Chapter 2
Mucins and Tumor Biology

2.1 Classification

Mucins, also known as MUC glycoproteins, belong to a gene family of over 20 members that are expressed on a tissue-specific basis by specialized epithelial cells at mucosal and secretory surfaces throughout the body (Corfield et al. 2001; Desseyn et al. 2008; Gipson 2005; Govindarajan and Gipson 2010; Hattrup and Gendler 2008; Kreda et al. 2012; Linden et al. 2008; McGuckin et al. 2011; Thornton et al. 2008; Voynow et al. 2006). The classification and distribution of mucin family is summarized in Table 2.1. Based on distinct structural and functional features, mucins are categorized into "membrane-associated" and "secreted" types, with the latter being divided into gel-forming and non-gel-forming subtypes (Rose and Voynow 2006; Williams et al. 2006). The membrane-associated mucins include typical monomeric glycoproteins that are anchored to the cell membrane whereas secreted mucins form extracellular homo-oligomeric structures that are secreted at mucosal and secretory surfaces (Corfield 2015). Both types contribute to the protection of epithelial cells from extracellular insults. The membrane-associated mucins possess a short cytoplasmic tail which participates in intracellular signal transduction (Brayman et al. 2004; Gendler 2001). Hence, these mucins mediate signaling cascades, communicate information about extracellular conditions, and contribute to morphological and behavioral characteristics of the epithelial cells (Hollingsworth and Swanson 2004; Rachagani et al. 2009). Secreted mucins provide a physical barrier for epithelial cells lining the respiratory and gastrointestinal tracts and form the ductal surfaces of organs such as liver, breast, pancreas, and kidney (Kufe 2009). Moreover, they are part of a defensive system at the mucosal surfaces, including intestinal mucosa (Corfield et al. 2000).

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Table 2.1 Mucin family: classification and distribution (Amini et al. 2014b; Corfield 2015)

Designation	Chromosomal location	Tandem repeat size (amino acids)	Main tissue expression
Membrane-associated			
MUC1	1q21	20	Breast, stomach, duodenum, ileum, colon, pancreas, trachea, bronchi, cornea, conjunctiva, middle ear, salivary gland, fallopian tubes, uterus, endometrium, endocervix, ectocervix, vagina
MUC3A/B	7q22	17	Small intestine, colon, gall bladder
MUC4	3q29	16	Breast, respiratory tract, stomach, small intestine, colon, conjunctiva, cornea, endocervix, ectocervix, vagina, endometrium, prostate
MUC11	7q22	28	Gastrointestinal, respiratory, reproductive and urinary tract, thymus, middle ear
MUC12	7q22	28	Colon, stom ach, pancreas, prostate, uterus
MUC13	3q21.2	27	Colon, small intestine, trachea, kidney, middle ear
MUC15	11p14.3	None	Colon, small intestine, esophagus, respiratory tract, salivary gland, thyroid gland, kidney, prostate, testis, placenta
MUC16	19p13.2	156	Cornea, conjunctiva, respiratory tract, endometrium, ovary, middle ear
MUC17	7q22	59	Stomach, duodenum, colon, conjunctiva, fetal kidney
MUC20	3q29	18	Kidney, placenta, colorectum, esophagus, liver, respiratory tract, prostate, middle ear
MUC21	6p21	15	Respiratory tract, thymus, colon, testis
Secreted, gel-forming mucins			
MUC2	11p15.5	23	Jejunum, ileum, colon, endometrium
MUC5AC	11p15.5	8	Respiratory tract, stomach, conjunctiva, lacrimal glands, endocervix, endometrium
MUC5B	11p15.5	29	Respiratory tract, submandibular salivary glands, esophagus, pancreatobiliary epithelia, endocervix
MUC6	11p15.5	169	Stomach, duodenum, ileum, hepatobiliary tract, pancreas, endocervix, endometrium
MUC19	12q12	19	Salivary glands, submucosal gland of the tracheal tissue, cornea, conjunctiva, lacrimal glands
Secreted, non-gel-forming mucins			
MUC7	4q13-q21	23	Oral cavity, sublingual and submandibular salivary gland, respiratory tract, submucosal glands of the bronchus, conjunctiva, pancreas
MUC8	12q24.3	13/41	Normal Human Nasal epithelial (NHNE) cells, middle ear, endocervix, endometrium
MUC9	1p13	15	Fallopian tubes

2.2 Molecular Structure

Attempts to characterize the molecular nature of mucins have been complicated by biophysical properties such as a relatively large mass (over 10^6 Da), a complex biochemical composition (50–80 % O-linked oligosaccharides), and a tendency to form higher-order structures through polymerization (Carlstedt et al. 1985). Through the cloning of mucin complementary DNAs (cDNAs) in the late 1980s, it was confirmed that some membrane-associated mucins were integral membrane proteins, that mucins contained both O-linked and N-linked oligosaccharides, and that the glycosylation of mucins produced by normal epithelial cells and their malignant counterparts were significantly different (Gendler et al. 1990; Gum et al. 2002; Lan et al. 1990a; Ligtenberg et al. 1990; Pallesen et al. 2002; Williams et al. 1999). Mucins are flexible macromolecular polypeptides identified by the characteristic organization of their monomeric peptide domains. Functions of each domain are described in Table 2.2 (Hollingsworth and Swanson 2004; Corfield 2015). The structural feature common to all mucins is the tandem-repeat domain, which contains tandem repeats of identical or highly similar sequences rich in serine, threonine, and proline residues (Gendler et al. 1987; Gupta and Jentoft 1989; Timpte et al. 1988). The specific sequence and number of tandem repeats is highly variable among different mucins and among orthologous mucins from different species. The tandem repeat provides a scaffold on which cells build oligosaccharide structures. These domains are highly O-glycosylated on serine and threonine residues. Mucin core protein contains from 5 to 500 repeats, and each repeat typically contains from 5 to 100 potential glycosylation sites. O-glycosylation with complex oligosaccharides is crucial to mucin structure and function. Mucin-type oligosaccharides are involved in specific ligand–receptor interactions (McDermott et al. 2001), confer hydroscopic properties (Carlstedt et al. 1985), and might bind various small molecules and proteins. Arrays of tandem repeats provide a high degree of multivalency for oligosaccharide structures, thereby providing a significant degree of stoichiometric power (McDermott et al. 2001). The largest mucins contain over 22,000 amino acids, 50 % of which might be O-glycosylated, which corresponds to a potential stoichiometric amplification of greater than 7500-fold for associated oligosaccharide side chains.

Different normal mucosal tissues within the same individual attach different oligosaccharides to the same mucin core proteins which reflect the distinct requirements of the epithelia (Lan et al. 1990a). Tumors also express oligosaccharide structures that are distinct from the normal epithelia and account for many of the tumor-associated carbohydrate antigens (TACAs) found on adenocarcinomas (Lan et al. 1990a, b). The density of glycosylation of tandem repeats among different normal and tumor cells is also highly variable and is believed to contribute significantly to the normal or aberrant functions observed (Hanisch and Muller 2000).

Table 2.2 Specific mucin domains and their function (Corfield 2015; Hollingsworth and Swanson 2004)

Peptide domain	Mucin	MUC type	Domain features and function
PTS-tandem repeat sequences (VNTR)	All MUCs	Secreted and membrane-associated	Heavily O-glycosylated domains rich in serine, threonine, and proline. Characteristic of mucin core protein. Can be highly polymorphic for length and sequence variability
Signal sequence	All MUCs	Secreted and membrane-associated	Directs insertion to the endoplasmic reticulum and mediates secretion or membrane delivery
Cysteine Rich, CYS domains	MUC2, 5AC, 5B and 19	Secreted	Non-glycosylated multiple copy domains adjacent to or inserted within tandem repeat domains. Important for various mucin-mucin interactions
Cysteine knot	MUC2, 5AC, 5B, 6, and 19	Secreted	Conserved with von Willebrand factor and the cysteine knot of TGF-β. Involved in dimerization
D domain (D1, D2, D′, D3)	MUC2, 5AC, 5B, C6 and 19	Secreted	Shows homology to the dimerization domain of von Willebrand factor and mediates oligomerization
D domain (D4)	MUC2, 4, 5AC, 5B and 6	Secreted and membrane-associated	Next to the VNTR domain, shows homology to D4 dimerization domain of von Willebrand factor and contains the GDPH autocatalytic cleavage site
Cytoplasmic tail	MUC1, 3, 4, 12, 13, 16, 17 and 21	Membrane-associated	Located on the cytoplasmic side of the cell surface membrane. Contains phosphorylation sites involved in signaling and might mediate association with cytoskeletal elements
SEA domain	MUC1, 3, 12, 13, 17 and 21	Membrane-associated	Widely distributed among heavily O-glycosylated cell surface proteins. Involved in protein binding to carbohydrate moieties. Contains autocatalytic proteolytic cleavage site
Epidermal growth factor (EGF)-like domains	MUC3, 4, 12, 13 and 17	Membrane-associated	Shows homology to EGF and related growth factors and cytokines and mediates interactions between mucin subunits and ErbB receptors
Transmembrane domain	MUC1, 3, 4, 12, 13, 16, 17, 20 and 21	Membrane-associated	Membrane-spanning sequence typical for membrane-associated mucins

(continued)

Table 2.2 (continued)

Peptide domain	Mucin	MUC type	Domain features and function
GDPH autocatalytic proteolytic site	MUC2, 4 and 5AC	Secreted and membrane-associated	Autocatalytic proteolysis site that cleaves between GD and PH residues, prior to formation of a unique covalent bond by which mucin subunits are linked to other secreted molecules
Proteolytic cleavage site	MUC1, 3, 4, 12, 13, 16 and 17	Membrane-associated	Found within the SEA domains of some mucins and outside of the SEA domains in others. Facilitates the creation of mucin subunits that remain associated

ErbB (Erythroblastic Leukemia Viral Oncogene Homolog): a protein family containing four receptor tyrosine kinases structurally related to epidermal growth factor receptor (EGFR), *MUC* mucin, *PTS* proline, threonine, serine, *SEA* (sea-urchin sperm protein, enterokinase and agrin): a domain named after the first three proteins in which it was identified (sperm protein, enterokinase, and agrin), *VNTR* variable number tandem repeat

2.3 Membrane-Associated Mucins

Mucins anchored to the apical cell surface form the largest group of mucins (Table 2.1). In contrast to the secreted mucins, membrane-associated mucins do not form oligomers and gels. These monomeric mucins contain characteristic membrane peptide domains (Table 2.2) and have properties typical of the membrane glycoproteins (Corfield 2015). They are bound to cells by an integral transmembrane domain and have relatively short cytoplasmic tails at the C-terminus that associate with cytoskeletal elements, cytosolic adaptor proteins and/or participate in signal transduction (Carraway et al. 2003). There are also common features that are seen in the extracellular juxtamembrane portions of the membrane-associated mucins. One common feature is a specific proteolytic cleavage that occurs during the intracellular post-translational processing on the juxtamembrane part of the protein that is destined to be expressed on the extracellular surface (Parry et al. 2001). This creates two subunits that remain associated during cellular transport through the endoplasmic reticulum and Golgi complex and at the cell surface. In several membrane-associated mucins, this cleavage is mediated by an unidentified intracellular protease in the SEA domain (Bork and Patthy 1995; Wreschner et al. 2002). Most membrane-associated mucins have juxtamembrane domains with homology to the epidermal growth factor (EGF) family (Gum et al. 1997b). These EGF-like domains are postulated to allow interaction with members of the EGF receptor (ErbB) family, thereby participating in the intracellular pathways related to growth, motility, differentiation, inflammation, or other higher-order functions (Carraway et al. 2000; Jepson et al. 2002). The number and general arrangement of EGF domains shows some conservation among membrane-associated mucins. Several mucins, including MUC3A, MUC3B, MUC4, MUC12, MUC13, and MUC17, have

two or three EGF domains. The EGF domains of MUC3A, MUC12, MUC13, and MUC17, but not that of MUC4, are separated by the SEA domain. One EGF domain is located on the extracellular subunit that contains the tandem-repeat domain, and a second (and, in some cases, third) EGF domain is located on the extracellular side of the membrane-associated subunit, proximal to the cell surface (Hollingsworth and Swanson 2004). MUC1 contains a SEA domain (Bork and Patthy 1995) and has been found to be associated with lipid rafts. However, it has no clearly defined extracellular EGF-like domains. Interestingly, it has been co-immunoprecipitated with ErbB1 (also known as EGFR) from human breast cancer cells (Li et al. 2001c) and all four ErbB members in mammary glands of MUC1-transgenic mice (Schroeder et al. 2001), indicating that they are associated directly or indirectly in molecular complexes. MUC1 also co-localizes with ErbB1 in lactating mammary glands and the stimulation of breast cancer cell lines with EGF, amphiregulin, or transforming growth factor-a (TGF-α) leads to phosphorylation of the MUC1 cytoplasmic tail on tyrosine and to its association with tyrosine phosphorylated proteins of 180 kDa (presumably one or more ErbB family members). It has been postulated that altered extracellular pH, ionic concentration, and hydration or other adverse conditions might lead to release of the extracellular domains, which might facilitate rapid clearance of cell surface-associated material (Hollingsworth and Swanson 2004). Autocatalytic peptide cleavage within the SEA domain leads to the formation of a non-covalent complex (Macao et al. 2006) that allows the release of the large extracellular mucin component into the mucus gel layer, while the membrane-specific domain is retained in the membrane (Thathiah et al. 2003; Thathiah and Carson 2004; Williams et al. 2001). The prototypical MUC1 is the membrane-associated mucin of relevance to the present project which is further discussed here.

2.3.1 MUC1

MUC1 (also known as episialin, PEM, H23Ag, EMA, CA15-3, and MCA) is a heterodimeric type I transmembrane protein with a heavily glycosylated extracellular domain that extends up to 200–500 nm from the cell surface (Nath and Mukherjee 2014). MUC1 is encoded by *MUC1* gene located on the long arm (q) of chromosome 1 at position 21. The human *MUC1* gene spans 4–7 kb and is comprised of 7 exons that can be alternatively spliced to form transcripts from 3.7 to 6.4 kb (Gendler and Spicer 1995; Lagow et al. 1999). In humans, there are several isoforms of MUC1 that result from alternative splicing, exon skipping, and intron retention. A recent study identified 78 isoforms of MUC1 (Zhang et al. 2013), with the most common isoforms being MUC1/A, MUC1/B, MUC1/C, MUC1/D, MUC1/X (or MUC1/Z), MUC1/Y, and MUC1/ZD. MUC1/A, MUC1/B, MUC1/C, and MUC1/D, encoding "full-length" MUC1, arise from alternative splicing between sites located in intron I and exon 2 and vary only by VNTR length (Ligtenberg et al. 1990; Obermair et al. 2001). MUC1/B is the so-called normal MUC1 mRNA. MUC1/X (or MUC1/Z), MUC1/Y, and MUC1/ZD isoforms are generated from alternative

splice acceptor sites located within exon 2, where VNTR encoding exon 2 is skipped (Oosterkamp et al. 1997; Zrihan-Licht et al. 1994). The MUC1/Y isoform is 54 bp shorter than MUC1/X and is highly expressed in breast, ovarian, and prostate cancer cells (Baruch et al. 1997; Hanisch and Muller 2000; Schut et al. 2003). MUC1/ZD also lacks the VNTR region and the flanking degenerate sequence, but contains a unique C terminal domain (43 amino acids) that results from a shift in the reading frame (Levitin et al. 2005a). A secreted isoform of MUC1 called MUC1/SEC that lacks both the TMD and CT binds to MUC1/Y causing phosphorylation of the tyrosine residues of MUC1/Y [38]. Presently, there is a lack of clear understanding of the functional significance of each of these spliced MUC1 variants [reviewed by (Nath and Mukherjee 2014)].

The MUC1 gene encodes a single polypeptide chain which, due to conformational stress, is autoproteolytically cleaved immediately after translation at the GSVVV motif, located within the SEA domain, into two peptide fragments: the longer N-terminal subunit (MUC1-N) and the shorter C-terminal subunit (MUC1-C) (Hattrup and Gendler 2008; Levitin et al. 2005b). Extracellularly, the two subunits remain associated through stable hydrogen bonds. MUC1-N is composed of the proline, threonine, and serine-rich (PTS) domain and the SEA domain. The PTS domain, also designated as the variable number tandem repeat (VNTR) region, is encoded by a highly polymorphic exon encoding for multiple 20–21 amino acid sequence repeats (Gendler et al. 1990). In northern Europeans, the VNTR is composed of 20–120 repeats, with 40–80 repeats being the most common (Hanisch and Muller 2000). The amino acid sequence of the VNTR region can vary in different cancer cell lines, consistent with the highly polymorphic nature of this motif (Muller et al. 1999). The VNTR region is flanked on both ends by a short degenerate sequence which bears subtle sequence similarity to the VNTR region (Hanisch and Muller 2000). MUC1 is extensively O-glycosylated and moderately N-glycosylated to yield mature functional mucin (Gendler 2001). MUC1 core protein and the mature glycosylated form have an estimated weight of 120–225 kDa and 250–500 kDa, respectively (Gendler and Spicer 1995; Lagow et al. 1999). The full-length protein contains three domains: short cytoplasmic (72 amino acids) and transmembrane (28 amino acid) domains that are highly conserved among species (Spicer et al. 1995), as well as a large extracellular domain (1000–2200 amino acids). The proline residues and glycosylation give rise to a rigid, extended structure that protrudes 200–500 nm above the cell surface, much farther than the distance spanned by most cell surface proteins, including syndecans and integrins [reviewed by (Brayman et al. 2004)]. Under normal conditions, MUC1 exists on the plasma membrane as a heterodimeric complex. However, the complex dissociates following stimulation with the proinflammatory cytokines interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α), and this is catalyzed by the sheddase activities of the enzymes including TNF-a converting enzyme (TACE, also called disintegrin and metalloprotease domain containing protein 17 (ADAM17)) and matrix metalloproteinases (MMPs). These enzymes cause release of MUC1-N from MUC1-C, and also catalyze the cleavage of the 58 amino acid ECD of MUC1-C, thereby generating smaller peptide fragments MUC1* and MUC1-CTF_{15} [reviewed by (Nath and

Mukherjee 2014)]. It has been reported that MUC1* can promote tumor growth (Mahanta et al. 2008) and also function as a growth factor receptor for a metastasis-associated protein (NM23-H1) in human embryonic stem cells (hESCs) (Smagghe et al. 2013).

2.4 Secreted Mucins

Lacking a transmembrane domain, these mucins are secreted into the extracellular space, remain at the apical surface, and form oligomers and gels (Table 2.1), with an evolutionary history going back to early metazoans (Lang et al. 2007). Genes that encode the gel-forming mucins are believed to have arisen by duplication from a common ancestor. They share some sequence homology and are clustered in the order *MUC6/MUC2/MUC5AC/MUC5B* on chromosome 11p15 (Pigny et al. 1996; Desseyn et al. 1998a). Secreted mucins show patterns of expression that are restricted to the secretory organs and cell types (Hollingsworth and Swanson 2004). The 5′ genomic regions of MUC2, MUC5AC, and MUC5B are composed of 29 or 30 exons that encode for cysteine-rich domains that are similar to structural domains, termed D1, D2, D′, and D3 domains, within von Willebrand factor (vWF) (Desseyn et al. 1998b; Escande et al. 2001; Gum et al. 1992). These D domains are important for the disulfide-mediated polymerization of this blood glycoprotein. Likewise, the 3′ genomic region of MUC2, MUC5AC, and MUC5B is composed of 18 exons that also code for cysteine-rich vWF-like domains (D4, B, C, and cysteine-knot (CK)) (Buisine et al. 1998; Desseyn et al. 1997; Gum et al. 1992). Each secretory mucin has a central region with a VNTR, but there is little similarity, among the different mucins, in the sequences of the VNTR-encoded threonine-, serine- and proline-rich repeat peptides. Furthermore, the exact sequence of the tandem repeats is poorly conserved between species, suggesting that it is the high content of Thr, Ser, and Pro, rather than the arrangement of these amino acids, that is most important for mucin function. Two structural features that are conserved are the presence of sequences homologous to vW D domains, thought to be involved in mucin oligomerization to form gels, and the C terminal CK motif, with likely involvement in the initial dimerization of apomucin monomers. MUC2, MUC5AC, and MUC5B also have sequences homologous to von Willebrand factor C domains involved in binding of trefoil factors (Tomasetto et al. 2000) and two to seven conserved 108-amino acid cysteine-rich domains (Byrd and Bresalier 2004). The C-terminal region of MUC6, in contrast, does not contain the vW D4, B, and C domains (Rousseau et al. 2004).

The core proteins of secreted gel-forming mucins are very large (typically greater than 5000 amino acids) and their overall structure is predicted to be complex. The ability to form mucin-type gels that are commonly found in the aerodigestive tract results from oligomerization of mucin core proteins. Oligomerization is mediated by D domains (Gum et al. 1994). The oligomeric secreted mucins show a characteristic linkage of monomers through disulphide bridges located in cysteine-rich CK

and vW C and D domains at the N- or C-terminus of the monomers. These domains flank centrally located VNTR sequences which are unique to each MUC gene and which are also PTS rich and serve to carry the glycan chains (Ambort et al. 2011). The molecular weights of mucins are characteristically very high, reflecting a large carbohydrate content, extensive oligomerization, and very large apomucin proteins. The complex, branching network of covalently linked mucin molecules is responsible for their gel-forming properties, which can be destroyed by reduction of the disulphide bonds. It is not known at present if intermolecular bonds are formed between different mucin core-protein backbones; however, it would not be surprising if these did occur and contribute to higher-order structures (Hollingsworth and Swanson 2004).

2.4.1 MUC2

MUC2 is the major structural molecule of the intestinal mucus. The assembly of this large and complex molecule is a major task for the intestinal goblet cell. The human MUC2 is still not fully sequenced, but recent next-generation sequencing suggests that MUC2 is 5100 amino acids long (Pelaseyed et al. 2014). Recent work on MUC2 (Ambort et al. 2011, 2012a, b; Johansson and Hansson 2012; Round et al. 2012) has established the detail of the peptide domain organization and its relation to mucin function and gel formation. MUC2 has two PTS domains and shows the following arrangement: ***N-terminus***, vW D1, D2, D′, D3, cysteine-rich D, small PTS, cysteine-rich D, large PTS, vW D4, vW B, vW C, CK, ***C-terminus*** (Corfield 2015). Rapid dimerization of the translated MUC2 peptide via the cysteine knot (CK) disulphide bridges occurs in the endoplasmic reticulum. Subsequent migration to the Golgi apparatus enables glycosylation of the PTS domain serine and threonine residues with mucin type O-linked glycans. In the trans-Golgi network, the third vW D domain in its N-terminal part is responsible for trimer formation and the macromolecules are concentrated in goblet cell vesicles. This process is analogous to the oligomerization and packing of vWF and is pH and Ca^{2+} ion concentration dependent. The creation of MUC2 trimers is necessary to permit the production of mucus networks at the cell surface and also provides a possible mechanism to account for the dramatic increase in volume seen during mucin secretion (Ambort et al. 2012a, b; Johansson and Hansson 2012; Corfield 2015). MUC2 is arranged in bundles having an association of N-terminal trimer rings linked at right angles to dimers stabilized by C-terminal CK and vW domains. On secretion and hydration of the condensed vesicular mucus granules, stacked planar networks are formed with a volume increase of approximately 3000-fold relative to the cellular granules (Ambort et al. 2011, 2012a, b; Johansson and Hansson 2012; Johnson et al. 2009; Verdugo 2012). The secreted mucins are packed in vesicles where a pH of 5.2, together with a high intragranular Ca^{2+} level, is found. A MUC2 isoform lacking the long TR2 tandem repeat portion designated MUC2.1 has been reported to be generated through alternative splicing (Sternberg et al. 2004).

2.4.2 *MUC5AC*

MUC5AC is one of the major structural molecules of the gastric (Ho et al. 2004) and respiratory tract mucus (Hovenberg et al. 1996a, b). *MUC5AC* gene is clustered with *MUC2*, *MUC5B,* and *MUC6* on chromosome 11p15.5. The 5′ region reveals high degree of sequence similarity with *MUC2* and *MUC5B* and codes for 1336 amino acids organized into a signal peptide, four N-terminal vW D domains (D1, D2, D′ and D3), and a short domain which connects to the central repetitive region. In the central region, coded by a single large exon, 17 major domains have been identified. Nine domains are cysteine-rich domains (Cys-domains 1–9) and exhibit high sequence similarity to the cysteine-rich domains described in the central region of MUC2 and MUC5B. Cys-domains 1–5 are interspersed by four PTS domains and Cys-domains 5–9 are interspersed by four MUC5AC-specific TR domains (TR1-TR4). The C-terminal region of MUC5AC has the cysteine-rich vWF-like domains D4, B, C, and CK (Escande et al. 2001). The CK domain mediates the formation of disulfide-linked dimmers by a pH-dependent, autocatalytic process. This cleavage may be important in pathological conditions, in which changes in pH within cells or at the epithelial surface may result in cross-linking of the mucins, potentially contributing to the aberrant properties in mucus (Desseyn 2009; Thornton et al. 2008). Similar to other secreted mucins, the biosynthesis of MUC5AC must ensure the gene translation, proper folding of the peptide, dimerization, appropriate O-glycosylation, polymerization, and storage. The initial stages of MUC peptide translation include N-glycosylation. The N-linked oligosaccharides direct the precursor peptides to their correct subcellular compartments for dimerization and subsequent O-glycosylation and oligomerization (Dekker and Strous 1990; van Klinken et al. 1998). MUC5AC dimerizes in the rough endoplasmic reticulum, similarly to MUC2 (Asker et al. 1998). However, these two structurally similar secretory mucins seem to have different chaperone requirements in the ER since no interaction of MUC5AC with ER lectins calnexin and calreticulin was detected at the stage of folding and oligomerization (McCool et al. 1999). Monomers and dimers are then transferred to the Golgi apparatus and undergo O-glycosylation (Asker et al. 1998; van Klinken et al. 1998). Once they reach the acidic trans-Golgi compartments, mucins are assembled into large covalent disulfide-linked oligomers/multimers. The process of MUC5AC packing and release is not completely known. It seems that the combination of low pH and high calcium ion concentration allows the packing of the mucin macromolecules in the vesicles and links with the remarkable volume expansion which occurs during secretion (Corfield 2015; Paz et al. 2003; Perez-Vilar et al. 2005).

2.5 Regulation of Mucin Expression

The expression of mucin genes is cell- and tissue-specific but is submitted to variations during cell differentiation and inflammatory process and altered during carcinogenesis. The molecular mechanisms responsible for the control of mucin transcription

and expression are beginning to be understood as mucin gene promoters and regulatory regions are characterized (Van Seuningen et al. 2001).

2.5.1 Regulation of MUC1 Expression

The regulation of MUC1 expression can be transcriptional or post-transcriptional (Nath and Mukherjee 2014). Studies on epigenetic regulation have shown that methylation of histone H3-K9 and the CpG islands in the MUC1 promoter (close to the transcriptional start site; −174 to −182 bp) causes transcriptional repression (Yamada et al. 2008). By contrast, H3-K9 acetylation is permissive of MUC1 expression. Thus, demethylation of CpG and H3-K9, and the acetylation of H3-K9 in the 50 flanking region lead to elevated MUC1 expression in cancer cells (Yamada et al. 2008). The MUC1 promoter contains several putative transcription start sites (Zaretsky et al. 1999) and several cis-acting elements such as binding sites for Sp1, AP1-4, NF-1, NF-κβ, an E-box, GC boxes, peroxisome proliferator-activated receptor (PPAR) responsive region, and estrogen and progesterone receptor sites [reviewed by (Gendler 2001)]. Proinflammatory cytokines such as TNF-α and IFN-γ also induce strong MUC1 induction through the independent actions of NF-κβ p65 and STAT1a (Lagow and Carson 2002). Furthermore, MUC1 expression is regulated post-transcriptionally. MUC1 mRNA contains the seed sequence for microRNA (miR)-125b in the 30 untranslated region (UTR), and loss of miR-125b expression in breast cancer cells contributes to MUC1 overexpression (Rajabi et al. 2010). Navabi et al. demonstrated that infection with *Helicobacter pylori* (HP) reduces the rate of mucin turnover and decreases the levels of Muc1 in the murine gastric mucosa (Navabi et al. 2013).

2.5.2 Regulation of MUC2 and MUC5AC Expression

It appears that *MUC2* and the *MUC5AC* genes have much in common both at the level of sequence homology and in molecular mechanisms responsible for the regulation of the expression (Van Seuningen et al. 2001). The expression of secreted mucins can be altered by methylation of the promoter (Gratchev et al. 2001; Hanski et al. 1997; Mesquita et al. 2003b). At the transcriptional level, 11p15 mucin genes are also regulated by different transcription factors, including ATF-1, cyclic AMP response element-binding protein (CREB), RAR-alpha (Van Seuningen et al. 2001), and Sp1/Sp3 family (Aslam et al. 2001; Gum et al. 1997a; Perrais et al. 2002), as well as by growth factors (EGF, TGF-α), proinflammatory cytokines (interleukin (IL)-1β, IL-6, TNF-α, INF-γ), pleiotropic cytokines (IL-4, IL-13, IL-9), bacterial lipopolysaccharide (LPS) and lipoteichoic acid (LTA), platelet-activating factor (PAF), retinoids, and hormones (Thai et al. 2008). In this regard, different intracellular signaling pathways, including MAPK, protein kinase A (PKA), PKC, PKG, NF-κβ, and Ca^{2+} signaling, have been shown to mediate the regulation of mucin

expression in response to an extracellular insult or during carcinogenesis (Van Seuningen et al. 2001).

Kageyama-Yahara et al. reported that MUC5AC expression is regulated by combination of multiple regulatory mechanisms such as universal transcription factors and epigenetic modulations. They found that Gli, a universal transcription factor, regulates MUC5AC gene expression via direct protein-DNA interaction through a highly conserved region containing a Gli-binding sequence (HCR-Gli) in the promoter of MUC5AC gene in gastrointestinal cells (Kageyama-Yahara et al. 2014). The induced MUC5AC expression was also observed after treatments with DNA demethylation reagent and/or histone deacetylase inhibitor in several cell lines that were deficient in MUC5AC expression. This epigenetic regulation of MUC5AC was in line with another study by Yamada et al. who indicated CpG methylation and histone H3-K9 modification of the MUC5AC promoter distal region as a regulatory mechanism in different cancer cells (Yamada et al. 2010). Perrais et al. showed that while Sp3 is a strong inhibitor of 11p15 mucin gene transcription, transcription factor Sp1 could not only bind and activate MUC2 and MUC5AC promoters, but also contributed to their EGF- and TGFα-mediated upregulation (Perrais et al. 2002). They reported that MUC2 and MUC5AC are two target genes of EGFR ligands in lung cancer cells, and up-regulation of these two genes goes through concomitant activation of the EGFR/Ras/Raf/Erk pathway and Sp1 binding to their promoters. Jonckheere et al. showed an important role for two transcription factors, GATA-4/-6 and HNF-1/-4 families of transcription, as regulators of expression of the murine MUC5AC mucin during stomach development and in epithelial cancer cells (Jonckheere et al. 2012). Kim et al. demonstrated that CREB activation via nonclassical retinoic acid (RA) signaling pathway may play an important role in regulating the expression of MUC2 and MUC5AC mucin genes and mediating the early biological effects of RA during normal mucous differentiation in normal human tracheobronchial epithelial (NHTBE) cells (Kim et al. 2007). Mesquita et al. indicated that human MUC2 mucin gene is transcriptionally regulated by the intestine-specific transcription factor CDX2 in gastrointestinal carcinoma cells (Mesquita et al. 2003a). It has also been described that MUC2 is transcriptionally activated by p53 in human CRC cells (Ookawa et al. 2002). Yang et al. showed that the cell fate determinant Numb promotes MUC2 protein expression and intestinal cell secretion of mucins and modulates intestinal epithelial cell differentiation toward goblet cell phenotype by inhibiting the Notch signaling pathway (Yang et al. 2011b).

In a study by Ho et al., the stimulation of PKA pathway appeared to upregulate MUC5AC (Ho et al. 2002). Hong et al. showed that MUC2 and MUC5AC gene expressions were stimulated by phorbol 12-myristate 13-acetate (PMA), an activator of PKC, in human colonic cell lines (Hong et al. 1999). The induced expression of MUC5AC protein and gene by PMA or EGF has also been confirmed in airway epithelial cells (Kim et al. 2012b). Investigating the regulatory role of IL-1β in human pulmonary epithelial cells, Kim et al. demonstrated that IL-1β activates extracellular signal-regulated kinase (ERK) or p38 to induce cyclooxygenase 2 (COX-2) production, which in turn induces MUC2 and MUC5AC expressions at both the mRNA and protein levels (Kim et al. 2002). IL-1β induction of MUC5AC

gene expression mediated by CREB and NF-κβ has also been observed by Chen et al. in respiratory cells (Chen et al. 2014). The role of pro-inflammatory cytokines such as TNF-α in the induction of MUC5AC gene and protein expression in airway epithelial cells (Fischer et al. 1999; Shao et al. 2003; Song et al. 2003) through NF-κβ signaling pathway has also been reported (Seo et al. 2014). In a study by Iwashita et al., MUC2 was upregulated at mRNA level by IL-4, IL-13, or TNF-α through a MAPK pathway in the human CRC cell lines (Iwashita et al. 2003). Upregulation of MUC2 gene expression by IL-4 and IL-13 in goblet cells has also been shown by Blanchard et al. (2004). In another study, Iwashita et al. also reported that cell attachment regulates MUC5AC production, which is upregulated by low adhesion to the ECM, and MUC5AC production is inversely proportional to the function of integrin β1, a major adhesion molecule between cells and the ECM (Iwashita et al. 2013). It has also been described that MUC2 is transcriptionally activated by LPS from *Pseudomonas aeruginosa* in tracheobronchial epithelial cells (Li et al. 1997, 1998). The downstream cascade known to activate mucin gene transcription was reported to be the Src/Ras/MAPK/pp90rsk cascade, which leads to the activation of the transcription factor NF-κβ. In another study by the same group, the similar mechanism of regulation by LPS was also found for MUC5AC (Dohrman et al. 1998). Induction of serum amyloid A3 protein (SAA3), an acute-phase protein, by Escherichia coli and LPS is also capable of upregulating MUC2 mucin production in colonic epithelial cells (Shigemura et al. 2014). Perrais et al. indicated that infection of GC cells by *Helicobacter pylori*, a causative agent in GC, alters 11p15 mucin gene transcription and induces MUC5AC expression (Perrais et al. 2014). Raja et al. has showed that Shigella dysenteriae-induced expression of interleukin-1β (IL-1β) upregulates MUC2 expression and the differential expression of MUC5AC through a cross talk between IL-1β and Akt wired by trefoil factor family peptide 3 (TFF3) in colonic epithelial cells (Raja et al. 2012). In an in vivo investigation on homozygous MUC1-deficient mice, Phillipson et al. found a thinner, firmly adherent mucus layer in both gastric and colon mucosa. These observations suggested a regulatory rather than structural role of MUC1 in the formation of the colonic and gastric mucus mainly composed of the gel-forming MUC2 and MUC5AC, respectively (Phillipson et al. 2008).

2.6 Mucins in Health and Cancer

2.6.1 Mucins and Gastrointestinal Physiology

Membrane-associated mucins are believed to serve as cell-surface receptors and sensors, and hence participate in signal transduction in response to changes in extracellular microenvironment and external stimuli that lead to coordinated cellular responses, including cell proliferation, differentiation and apoptosis, or secretion of specialized cellular products. They also associate with the secreted mucin layer by covalent and non-covalent bonds and contribute to physicochemical protection of

the epithelial cell surface from adverse conditions (Gipson et al. 2014; Hollingsworth and Swanson 2004). MUC1, MUC3, MUC4, MUC12, MUC13, and MUC17 are all found in the gastrointestinal tract (Pelaseyed et al. 2014). As the first to be characterized, MUC1 is the most extensively studied membrane-associated mucin. In the gastrointestinal tract, MUC1 is expressed abundantly in the stomach and only in small amounts in the intestine (Linden et al. 2007). MUC1 is found in the surface foveolar cells in the entire stomach, in mucous neck cells and in chief cells of the gastric fundus and antrum, as well as in the pyloric gland. In normal gastric mucosa, MUC1 is believed to protect gastric epithelial cells from a variety of external insults that cause inflammation and carcinogenesis [reviewed by (Saeki et al. 2014)].

Secreted mucins are expressed at mucosal surfaces with secretory and/or absorptive functions, including gastrointestinal tract. They have a central role in maintaining homeostasis in these sites and providing protection against insults by endogenous and exogenous agents in a relatively harsh environment with diverse, variable conditions. In the gastrointestinal tract, MUC2 and MUC5AC are the major components of mucus in the stomach and intestines, respectively. Secreted mucins not only protect and lubricate the lining of the alimentary canal for enhanced digestive functionality (Cone 2009), but also contribute to the specialized tasks of these organs. In the stomach, the mucous layer consists primarily of MUC5AC extending in layered sheets with MUC6 in between (Ho et al. 2004). MUC5AC along with MUC6 forms a protective layer over the surface epithelium and acts as a selective diffusion barrier for HCl (Bhaskar et al. 1992). The intestinal mucin MUC2 participates in the front line of the enteric host defense generated by the alliance of the epithelial cells, immune cells, and resident microbiota (Lievin-Le Moal and Servin 2006). This interactive ecosystem is essential for the maintenance of intestinal homeostasis and the normal function and activity of digestive system (McCracken and Lorenz 2001). Colonic mucus is composed of two layers. The outer, loose layer is the habitat of the microbial flora. The inner, dense layer is bacteria-free and firmly attached to the epithelium. This organization keeps the flora well separated from the mucosal surface. The gel-forming MUC2 comprises the substantial component of this double-layered mucus compartment. MUC2 is uncleaved in the inner layer and undergoes proteolytic cleavage to allow expansion of the polymeric structure, hence formation of the outer layer (Johansson et al. 2011).

The first stage in the biosynthesis of MUC2 is the formation of MUC2 monomer as an N-glycosylated apoprotein in the endoplasmic reticulum. Subsequently, MUC2 dimers are formed when intermolecular disulfide bonds bridge between the C-terminal cysteine knot domains. During transit through the Golgi apparatus, MUC2 dimers become heavily O-glycosylated. Complete glycosylation of the dimers occurs in the Golgi where trimerization through disulfide bonds at the N-terminus forms protease-resistant trimers. The fully glycosylated and processed MUC2 mucin is densely packed and stored in secretory granules/vesicles and released through constitutive or stimulated secretory mechanisms. Once released, MUC2 is organized into the firmly adherent inner layer. At a certain distance from the epithelium, this layer is converted into the loose outer layer through proteolytic cleavage and expansion. Mucus also contains immunoglobulins and other proteins (Amini et al. 2014b).

2.6.2 Mucins in Cancer

Mucins have been implicated in the pathophysiology of cancer. Malignant tumors, especially adenocarcinomas, express aberrant forms and/or amounts of mucins. At the simplest level, cancer cells use mucins in much the same way as normal epithelia to control their local microenvironment and to protect themselves from adverse growth conditions. Aberrant production, composition, and structure of tumor-associated (TA) mucins enhance growth and survival of tumor cells in otherwise inhospitable conditions and provide them with an effective means for invasion, metastasis, and immune evasion. In addition, many lines of evidence support the involvement of TA mucins in diverse biological mechanisms underlying resistance to chemotherapy, including their implications in physical barrier formation, resistance to apoptosis, drug metabolism, cell stemness, and epithelial–mesenchymal transition (EMT) (Jonckheere et al. 2014; Nath and Mukherjee 2014; Singh and Settleman 2010). Role of membrane-associated and secreted mucins in cancer is reviewed here (Byrd and Bresalier 2004; Hollingsworth and Swanson 2004; Nath and Mukherjee 2014).

2.6.2.1 Membrane-Associated Mucins

Membrane-associated mucins of cancer cells differ from those expressed by normal cells in both the expression status (amount and arrangement) and biochemical features. Overexpression, redistribution, and aberrant glycosylation of membrane-associated mucins contribute to the invasive and metastatic properties of adenocarcinomas by simultaneously configuring the adhesive and anti-adhesive properties of tumor cell surface (Hollingsworth and Swanson 2004). Upon loss of polarity associated with transformation, overexpressed TA-MUC1 is redistributed over the entire surface (Gendler 2001) and inhibits integrin-mediated cell adhesion to extracellular matrix components, thereby promoting cell detachment and increasing cancer cell invasiveness (Wesseling et al. 1995). The anti-adhesive properties of the overexpressed MUC1 also prevent tumor cells from conjugating with the effector cells of the immune system and allow them to evade immune surveillance (van de Wiel-van Kemenade et al. 1993). Aberrant glycosylation of TA-MUC1, on the other hand, exposes some epitopes otherwise masked in the normal mucin, resulting in the expression of a number of glycans serving as tumor-associated carbohydrate antigens (TACA) and potential ligands for interaction with other receptors. These antigens, such as Thomsen-Friedenreich (TF), Tn, sialyl-Tn (STn), sialyl Lewis^{A} (sLe^{A}, also termed CA19.9), and sialyl Lewis^{X} (sLe^{X}), are believed to facilitate tumor invasion and metastasis. In CRC cells, for example, MUC1 overexpresses sLe^{X} and sLe^{A} epitopes, resulting from a decrease in O-acetylation (Mann et al. 1997).

With loss of the MUC1 restricted localization to the apical membrane along with the redistribution of cell surface growth factors normally restricted to the basolateral

surface of epithelial cells, TA-MUC1 forms complexes with EGFR and other members of the ErbB family. Growth factors juxtaposed to MUC1 and intracellular kinases, such as ZAP-70, PKC-g, GSK-3b, and c-Src, phosphorylate serine, tyrosine, and threonine residues on MUC1. It is also thought that hypoglycosylation unmasks the core peptide and allows MUC1-N cleavage and release by extracellular proteases. MUC1-N release induces conformational changes in MUC1-C that alter its ligand status and subsequently activates downstream cell signaling pathways such as MAPK, phosphatidylinositol 3-kinase (PI3K)/Akt, and wingless-type (Wnt) pathways (Hollingsworth and Swanson 2004; Nath and Mukherjee 2014).

In addition, hypoglycosylation impacts the stability and subcellular localization of MUC1 (Altschuler et al. 2000). Compared with fully glycosylated MUC1, hypoglycosylated MUC1 shows increased intracellular uptake by clathrin-mediated endocytosis, without any enhanced degradation. Thus, hypoglycosylation may potentiate MUC1 oncogenic signaling by decreasing its cell surface levels and increasing intracellular accumulation (Altschuler et al. 2000). TA-MUC1-C can generate functional homodimers that translocate to the nucleus via importin-β and nucleoprotein 62 (Nup62) and act as a co-transcription factor (Raina et al. 2012; Kufe 2013). Several studies have indicated that MUC1 plays a critical role in the transcriptional regulation of genes associated with tumor cell proliferation, survival, invasion, metastasis, angiogenesis, drug resistance, inflammation, and immune regulation (Ahmad et al. 2009; Behrens et al. 2010; Cascio et al. 2011; Hattrup and Gendler 2006; Kufe 2009; Nath et al. 2013; Roy et al. 2011; Sahraei et al. 2012). Evidence also indicates that MUC1 causes transcriptional alterations that result in metabolic reprogramming in cancer cells (Mehla and Singh 2014). MUC1 interacts with both p53 and hypoxia-inducible factor 1α (HIF-1α), two key transcription factors that directly regulate metabolic gene expression. Serving as a transcriptional co-activator, MUC1 directly regulates expression of genes involved in multiple nutrient uptake and metabolic pathways (Chaika et al. 2012; Wei et al. 2005). MUC1 expression leads to changes in metabolic flux during glycolysis, as well as in the pentose phosphate pathway (PPP), the tricarboxylic acid (TCA) cycle, and fatty acid biosynthesis pathways (Chaika et al. 2012; Pitroda et al. 2009). PPP leads to the production of ribose, an essential building block for de novo DNA and RNA synthesis. As a consequence of MUC1 expression, the production of biosynthetic intermediates required for cell growth (i.e., biomass) is increased in cancer cells and cell proliferation is enhanced (Chaika et al. 2012). In addition to the transcriptional co-activator functions, MUC1 also directly modulates enzymatic functions of metabolic enzymes to regulate carbon flux (Kosugi et al. 2011). Metabolic alterations are a hallmark feature of cancer and provide tumorigenic properties to cancer cells (Hanahan and Weinberg 2011). Additionally, by modulation of autophagy, levels of reactive oxygen species, and metabolite flux, MUC1 facilitates cancer cell survival under hypoxic and nutrient-deprived conditions [reviewed by (Mehla and Singh 2014)]. Moreover, it has been hypothesized that the sugar branches sequester proinflammatory factors, such as transforming growth factor α (TGF-α), IL-1, IL-4, IL-6, IL-9, and IL-13, which are released upon MUC1-N shedding, thereby triggering inflammation (Hollingsworth and Swanson 2004). Smoldering inflammation in the

tumor microenvironment enhances proliferation and survival of malignant cells, promotes angiogenesis and metastasis, suppresses adaptive immune responses, and alters responses to hormones and chemotherapeutic agents (Mantovani et al. 2008).

2.6.2.2 Secreted Mucins

The mucus layer covering tumor cells is believed to serve as an impenetrable physicochemical barrier that helps them evade immune and inflammatory responses. In so doing, it is hypothesized that the viscous mucin coating, equipped with several ligands for adhesion molecules as well as with sequestered suppressive cytokines, prevents the approach of antigen-presenting and effector cells and suppresses their motility and activation. This mucus layer is also thought to capture biologically active molecules, including growth factors or cytokines, which might contribute to tumor growth (Hollingsworth and Swanson 2004). Secreted mucins are also implicated in the development of tumor chemoresistance. With the contribution of the aberrant membrane-associated mucins, they form a physical barrier that can act as either a size filter allowing entrance of particles smaller than the mucus network porosity, or an interaction filter via electrostatic or hydrophobic forces (Shaw et al. 2005; Sigurdsson et al. 2013). Secreted mucins can largely contribute to the biological behavior of cancer cells. As such, MUC2 has been identified as a major carrier of STn and sLe^X antigens, with implications in tumorigenesis and metastasis of gastrointestinal cancer (Conze et al. 2010; Izumi et al. 1995; Mann et al. 1997). Similarly, Bara et al. provided evidence that M1 antigen, an early oncofetal marker of colonic carcinogenesis, is indeed the product of the MUC5AC gene (Bara et al. 1998). They consistently reported later that M1/MUC5AC mucin is abnormally expressed by colonic goblet cells during colon carcinogenesis (Bara et al. 2003). In agreement, de novo expression of MUC2 and MUC5AC (Conze et al. 2010; Wakatsuki et al. 2008; Walsh et al. 2013) or a mucinous phenotype (Koseki et al. 2000; Wakatsuki et al. 2008; Thota et al. 2014; Tung et al. 1996) can be indicative of a more aggressive phenotype. In addition, overproduction or ectopic secretion of gel-forming mucins may largely contribute to tumor pathogenesis and clinicopathological features observed. A typical example is the peritoneal adenomucinosis or mucinous carcinomatosis from different primary sites, including the appendix, stomach, small and large bowel, urachus, pancreas, gallbladder, and ovary. In this regard, PMP syndrome is a paradigm (Sugarbaker 2006a; O'Connell et al. 2002b). Role of secreted mucins in PMP is discussed here [also reviewed elsewhere (Amini et al. 2014b, 2015c)].

2.6.2.3 Role of Secreted Mucins in PMP

Under normal conditions, metabolic turnover of intestinal mucin is maintained by the constitutive expression against enzymatic degradation, and, elimination. In PMP, however, mucin is ectopically secreted and increasingly deposited in the

peritoneal cavity where it is unable to degrade or drain away. Accumulating mucin causes a major part of the PMP morbidity. The typical syndrome develops after secreted mucin forms voluminous gels over months and years. Mucin also plays a key role in the biology of the PMP tumor. Most of the tumor cells are surrounded by a mucin coat that allows them to freely move, disseminate, and redistribute within the peritoneal cavity to create the distinctive feature of PMP (Sugarbaker 1994). As mentioned earlier, this coating also appears to protect tumor cells against extracellular insults, immune recognition, and chemotherapy. MUC2, MUC5AC, and MUC5B are the gel-forming mucins reportedly found in the PMP secretions (Mall et al. 2007; O'Connell et al. 2002b). MUC2, however, is known as the PMP-specific mucin. According to O'Connell et al. (2002a, b), primary ovarian mucinous tumors essentially express MUC5AC whereas solitary appendiceal mucinous tumors and different categories of PMP express MUC2 along with MUC5AC. This finding also supports the notion that PMP is a neoplasm of the appendiceal origin. MUC2 plays the key role in the pathogenesis of PMP. In their studies, O'Connell et al. showed that MUC2 is behind the high degree of gelation formed in PMP. Since MUC2 becomes more extensively glycosylated, it sterically occupies a greater volume than MUC5AC on an equimolar basis. Thus, ectopic production and accumulation of MUC2 lead to the formation of copious mucinous collections (O'Connell et al. 2002a). Widespread collection of massive gels increases the intra-abdominal pressure, compresses visceral organs, and triggers inflammatory and fibrotic responses, with major contribution to morbidity and eventual development of fatal complications, including bowel obstruction (Sugarbaker 1996c). PMP inflammatory microenvironment with a unique profile of cytokines (Lohani et al. 2014) has been shown to upregulate MUC2 expression and thus increase mucin secretion (Enss et al. 2000; Kim et al. 2000a; Iwashita et al. 2003). Apart from MUC2 and MUC5AC with definitive roles in PMP pathogenesis, Mall et al. reported that MUC5B is also present in the PMP material (Mall et al. 2007, 2011). Based on the investigations by Sheehan et al. implicating a low-charge glycoform of MUC5B in the production of a tenacious respiratory mucus plug (Sheehan et al. 1995, 1999), Mall et al. speculated that it may be MUC5B that is responsible for the semisolid material found in some PMP patients. Given the high protein content of the PMP secretions, they also raised the possibility that interactions between mucin and non-mucin proteins could contribute to the viscous nature of the PMP exudates (Mall et al. 2007, 2011). The expression status of MUC2 and other mucins reported by a number of investigators are summarized in Table 2.3.

Table 2.3 Expression of MUC2 and other mucins in PMP

Study	Year	Number of PMP cases	Percentage of cases exhibiting the expression of mucins	
			MUC2	Other forms of mucins
O'Connell et al. (2002b)	2002	100	98 %	MUC5AC 95 %
O'Connell et al. (2002a)	2002	25	96 %	MUC5AC 92 %
Mohamed et al. (2004)	2004	33	97 %	MUC1 57.5 %
Nonaka et al. (2006)	2006	42	100 %	MUC5AC 100 %
Mall et al. (2007)	2007	1	100 %	MUC5AC 100 %
				MUC5B 100 %
Ferreira et al. (2008)	2008	7	100 %	MUC1 28.6 %
				MUC5AC 100 %
				MUC6 28.6 %
Semino-Mora et al. (2008)*	2008	16	N/A‡	N/A‡‡
Baratti et al. (2009)**	2009	85	100 %	MUC5AC 87.5 %
Flatmark et al. (2010)	2010	5	100 %	MUC1 0 %
				MUC5AC 40 %
				MUC4 100 %
Guo et al. (2011)	2011	35	94.3 %	MUC1 0 %
Mall et al. (2011)	2011	1	100 %	MUC1 0 %
				MUC4 100 %
				MUC5AC 100 %
				MUC5B 100 %
				MUC6 0 %
Chang et al. (2012)***	2012	4	64 %	MUC5AC 43 %

*This study reports the expression of MUC2 and MUC5A in DPAM and PMCA tissues as apomucin volumetric density (Vvi/10^4 μm) in epithelium, lymphoid aggregates, stroma vessels and free mucin compartments, respectively, as follows

‡MUC2, DPAM: 264 ± 60, 47 ± 16, 31 ± 14 and 261 ± 51; PMCA: 356 ± 90, 170 ± 26, 117 ± 25 and 1043 ± 282

‡‡MUC5AC, DPAM: 90 ± 13, 345 ± 20, 65 ± 17, 37 ± 6; PMCA: 56 ± 12, 246 ± 17, 50 ± 15 and 48 ± 9

**The percentages shown are numerical estimations of data originally presented by a column graph

***Four out of 14 patients with mucinous adenocarcinoma were PMP cases. With no individual data reported for PMP, results shown are indicative of MUC2 and MUC5AC expressions in the whole group. *N/A* not available

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Utility of Bromelain and N-Acetylcysteine in Treatment of Peritoneal Dissemination of Gastrointestinal Mucin-Producing Malignancies

Amini, A.; Masoumi-Moghaddam, S.; Morris, D.L.

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