secreted mucus layer in the colon, which mainly consists of MUC2 and to a lesser extent of MUC5B (Appendix 2), is essential in suppressing an inflammatory response by preventing the penetration of bacteria to the underlying epithelium. This was clearly demonstrated in *Muc2*-deficient mice who develop colitis, as characterized by increased colonic infiltration of lymphocytes and elevated levels of pro-inflammatory cytokines, and adenocarcinoma spontaneously or in the presence of *Salmonella* infection.[3,5] Expression of MUC5AC, which is rarely secreted in the healthy colon, has been consistently observed to be increased in the inflamed mucosa of IBD patients.[6] Similarly, depletion of

Muc5AC in mice also resulted in an aggravation of colitis.[6] In addition, it is important to note that impaired mucin glycosylation or defects in goblet cell functioning can also aggravate colitis severity as well as promoting the development of colitis-associated cancer.[7]

Given the involvement of transmembrane mucins in epithelial cell signalling pathways, they are also excellent candidates to mediate barrier function upon health and disease. Indeed, loss of MUC1 and MUC13 expression has been shown to aggravate acute murine colitis, characterized by an increase in colonic barrier permeability and reduced mucosal healing. During chronic colitis, however, their absence decreased the risk of tumour formation.[8,9] Reduction of MUC17 expression also resulted in a profound loss of colonic epithelial barrier function, in terms of changes in transepithelial electrical resistance (TEER) and permeability to 10 kDa fluorescein isothiocyanate (FITC)-dextran.[10] On the contrary, depletion of MUC4 improved disease resistance.[2] Furthermore, growing evidence now shows that inappropriate overexpression of transmembrane mucins can affect barrier integrity by modulating junctional protein function and subsequent signalling pathways affecting cell invasiveness, migration, proliferation, and survival and may thus be responsible for the progression towards diseases, including chronic inflammatory diseases and cancer.[2]

3. Mucins and the microbiota – a Bidirectional communication

Mucins are uniquely positioned to interact with the extracellular environment and an imbalance in mucin-microbiota interactions will dictate the onset and course of disease.[11,12] In healthy conditions, gastrointestinal bacteria are not able to penetrate the mucus layer and reach the transmembrane mucins. Nevertheless, impairment of mucin expression, resulting in a reduction of mucus secretion and thickness, an increase in mucus degradation and penetrability and an altered mucin glycosylation profile, allows commensal and pathogenic bacteria to reach the surface epithelium thereby inducing infection and inflammation as described in many GI diseases.[3] Indeed, CRC, IBD and pathogen susceptibility are linked to excessive degradation of secreted mucins by the microbiota and aberrant transmembrane mucin overexpression.[1,3] In the stomach the interplay between mucins and

150 *Helicobacter pylori*, a major driver of gastric inflammation and cancer, is known to be important for
151 pathogenesis.[13]

152 Furthermore, antimicrobial treatment in mice causes a shift in microbial composition leading to an
153 alteration of goblet cell function and subsequently a decreased MUC2 production and thinning of the
154 inner mucus layer.[3,14–17] Similarly, when comparing conventionally housed mice with germ free (GF)
155 mice, fewer and smaller goblet cells together with an impaired mucus layer due to decreased *Muc2*
156 levels were found in the GI tract of GF mice.[18] All this confirms that the presence of a microbial
157 homeostasis is important for allowing a fully functional mucus layer to develop and mature. In
158 summary, mucins and the microbiota mutually influence each other, an extended overview of known
159 bacterial-mucin interactions is shown in Table 1, highlighting an intimate bidirectional crosstalk at the
160 level of the GI tract.[18]

161 Specific bacterial species can bind to mucins through various structures, including mucus binding
162 proteins, outer membrane proteins, adhesins, lectins and appendages such as pili, flagella and
163 fimbriae (Table 1).[1,22,50] In addition to supplying attachment sites, mucins also serve as important
164 carbon sources for the mucus-associated microorganisms, the so-called mucobiota, favouring their
165 replication (Table 1).[11,51] *Akkermansia muciniphila, Bacteroides thetaiotaomicron* and
166 *Bifidobacterium bifidum* are three well characterised species who are known to be beneficial for
167 mucosal homeostasis and digest host glycans (Table 1). *B. bifidum* is able to strengthen tight
168 junctions and prevent the disruption of the epithelial barrier, whereas *A. muciniphila* and *B.*
169 *thetaiotaomicron* play an important role in maintaining gut homeostasis by supporting butyrate-
170 producing bacteria through degradation of mucins to acetate and by stimulating goblet cell
171 proliferation and mucin gene expression in the GI tract, respectively.[3] Additionally, pathogens such as
172 enterohemorrhagic *E. coli*, *Citrobacter rodentium, Salmonella typhimurium, Clostridium difficile* and
173 *rotavirus* have the ability to hijack the beneficial effects of *A. muciniphila* and *B. thetaiotaomicron* to
174 enhance their pathogenicity.[52–55] Other enteric pathogens such as *Vibrio Cholerae* and *Salmonella*

typhimurium are able to directly utilise mucin glycans as a carbon source (Table 1).[56,57] Specific enzymes required for mucin degradation are the sulfatases, glycoside hydrolyses, and proteases (Table 1). The sulfatases desulfate mucin terminal glycans which are subsequently degraded by glycoside hydrolases and released as mono- and oligosaccharides which can then be used as energy source by the microbiota.[58] When all glycans are digested, the mucin protein core becomes available for degradation by proteases.[3] Degradation of host glycans provides the required nutrients for the establishment of the mucobiome but can also be an important mechanism in mucus thinning and decreasing viscosity thus playing a role in GI infections. For example, *A. muciniphila* has been associated with mucus thinning under dysbiotic conditions and dietary fiber deprivation.[3,59] Besides using glycans as an energy source, bacteria with mucolytic properties also generate short-chain fatty acids (SCFAs) through fermentation. These SCFAs can serve as food source for non-mucolytic bacteria through cross-feeding or can be absorbed by enterocytes to recover part of the energy spent through synthesis and secretion of mucins. Additionally, SCFAs can increase colonic MUC2 expression further confirming the mutualistic relationship between the host mucins and its resident microbiota.[60–62]

Both commensal and pathogenic bacteria are able to degrade mucins or use them as attachment sites, promoting their replication and colonisation (Table 1). The host distinguishes commensal and pathogenic bacteria via the presence of pattern recognition receptors (PRRs).[63] These PRRs recognise conserved bacterial molecular structures, known as pathogen- or damage-associated molecular patterns (PAMPs and DAMPs), resulting in the activation of specific pathways that lead to inflammation.[63] Interestingly, some mucins can modulate the properties of host antimicrobial peptides to maintain homeostasis and avoid pathogen invasion and infection. For example, MUC2 has been shown to induce the expression of β-defensin 2, a broad spectrum anti-microbial peptide, in HT-29 CRC cells.[64] Commensal *E. coli* which are susceptible to β-defensin 2, are then protected by MUC2, highlighting a dual role of this mucin to induce defence mechanisms against pathogens while also shielding commensal microbes.[64] Furthermore, MUC2 also imprints dendritic cells (DCs) with anti-inflammatory properties contributing to the tolerogenic tone of the gut environment.[65] Another

defence mechanism of MUC2 is the inhibition of pathogenic infection in the gut by reducing colonisation through mucus clearance.[66] Similarly, MUC16 and MUC17 have also the ability to prevent colonisation of the GI tract by enteroinvasive *E. coli* and *Staphylococcus aureus,* respectively.[10,67] Nevertheless, there are some situations in which pathogenic bacteria can penetrate the mucus layer by degrading mucins, influencing mucus viscosity or affecting mucin expression, synthesis and/or secretion (Table 1). *Campylobacter jejuni*, for instance, uses MUC2 as an environmental trigger to increase expression of mucin degrading proteins to decrease mucus viscosity.[68] *H. pylori* can penetrate the mucus layer by increasing the pH which reduces mucus viscosity and by decreasing MUC5AC and MUC1 expression resulting in a disruption of the mucus structure.[13] In the colon, *Lactobacillus, B. thetaiotaomicron*, *Bifidobacteria* and *Faecalibacterium prausnitzii* modulate mucus secretion by influencing goblet cell differentiation.[21,33] Also probiotic strains, such as *B. breve*, *B. dentium*, *E. coli Nissle, L. casei*, *L. plantarum*, *L. rhamnosus*, and *Streptococcus thermophilus*, have been shown to regulate colonic mucus production in both animal and cell culture models.[33,38,42,69] Additionally, PAMPs, such as lipopolysaccharide (LPS) or peptidoglycan, are known to induce the production of MUC2, MUC5AC, and MUC5B through activation of the Ras-signalling pathway.[70]

4. Cell signalling pathways mediated by mucins

4.1. Inflammation

In the connective tissue beneath the epithelium (i.e. the lamina propria), immune cells from both the innate and adaptive immune system reside (Appendix 1). During an inflammatory response, foreign antigens are presented by DCs to $CD4^+$ T cells which produce cytokines to enhance barrier function (e.g. IL-17, IL-22) and promote the recruitment of immune cells to prevent pathogen invasion in a robust and well-coordinated way. Nevertheless, abnormally activated effector $CD4^+$ T cells produce different inflammatory mediators that sustain inflammation leading to chronic epithelial damage and thus promote the development of inflammatory or autoimmune diseases.[7] In addition, chronic

inflammation can induce abnormal cell growth and can also lead to local immunosuppression, mediated by myeloid-derived suppressor cells (MDSCs) and Tregs, thereby promoting tumourigenesis.[8] Interestingly, transmembrane mucins can modulate the inflammatory microenvironment and cell signalling as further highlighted below.[2,8,9]

MUC1. MUC1 has been shown to modulate immune cell populations during colonic inflammation, characterised by the inhibition of an excessive microbiota-driven Th17 immune response, an increase in neutrophils in the colon and in MDSCs in the spleen and loss of $CD8^+$ T lymphocytes in the spleen and lymph nodes during experimental murine colitis.[2,9] Such MUC1-mediated increase in MDSCs and neutrophils can also create a tumour-promoting environment as shown by the higher prevalence of colitis-associated colon cancer in Muc1-overexpressing mice.[71] Since MUC1 has been identified as an IBD susceptibility gene and its expression is upregulated by Th17-produced cytokines, its aberrant expression might dysregulate an important feedback mechanism that is necessary to prevent an excessive and abnormal Th17-response.[2,7,9] Furthermore, the cytoplasmic tail of MUC1 (MUC1-C) can activate nuclear factor kappa B (NF-κB) and β-catenin/T-cell factor (TCF)4 pathways in the colon of mice, via induction and facilitation of the complex formation of TNF receptor-associated factor 6 (TRAF6) and transforming growth factor-β-activated kinase 1 (TAK1), thereby promoting intestinal inflammation and cancer progression (Figure 2).[9] In the stomach, however, MUC1 blocks IL-8 secretion by suppressing NF-κB p65 activity in human gastric epithelial cells, and reduces oxidative stress, as shown by the sustained increase in 3-nitrotyrosine levels in the gastric epithelium of mice lacking epithelial MUC1 upon acetylsalicylic acid-induced inflammation.[2,72]

MUC4. MUC4 depletion resulted in a reduced infiltration of proinflammatory immune cells (T cells and macrophages) and a reduced expression of interleukin (IL)-1, tumour necrosis factor (TNF)-α and anti-microbial genes (Lysozyme M, SLP1) in the colon of DSS-induced colitis mice, which was associated with an increased resistance against developing cancer.[2]

MUC13. In Muc13-deficient mice, fewer colonic tumours are seen in the azoxymethane (AOM)/dextran sodium sulphate (DSS) CRC mice model with marked reduction in β-catenin/Wnt signalling, an increase in $CD103^+$ DCs (which are essential for tumour antigen cross-presentation) and $CD8^+$ T lymphocytes and a decrease in $CD11b^+Lys6G^{hi}F4/80^-$ granulocytic-MDSCs.[8] MUC13 has also the ability to induce IL-8 secretion upon TNF-α stimulation by promoting p65 activity in human CRC cells, further suggesting a pro-inflammatory role for MUC13.[2] Furthermore, in acutely inflamed or dysplastic regions of the murine colon, the presence of Muc13 has been associated with decreased production of pro-inflammatory cytokines, thus also suggesting an immunosuppressive role.[8]

MUC17. In human colonic epithelial cells, silencing of MUC17 expression aggravated the proinflammatory response upon *E. coli* infection by increasing inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)2 protein expression.[10]

Overall, activation of mucin signalling beneficially modulates the immune response during acute inflammation, but sustained activation during chronic inflammation results in the promotion of tumour development.

4.2. Cell-cell interactions, cell differentiation, motility and invasion

In the healthy gut, epithelial cells are characterised by a polarized columnar shape, strong cell-cell interactions and limited migratory capacity (Appendix 1). Loss of intestinal epithelial barrier function through a disruption of intercellular junctional proteins, is considered as an important feature of the pathogenesis of IBD and CRC (Appendix 1).[2] Furthermore, several biochemical changes are induced in epithelial cells known as epithelial-to-mesenchymal transition (EMT) during inflammation and cancer progression. Upon EMT, epithelial cells gain mesenchymal characteristics, resulting in cell morphology alterations, loss of cell adherence and increased cell motility.[9]

MUC1. Aberrant expression of *Muc1* correlated with the altered expression levels of the tight junction protein genes *claudin* (*Cldn)1* and *occludin (Ocln)* in the T-cell transfer colitis mouse model.[4]

MUC1 depletion in breast cancer cells also affected expression of *CLDN1*, *OCLN*, *zonula-occludens* (*ZO)-1* and *E-cadherin* (*CDH1)*, further underlining a functional role for MUC1 in regulating junctional protein expression during inflamation and carcinogenesis.[9] Regarding EMT, MUC1 associates with the SH3 domain containing kinase binding protein 1 (SH3KBP1) and Cbl proto-oncogene (CBL), a protein complex involved in the reorganization of the actin cytoskeleton, cell adhesion, migration, and invasion in human CRC (Figure 3).[2,9] MUC1 was also found to form a complex with MYC Proto-Oncogene, BHLH Transcription Factor (MYC), thereby promoting stemness and pluripotency in the murine colon during the progression of colitis to dysplasia and cancer (Figure 3).[9] Another MUC1 interaction partner to induce EMT in CRC cells is zinc finger E-box binding homeobox 1 (ZEB1), via phosphorylation of AKT serine/threonine kinase 1 (AKT) (Figure 3).[9,72] ZEB1 stimulates the expression of vimentin and N-cadherin and suppresses the expression of E-cadherin, resulting in the loss of cell-cell adherence and an increased cell motility.[9] Ligation of MUC1 and ZEB1 also inhibits CRB3 expression in breast cancer cells, leading to the activation of the yes-associated protein 1 (YAP), which in turn activates Wnt/β-catenin/TCF4 signalling.[9] A direct interaction between MUC1-C and β-catenin has also been shown at the invasive front of colorectal tumours (Figure 3), which associates with low grade differentiation and worse patient survival.[2,73] Interestingly, depletion of MUC1 in CRC cells suppresses the nuclear translocation of β-catenin and decreased ZEB1 expression.[9,73] The erb-b2 receptor tyrosine kinases (ERBB) are also key modulators of many cellular characteristics, including cell migration. As such, MUC1 is able to interact with epidermal growth factor receptor (EGFR) in colon cancer cells (Figure 3).[74]

MUC4. In CRC, most patients have decreased colonic MUC4 expression. Nevertheless, high MUC4 expression in early-stage CRC is associated with a worse prognosis. Indeed, aberrant MUC4 signalling induces loss of cell-cell contact in different human epithelial cell lines which is probably mediated by the activation of mitogen-activated protein kinase (MAPK)3/ERK1 and MAPK1/ERK2 and the upregulation of Cyclin D1 (CCND1), resulting in the relocalisation of E-cadherin.[75] Similar to MUC1, loss of MUC4 promotes epithelial differentiation in pancreatic cancer cells by increasing the

303 binding of β-catenin to E-cadherin at the cell membrane which stimulates tight junction expression.[73]
304 Conversely, β-catenin represses MUC4 expression in CRC cells, probably through the suppression of
305 the transcription factor atonal BHLH transcription factor 1 (HATH1).[76] In gastric adenocarcinoma,
306 MUC4 overexpression is also observed, yet it does not correlate with tumour differentiation or stage.
307 However, MUC4 promotes the activation of ERBB2, thereby reducing cell-cell aggregation and
308 enhancing the motility of human gastric cancer cells.[77]

309 *MUC13*

310 In the DSS-induced murine colitis model, aberrant expression of *Muc13* correlates with intestinal
311 permeability and the expression of *Cldn1*, *junctional adhesion molecule (Jam)2*, and *tight junction*
312 *protein (Tjp)2*.[4] In addition, MUC13 stabilizes β-catenin localisation in the nucleus, resulting in
313 increased levels of Jun proto-oncogene (JUN), CCND1, matrix metallopeptidase (MMP)7, and SMAD
314 family member (SMAD)2/3 in CRC cells (Figure 4).[8]

315 *MUC17*. MUC17 increases ERK phosphorylation, thereby stimulating migration and cell
316 adherence of human CRC cells and promoted healing following experimental colitis.[78] On the
317 contrary, loss of MUC17 expression in CRC cells was associated with reduced phosphorylation of
318 OCLN and decreased expression of ZO-1.[10]

319 Aberrantly expressed MUC1, MUC4 and MUC13 seem to induce EMT signalling pathways in GI
320 epithelial cells upon inflammation and cancer, by interacting with amongst other β-catenin, ZEB1,
321 ERK and ERBB. As a result, cell-cell interactions are disturbed and cell motility and invasion are
322 stimulated, further promoting tumorigenicity. In contrast, MUC17 signalling appears to stimulate
323 cell-cell interactions as well as cell motility, but the evidence is still limited.

324

325 *4.3. Cell proliferation, growth and survival*

326 A breach in the GI mucosal barrier further results in disruption of regulated cell death and the vicious
327 cycle of continuous barrier and cell death dysfunction promotes cancer development. Diverse human

malignancies, including GI cancers, overexpress transmembrane mucins over the surface of the tumour to exploit their role in signalling cell proliferation, growth, and survival.[2]

MUC1. MUC1-C is able to enhance cell growth in CRC cells via phosphorylation of AKT and induction of the kinase S6K1 thereby affecting translation of TP53-induced glycolysis regulatory phosphatase (TIGAR), a major regulator of cellular redox homeostasis (Figure 2).[9] The regulatory effect of MUC1-C on CRC cell growth can also occur through the β-catenin-mediated upregulation of CCND1 and MYC.[73] MUC1 can then bind directly to the MYC promotor or β-catenin resulting in suppression of β-catenin phosphorylation and degradation by glycogen synthase kinase 3β (GSK3β) (Figure 2).[9] Additionally, MUC1 has also the ability to enhance homo-dimerization and phosphorylation of EGFR for the activation of ERK1/2, a signalling pathway involved in cell proliferation and survival. The interaction between MUC1 and EGFR is then stimulated by galectin-3, a carbohydrate-binding protein elevated in GI cancers (Figure 2).[74]

MUC1 also interferes with cell death mechanisms making it an important hurdle for cytotoxic cancer therapies by promoting chemoresistance in GI cancers (Figure 2). In CRC cells, multiple pathways are modulated by MUC1 signalling to confer resistance to DNA damage-induced apoptosis. Firstly, MUC1-C disrupts Cullin 4A (CUL4A) signalling by sequestering ABL proto-oncogene 1 (ABL1) to the cytoplasm and blocking CUL4A-mediated translocation of Cdc6 to the cytoplasm. As a result, MDM2-induced proteasomal degradation of TP53 is suppressed. Simultaneously, homeodomain interacting protein kinase 2 (HIPK2) expression is downregulated by MUC1-C which decreases TP53 phosphorylation and activates cyclin dependent kinase inhibitor 1A (CDKN1A), resulting in cell cycle arrest instead of cell death. In addition, MUC1-C also directly interacts with TP53 and co-activates CDKN1A transcription (Figure 2).[79] Secondly, JNK, activated by MUC1-C, stimulates transcriptional activity of JUN thereby attenuating the activation of apoptotic effectors (Figure 2).[80] Thirdly, MUC1-C associates with BCL2 associated X (BAX) protein to block its dimerization and prevent leakage of apoptotic factors from the mitochondria (Figure 2).[80] Fourthly, MUC1 also activates the NF-κB

pathway through release of p65 promoting cell survival (Figure 2).[2] Lastly, MUC1-C has also been shown to induce PI3K-AKT-S6K1 signalling, inducing TIGAR translation (Figure 2).[9]

In gastric tumour cells, however, ERBB2-mediated phosphorylated MUC1-C suppresses apoptosis by modulating β-catenin translocation or by associating with heat shock protein (HSP)90 and HSP70 leading to an attenuated release of pro-apoptotic factors (Figure 2).[2,9,72,81]

Tumours often have a hypoxic environment which can stimulate cells to undergo apoptosis. MUC1 signalling can block this hypoxia-mediated cell death in CRC cells by attenuating AKT activity via PI3K, resulting in forkhead box O3 (FOXO3)-induced gene transcription which is involved in oxidant scavenging and DNA damage repair (Figure 3).[9,80] Furthermore, MUC1 upregulates prolyl hydroxylase domain protein (PHD)3 expression in CRC cells, suppressing the activation of hypoxia-inducible factor (HIF)1A by marking it for proteasomal degradation. MUC1 then reduces the accumulation of ROS by stimulating the expression of antioxidant enzymes, further potentiating PHD3-mediated inhibition of HIF1A. Although HIF1A initially induces invasion and resistance to apoptosis, it will promote apoptosis under severe and/or prolonged hypoxia via TP53 (Figure 3).[9,80]

Finally, MUC1 also has the ability to block anoikis, a process where apoptosis is induced upon loss of cell attachment. More specifically, the extracellular domain of MUC1 suppresses activation of integrin β1 and subsequently prevents cell surface death receptors to bind their ligands through steric hindrance.[2]

MUC13. Janus kinase (JAK)2/signal transducer and activator of transcription (STAT)5 induce expression of MUC13 in CRC cells. This in turn modulates various cancer-associated proteins resulting in the activation of the anti-apoptotic protein BCL2 like 1 (BCL2L1) and subsequent blocking of apoptosis (Figure 4).[2,8] This activation of BCL2L1 via MUC13 can also be mediated by the NF-κB pathway in two distinct ways. Firstly, MUC13 interacts with tumour necrosis factor receptor (TNFR)1 and baculoviral IAP repeat containing 2 (cIAP1) to increase activation of inhibitor of NF-κB kinase regulatory subunit gamma (IKBKG) (Figure 4). Secondly, DNA damage induces MUC13 translocation to the cytosol and nucleus, increasing activation of IKBKG which in turn leads to activation of

inhibitor of NF-κB kinase (IKK)s and NF-κB (Figure 4).[8] Similar to MUC1, MUC13 can inhibit GSK3B-mediated phosphorylation of β-catenin, promoting its nuclear translocation, activating transcription of genes involved in survival and cell cycle control (i.e. CCND1 and MYC) (Figure 4).[8]

MUC17. MUC17 has the ability to inhibit apoptosis through promotion of ERK1/2 signalling in the colonic epithelium.[78]

It is clear that transmembrane mucins possess various mechanisms to affect cell growth and survival in inflammation and cancer. A better understanding of their regulatory role on DNA-damage induced cell death could be of major importance to overcome chemoresistance in GI cancers.

5. Clinical and therapeutic potential of mucins

It is clear that mucins are aberrantly expressed during gastrointestinal inflammation and cancer progression, resulting in the modulation of the mucobiota and key cellular pathways involved in cell-cell communication, cell differentiation, proliferation, growth, and survival. As such, mucins could be interesting targets for the diagnosis, follow-up, and treatment of many diseases.

Several studies have already highlighted that targeting mucins by different means could exert beneficial effects on cellular homeostasis and enhance the efficacy of treatment (Table 2). For instance, since loss of MUC13 is associated with elevated levels of $CD103^+$ DCs and CD8 T cells at the tumour site, an increased sensitivity of MUC13-deficient colorectal tumours to checkpoint inhibitor anti-PD-L1 antibodies was shown.[8] Furthermore, coupling MUC1-antibodies with PE38-exotoxins induced tumour cell killing proportional to MUC1 expression in breast and pancreas tumours.[82] Intraperitoneal injection of GO-203, a MUC1-C inhibitor peptide, has been shown to protect against colitis and the progression to dysplasia and colitis-associated colon cancer in the AOM/DSS mouse model.[9] This effect could be partially mediated by reversing MUC1-C-induced EMT, as has been shown in CRC cells.[9,83] Alternatively, the intrarectal administration of recombinant MUC17 protein improved intestinal healing after chemical induction of murine colitis by the intrarectal administration of 5% acetic acid, of which the biological activity was influenced by the amount of

cysteine-rich like domains and the presence of a functional SEA domain.[78,89] Besides directly targeting mucins for therapeutic purposes, probiotic bacteria can also be used to promote the expression of particular mucins and in this way modulate the gastrointestinal mucus barrier and prevent the adherence of potential pathogenic organisms. For instance, the administration of *L. plantarum* and *L. rhamnosus* can effectively increase MUC2 and MUC3 expression in intestinal epithelial cells, strengthening the mucosal barrier and decrease pathogen invasion susceptibility. Administration of these *Lactobacilli* has already been proven to inhibit EPEC adhesion and infection of the intestinal epithelium.[26] Another strategy to manipulate the microbiota is through faecal microbiota transplantation (FMT). Although FMT is gaining interest for treating *C. difficile* infection and IBD, the mechanisms behind the therapy remain largely unknown.[84] In a piglet model infected with *E. coli* K88, the number of goblet cells and the expression of MUC2 and tight junction proteins significantly increased after FMT while the abundance of beneficial bacteria such as *Lactobacilli* also increased.[84] FMT could prove to be a viable treatment option to alleviate mucosal injury, increased gastrointestinal permeability and disease-associated dysbiosis.

Incorporating mucin antigens in vaccines is also a promising development within tumour immunotherapy (Table 2). The use of several types of MUC1-containing vaccines (e.g. injection of cancer stem cells, DNA vaccines, subunit vaccines and DC vaccines) has been associated with protection against colitis and smaller CRC tumours and longer tumour-free periods. Mechanistically this has been shown to be achieved through enhanced activity of DCs, natural killer cells, cytotoxic T lymphocytes, and antibody production as well as suppression of MDSCs and Treg cells.[71,85,86] Remarkably, combinatory therapy (DNA vaccine with oxaliplatin) was shown to suppress tumour growth by almost 75%.[86] Additionally, the intratumoural injection of MUC1-loaded DCs resulted in the complete disappearance of gastric cancer lesions in an 80-year-old male patient.[88] Numerous clinical studies have already highlighted the potential and safety of MUC1-based vaccines for the treatment of various cancers by boosting anti-tumour immunity.[86,87] For instance, a phase 2 study investigating CRC patients with resected lung or liver metastases showed a similar 2-year recurrence

free survival after treatment with autologous DCs loaded with a poxvector vaccine encoding carcinoembryonic antigen (CEA) and MUC1 (PANVAC) (47%) or with the PANVAC vaccine in combination with granulocyte-macrophage colony-stimulating factor (GM-CSF) (55%). Interestingly, although there were no significant differences in the 2-year recurrence-free survival between vaccinated patients and a contemporary group of unvaccinated patients, overall survival was significantly improved upon vaccination.[87] Another MUC1-based vaccine combined with the immunomodulator poly-ICLC was well tolerated and showed a strong immunogenic response in 43·6% of patients with a history of premalignant lesions in the colon, highlighting its potential as a strategy for CRC prevention.[86]

Finally, mucin mRNA and protein expression signatures could also be used as biomarkers for disease diagnosis and prognosis at early stage and follow-up (Table 2). High expression of MUC1 or MUC13 in tumour tissue is associated with a poor prognosis in oesophageal, gastric and/or CRC.[8,9,91] High MUC4 expression has also been reported to be predictive for poor survival in early stage colorectal tumours.[89] The detection of *MUC1* expression in blood has proven its role in the prediction of 2-year progression-free survival of CRC patients.[92] In addition, fluorescently and radiolabelled MUC1 is also used to monitor therapy response by MRI and to visualise colon tumour xenografts by molecular imaging.[98,99]

Genetic variation such as single nucleotide polymorphisms (SNPs) in mucin genes are frequently reported in GI pathologies and often correlate with clinical outcomes.[7,89,94,95] Indeed, SNPs in *MUC1*, *MUC4* and *MUC16* are associated with the prognosis, tumour mutation burden, and efficacy of immunotherapy in CRC and/or gastric cancer patients (Table 2).[93–95,97,100] In addition, CRC patients with a TT to CC tandem substitution of rs886403 in *MUC21* had a worse survival and higher recurrence risk. Carrying the CC genotype of rs4729655 in *MUC17*, on the contrary, increased survival in rectal cancer patients.[100] Genetic polymorphisms in *MUC1*, *MUC3A*, *MUC4*, *MUC13*, *MUC16*, *MUC19*, *MUC21*, and *MUC22* have also been associated with Crohn's disease and ulcerative colitis.[7]

456 The presence of genetic variants in mucin genes can affect their expression pattern resulting in
457 different mRNA isoforms via alternative splicing. While most mRNA isoforms produced from the
458 same mucin gene locus encode similar biological functions, others can alter the protein function.[7,97]
459 In oesophageal cancer, for instance, different mRNA isoform expression patterns of *MUC1* have been
460 found between normal and tumour tissue. Here, the expression of mRNA isoform MUC1/B was lower
461 in tumour tissue as compared to control tissue and inversely correlated with tumour stage and lymph
462 node metastasis. On the contrary, the concomitant expression of the mRNA isoforms MUC1/C,
463 MUC1/D and MUC1/Z positively associated with cancer progression.[97] Also, in CRC tissue and
464 adjacent normal tissue, two distinct isoforms of *MUC2* have been identified. A higher positivity for
465 the variant MUC2TR2 was observed as compared to MUC2.1 but their clinical role is still unclear.[97]
466 Although the findings in the GI tract are still limited, alternative mucin transcripts have been
467 identified in several pathologies outside the GI tract, such as ovarian and pancreatic cancer and dry
468 eye disease[97], highlighting the role of mucin mRNA isoforms in disease progression and their
469 potential use as biomarkers or novel treat-to-target strategies.
470

471 ## 6. Concluding remarks

472 Although mucins are indispensable for GI mucosal barrier homeostasis, they are dual-faceted
473 proteins. They provide an essential nutrient source for the gut microbiota, shaping community
474 composition and function. However, some bacterial members have evolved mechanisms to
475 penetrate the secreted mucus layer and/or to use mucins as docking sites to infect the host and
476 induce an inflammatory response. Furthermore, it has also been shown that transmembrane mucins
477 affect epithelial cell behaviour via ERBB, NF-κB, Wnt/β-catenin, PI3K/AKT, MAPK/ERK, and JAK/STAT
478 signalling, which are major signalling pathways in the context of wound healing and the restoration
479 of GI barrier integrity. However, constitutive activation of these pathways compromises GI barrier
480 function promoting chronic inflammation and tumourigenesis. Since aberrant expression and genetic
481 variants of mucins are frequently observed in GI pathologies, they must be considered as important

players in the modulation of these pathways and thus also in the onset and course of these diseases. As such, aberrant mucin expression patterns have proven their worth in the diagnosis and prognosis of GI inflammatory disorders and cancers. Concerning the prevention and treatment of GI diseases, most clinical research so far has successfully focused on the potential of MUC1. Nevertheless, future research should extent to other mucins as well and further improve our understanding of their role in GI epithelial cell signalling since they are promising candidates for the management and treatment of many GI diseases.

7. Contributors

AS, BDW, GH, TB and BO contributed to the concept and design of this review. TB and BO performed the literature search and reviewed and selected the papers for inclusion in this review. TB, BO and AS wrote and edited the manuscript. BO, TB and AS created the figures (BO: figures 1 – 4; TB: Appendix 1; AS: Appendix 2). AS, BDW, GH, WA, HC and JDM critically revised the manuscript, the figures, and the tables. All authors approved the final draft.

8. Declaration of interests

TB, BDW and AS are inventors on a patent related to mucin isoforms in diseases characterized by barrier dysfunction, including IBD, IBS, gastrointestinal infections and cancer (WO/2021/013479). The other authors declared no conflicting interests.

9. Acknowledgements

Figures 2 – 4 and appendices 1 – 2 were created with Biorender.com. The work was supported by the Antwerp University Research Fund (BOF DOCPRO4 n°34782 (TB) and n° 39958 (BO)), the Antwerp University valorisation fund (IOF-SBO: n° 42601 (WA)) and the Research Foundation Flanders (FWO n° 1244421N (HC)).

10. References

1. Linden S, Putton P, Karlsson N, Korolik V, McGuckin M. Mucins in the mucosal barrier to infection. Mucosal Immunol. 2008; **1**(3): 183–97.
2. van Putten JPM, Strijbis K. Transmembrane Mucins: Signaling Receptors at the Intersection of Inflammation and Cancer. J Innate Immun. 2017; **9**(3): 281–99.
3. Paone P, Cani PD. Mucus barrier, mucins and gut microbiota: the expected slimy partners? Gut. 2020; **69**(12): 2232–43.
4. Breugelmans T, Van Spaendonk H, De Man JG, et al. In-Depth Study of Transmembrane Mucins in Association with Intestinal Barrier Dysfunction During the Course of T Cell Transfer and DSS-Induced Colitis. J Crohns Colitis. 2020; **14**(7): 974–94.
5. Zarepour M, Bhullar K, Montero M, et al. The mucin muc2 limits pathogen burdens and epithelial barrier dysfunction during salmonella enterica serovar typhimurium colitis. Infect Immun. 2013; **81**(10): 3672–83.
6. Olli KE, Rapp C, O'Connell L, et al. Muc5ac Expression Protects the Colonic Barrier in Experimental Colitis. Inflamm Bowel Dis. 2020; **26**(9): 1353–67.
7. Sheng YH, Hasnain SZ, Florin THJ, McGuckin MA. Mucins in inflammatory bowel diseases and colorectal cancer. J Gastroenterol Hepatol. 2012; **27**(1): 28–38.
8. Sheng Y hua, Wong KY, Seim I, et al. MUC13 promotes the development of colitis-associated colorectal tumors via β-catenin activity. Oncogene. 2019; **38**(48): 7294–310.
9. Kufe DW. MUC1-C in chronic inflammation and carcinogenesis; emergence as a target for cancer treatment. Carcinogenesis. 2020; **41**(9): 1173–83.
10. Resta-Lenert S, Das S, Batra SK, Ho SB. Muc17 protects intestinal epithelial cells from enteroinvasive E. coli infection by promoting epithelial barrier integrity. Am J Physiol - Gastrointest Liver Physiol. 2011; **300**(6): G1144-55.
11. Johansson MEV, Phillipson M, Petersson J, Velcich A, Holm L, Hansson GC. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. Proc Natl Acad Sci U S

A. 2008; **105**(39): 15064–9.

12. Belzer C, Chia LW, Aalvink S, et al. Microbial metabolic networks at the mucus layer lead to diet-independent butyrate and vitamin B12 production by intestinal symbionts. MBio. 2017; **8**(5): e00770-17.

13. Cornick S, Tawiah A, Chadee K. Roles and regulation of the mucus barrier in the gut. Tissue Barriers. 2015; **3**(1): e982426.

14. Wlodarska M, Willing B, Keeney KM, et al. Antibiotic treatment alters the colonic mucus layer and predisposes the host to exacerbated Citrobacter rodentium-induced colitis. Infect Immun. 2011; **79**(4): 1536–45.

15. Chen C-Y, Hsu K-C, Yeh H-Y, Ho H-C. Visualizing the effects of antibiotics on the mouse colonic mucus layer. Tzu-Chi Med J. 2020; **32**(2): 145.

16. Pélissier M-A, Vasquez N, Balamurugan R, et al. Metronidazole effects on microbiota and mucus layer thickness in the rat gut. FEMS Microbiol Ecol. 2010; **73**(3): 601–10.

17. Kaur K, Saxena A, Debnath I, et al. Antibiotic-mediated bacteriome depletion in ApcMin/+ mice is associated with reduction in mucus-producing goblet cells and increased colorectal cancer progression. Cancer Med. 2018; **7**(5): 2003.

18. Johansson MEV, Jakobsson HE, Holmén-Larsson J, et al. Normalization of host intestinal mucus layers requires long-term microbial colonization. Cell Host Microbe. 2015; **18**(5): 582–92.

19. van Tassell ML, Miller MJ. Lactobacillus adhesion to mucus. Nutrients. 2011; **3**(5): 613–36.

20. Etienne-Mesmin L, Chassaing B, Desvaux M, et al. Experimental models to study intestinal microbes–mucus interactions in health and disease. FEMS Microbiol Rev. 2019; **43**(5): 457–89.

21. Sicard JF, Bihan G Le, Vogeleer P, Jacques M, Harel J. Interactions of intestinal bacteria with components of the intestinal mucus. Front Cell Infect Microbiol. 2017; **7**: 387.

22. Juge N. Microbial adhesins to gastrointestinal mucus. Trends Microbiol. 2012; **20**(1): 30–9.

23. Martens EC, Neumann M, Desai MS. Interactions of commensal and pathogenic

559 microorganisms with the intestinal mucosal barrier. Nat Rev Microbiol. 2018; **16**(8):457-470.
560 24. Boll EJ, Ayala-Lujan J, Szabady RL, et al. Enteroaggregative Escherichia Coli adherence fimbriae
561 drive inflammatory cell recruitment via interactions with epithelial MUC1. MBio. 2017; **8**(3):
562 e00717-17.
563 25. Kumar P, Kuhlmann FM, Bhullar K, et al. Dynamic Interactions of a Conserved Enterotoxigenic
564 Escherichia coli Adhesin with Intestinal Mucins Govern Epithelium Engagement and Toxin
565 Delivery. Infect Immun. 2016; **84**(12): 3608.
566 26. Fang J, Wang H, Zhou Y, Zhang H, Zhou H, Zhang X. Slimy partners: the mucus barrier and gut
567 microbiome in ulcerative colitis. Exp Mol Med. 2021; **53**(5): 772.
568 27. Huynh DTN, Kim A-Y, Kim Y-R. Identification of Pathogenic Factors in Klebsiella pneumoniae
569 Using Impedimetric Sensor Equipped with Biomimetic Surfaces. Sensors (Basel). 2017; **17**(6):
570 1406.
571 28. Tytgat HLP, Douillard FP, Reunanen J, et al. Lactobacillus rhamnosus GG Outcompetes
572 Enterococcus faecium via Mucus-Binding Pili: Evidence for a Novel and Heterospecific
573 Probiotic Mechanism. Appl Environ Microbiol. 2016; **82**(19): 5756.
574 29. Houeix B, Synowsky S, Cairns MT, Kane M, Kilcoyne M, Joshi L. Identification of putative
575 adhesins and carbohydrate ligands of Lactobacillus paracasei using a combinatorial in silico
576 and glycomics microarray profiling approach. Integr Biol. 2019; **11**(7): 315–29.
577 30. Martins M, Porrini C, Merle L du, et al. The Pil3 pilus of Streptococcus gallolyticus binds
578 tointestinal mucins and to fibrinogen. Gut Microbes. 2016; **7**(6): 526.
579 31. Li X, Bleumink-Pluym NMC, Luijkx YMCA, Wubbolts RW, Putten JPM van, Strijbis K. MUC1 is a
580 receptor for the Salmonella SiiE adhesin that enables apical invasion into enterocytes. PLoS
581 Pathog. 2019; **15**(2): e1007566.
582 32. He F, Ouwehand AC, Hashimoto H, Isolauri E, Benno Y, Salminen S. Adhesion of
583 Bifidobacterium Spp. to Human Intestinal Mucus. Microbiol Immunol. 2001; **45**(3): 259–62.
584 33. Engevik MA, Luk B, Chang-Graham AL, et al. Bifidobacterium dentium Fortifies the Intestinal

Mucus Layer via Autophagy and Calcium Signaling Pathways. MBio. 2019; **10**(3): e01087-19.

34. Westermann C, Gleinser M, Corr SC, Riedel CU. A Critical Evaluation of Bifidobacterial Adhesion to the Host Tissue. Front Microbiol. 2016; **7**: 1220.

35. Engevik MA, Danhof HA, Ruan W, et al. Fusobacterium nucleatum Secretes Outer Membrane Vesicles and Promotes Intestinal Inflammation. MBio. 2021; **12**(2): 1–17.

36. Tailford LE, Crost EH, Kavanaugh D, Juge N. Mucin glycan foraging in the human gut microbiome. Front Genet. 2015; **6**: 81.

37. Shon DJ, Kuo A, Ferracane MJ, Malaker SA. Classification, structural biology, and applications of mucin domain-targeting proteases. Biochem J. 2021; **478**(8): 1585–603.

38. Shastri MD, Chong WC, Vemuri R, et al. Streptococcus Thermophilus UASt-09 Upregulates Goblet Cell Activity in Colonic Epithelial Cells to a Greater Degree than other Probiotic Strains. Microorganisms. 2020; **8**(11): 1–15.

39. JL M, SK L, CW P, et al. MUC1 cell surface mucin is a critical element of the mucosal barrier to infection. J Clin Invest. 2007; **117**(8): 2313–24.

40. Lindén SK, Florin THJ, McGuckin MA. Mucin dynamics in intestinal bacterial infection. PLoS One. 2008; **3**(12): e3952.

41. Hill DR, Huang S, Nagy MS, et al. Bacterial colonization stimulates a complex physiological response in the immature human intestinal epithelium. Elife. 2017; **6**: e29132.

42. Hafez MM. Upregulation of Intestinal Mucin Expression by the Probiotic Bacterium E. coli Nissle 1917. Probiotics Antimicrob Proteins. 2012; **4**(2): 67–77.

43. Flores-Sanchez F, Chavez-Dueñas L, Sanchez-Villamil J, Navarro-Garcia F. Pic Protein From Enteroaggregative E. coli Induces Different Mechanisms for Its Dual Activity as a Mucus Secretagogue and a Mucinase. Front Immunol. 2020; **11**: 564953.

44. Navarro-Garcia F, Gutierrez-Jimenez J, Garcia-Tovar C, Castro LA, Salazar-Gonzalez H, Cordova V. Pic, an autotransporter protein secreted by different pathogens in the Enterobacteriaceae family, is a potent mucus secretagogue. Infect Immun. 2010; **78**(10): 4101–9.

611 45. Dharmani P, Leung P, Chadee K. Tumor necrosis factor-α and Muc2 mucin play major roles in
612 disease onset and progression in dextran sodium sulphate-induced colitis. PLoS One. 2011;
613 **6**(9): e25058.
614 46. Wang L, Cao H, Liu L, et al. Activation of Epidermal Growth Factor Receptor Mediates Mucin
615 Production Stimulated by p40, a Lactobacillus rhamnosus GG-derived Protein. J Biol Chem.
616 2014; **289**(29): 20234.
617 47. Zhou X, Zhang K, Qi W, et al. Exopolysaccharides from Lactobacillus plantarum NCU116
618 Enhances Colonic Mucosal Homeostasis by Controlling Epithelial Cell Differentiation and c-
619 Jun/Muc2 Signaling. J Agric Food Chem. 2019; **67**(35): 9831–9.
620 48. Martín R, Chamignon C, Mhedbi-Hajri N, et al. The potential probiotic Lactobacillus
621 rhamnosus CNCM I-3690 strain protects the intestinal barrier by stimulating both mucus
622 production and cytoprotective response. Sci Reports 2019 91. 2019; **9**(1): 1–14.
623 49. Graziani F, Pujol A, Nicoletti C, et al. Ruminococcus gnavus E1 modulates mucin expression
624 and intestinal glycosylation. J Appl Microbiol. 2016; **120**(5): 1403–17.
625 50. Etzold S, Juge N. Structural insights into bacterial recognition of intestinal mucins. Curr Opin
626 Struct Biol. 2014; **28**(1): 23–31.
627 51. Marcobal A, Southwick AM, Earle KA, Sonnenburg JL. A refined palate: Bacterial consumption
628 of host glycans in the gut. Glycobiology. 2013; **23**(9): 1038–46.
629 52. Curtis MM, Hu Z, Klimko C, Narayanan S, Deberardinis R, Sperandio V. The gut commensal
630 Bacteroides thetaiotaomicron exacerbates enteric infection through modification of the
631 metabolic landscape. Cell Host Microbe. 2014; **16**(6): 759–69.
632 53. Engevik MA, Engevik AC, Engevik KA, et al. Mucin-Degrading Microbes Release
633 Monosaccharides That Chemoattract Clostridioides difficile and Facilitate Colonization of the
634 Human Intestinal Mucus Layer. ACS Infect Dis. 2021; **7**(5): 1126.
635 54. Engevik MA, Banks LD, Engevik KA, et al. Rotavirus infection induces glycan availability to
636 promote ileum-specific changes in the microbiome aiding rotavirus virulence. Gut Microbes.

2020; **11**(5): 1324.

55. Alvarado I, Abel-Santos E. How enteric pathogens know they hit the sweet spot. Future Microbiol. 2014; **9**(1): 13–6.

56. Mondal M, Nag D, Koley H, Saha DR, Chatterjee NS. The Vibrio cholerae extracellular chitinase ChiA2 is important for survival and pathogenesis in the host intestine. PLoS One. 2014; **9**(9): e103119.

57. Arabyan N, Park D, Foutouhi S, et al. Salmonella Degrades the Host Glycocalyx Leading to Altered Infection and Glycan Remodeling. Sci Rep. 2016; **6**: 29525.

58. Rey FE, Gonzalez MD, Cheng J, Wu M, Ahern PP, Gordon JI. Metabolic niche of a prominent sulfate-reducing human gut bacterium. Proc Natl Acad Sci U S A. 2013; **110**(33): 13582–7.

59. Yoshihara T, Oikawa Y, Kato T, et al. The protective effect of Bifidobacterium bifidum G9-1 against mucus degradation by Akkermansia muciniphila following small intestine injury caused by a proton pump inhibitor and aspirin. Gut Microbes. 2020; **11**(5): 1385.

60. Birchenough G, Schroeder BO, Bäckhed F, Hansson GC. Dietary destabilisation of the balance between the microbiota and the colonic mucus barrier. Gut Microbes. 2019; **10**(2): 246–50.

61. Burger-van Paassen N, Vincent A, Puiman PJ, et al. The regulation of intestinal mucin MUC2 expression by short-chain fatty acids: Implications for epithelial protection. Biochem J. 2009; **420**(2): 211–9.

62. Pearce SC, Weber GJ, Sambeek DM van, Soares JW, Racicot K, Breault DT. Intestinal enteroids recapitulate the effects of short-chain fatty acids on the intestinal epithelium. PLoS One. 2020; **15**(4).

63. Bersch KL, DeMeester KE, Zagani R, et al. Bacterial Peptidoglycan Fragments Differentially Regulate Innate Immune Signaling. ACS Cent Sci. 2021; **7**(4): 688–96.

64. Cobo ER, Kissoon-Singh V, Moreau F, Chadee K. Colonic MUC2 mucin regulates the expression and antimicrobial activity of β-defensin 2. Mucosal Immunol. 2015; **8**(6): 1360–72.

65. Shan M, Gentile M, Yeiser JR, et al. Mucus enhances gut homeostasis and oral tolerance by

663 delivering immunoregulatory signals. Science (80-). 2013; **342**(6157): 447–53.
664 66. Bergstrom KSB, Kissoon-Singh V, Gibson DL, et al. Muc2 Protects against Lethal Infectious
665 Colitis by Disassociating Pathogenic and Commensal Bacteria from the Colonic Mucosa. Roy
666 CR, editor. PLoS Pathog. 2010; **6**(5): e1000902.
667 67. Gipson IK, Spurr-Michaud S, Tisdale A, Menon BB. Comparison of the Transmembrane Mucins
668 MUC1 and MUC16 in Epithelial Barrier Function. Batra SK, editor. PLoS One. 2014; **9**(6):
669 e100393.
670 68. Tu Q V., McGuckin MA, Mendz GL. Campylobacter jejuni response to human mucin MUC2:
671 Modulation of colonization and pathogenicity determinants. J Med Microbiol. 2008; **57**(7):
672 795–802.
673 69. Caballero-Franco C, Keller K, Simone C De, Chadee K. The VSL#3 probiotic formula induces
674 mucin gene expression and secretion in colonic epithelial cells. Am J Physiol - Gastrointest
675 Liver Physiol. 2007; **292**(1): 315–22.
676 70. McNamara N, Basbaum C. Signaling networks controlling mucin production in response to
677 Gram-positive and Gram-negative bacteria. Glycoconj J. 2001; **18**(9): 715–22.
678 71. Beatty PL, Narayanan S, Gariépy J, Ranganathan S, Finn OJ. Vaccine against MUC1 antigen
679 expressed in inflammatory bowel disease and cancer lessens colonic inflammation and
680 prevents progression to colitis-associated colon cancer. Cancer Prev Res. 2010; **3**(4): 438–46.
681 72. Fernandez HR, Lindén SK. The aspirin metabolite salicylate inhibits lysine acetyltransferases
682 and MUC1 induced epithelial to mesenchymal transition. Sci Rep. 2017; **7**(1): 5626.
683 73. Pai P, Rachagani S, Dhawan P, Batra SK. Mucins and Wnt/β-catenin signaling in
684 gastrointestinal cancers: an unholy nexus. Carcinogenesis. 2016; **37**(3): 223.
685 74. Piyush T, Chacko AR, Sindrewicz P, Hilkens J, Rhodes JM, Yu L-G. Interaction of galectin-3 with
686 MUC1 on cell surface promotes EGFR dimerization and activation in human epithelial cancer
687 cells. Cell Death Differ. 2017; **24**(11): 1937–47.
688 75. Pino V, Ramsauer VP, Salas P, Carothers Carraway CA, Carraway KL. Membrane mucin Muc4

induces density-dependent changes in ERK activation in mammary epithelial and tumor cells ROLE in reversal of contact inhibition. J Biol Chem. 2006; **281**(39): 29411–20.

76. Pai P, Rachagani S, Dhawan P, et al. MUC4 is negatively regulated through the Wnt/β-catenin pathway via the Notch effector Hath1 in colorectal cancer. Genes Cancer. 2016; **7**(5–6): 154–68.

77. Senapati S, Chaturvedi P, Sharma P, et al. Deregulation of MUC4 in gastric adenocarcinoma: Potential pathobiological implication in poorly differentiated non-signet ring cell type gastric cancer. Br J Cancer. 2008; **99**(6): 949–56.

78. Luu Y, Junker W, Rachagani S, et al. Human intestinal MUC17 mucin augments intestinal cell restitution and enhances healing of experimental colitis. Int J Biochem Cell Biol. 2010; **42**(6): 996–1006.

79. Raina D, Ahmad R, Chen D, Kumar S, Kharbanda S, Kufe D. MUC1 oncoprotein suppresses activation of the ARF-MDM2-p53 pathway. Cancer Biol Ther. 2008; **7**(12): 1959–67.

80. Supruniuk K, Radziejewska I. MUC1 is an oncoprotein with a significant role in apoptosis (Review). Int J Oncol. 2021; **59**(3): 68.

81. Benjamin JBE, Jayanthi V, Devaraj H. MUC1 expression and its association with other aetiological factors and localization to mitochondria in preneoplastic and neoplastic gastric tissues. Clin Chim Acta. 2010; **411**(23–24): 2067–72.

82. Pichinuk E, Chalik M, Benhar I, et al. In vivo anti-MUC1+ tumor activity and sequences of high-affinity anti-MUC1-SEA antibodies. Cancer Immunol Immunother. 2020; **69**(7): 1337–52.

83. Li W, Zhang N, Jin C, et al. MUC1-C drives stemness in progression of colitis to colorectal cancer. JCI Insight. 2020; **5**(12): e137112.

84. Pothuraju R, Chaudhary S, Rachagani S, et al. Mucins, gut microbiota, and postbiotics role in colorectal cancer. Gut Microbes. 2021; **13**(1): 1974795.

85. Guo M, Luo B, Pan M, Li M, Zhao F, Dou J. MUC1 plays an essential role in tumor immunity of colorectal cancer stem cell vaccine. Int Immunopharmacol. 2020; **85**: 106631.

86. Gao T, Cen Q, Lei H. A review on development of MUC1-based cancer vaccine. Biomed Pharmacother. 2020; **132**: 110888.

87. Morse MA, Niedzwiecki D, Marshall JL, et al. A randomized phase II study of immunization with dendritic cells modified with poxvectors encoding CEA and MUC1 compared with the same poxvectors plus GM-CSF for resected metastatic colorectal cancer. Ann Surg. 2013; **258**(6): 879–86.

88. Kobayashi M, Sakabe T, Chiba A, et al. Therapeutic effect of intratumoral injections of dendritic cells for locally recurrent gastric cancer: A case report. World J Surg Oncol. 2014; **12**(1): 1–6.

89. Almasmoum H. The Roles of Transmembrane Mucins Located on Chromosome 7q22.1 in Colorectal Cancer. Cancer Manag Res. 2021; **13**: 3271.

90. Song ZB, Gao SS, Yi XN, et al. Expression of MUC1 in esophageal squamous-cell carcinoma and its relationship with prognosis of patients from Linzhou city, a high incidence area of northern China. World J Gastroenterol. 2003; **9**(3): 404–7.

91. Lee HJ, Nam KT, Park HS, et al. Gene Expression Profiling of Metaplastic Lineages Identifies CDH17 as a Prognostic Marker in Early Stage Gastric Cancer. Gastroenterology. 2010; **139**(1): 213.

92. Shou X, Li Y, Hu W, et al. Six-gene Assay as a new biomarker in the blood of patients with colorectal cancer: establishment and clinical validation. Mol Oncol. 2019; **13**(4): 781–91.

93. Ge W, Hu H, Cai W, et al. High-risk Stage III colon cancer patients identified by a novel five-gene mutational signature are characterized by upregulation of IL-23A and gut bacterial translocation of the tumor microenvironment. Int J Cancer. 2020; **146**(7): 2027–35.

94. Peng L, Li Y, Gu H, et al. Mucin 4 mutation is associated with tumor mutation burden and promotes antitumor immunity in colon cancer patients. Aging (Albany NY). 2021; **13**(6): 9043–55.

95. Yang Y, Zhang J, Chen Y, Xu R, Zhao Q, Guo W. MUC4, MUC16, and TTN genes mutation

correlated with prognosis, and predicted tumor mutation burden and immunotherapy efficacy in gastric cancer and pan-cancer. Clin Transl Med. 2020; **10**(4): e155.

96. Zheng L, Zhu C, Gu J, Xi P, Du J, Jin G. Functional polymorphism rs4072037 in MUC1 gene contributes to the susceptibility to gastric cancer: evidence from pooled 6,580 cases and 10,324 controls. Mol Biol Reports 2013 4010. 2013; **40**(10): 5791–6.

97. Kumar S, Cruz E, Joshi S, et al. Genetic variants of mucins: unexplored conundrum. Carcinogenesis. 2017; **38**(7): 671–9.

98. Zhao H, Richardson R, Talebloo N, Mukherjee P, Wang P, Moore A. uMUC1-Targeting Magnetic Resonance Imaging of Therapeutic Response in an Orthotropic Mouse Model of Colon Cancer. Mol Imaging Biol. 2019; **21**(5): 852–60.

99. Maleki F, Masteri Farahani A, Sadeghzadeh N, Mardanshahi A, Abediankenari S. Preparation and evaluation of 99mTc-HYNIC-D(TPPE) as a new targeted imaging probe for detection of colon cancer: Preclinical comparison with 99mTc-HYNIC-EPPT. Chem Biol Drug Des. 2020; **96**(5): 1223–31.

100. Lu S, Catalano C, Huhn S, et al. Single nucleotide polymorphisms within MUC4 are associated with colorectal cancer survival. PLoS One. 2019; **14**(5): e0216666.

Figure legends

Figure 1. Structural overview of secreted and transmembrane mucins. The structure of the canonical sequence is shown here, but for most mucins, multiple (mRNA/protein) isoforms exist. *MUC20 localises to the cell membrane, yet it does not contain a well-defined transmembrane region.

Figure 2. Known molecular mechanisms of MUC1 mediating GI epithelial cell signalling pathways during inflammation and cancer. MUC1 can induce EMT-features by (in)directly interacting with (1) SH3KBP1 and CBL, (2) MYC, (3) ZEB1, (4) β-catenin and (5) NF-κB. MUC1 mediates cell proliferation

and resistance to (genotoxic stress-induced) apoptosis through (in)direct modulation of (1) HSP90 and HSP70, (2) ERBB (EGFR), (3) ABL1, (4) HIPK2, (5) TP53, (6) JNK, (7) NF-κB and (8) PI3K-AKT-S6K.

Figure 3. Known molecular mechanisms of MUC1 mediating hypoxia-induced cell death in GI cancer. 1) MUC1 indirectly induces FOXO3a-induced gene transcription which is involved in oxidant scavenging and DNA damage repair, both important mechanisms to survive in hypoxic conditions. 2) MUC1 will prevent apoptotic/necrotic responses under prolonged or severe hypoxic conditions by inhibiting HIF-1α-mediated stabilization of TP53 and the subsequent induction of pro-death proteins.

Figure 4. Known molecular mechanisms of MUC13 mediating GI epithelial signalling pathways during inflammation and cancer. 1) MUC13 promotes the nuclear translocation of β-catenin, thereby promoting EMT and cell survival. 2) MUC13 can inhibit apoptosis through activation of the anti-apoptotic protein BCL2L1 by modulating various cancer-associated proteins or by mediating NF-κB signalling via IKBKG.

Appendix 1. **Schematic representation of the gastrointestinal mucosal barrier.** The gastrointestinal barrier comprises a mucus barrier, a monolayer of epithelial cells and the inner lamina propria hosting immune cells. Secreted and transmembrane mucins represent the major components of the mucus barrier. Gastrointestinal epithelial cells are tightly linked to each other by intercellular junctions: i.e. tight junctions, adherens junctions and desmosomes. The PAR, Crumbs, and Scribble polarity complexes regulate the polarized expression of membrane proteins in the epithelial cells. DC: dendritic cell; NK cell: natural killer cell; AMPs: antimicrobial peptides.

Appendix 2. The organization of the epithelium and the two-layered mucus system (i.e. an inner, attached sterile mucus and an outer, loose mucus layer) in the stomach and colon. The outer mucus layer of the stomach contains low numbers of bacteria (i.e. $<10^4$/ml) whereas the numbers of bacteria are greater in the colonic outer mucus layer (i.e. $10^9 – 10^{12}$/ml).

Figure 1

Click here to access/download;Figure;Figure1_R1.png

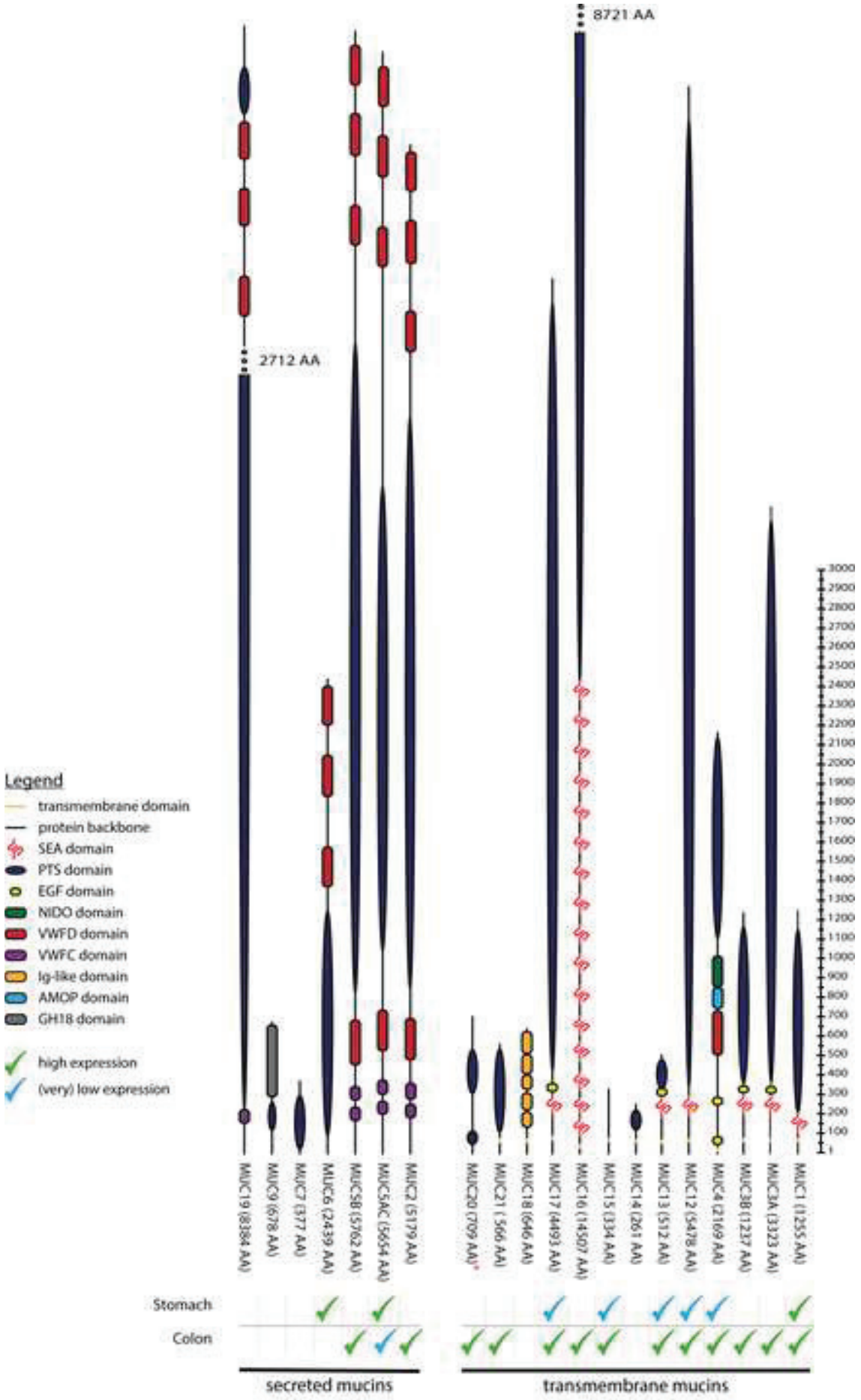
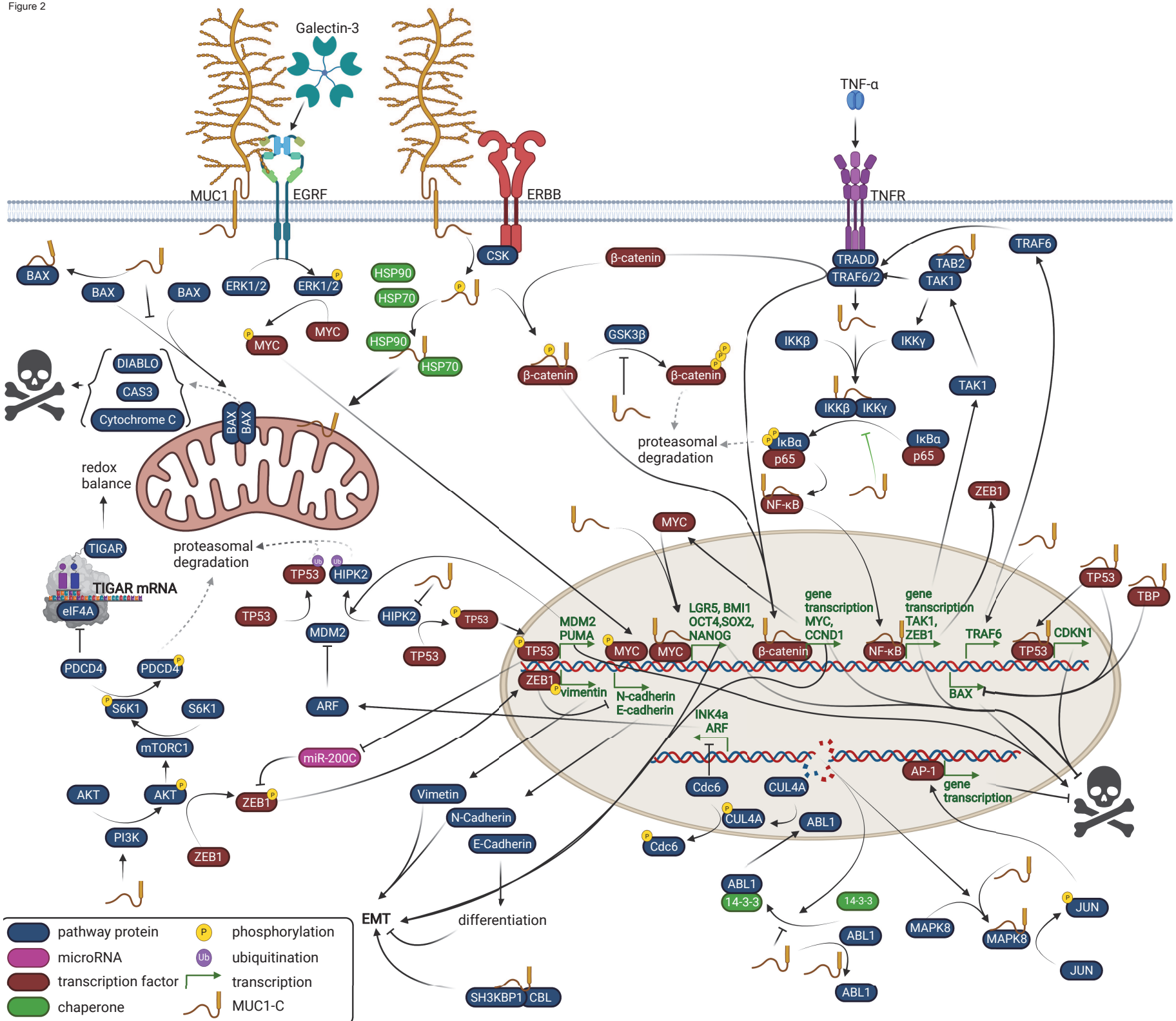


Figure 2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EGRF
ERBB
CSK
TNF-α
TNFR
TRADD
TRAF6/2
TAB2
TAK1
TRAF6
β-catenin
GSK3β
proteasomal degradation
IKKβ
IKKγ
IκBα
p65
NF-κB
BAX
ERK1/2
HSP90
HSP70
MYC
DIABLO
CAS3
Cytochrome C
redox balance
TIGAR
TIGAR mRNA
eIF4A
PDCD4
S6K1
mTORC1
AKT
PI3K
TP53
HIPK2
MDM2
ARF
miR-200C
ZEB1
Vimetin
N-Cadherin
E-Cadherin
EMT
differentiation
SH3KBP1
CBL
MDM2
PUMA
LGR5, BMI1
OCT4,SOX2,
NANOG
gene transcription
MYC, CCND1
TAK1, ZEB1
TRAF6
CDKN1
TBP
vimentin
N-cadherin
E-cadherin
INK4a
ARF
Cdc6
CUL4A
AP-1
gene transcription
ABL1
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JUN
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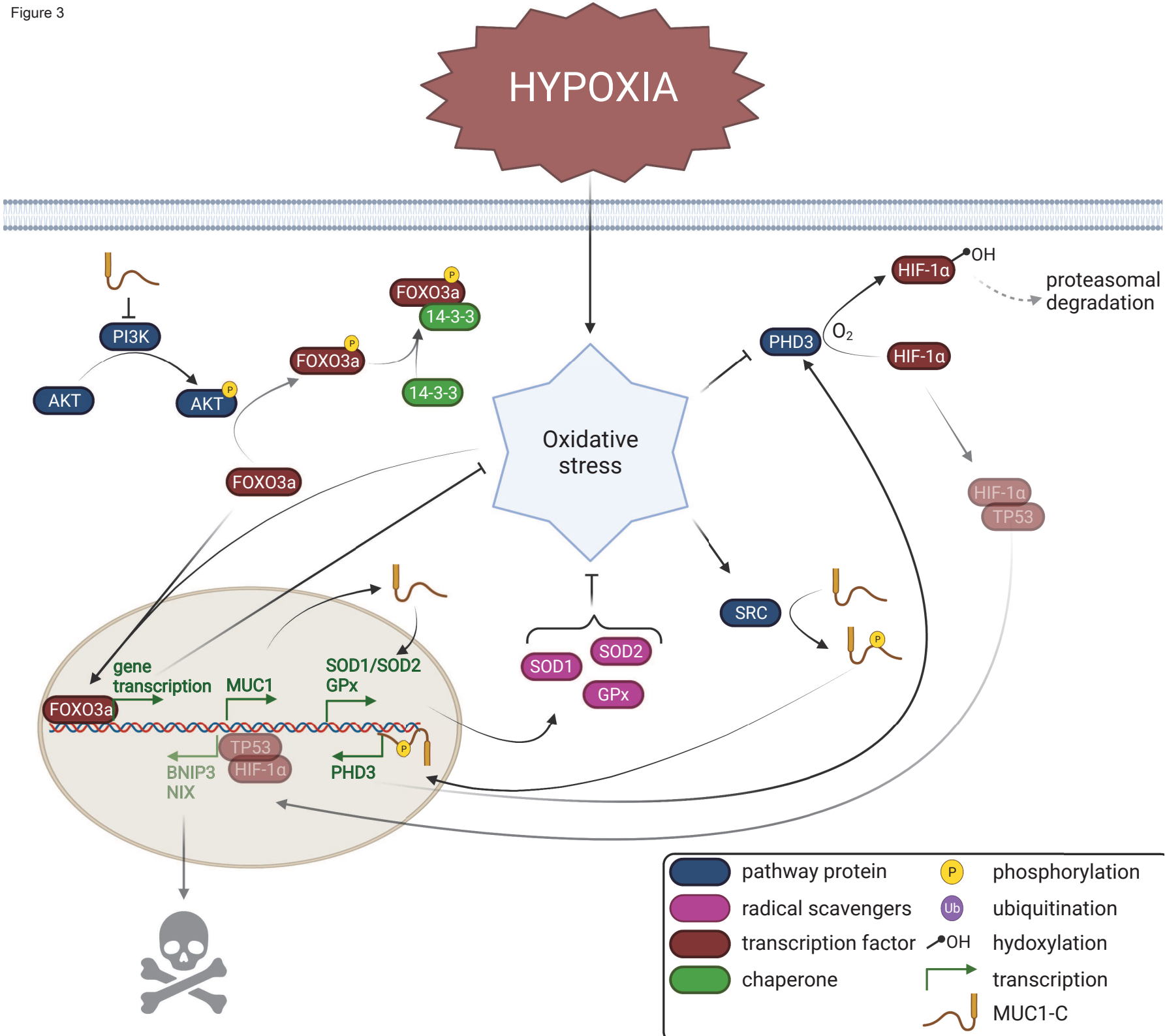
microRNA
transcription factor
chaperone
phosphorylation
ubiquitination
transcription
MUC1-C

Figure 3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PI3K
AKT
AKT
FOXO3a
FOXO3a
14-3-3
FOXO3a
14-3-3
FOXO3a
Oxidative stress
PHD3
O2
HIF-1α
OH
proteasomal degradation
HIF-1α
TP53
SRC
SOD1
SOD2
GPx
gene transcription
MUC1
SOD1/SOD2
GPx
FOXO3a
TP53
HIF-1α
BNIP3
NIX
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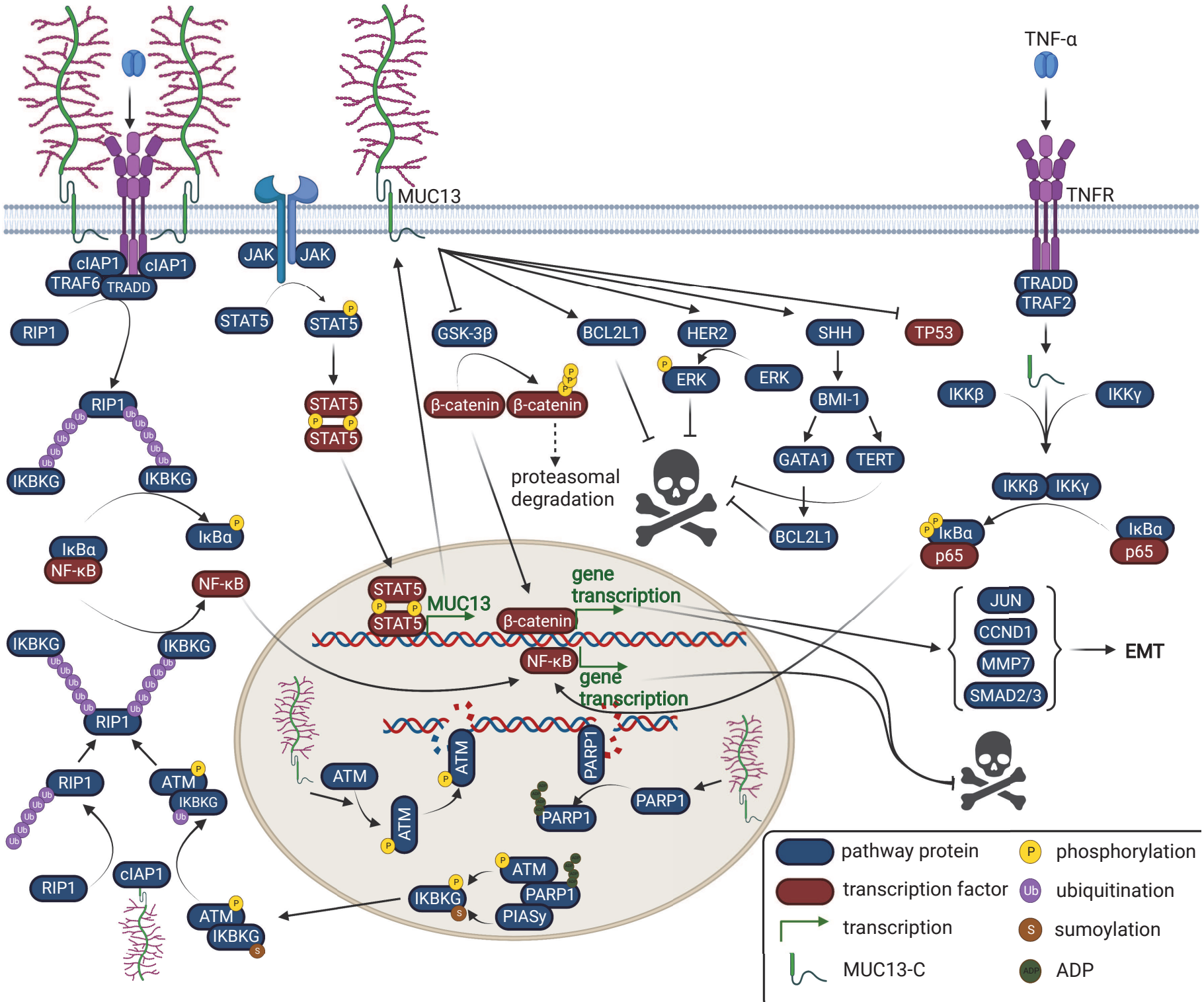
radical scavengers
transcription factor
chaperone
phosphorylation
ubiquitination
hydoxylation
transcription
MUC1-C

Figure 4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MUC13
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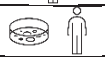
cIAP1
TRAF6
TRADD
RIP1
JAK
JAK
STAT5
STAT5
STAT5
STAT5
GSK-3β
β-catenin
β-catenin
proteasomal degradation
BCL2L1
HER2
ERK
ERK
SHH
BMI-1
GATA1
TERT
BCL2L1
TP53
TRADD
TRAF2
IKKβ
IKKγ
IKKβ
IKKγ
IκBα
p65
IκBα
p65
RIP1
IKBKG
IKBKG
IκBα
NF-κB
NF-κB
STAT5
STAT5
MUC13
β-catenin
gene transcription
NF-κB
gene transcription
JUN
CCND1
MMP7
SMAD2/3
EMT
IKBKG
IKBKG
RIP1
RIP1
ATM
IKBKG
ATM
ATM
PARP1
PARP1
PARP1
RIP1
cIAP1
ATM
IKBKG
IKBKG
ATM
PARP1
PIASy
pathway protein
transcription factor
transcription
MUC13-C
phosphorylation
ubiquitination
sumoylation
ADP

Table 1. Overview of known bacterial-mucin interactions in the GI tract.

Bacterial species	Pathogen /commensal	Bacterial protein	Target	Model	Ref.
MUCIN ADHESION					
Mucus binding protein					
Lactobacillus fermentum	commensal	MucBP	GalNAc; GlcNAc;Gal; Neu5Ac		19
Lactobacillus lactis	commensal	MbpL	MUC3, MUC5AC		20
Lactobacillus reuteri	commensal	MucBP	GalNAc; GlcNAc;Gal; Neu5Ac		19-21
Lactobacillus plantarum	commensal	MucBP	mucus		20
Mucin lectins					
Bifidobacterium longum (infantis)	commensal	F1SBPs	O-glycans		21
Vibrio cholerae	pathogen	GbpA	GlcNAc		20,21
Flagella, pili, fimbriae					
Bacillus cereus	commensal	flagellin	mucus		22
Clostridium difficile	pathogen	flagellin (FliC; FliD)	mucus		20,21,23
*Escherichia coli (*nissle)	commensal	flagellin	O-glycans		21
Escherichia coli (EHEC)	pathogen	flagellin (H7)	core 2 O-glycans		20,21
Escherichia coli (EPEC)	pathogen	flagellin (H6)	core 2 O-glycans		20,21
Pseudomonas aeruginosa	pathogen	flagellin	MUC1		21
Escherichia coli (EAEC)	pathogen	fimbriae	MUC1		24
Escherichia coli (EHEC)	pathogen	fimbriae (FimH)	mannose		22
Escherichia coli (ETEC)	pathogen	fimbriae (F17G; EtpA; CfaE)	resp. GlcNAc; O-glycans; sialylated mucin		25,26
Escherichia coli (STEC)	pathogen	fimbriae (FedF)	H antigens of type 1		26
Escherichia coli (UPEC)	uropathogen	fimbriae (F9; FimH)	resp. Galβ1,3N-GalNAc; mannose		26
Klebsiella pneumoniae	pathogen	fimbriae (fimA)	mannose		27
*Salmonella enterica (*typhimurium*)*	pathogen	fimbriae (Std, FimH)	resp. α1->2fucosylation; mannose		26
Enterococcus faecium	pathogen	pilus (PilB)	mucus		28
Lactobacillus johnsonii	commensal	pilus (SpaC)	mucus		19,21
Lactobacillus paracasei	commensal	pilus	α2->3sialylation; α1->2fucosylation; blood groups A, B, O; Lewis x, y and b		29
Lactobacillus rhamnosus	commensal	pilus (SpaC)	mucus		19,21
Streptococcus gallolyticus	commensal	pilus (Pil3)	MUC5AC		30
Blood group binding adhesins					
Campylobacter jejuni	pathogen	Carbohydrate-lectin, FlaA, MOMP	Fucα1, 2; Gal1, 4; GlcNAc		20,21
Helicobacter pylori	pathogen	BabA; SabA; LabA	resp. lewis b; lewis x and a; LacdiNAc		20
Lactobacillus mucosae	commensal	Lam29	blood groups A and B antigens		20
Salmonella enterica	pathogen	SiiE adhesin	MUC1		31
Other					
Bacteroides fragilis	commensal	NanU	Neu5Ac		21,23
Bacteroides thetaiotaomicron	commensal	SusD-like protein	LacNAc		23

Bifidobacterium breve	commensal		mucus		32
Bifidobacterium bifidum	commensal	transaldose, sialidase	type A antigen		20,26
Bifidobacterium dentium	commensal		MUC2		33
Bifidobacterium longum (longum)	commensal	transaldolase	mucus		34
Fusobacterium nucleatum	pathogen		MUC2		35
Klebsiella pneumoniae	pathogen	LPS, capsular polysaccharide	mucus		27
Lactobacillus acidophilus	commensal	SlpA, GAPDH	mucus		19,20
Lactobacillus fermentum	commensal	MapA	mucus		20
Lactobacillus lactis	commensal	AggL	mucus		20
Lactobacillus plantarum	commensal	Msa, GAPDH	resp. mannose, blood groups A and B antigens		19,20
Lactobacillus reuteri	commensal	MapA, CmbA, EF-Tu	resp. mucus, mucus, sulphated carbohydrates		19,20
Lactobacillus rhamnosus	commensal	MBF	mucus		20
Listeria Monocytogenes	pathogen	LmiA	MUC2		21,26
Ruminococcus gnavus	pathogen	RgNanH (trans-sialidase)	α2,3- or α2,6-Sialyllactose		23
MUCIN DEGRADATION					
Akkermansia muciniphila; Bacteroides fragilis; Bacteroides thetaiotaomicron; Bifidobacterium bifidum	commensal	glycohydrolases*			26,36
Clostridium perfringens; Clostridium difficile; Ruminococcus gnavus; Salmonella typhimurium	pathogen	glycohydrolases*			26,36
Akkermansia muciniphila; Bacteroides fragilis; Bacteroides ovatus; Bacteroides thetaiotaomicron; Bifidobacterium breve; Bifidobacterium fragilis; Ruminococcus torques	commensal	sulfatases			20,26,36
Helicobacter pylori; Prevotella RS2; Pseudomonas Aeruginosa; Streptococcus oralis	pathogen	sulfatases			20,26,36
*Akkermansia muciniphila; Bacteroides thetaiotaomicron; Acinetobacter; Escherichia coli (*SE-11*;* nissile)	commensal	proteases			37
Citrobacter rodentium; Clostridium perfringens; Escherichia coli (AIEC; EAEC; EHEC; ETEC; STEC; UPEC); *Pseudomonas aeruginosa; Shigella flexneri; Streptococcus pneumoniae; Vibrio cholerae*	pathogen	proteases			37
MUCIN EXPRESSION/INHIBITION					
Bacteroides thetaiotaomicron	commensal		increase MUC2, MUC4		20,23
Bifidobacterium animalis (lactis)	commensal		increase MUC2		38
Bifidobacterium dentium	commensal	acetate	increase MUC2		33
Campylobacter jejuni	pathogen		increase MUC1		39,40
Clostridium difficile	pathogen	C difficile toxin A	decrease MUC2; increase MUC1		13,40
Citrobacter rodentium	pathogen		increase MUC1; decrease MUC2		40
Escherichia coli	commensal	LPS	increase MUC5AC; MUC2; MUC5B		13,41
*Escherichia coli (*Nissile)	commensal		increase MUC2, MUC3, MUC5AC, MUC5A		42

atypical *EPEC*	pathogen		increase MUC2, MUC5AC, MUC3,MUC4		2
EAEC	pathogen	Pic	increase MUC2, MUC5AC		43,44
UPEC	pathogen	Pic	increase MUC2, MUC5AC		43,44
Faecalibacterium prausnitzii	commensal		increase MUC2, MUC4		20,23
Fusobacterium nucleatum	pathogen		increase MUC2		45
Helicobacter pylori	pathogen	LPS	decrease MUC5AC and increase MUC1		13
Lactobacillus	commensal	p40	increase MUC2, MUC3		13,46
Lactobacillus acidophilus	commensal		increase MUC2		38
Lactobacillus casei	commensal		increase MUC2		19,42
Lactobacillus plantarum	commensal	exopolysaccharide 116	increase MUC2		38,47
Lactobacillus rhamnosus	commensal		increase MUC2		48
Listeria monocytogenes	pathogen	Listerio-lysin 0	increase MUC3, MUC4, MUC12		13
Pseudomonas aeruginosa	pathogen	LPS	increase MUC5AC; MUC2; MUC5B		13
Ruminococcus gnavus	pathogen	rgE1	increase MUC1, MUC2		49
Shigella flexneiri	pathogen	Pic	increase MUC2, MUC5AC		43,44
Streptococcus thermophilus	commensal		increase MUC2		38
Vibrio cholerae	pathogen	cholera toxin	increase MUC2		13

*It is suggested that up to 40% of the gut microbiota encodes glycohydrolases.[4]

[#] Data was obtained from a cell culture model (), animal model (mouse (), rabbit () or bovine ()), and/or human patients ().

Table 2_R1

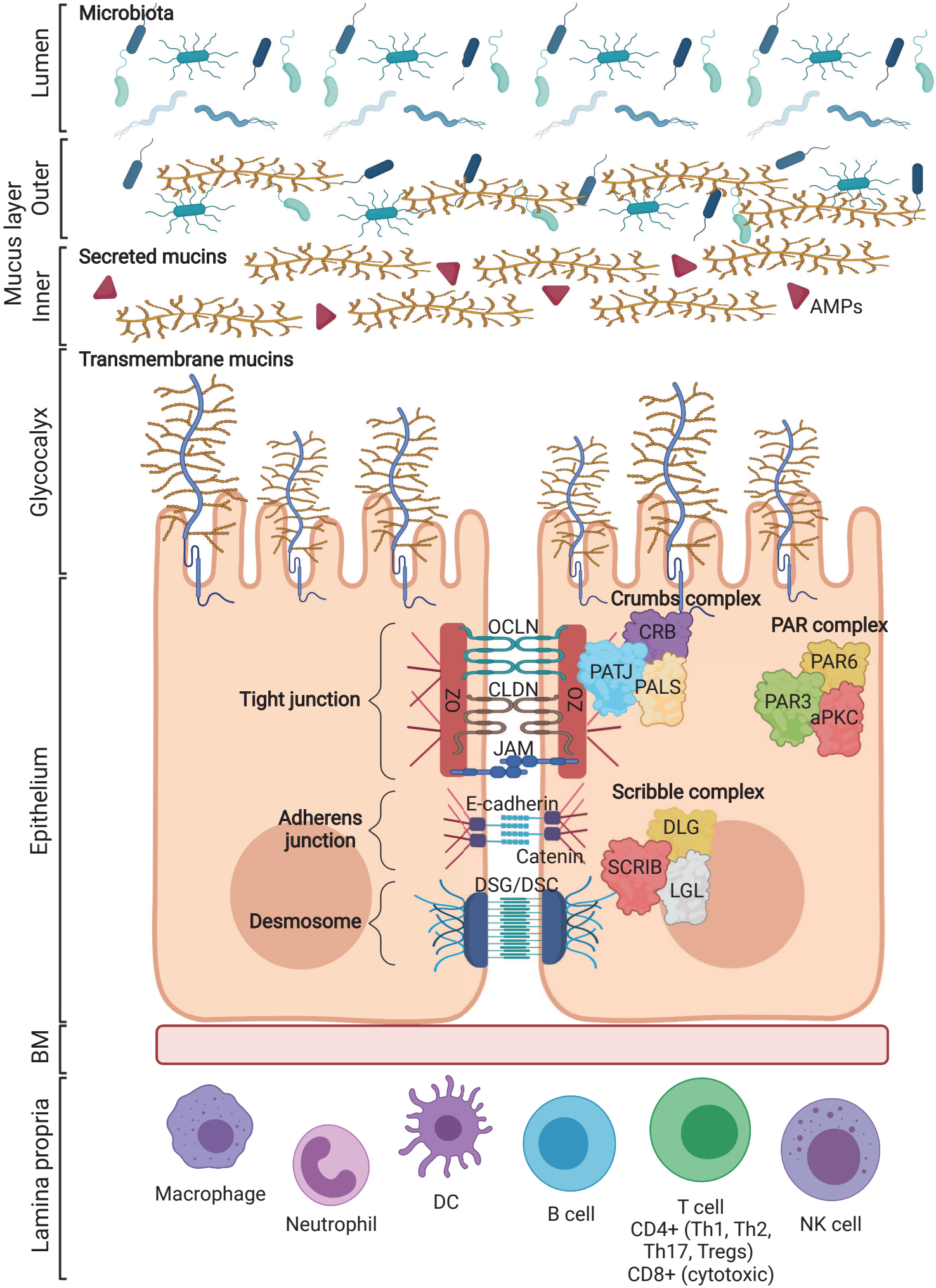
Table 2. Overview of clinical applications involving mucins in the GI pathologies.

Clinical application	Specific effects	Model[#]	Ref.
TARGETING MUCINS FOR THERAPY			
Antibody-based immunotherapy	Antibodies targeting the **MUC1**-SEA domain coupled with PE38-exotoxins induces tumour cell killing proportional to MUC1 expression	C, A	[82]
Mucins as a potential therapeutic target	• Treatment with GO-203, a **MUC1**-C inhibitor peptide, protects against colitis and the progression to colon cancer;	A	[83]
	• Increased sensitivity of **MUC13**-deficient CRC to immune checkpoint inhibitors.	A	[8]
Mucins as a therapeutic agent	Intrarectal administration of recombinant **MUC17** improves intestinal healing post-colitis.	A	[78]
Stimulation of mucin production to enhance the gastrointestinal mucus barrier	• The administration of *Lactobacillus plantarum* and *rhamnosus* increases **MUC2** and **MUC3** expression.	C	[26]
	• FMT re-introduces essential bacterial species for stimulating production of **MUC2** and tight junction proteins.	A	[84]
USING MUCINS IN VACCINES TO ENHANCE TUMOUR IMMUNOTHERAPY			
Vaccination with cancer stem cells	Injection of CRC stem cells with high **MUC1** expression enhances anti-tumour efficacy.	A	[85]
DNA vaccine	Vaccination with a plasmid carrying the fusion gene of survivin/**MUC1** potentiated immunogenicity and the anti-tumour effect in CRC, and prolonged survival. Moreover, the combinatory therapy with oxaliplatin enhanced these effects.	A	[86]
DC vaccines	• The administration of patient-derived DCs mixed with viral vectors carrying CEA, **MUC1**, B7·1, ICAM-1, and LFA-3 promoted overall survival after surgical resection of CRC metastases;	P	[87]
	• The intratumoural injection of MUC1-loaded DCs resulted in the complete disappearance of gastric cancer lesions in 80-year old male patient.	P	[88]
Subunit vaccines	The subcutaneous administration of a peptide mixture containing 100-amino acid synthetic **MUC1** and a TLR3 agonist induced immunogenicity in 43·6% of patients with a history of advanced colonic adenomas.	P	[86]
MUCINS AS BIOMARKERS			
Mucin expression to predict therapy response	Increased sensitivity of **MUC13**-deficient CRC to immune checkpoint inhibitors	A	[8]
Prognosis	• High tumoural **MUC1** or **MUC13** expression is associated with poor survival in CRC;	P	[8,89]
	• High tumoural **MUC4** expression is associated with poor survival in CRC patients with early stage tumours;	P	[89]
	• High tumoural **MUC1** expression is associated with metastasis and survival in oesophageal squamous cell carcinoma;	P	[90]
	• High cytoplasmic expression of **MUC13** in gastric cancer is associated with tumour stage and survival.	P	[91]
	• Algorithmic determination of circulating tumour cells by evaluating the expression of six genes (CEA, EpCAM, CK19, **MUC1**, EGFR and C-Met) enabled a prediction of progression-free survival in CRC patients.	P	[92]
	• The mutational profile of five genes (SMAD4, **MUC16**, COL6A3, FLG and LRP1B) independently predicts recurrence and prognosis in patients with advanced stage colon cancer;	P	[93]
	• **MUC4** mutation in colon cancer is associated with tumour mutation burden and patient prognosis;	P	[94]
	• Mutation status and mutation number of **MUC4**, **MUC16** and **TTN** are predictors for tumour mutation burden, prognosis and immunotherapy efficacy in gastric cancer;	P	[95]
	• The **MUC1** rs4072037 polymorphism is associated with an increased risk of developing gastric cancer as well as recurrence and disease-related death.	P	[96]
	• Specific **MUC1** splice variants are associated with tumour progression and outcome in oesophageal squamous cell carcinoma.	P	[97]
Diagnosis	RT-PCR assay evaluating the expression of six genes (CEA, EpCAM, CK19, **MUC1**, EGFR, and C-Met) correctly diagnoses CRC (87% sensitivity, 85% specificity).	P	[92]
Tumour imaging and therapy response	Magnetic resonance imaging using a dual-modality imaging probe consisting of dextran-coated iron oxide nanoparticles conjugated to the fluorescent dye Cy5·5 and to a underglycosylated **MUC1**-peptide enables the assessment of 5-fluouracil response in CRC.	M	[98]
Imaging for tumour detection	A radiolabeled peptide targeting MUC1 (99mTc-HYNIC- D(TPPE)) can visualize tumours in nude mice bearing HT29 tumours.	M	[99]

DC: dendritic cell; CRC: colorectal cancer

[#]Data was obtained from a cell culture model (C), animal model (A) or human patients (P).

Appendix 1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Inner
Secreted mucins
AMPs
Glycocalyx
Transmembrane mucins
Epithelium
Crumbs complex
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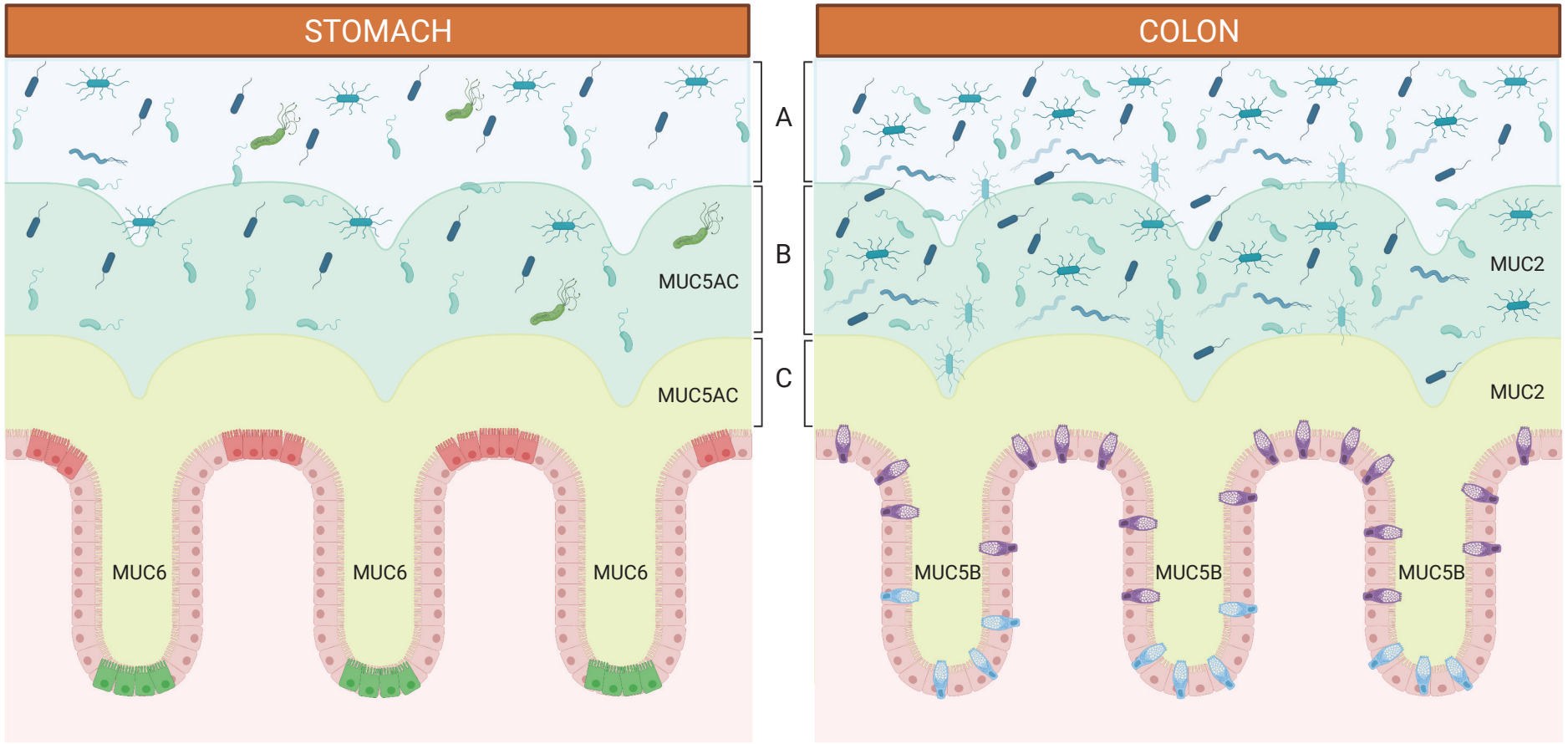
PALS
PAR complex
PAR6
PAR3
aPKC
OCLN
CLDN
JAM
ZO
ZO
Tight junction
Adherens junction
E-cadherin
Catenin
Scribble complex
DLG
SCRIB
LGL
DSG/DSC
Desmosome
BM
Lamina propria
Macrophage
Neutrophil
DC
B cell
T cell
CD4+ (Th1, Th2, Th17, Tregs)
CD8+ (cytotoxic)
NK cell

Appendix 2



MUC5AC-producing mucous cells

MUC6-producing mucous cells

MUC2-producing goblet cell

MUC5B-producing goblet cell

A = lumen

B = outer mucus layer

C = inner mucus layer