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Membrane-bound mucins: the mechanistic basis for alterations in the growth and survival of cancer cells

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Abstract

Mucins (MUC) are high molecular weight O-linked glycoproteins whose primary functions are to hydrate, protect, and lubricate the epithelial luminal surfaces of the ducts within the human body. The MUC family is comprised of large secreted gel forming and transmembrane (TM) mucins. MUC1, MUC4, and MUC16 are the well-characterized TM mucins and have been shown to be aberrantly overexpressed in various malignancies including cystic fibrosis, asthma, and cancer. Recent studies have uncovered the unique roles of these mucins in the pathogenesis of cancer. These mucins possess specific domains that can make complex associations with various signaling pathways, impacting cell survival through alterations of cell growth, proliferation, death, and autophagy. The cytoplasmic domain of MUC1 serves as a scaffold for interaction with various signaling proteins. On the other hand, MUC4 mediates its effect by stabilizing and enhancing the activity of growth factor receptor ErbB2. MUC16, previously known as CA125, is a well-known serum marker for the diagnosis of ovarian cancer and has a key role in stimulation and dissemination of ovarian cancer cells by interacting with mesothelin and galectin. Therefore, herein we discuss the function and divergent mechanisms of MUC1, MUC4, and MUC16 in carcinogenesis in the context of alteration in cell growth and survival.

Keywords

MUC4; MUC1; MUC16 (CA125); cancer; cell survival; apoptosis

Introduction

Conventionally, mucins are regarded as an extracellular secretion of goblet cells mainly involved in coating, lubricating, and protecting the epithelial surfaces of the internal tracts of the body from foreign insults by forming a gel (Mall, 2008). The epithelial membrane-tethered mucins are quite distinct from the classic extracellular complex mucins forming the mucous layers of the gastrointestinal and respiratory tracts. These epithelial membrane-tethered mucins are the transmembrane (TM) molecules, expressed by most glandular and ductal epithelial cells (Taylor-Papadimitriou *et al.*, 1999). Several TM mucins (MUC1, MUC3A, MUC3B, MUC4,

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Conflict of interest
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MUC 12, MUC13, MUC15, MUC16, MUC17, MUC20, and MUC21) (human mucins are designated as MUC, whereas other species mucins are designated as Muc) have been identified so far (Moniaux *et al.*, 2001; Chaturvedi *et al.*, 2008a; Itoh *et al.*, 2008). Figure 1 illustrates the prototypic structure of membrane-bound mucins. In general, these mucins have a single membrane-spanning domain and a cytoplasmic tail (CT) (varies from 22 amino-acid residues in MUC4 to 80 amino acids in MUC17) in addition to the extensive extracellular domain (Moniaux *et al.*, 1999, 2001, 2006). The extracellular domain of TM mucins is mainly composed of a variable number of the tandem-repeat (TR) domain, sperm protein, enterokinase, and agrin (SEA) domain or epidermal growth factor (EGF)-like domain. Centrally located TR regions are the characteristic feature of all mucins, differentiating them from other membrane-bound glycoproteins. The biochemical and biophysical properties of mucins are largely governed by the extent and nature of their glycosylation. Arrays of TRs provide a high degree of multivalency for oligosaccharide structures, thereby providing a significant stoichiometric power (Hollingsworth and Swanson, 2004). *N*-acetylgalactosamine is linked to a serine or threonine residue during the *O*-glycan chain synthesis, and the subsequent carbohydrate moieties, however, may vary depending on the mucin type, the site of mucin expression, and the physiological or pathological conditions (Chaturvedi *et al.*, 2008a). By their characteristic pattern of glycosylation, mucins can modulate immunological response, facilitate cell adhesion during tumor metastasis, and also alter the functions of proteins interacting with the mucin carbohydrate moieties. CT and extracellular domain sequences of the TM mucins are highly variable and, therefore, may impart unique functions to each mucin. Out of all of the membrane-bound mucins, MUC1, MUC4, and MUC16 are well-characterized MUCs; therefore, this review focuses on various mechanisms affected by these mucins during cancer progression. Figure 2 illustrates the schematic presentation of the MUC1, MUC4, and MUC16 CT and its interacting partners. In addition, all membrane-tethered mucins have been discussed shortly in Table 1 (Williams *et al.*, 2001; Pallesen *et al.*, 2002; Higuchi *et al.*, 2004a, b; Li *et al.*, 2005; Moniaux *et al.*, 2006; Shyu *et al.*, 2007; Walsh *et al.*, 2007; Itoh *et al.*, 2008; Malmberg *et al.*, 2008; Chauhan *et al.*, 2009).

Cancer is a disease of the deregulation of tissue growth. Normal cell growth is maintained by the balance between cell proliferation and cell death. In order for a normal cell to transform into a cancer cell, genes that regulate cell growth and cell death must be altered (Croce, 2008). Mucins have been shown to contain complex associations with various cellular pathways, impacting cell growth, proliferation, and apoptosis. Historically, transformation events in cancer have been defined as initiation events (contributing to the early stages of neoplastic transition) or progression events (referring to the subsequent transformative processes) (Croce, 2008). Using human tumor xenograft models, MUC1 and MUC4 have been shown to cause transformation of fibroblast cells. Also, alteration of the tumorigenicity and metastasis of various cancer cell lines by overexpression and downregulation of mucins demonstrates their role in the pathogenesis of different malignancies (Li *et al.*, 2003; Singh *et al.*, 2004; Chaturvedi *et al.*, 2007; Moniaux *et al.*, 2007; Bafna *et al.*, 2008). Recent studies suggest that MUC1 and MUC4 modulate various pathways contributing to cell growth. MUC16, previously known as CA125, is the largest membrane-bound mucin (Hattrup and Gendler, 2008) and well-known serum marker for ovarian cancer. However, there is a lacuna of information on the signaling mediated by this mucin due to its recent identification and huge size. The present review summarizes the function and divergent mechanism of MUC1, MUC4, and MUC16 in carcinogenesis in the context of alteration in cell growth and survival.

Mechanisms associated with MUC1-mediated survival of cancer cells

MUC1 is a membrane-bound *O*-glycoprotein that is expressed at the basal level in most epithelial cells (Patton *et al.*, 1995). On the other hand, deregulated expression of MUC1 is a prominent characteristic of various types of cancers and inflammatory diseases. In addition,

MUC1 mucin has long been viewed as a tumor-associated molecule because of its frequent overexpression and aberrant glycosylation in most carcinomas. MUC1 is overexpressed in >90% of breast carcinomas and frequently in other types of cancer, including ovarian, lung, colon, and pancreatic carcinomas (Gendler, 2001). Ectopic MUC1 expression has been shown to induce transformation and increased tumorigenicity in a number of systems: pancreatic, breast, and myeloma cell lines, including the MUC1 transgenic model of human breast cancer and MUC1-transfected 3Y1 rat fibroblasts (Li *et al.*, 2003; Schroeder *et al.*, 2004; Hattrup and Gendler, 2006; Singh *et al.*, 2007; Kawano *et al.*, 2008). Recently, MUC1 has been shown to serve as a natural ligand of galectin-3 in human cancer cells, and the interaction between circulating galectin-3 and cancer-associated MUC1 enhances cancer cell-endothelial adhesion and, hence, promotes metastasis (Zhao *et al.*, 2009). Structurally, the MUC1 mucin consists of a large extracellular subunit comprised of a mucin-type identical 20 amino acids TRs and a smaller subunit that includes a small extracellular domain and a TM domain plus a cytoplasm tail (MUC1-CT). The oncogenic effects of MUC1 are believed to occur through the interaction of its CT with various signaling molecules. The CT of MUC1 is 69 amino acids long (Figure 2), and has several tyrosine, serine, and threonine phosphorylation sites that can bind to several proteins implicated in the cancer regulation by affecting the proliferation, apoptosis, and transcription of various genes (Lan *et al.*, 1990; Singh and Hollingsworth, 2006; Hattrup and Gendler, 2008).

MUC1-mediated enhanced cell growth and alterations in various signaling pathway

Growth factor receptors are key regulators of growth in both normal and transformed cells. Constitutive activation of a growth factor signaling leads to uncontrolled proliferation and contributes to malignant transformation (Croce, 2008). Figure 3 elucidates the role of MUC1 in tumor progression through its interaction with various growth factor receptors and other signaling molecules. Through its CT, MUC1 binds with the ErbB family of growth factor receptor tyrosine kinases (RTKs) and potentiates ErbB-dependent signal transduction in the MUC1 transgenic breast cancer mouse model (Li *et al.*, 2001; Schroeder *et al.*, 2001; Pochampalli *et al.*, 2007). The ErbB family is comprised of the ErbB1/EGF receptor (EGFR), ErbB2/HER2/Neu, ErbB3, and ErbB4. Following ligand binding and receptor activation, these receptors are endocytosed and transported to lysosomes where the receptor is degraded (Roepstorff *et al.*, 2008). This downregulation of growth factor receptors is a complex and tightly regulated process. MUC1 has been shown to enhance ErbB1 signaling in breast cancer cells by inhibiting the growth factor-mediated ubiquitination and degradation of ErbB1 and by enhancing the internalization and recycling of ErbB1 (Pochampalli *et al.*, 2007). Through stabilizing and enhancing the ErbB signaling by MUC1-ErbB kinase interaction, MUC1 activates extracellular signal-regulated kinases (Erks) 1 and 2 and thereby increases cell proliferation (Schroeder *et al.*, 2001). MUC1 also regulates ErbB-independent ERK signaling through modulating the transcription of the genes encoding MEK1, Raf-1, and c-jun (Hattrup and Gendler, 2006). The role of MUC1 in the regulation of ERK signaling was also supported in COS-7 cells using MUC1-CT in conjunction with the extracellular and TM domains of the CD-8 T-cell receptor (Meerzaman *et al.*, 2001).

In addition to the ErbB family, MUC1 also interacts with fibroblast growth factor receptor 3 (FGFR3) (Ren *et al*., 2006). Stimulation of breast cancer cells with FGF induces tyrosine phosphorylation of MUC1-CT, and finally increases the binding of MUC1 to β-catenin and targeting of MUC1 and β-catenin to the nucleus (Ren *et al*., 2006). β-catenin acts as a transcriptional co-activator to increase the expression of cell-cycle progression genes *cyclin-D1* and *c-myc*. Dysregulation of β-catenin is of great importance to the development of diverse human malignancies (Huang *et al*., 2005). MUC1 increases cytoplasmic and nuclear β-catenin levels by inhibiting GSK3β-mediated phosphorylation and degradation of β-catenin (Huang *et al*., 2005). Src family members, such as c-Src, Lyn, and Lck, have been shown to bind and

phosphorylate the CT of MUC1 at the YEKV motif (Singh and Hollingsworth, 2006). This, in turn, inhibits the binding of GSK3β with MUC1-CT, which leads to better binding of MUC1-CT with β-catenin and the inhibition of GSK3β-mediated degradation of β-catenin. Importantly, disruption of the MUC1-β-catenin interaction in rat fibroblast cells attenuated MUC1-induced anchorage-dependent and -independent growth and delayed MUC1-mediated tumorigenicity (Huang *et al*., 2005). Further, by blocking the intracellular interactions between MUC1/β-catenin and MUC1/EGFR using MUC1 inhibitory peptide (MIP), Bitler *et al*. (2009) have shown a significant decrease in the proliferation, migration, and invasion of metastatic breast cancer cells *in vitro*, and also decreased tumor growth and recurrence in an established MDA-MB-231 immunocompromised (SCID) mouse model.

The MUC1 also controls cell proliferation by interacting with estrogen receptor α (ERα). The MUC1-CT subunit is found in the nucleus and thereby it can modulate transcription directly (Hattrup and Gendler, 2006; Wei *et al*., 2006). MUC1 binds directly to the ERα DNA-binding domain and stabilizes ERα by blocking its ubiquitination and degradation. This interaction is stimulated by the presence of estrogen (Wei *et al*., 2006). Further, it has been shown that MUC1 stimulates ERα-mediated transcription and contributes to estrogen-mediated growth and the survival of breast cancer cells (Wei *et al*., 2006).

Relationship between MUC1 overexpression and inhibition of cancer cell death

Recent studies suggest that MUC1 confers a protective function against oxidative stress-induced cell death. Figure 4 illustrates the different mechanisms involved in MUC1-induced decreased cell death. In response to genotoxic stress, MUC1 regulates p53-responsive genes and thereby cell fate (Wei *et al*., 2005). The p53 tumor suppressor functions in the cellular response to stress by inducing growth arrest, DNA repair, senescence, differentiation, or apoptosis (Levine, 1997). Selective transactivation of p53 target genes dictates the induction of apoptosis or a growth arrest and repair response (Chao *et al*., 2000). MUC1 directly binds to the p53 regulatory domain and selectively promotes transcription of growth arrest genes and decreases transcription of apoptotic genes as a survival response to stress and thereby decreases cell death (Wei *et al*., 2005). In addition, MUC1 increases anti-apoptotic Bcl-X_L and PI3K/Akt pathways to attenuate genotoxin-induced apoptosis (Raina *et al*., 2004).

MUC1 also activates the survival-related FOXO3a transcription factor in response to oxidative stress (Yin *et al*., 2004). FOXO3a, a member of the fork head family of transcription factors, induces reactive oxygen species scavenging and resistance to oxidative stress (Nemoto and Finkel, 2002). FOXO3a is deactivated by its phosphorylation by the phosphoinositide 3-kinase (PI3K) phospho-Akt/PKB pathway and retained in the cytoplasm. Dephosphorylation of FOXO3a induces its nuclear localization and FOXO3a-mediated transactivation of gene transcription (Nemoto and Finkel, 2002). Yin *et al*. (2004) have found that MUC1 attenuates activation of the PI3K phospho-Akt/PKB pathway in HCT116 colon carcinoma cells and thereby decreases FOXO3a phosphorylation. In addition, stable downregulation of endogenous MUC1 in breast cancer cells has been found to inactivate FOXO3a, increase intracellular oxidant levels, and sensitize cells to oxidative stress-induced necrosis.

As discussed earlier, the CT of MUC1 gets phosphorylated by various growth factors and thereby provides a docking site for different signaling molecules. It has been shown that FGF1 induces tyrosine phosphorylation of MUC1-CT and facilitates the binding of MUC1 to the heat shock protein (HSP)90 chaperone, finally targeting MUC1-CT to the mitochondria (Ren *et al*., 2006). The localization of MUC1-CT to the mitochondria is shown to be associated with the attenuation of a stress-induced loss of mitochondrial TM potential, release of mitochondrial apoptogenic factors, activation of caspase-3, and cell death (Yin *et al*., 2003). This suggests that by interacting with HSP90, MUC1-CT transduces signals from the cell membrane to

mitochondria that attenuate activation of the intrinsic apoptotic pathway in response to stress (Ren *et al*., 2006).

MUC1-CT also binds directly to c-Abl and sequesters it in the cytoplasm (Raina *et al*., 2006). The c-Abl is a non-RTK, binds to cytosolic 14-3-3 proteins and targeted to the nucleus, where it activates MEK kinase-1 and thereby the pro-apoptotic c-Jun N-terminal kinase (JNK) pathway (Kharbanda *et al*., 1995a, b). Raina *et al*. have shown that MUC1 blocks nuclear targeting of c-Abl and thereby the apoptotic response to genotoxic anticancer agents. MUC1 also interacts with the IκB kinase complex and increases the phosphorylation and degradation of IκBα (Ahmad *et al*., 2007) and helps in the constitutive activation of nuclear factor-κB (NF-κB) and thereby blocks apoptosis and induces transformation.

Recently, Agata *et al*. (2008) have shown a constitutive interaction of MUC1 with caspase-8 and the death effector domain of FADD in MCF-10A breast epithelial cells. MUC1 binds directly to caspase-8 and FADD, blocks recruitment of caspase-8 to the death-inducing signaling complex and thereby prevents activation of the death receptor-induced extrinsic apoptotic pathway. The functional significance of this interaction in normal epithelial cells is to inhibit caspase-8 activation as a protective mechanism, whereas malignant cells exploit this phenomenon for survival under adverse conditions by having an overexpression of MUC1.

Taken together, these studies support the role of MUC1 in tumor progression, because it can stimulate cell proliferation through growth factor receptor, β-catenin and ERα, and also suppress apoptosis through the regulation of JNK, NF-κB, HSP90, and extrinsic apoptotic pathways.

MUC4 and tumor progression

MUC4 is normally expressed by the epithelial surface of the eye, oral cavity, middle ear, lachrymal glands, salivary glands, female reproductive tract, prostate gland, stomach, colon, lung, trachea, and mammary gland to lubricate and protect these surfaces. Although similar to MUC1, qualitative and quantitative alterations in the expression of MUC4 have also been observed in various preneoplastic and neoplastic lesions (Buisine *et al*., 2001; Copin *et al*., 2001; Corfield *et al*., 2001; Singh *et al*., 2006). MUC4 is normally absent in the pancreas but an aberrant expression of MUC4 in pancreatic cancer is detected early pancreatic intraepithelial neoplastic lesions and correlates with the disease advancement (Swartz *et al*., 2002; Park *et al*., 2003). In addition to the aberrant expression of MUC4 in pancreatic cancer, it is frequently overexpressed in various cancers like lung, breast, colon, and ovarian carcinomas (Andrianifahanana *et al*., 2001; Hanaoka *et al*., 2001; Shibahara *et al*., 2004; Davidson *et al*., 2007). Furthermore, the association of MUC4 with the poor prognosis of pancreatic, lung, and bile duct cancer patients has also been reported (Saitou *et al*., 2005; Tamada *et al*., 2006; Tsutsumida *et al*., 2007). In addition, ectopic MUC4 expression has been shown to induce transformation in mouse embryonic fibroblast NIH3T3 cells (Bafna *et al*., 2008). Using MUC4 knockdown and overexpression cancer cell models, MUC4 has been shown to alter tumorigenicity and metastasis by altering the behavioral properties of the tumor cells (Singh *et al*., 2004; Chaturvedi *et al*., 2007; Moniaux *et al*., 2007). MUC4 mucin, which is also implicated in the pathogenesis of various cancers, is synthesized as a single polypeptide chain of ~930 kDa. MUC4 is hypothesized to be cleaved at a GDPH proteolytic cleavage site generating two subunits (the mucin-type subunit MUC4α, and a TM subunit MUC4β). MUC4α possesses three putative functional domains, TR, nidogen-like (NIDO), and adhesion-associated domain in MUC4 and other proteins (AMOP), whereas MUC4β has three EGF-like domains and a short 22 amino acids long CT (Chaturvedi *et al*., 2008a). The oncogenic effects of MUC4 are believed to occur through its interaction with the growth factor receptor, which has been discussed in later sections.

Tumor progression through MUC4-induced increased cell proliferation and growth

Like MUC1, MUC4 has also been implicated in the regulation of cellular growth signaling through its interaction with the ErbB family of growth factor RTKs. Figure 3 illustrates the role of MUC4 in tumor progression through its interaction with growth factor receptors. The EGF-like growth factor family of ligands binds with the extracellular domain of the ErbB receptors, leading to the formation of both homo and heterodimers (Riese and Stern, 1998). Ligand-induced dimer formation causes cross-phosphorylation of specific tyrosine residues in the CT, which serve as docking sites for the activation of various signaling proteins to control cell proliferation, differentiation, apoptosis, and survival (Schlessinger, 2000). No ligand, however, has been ascribed to the ErbB2/HER2/Neu growth factor receptor. The MUC4 mucin has been shown to act as an intramembrane ligand and activator for the receptor ErbB2 and thus facilitates its dimerization with other ErbB receptors (Carraway *et al*., 2002;Jepson *et al*., 2002;Singh *et al*., 2004). The MUC4/ErbB2 complex has been observed in many tissues where MUC4 is normally expressed (Arango *et al*., 2001;Price-Schiavi *et al*., 2005) and also in various tumors and cancer cell lines where MUC4 aberrantly gets overexpressed (Ramsauer *et al*., 2006;Chaturvedi *et al*., 2008b). This suggests that MUC4 modulates ErbB2 signaling in both normal and malignant epithelia but overexpression of MUC4 and ErbB2 provides a scenario for the promotion of tumor progression. Silencing of MUC4 in pancreatic cancer cells was associated with a downregulation of HER2 and a concomitant decrease in its phosphorylated form (pY1248-HER2), which is one of the major autophosphoryalation sites in the cytoplasmic region of HER2 (Singh *et al*., 2004;Chaturvedi *et al*., 2008b). The tumorigenic property of HER2 contributes to its constitutive activation in tumor cells (Hynes and Lane, 2005). Our recent studies have revealed that MUC4 interacts with the HER2 and stabilizes its expression and activity by post-translational mechanisms (Chaturvedi *et al*., 2008b). HER2 can regulate cell proliferation and metastasis by activating its downstream mitogen-activated protein kinase (MAPK), phosphoinositide-3-kinase/Akt, FAK, and c-src family kinase pathways (Holbro and Hynes, 2004). Enhanced stabilization of HER2 by MUC4 interaction was associated with enhanced activation of extracellular signal-regulated kinases (Erks) 1 and 2 MAPK (Chaturvedi *et al*., 2008b). The HER2-mediated activation of the ERK pathway has a crucial role in mediating cancer cell growth and proliferation.

Interaction of rat Muc4 (rMuc4) with ErbB2 has been shown to cause context-dependent epithelial differentiation or cell proliferation. Binding of rMuc4 with ErbB2 induces limited phosphorylation at 1248 tyrosine residues, which subsequently leads to the upregulation of cell-cycle inhibitor $p21^{kip}$ and did not activate the MAPK pathway (Jepson *et al*., 2002). On the contrary, when Muc4 potentiates the neuregulin-mediated activation of ErbB3 and ErbB2 complex then it leads to an enhanced activation of the Erk MAPK pathway and thereby increased cell proliferation. Muc4, through its interaction with ErbB2, also enhances the recruitment of PI3K to activated ErbB3 (Carraway *et al*., 2007). It has been shown that PI3K is essential for ErbB2/ErbB3-mediated breast cancer cell proliferation (Holbro *et al*., 2003). In addition, Muc4 has also been shown to cause relocalization of ErbB2 to the apical cell surface in polarized colon cancer cells CACO-2, where MUC4 phosphorylates ErbB2 at 1139 and 1248 tyrosine residues and activates p38 MAPK with a consequent activation of Akt (Ramsauer *et al*., 2006). Growth factor-induced activation of Akt via p38 has previously been shown as a pro-survival pathway in lung cells (Horowitz *et al*., 2004). Ramsauer *et al*. (2006) have also demonstrated the role of p38 activation via Muc4–ErbB2 interaction in cell survival and maintenance. Hence, MUC4-mediated activation of ErbB2/HER2 has an important role in tumor progression.

Tumor progression through MUC4-induced decreased cell death

MUC4 overexpression has been shown to impart apoptotic resistance to tumor cells. Upregulation of rat Muc4/sialo mucin complex (SMC) expression accelerates growth of the

A375 tumor in the xenotransplanted tumor model (Komatsu *et al*., 2001). Further, *in vitro* studies suggested that this effect was not due to the increased proliferative potential of A375 cells, but was rather due to the suppression of apoptosis. By knockdown and overexpression of MUC4 in pancreatic cancer cells, our laboratory has also demonstrated the anti-apoptotic function of MUC4 (Chaturvedi *et al*., 2007; Moniaux *et al*., 2007). In addition, Muc4 renders drug resistance to melanoma cell lines (Hu *et al*., 2003). Upon treatment with doxorubicin, Muc4-expressing melanoma cells were blocked less frequently in G2 and underwent less apoptosis and necrosis than Muc4-negative melanoma cells (Hu *et al*., 2003). Activation of caspase-9 was decreased in Muc4-expressing melanoma cells when treated with the apoptotic-inducing agent, actinomycin D. Further, in contrast with MUC1, Muc4 did not cause suppression of the extrinsic apoptotic pathway (Hu *et al*., 2003).

A possible mechanism of the anti-apoptotic function of MUC4 is not yet clear, but it will be interesting to explore the relationship between signaling from the MUC4/ErbB2 complex and apoptosis. Figure 4 summarizes the possible mechanisms involved in MUC4-induced decreased cell death. Studies have shown that ErbB2 expression in tumors can lead to resistance to multiple drugs through ErbB2-mediated upregulation of cell-cycle inhibitor $p21^{Cip1}$, which further inhibits activation of cdk1/cyclin B and thus blocks its normal pathway to cellular apoptosis (Yu and Hung, 2000). Studies with rat Muc4 have shown that Muc4 induces limited and specific phosphorylation of ErbB2 at Tyr^{1248} in melanoma cells, which is associated with an upregulation of cell-cycle inhibitor $p27^{kip}$ and a repression of apoptosis (Jepson *et al*., 2002). Recently, we have shown the role of MUC4 in the protection of pancreatic cancer cells from gemcitabine-induced apoptosis through HER2/ERK-dependent phosphorylation and inactivation of the pro-apoptotic protein Bad (Bafna *et al*., 2009). This suggests that MUC4 might exert its anti-apoptotic function through ErbB2 downstream signaling. However, MUC4 causes downregulation of $p27^{kip}$ in the presence of neuregulin, a ligand for ErbB3. In addition, a recent study has shown that the anti-apoptotic effect of rat Muc4 is dependent on ErbB2 in JIMT-1 breast cancer cells, whereas it is independent of ErbB2/HER2 in A375 melanoma and MCF-7 breast cancer cells (Workman *et al*., 2009). These findings indicate that the role of MUC4 in the resistance to apoptotic cell death is not only regulated by MUC4 and ErbB2 interaction but also implicates other signaling pathways regulated by MUC4. Hence, the mechanism of the anti-apoptotic function of MUC4 is still an open question and needs to be elucidated by future studies.

Biological significance of MUC16 in cancer progression

MUC16 is the largest membrane-bound mucin and is normally expressed by epithelium of the ocular surface, upper respiratory tract, the mesothelium lining body cavities (pleural, peritoneal, and pelvic cavities), the internal organs, and male and female reproductive organs (Matsuoka *et al*., 1990; Zeimet *et al*., 1998; Argueso *et al*., 2003; Gipson, 2005) to lubricate and protect these surfaces. In contrast, numerous studies on human tumors and serum have shown a deregulated expression of MUC16 in ovarian cancer. MUC16, previously known as CA125, is a tumor-associated antigen that is cleaved from the surface of ovarian cancer cells and shed into blood and used as a well-established biomarker for monitoring the growth of ovarian cancer (Bast *et al*., 1998). It has been shown that disease progression is associated with an increase in serum CA125/MUC16 level in >80% of ovarian cancer patients, whereas a decline in serum CA125/MUC16 level is associated with response to therapy (Capstick *et al*., 1991). Studies of MUC16 have focused primarily on clinical applications, whereas the biological significance of MUC16 overexpression in ovarian cancer is still poorly understood.

MUC16 is a very large mucin with an average molecular weight between 2.5 and 5 million Da (O'Brien *et al*., 2001, 2002). The peptide backbone of MUC16 is composed of an N-terminal region, an extensive TR domain, and a C-terminal region with a short CT (O'Brien *et al*.,

2001). The N-terminal domain is rich in serine/threonine residues and accounts for the major *O*-glycosylation sites known to be present in MUC16. The TR region is composed of 18–60 repeats, each of which contains 156 amino acids. The C-terminal region can be divided into three major domains: extracellular domain, TM domain, and CT. Human MUC16 is unique among the membrane-bound mucins by having 16 SEA domains and unlike MUC1 and MUC4, it does not have an EGF-like domain (Hattrup and Gendler, 2008). In addition to its extensive protein structure, MUC16 is heavily glycosylated with both O-linked and N-linked oligosaccharides (Kui *et al*., 2003). As a large heavily glycosylated molecule, MUC16 extends from the surface of ovarian cancer cells and binds to mesothelin, a protein that is found on the surface of the mesothelial cells that line the peritoneum (Rump *et al*., 2004; Gubbels *et al*., 2006; Scholler *et al*., 2007). These studies suggest that MUC16 provides contact and adhesion for metastasizing epithelial ovarian cancer cells and thus have a biological role in the metastasis of ovarian cancer cells. In addition, MUC16 has also been shown to bind with galectin-1, a mammalian lectin expressed on human immune cells, and prevent anti-tumor immune responses (Seelenmeyer *et al*., 2003). Recently, Boivin *et al*. (2009) have shown increased sensitivity to various genotoxic agents in epithelial ovarian carcinoma cells by downregulation of MUC16. Further, Gubbels *et al*. (2010) have shown that MUC16 protects ovarian tumor cells from natural killer cell mediated anti-tumor cytotoxic responses. The protection is mediated mainly by inhibition of synapse between tumor and NK cells. It also potentiates peritoneal metastasis of ovarian cancer cells (Gubbels *et al*., 2006). The CT of MUC16 contains polybasic amino acids, and it is predicted to interact with ezrin/radixin/moesin (ERM) actin-binding proteins (Blalock *et al*., 2007). Although the MUC16 CT has many possible phosphorylation sites (Hattrup and Gendler, 2008), the functional characteristics and signaling capabilities of MUC16 that contribute to cell growth in cancer are still unknown.

Mucins in cell survival and autophagy

Solid tumors and specifically pancreatic tumor is clinically hypovascular and its three-dimensional growth causes biologically different zones within the tumor: central and peripheral zones of the tumor (Nakamura *et al*., 2007). Nutrient level is frequently more reduced in the center of locally advanced tumors than in the peripheral zone of the tumor. In addition, to metasta-size, tumor cells must survive in the circulatory or lymphatic systems in the absence of stromal-derived survival growth factors and limited nutrients. In these conditions, tumor cells need to adapt to the environment that is deprived of nutrients and acquire alternative energy sources. Autophagy is a cellular response to stress or nutrient deprivation, which is a way to supply amino acids as an alternative energy source by degradation of damaged cytoplasmic organelles or protein (Hait *et al*., 2006). Recently, the *MUC4* gene has been shown to be upregulated in the central zone of the pancreatic tumor compared with the peripheral zone of the tumor. This implicates the potential role of MUC4 in nutrient deprivation-induced mechanisms (Nakamura *et al*., 2007). MUC1 has also been shown to protect cells against oxidative stress-induced cell death (Yin *et al*., 2003). Recently, MUC1 is shown as a new target of the hypoxia inducible factor (HIF)-signaling pathway, which is the main renal carcinogenetic pathway and have a role in migration and invasive properties in renal cancer cells (Aubert *et al*., 2009). Mucins are very large glycoprotein and found to be overexpressed in various cancers. The aberrant overexpression and large size of mucins might also have a survival advantage under nutrient deprivation in cancer cells by undergoing autophagy. Therefore, it would be interesting to determine the association of mucins and autophagy as a survival advantage in cancer cells under nutrient-deprivation conditions. In a recent study, MUC1 has been shown to inhibit the induction of necrosis in response to the deprivation of glucose with the induction of autophagy (Yin *et al*., 2009).

Conclusion and Perspectives

In recent years, it has become clear that the deregulation of mucin expression creates a favorable environment for tumor progression. As described herein, enormous progress has been made regarding the mechanisms of membrane-bound mucins in tumor progression. Both MUC1 and MUC4 have many unique domains, which enhance or inhibit various signaling pathways involved in cellular proliferation and cell death. MUC16 is a well-established serum marker for ovarian cancer patients but the functional characteristics and signaling capabilities of MUC16 to contribute to cell growth in cancer are still unknown. Further, the aberrant overexpression and large size of mucins might provide survival advantage to cancer cells under stress or nutrient deprivation by promoting autophagy. Figures 3 and 4 illustrate the various mechanisms of MUC1 and MUC4 responsible for tumor progression. MUC1 has a distinct role in tumor progression because it can stimulate cell proliferation through its interaction with growth factor receptor, β-catenin, and ERα. MUC1 also suppresses apoptosis through the regulation of various pathways as described in the review. The tumorigenic potential of MUC4 contributes to its interaction with the ErbB2 receptor and regulation of ErbB2 downstream signaling. However, future studies are needed to corroborate and clarify the contribution of these interactions in tumor progression. If the aforementioned mucin interactions are required to promote tumor progression, then it could be useful to target these interactions for the treatment of cancer. Further, disruption of these interactions will elucidate the contribution of each process and the intervention of these pathways may be helpful in controlling tumor progression.

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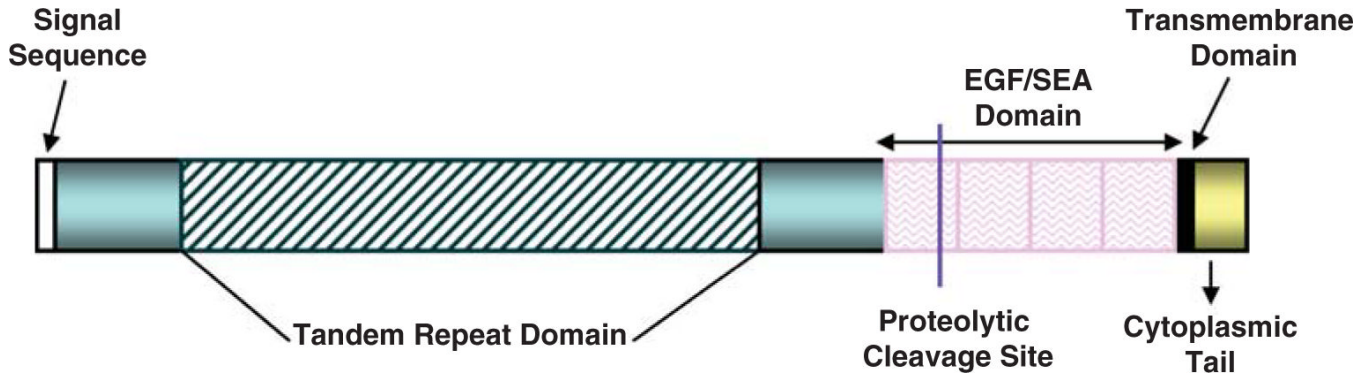

Figure 1.
Prototype structure of the membrane-bound mucins. These mucins typically compose two subunits, based on the putative proteolytic cleavage site. The larger subunit is extracellular and predominantly composed of variable number of tandem repeats. The smaller subunit consists of short extracellular region (containing either sperm protein, enterokinase, and agrin (SEA) domain or epidermal growth factor (EGF)-like domain), single transmembrane domain, and the cytoplasmic tail.

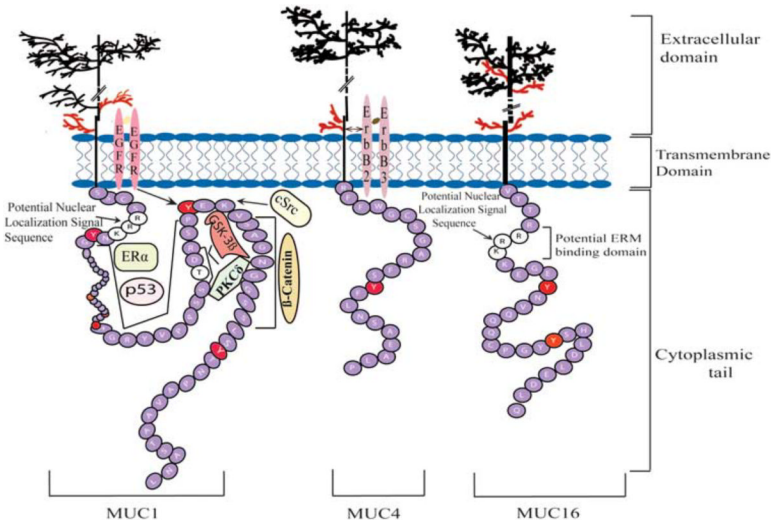

Figure 2.

Schematic representation of MUC1, MUC4, and MUC16 cytoplasmic tails (CT). The sequence of cytoplasmic tails of different MUCs is shown with emphasis on tyrosine residue (red) and their known binding partners. MUC1 cytoplasmic tail is the most well-studied and known to interact with proteins with kinase activity (PCKδ, GSK3β, EGFR, and c-Src) and without kinase activity (p53, ERα, β-catenin). The MUC16 cytoplasmic tail has a motif containing polybasic amino-acid sequence, which is a potential site for interaction with cytoskeleton through ERM protein. Both categories of interacting partners are involved in different signaling pathways emphasizing on the critical role of mucin cytoplasmic tail in intracellular signaling events. MUC4 cytoplasmic tail also contains tyrosine residue but till date nothing is known about their intracellular-binding partners. In addition, both MUC1 and MUC16 contain positively charged lysine-arginine rich potential nuclear localization motif in the region juxtaposed to the plasma membrane. In extracellular domain, N and O-linked glycosylations are shown schematically with red and black color, respectively.

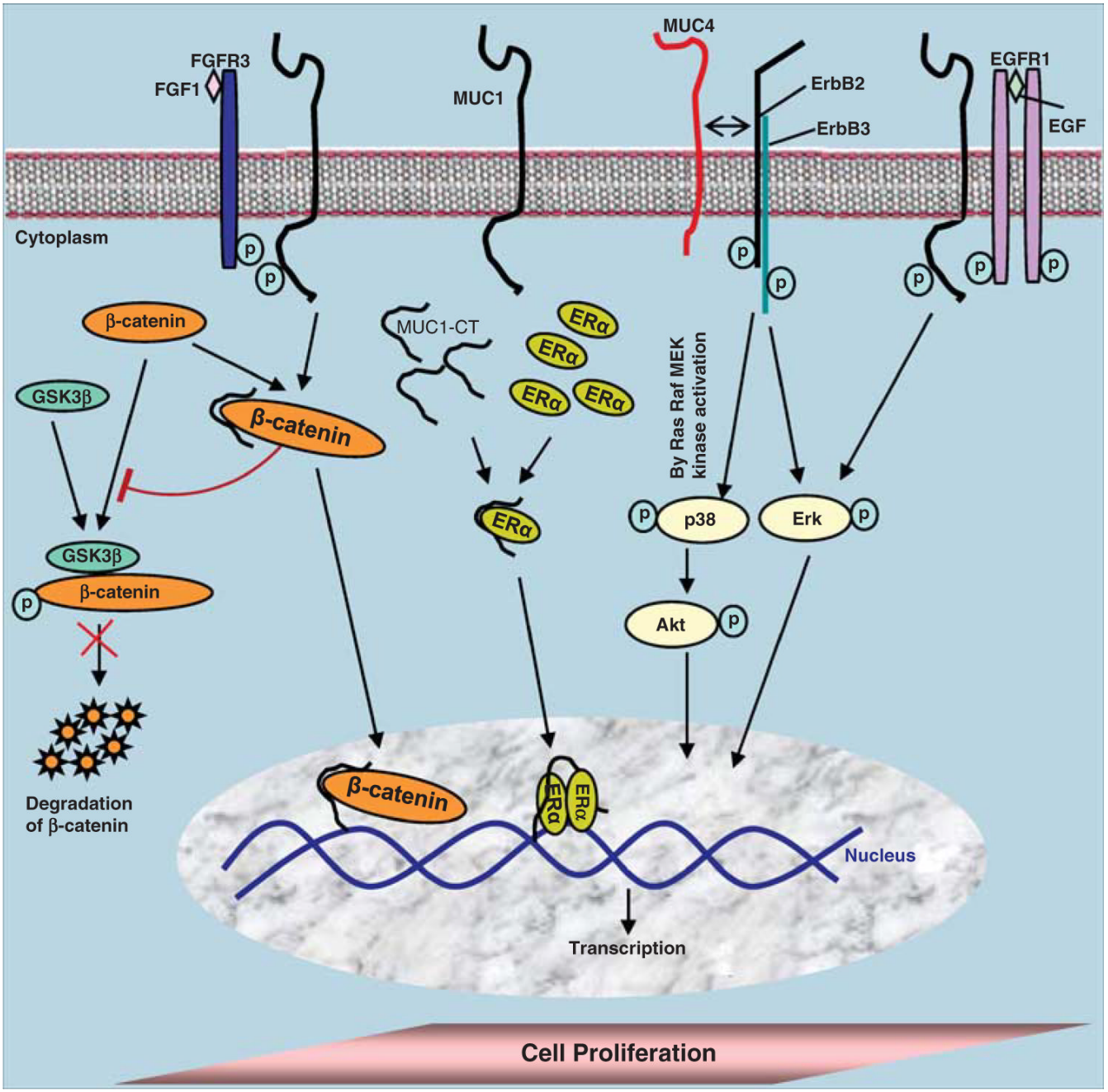

Figure 3.

Divergent mechanisms of MUC1 and MUC4 for enhanced cancer cell proliferation. MUC1 through its cytoplasmic tail (CT) interacts with the ErbB1 epidermal growth factor receptor tyrosine kinase and increases cell proliferation via activation of extracellular signal-regulated kinases (Erks). MUC4 also contributes to enhanced cellular proliferation through its interaction with another epidermal growth factor receptor tyrosine kinase ErbB2 with subsequent activation of Erk and Akt signaling pathways. By interacting with β-catenin, MUC1-CT inhibits GSK3β-mediated degradation of the β-catenin and increases the expression of cell-cycle progression genes by increasing the nuclear pool of β-catenin. MUC1 also controls cell proliferation by stabilizing estrogen receptor α (ERα).

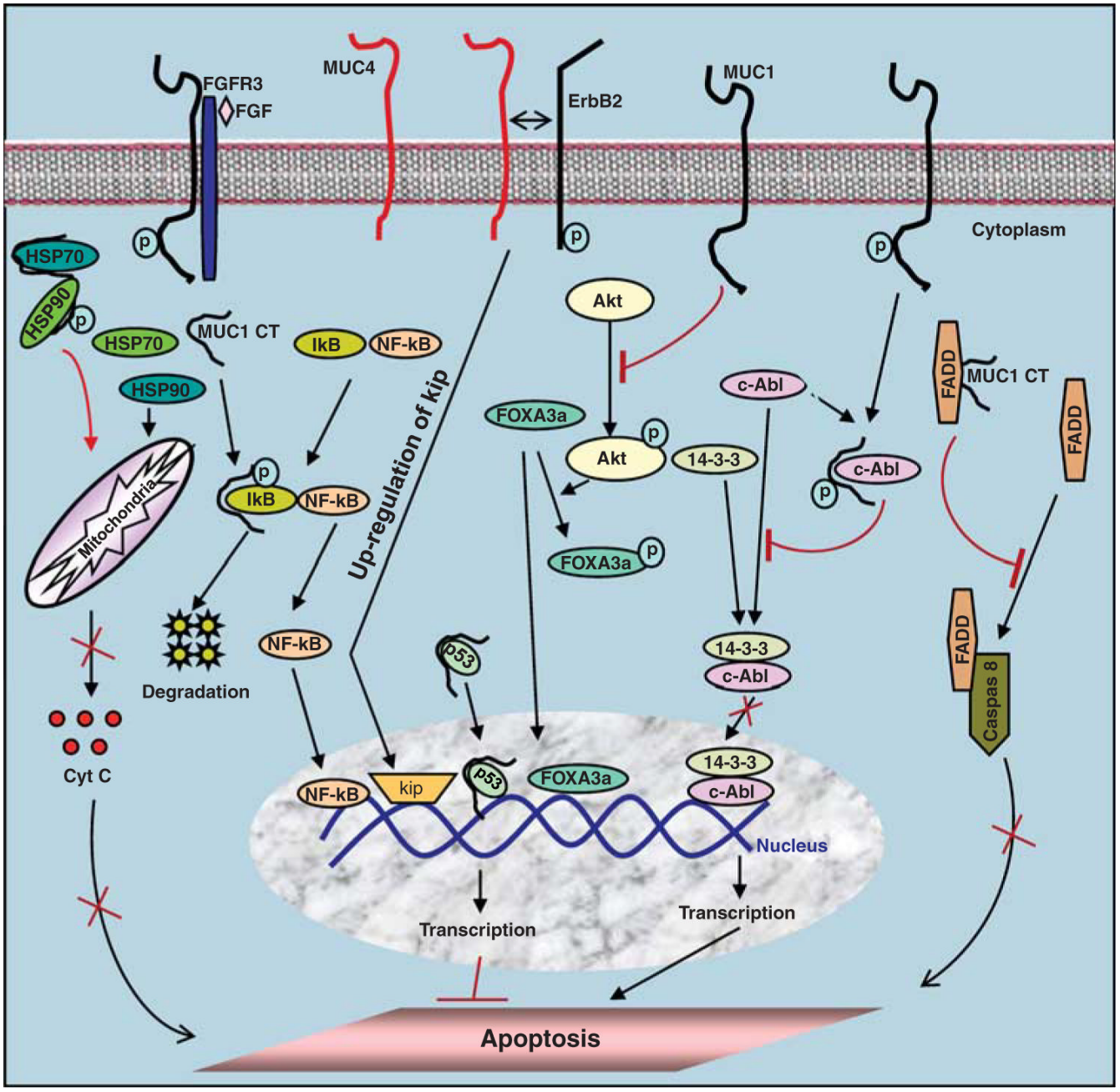

Figure 4.

Illustration of different mechanisms of MUC1 and MUC4 for the repression of apoptosis. MUC1 selectively transactivates p53, FOXA3a, and NF-κB transcription factors and suppresses induction of apoptosis. In contrast, MUC1 blocks nuclear targeting of c-Abl and blocks apoptosis. Activation of MUC1-CT by growth factor signaling causes its binding with HSP90, which transduces signals from the cell membrane to mitochondria that attenuates activation of the intrinsic apoptotic pathway. In addition, MUC1 interacts with the death effector domain of FADD and thereby prevents an activation extrinsic apoptotic pathway. MUC4 has also been proposed to represses apoptosis through the upregulation of kip.

Table 1

Salient features of membrane-bound mucins

MUCs	Salient features
MUC1	MUC1 is normally expressed in most epithelial cells and aberrantly overexpressed in various carcinomas. Around 200 kDa of MUC1 protein comprises 20-residue tandem repeats at the N-terminus of extracellular subunit, an SEA module, a TM domain, and 69 amino acids long C-terminal cytoplasmic domain
MUC3	MUC3 is the product of two genes MUC3A and MUC3B and both are normally expressed in gastrointestinal epithelia. MUC3 consists of 17-residue tandem repeats mucin domain at the N-terminus of the extracellular subunit, an SEA module, two cysteine-rich EGF-like domains, one to either side of the SEA module, a TM domain, and a 72 amino acids long C-terminal cytoplasmic domain (CT)
MUC4	A 930 kDa of MUC4 protein has two subunits, MUC4α and MUC4β. MUC4α comprises three putative functional domains, 16 residue tandem-repeats, nidogen-like (NIDO), and adhesion-associated domain in MUC4 and other proteins (AMOP), whereas MUC4b has three EGF-like domains, a TM domain and a 22-amino acids long CT
MUC12	MUC12 is normally expressed by stomach and colon. It is a 5478-residue membrane-anchored protein and comprises a large N-terminal mucin domain, an SEA module, two cysteine-rich EGF-like domains, one to either side of the SEA module, a TM domain, and a 75 residue C-terminal cytoplasmic domain
MUC13	MUC13 is highly expressed in the epithelium of gastrointestinal and respiratory tracts and aberrantly expressed in gastric, colorectal, pancreatic, lung, and ovarian carcinomas. It comprises of an N-terminal large 151-amino acid tandem repeat mucin domain, three EGF-like domains, an SEA module, a TM domain, and a 69 amino acids CT
MUC15	MUC15 was originally isolated from bovine milk fat globule membranes. It is most abundantly expressed in the placenta, salivary gland, thyroid gland, and moderately in the kidney and lung. 311 amino acids protein of MUC15 contains a signal sequence, an extracellular mucin domain, a small TM domain, and a 74 amino acids CT
MUC16	The largest membrane-bound mucin is expressed by epithelium of the mesothelium lining body cavities (pleural, peritoneal, and pelvic cavities). The peptide backbone of MUC16 is composed of N-terminal region, an extensive 52 residue tandem repeat domain, 16 SEA domains, a C-terminal region with a 32 amino acids CT
MUC17	It is a 4493 amino acids long gastrointestinal tract mucin and comprises a signal sequence, a large N-terminal mucin domain with 59 residue amino acids tandem repeats, two cysteine-rich EGF-like domains, an SEA module, a hydrophobic TM domain, and a C-terminal 80 amino acids long CT
MUC20	A small 503 amino acids protein is markedly upregulated in renal injuries. Two isoforms of the MUC20 exist with different N-terminal sequences. According to the number of mucin repeats, these isoforms are MUC20-S (repeat times <3) and MUC20-L (repeat times=/>3). 53 amino acids long CT has two functional domains: one involved in MUC20 oligomerization and the other involved in MUC20-Met binding. In addition, as a membrane protein, it also comprises several hydrophobic domains
MUC21	A novel 535 amino acids long TM mucin is considered as a marker for lung adenocarcinomas. It comprises a signal sequence, 28 tandem repeats of 15 amino acids, 22 amino acids of stem domain, 23 amino acids of TM domain, and a CT of 64 amino acids

Abbreviations: CT, cytoplasmic tail; EGF, epidermal growth factor; MUC, mucins; TM, transmembrane.