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The Membrane-Bound Mucins: How Large O-Glycoproteins Play Key Roles in Epithelial Cancers and Hold Promise as Biological Tools for Gene and Immunotherapies

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ABSTRACT: Membrane-bound mucins belong to an ever-increasing family of *O*-glycoproteins that share a structure conserved throughout evolution. Typically, membrane-bound mucins contain a long extracellular domain, a hydrophobic transmembrane domain, and a short cytoplasmic tail. They are modular proteins and have a structural organization containing Pro/Thr/Ser-rich O-glycosylated domains and EGF-like domains. The biological roles of mucins arise from their complex structures. MUC1 and MUC4 modulate biological properties of the cell, alter its behavior and modulate cell signaling pathways associated with tumorigenesis. Altered expression and post-translational modifications confer an important role to MUC1 and MUC4 in tumor progression, metastasis, and cancer cell chemoresistance. Moreover, increasing knowledge about their animal counterparts has made possible a greater understanding of their pathophysiological role *in vivo*. Most biological functions attributed to MUC4 are based on the structural homology with its rat homologue. From these results, the development of new biological tools targeting mucins has been increasing and the recent attention given to these complex molecules may bring hope for improved cancer treatments in the future. This review discusses the structure/function of MUC1 and MUC4 membrane-bound mucins in relation to cancer cell behavior and cell signaling pathways associated with tumorigenesis, as well as their potential as biological tools for gene therapy and immunotherapy approaches.

KEY WORDS: mucin, cancer, MUC1, MUC4, proliferation, tumor progression, therapeutic target, cell signaling, chemoresistance

I. INTRODUCTION TO MEMBRANE-BOUND MUCINS

Mucins are a heterogeneous family of large *O*-glycoproteins composed of a long peptidic chain called apomucin on which are linked hundreds of oligosaccharidic chains. On the basis of biochemical studies, mucins were initially defined as high molecular weight (MW) molecules secreted by epithelia, which are able to form viscoelastic gels and are responsible for the rheological properties of mucus. They were additionally characterized by a high content in serine (Ser), threonine (Thr), and proline (Pro) residues.

The advent of molecular biology in the 1990s enabled the identification of high MW *O*-glycoproteins with structural characteristics of mucins that contained a trans-membrane (TM) domain. Today, the family of membrane-bound mucins includes MUC1, MUC3A/B, MUC4, MUC12, MUC13, MUC15, MUC16, MUC17, MUC20, and MUC21.¹ Among them, MUC1 and MUC4 are best characterized. Overexpression of membrane-bound mucins in numerous cancers is often associated with a poor prognosis. Because of their structure and cellular localization, they play biological roles in extracellular cell-cell, cell-matrix interactions, and in cell signaling, and they modulate biological properties of cancer cells. Thus, membrane-bound mucins offer exciting opportunities and stay under intense investigation as both potential biomarkers and therapeutic targets in epithelial cancers.

This review focuses on the structure/function of MUC1 and MUC4 membrane-bound mucins in relation to cancer cell behavior and cell signaling pathways associated with tumorigenesis. In addition, we discuss the potential of membrane-bound mucins as biological tools for both gene- and immunobased therapeutical approaches.

II. MUC1

II.A. Structure of the *MUC1* Gene

MUC1 was the first mucin gene characterized in 1991.²⁻⁴ *MUC1* is localized on chromosome 1 at q21-q24, contains 7 exons, and has a 4- to 7-kb size because of a variable number of tandem repeat (VNTR) polymorphisms varying in size from 400 to 2400 residues.⁵ *MUC1* is structurally organized in domains: exon 1 encodes the signal peptide, and exon 2 encodes the central domain. This central domain comprises a sequence repeated in tandem with a unit of 20 amino acids (aa). Variations in the number of tandem repeats and sequences of the individual TRs are observed and could have potential functional consequences.⁶ Therefore, it was shown that *MUC1* small allele distribution is increased in gastric carcinoma patient populations.⁷ Exons 6 and 7 encode the TM domain and cytoplasmic tail, respectively. (For reviews on the structure of *MUC1* gene, see Ref. 8.)

II.B. Structure of the *MUC1* Protein

The *MUC1* mucin is also known as polymorphic epithelial mucin (PEM), episialin, DUPAN-2, DF3, human milk fat globule (HMFG), epithelial membrane antigen

(EMA), and CD227.⁹ This mucin lacks both the domain responsible for polymerization and EGF domains usually found in the other membrane-bound mucins (Fig. 1). The MUC1 apomucin contains an O-glycosylated repeated central domain and a 72-aa cytoplasmic tail. MUC1 also contains five potential N-glycosylation sites located in the SEA domain following the tandem repeat (TR).¹⁰ The MUC1 SEA module is a self-cleavage domain.¹¹ It was recently shown that extracellular and TM subunits of MUC1 are cleaved in the rough endoplasmic reticulum (RER) at the FRPG/SVVV site localized between the SEA domain and the TM unit.¹² The SEA module occurs in many membrane-associated proteins that are released from the cell surface and has been implicated in both cleavage events and association of the subunits, which are noncovalently linked. MUC1 resides at the apical side of the cell because of a site contained within the extracellular domain and a CQC site located at the junction between the cytoplasmic and TM domains (Fig. 2).¹³

Diverse glycosylation events at the MUC1 extracellular domain provide tissue-specific interactions with the host environment. Changes in the carbohydrate epitopes carried by MUC1 have been detected in many carcinomas, such as those of the colon

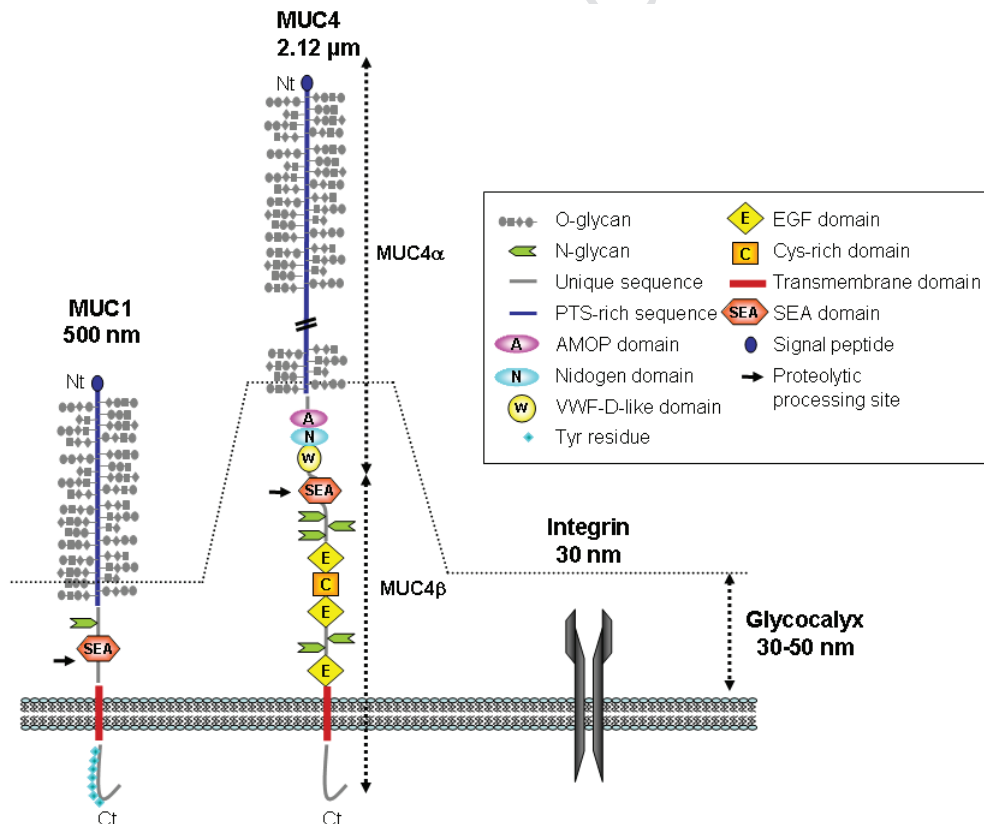


FIGURE 1. Conserved modular organization of MUC1 and MUC4 membrane-bound mucins.

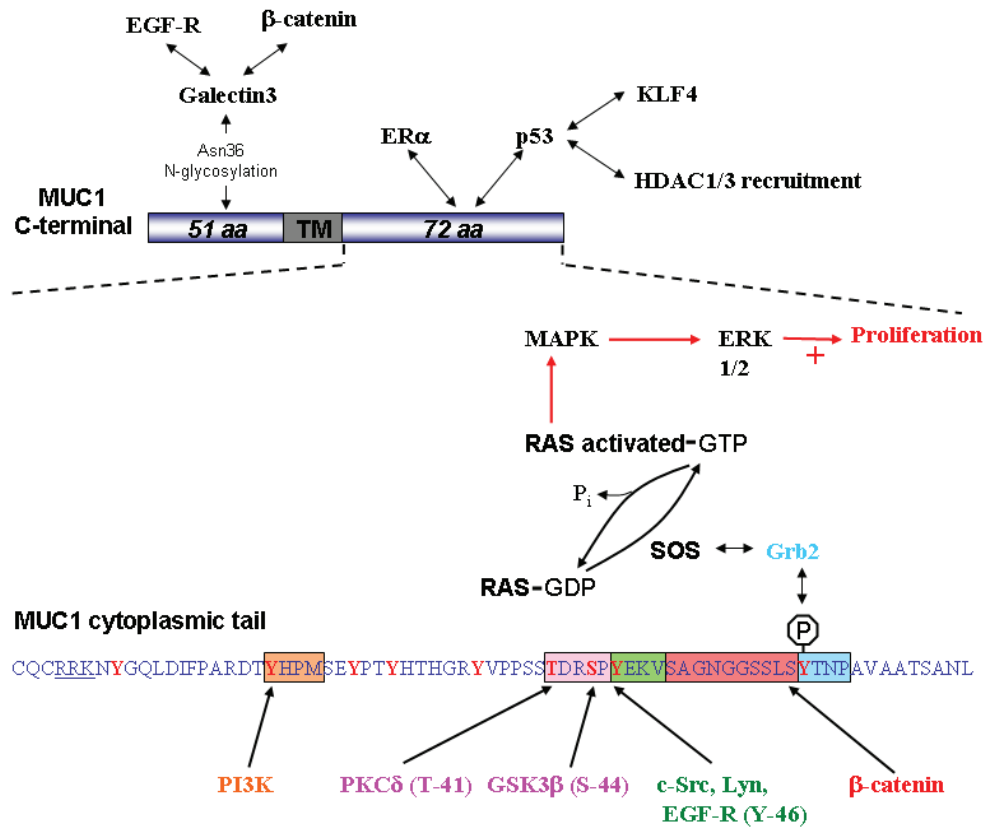


FIGURE 2. MUC1 and cell signaling. The MUC1 C-terminal subunit and cytoplasmic tail allow the interaction of MUC1 with a wide array of signaling pathways in tumor cells.

or breast. In malignant epithelial cells, the MUC1 glycosylation pattern and distribution on the polypeptide are both altered. These alterations are associated with metastatic progression and generation of immunogenic epitopes.¹⁴

MUC1 can also undergo alternative splicing, which leads to splice variants such as *MUC1/SEC* or *MUC1/Y*. *MUC1/SEC* results from an alternative splicing event occurring in the 3'-end of the TR array and consequently does not possess the TM sequence and cytoplasmic tail.¹⁵ *MUC1/Y* is an alternative splice variant with a 1.2-kb full length cDNA resulting from deletion of the mucin-like central domain.¹⁶ *MUC1/Y* appears to act as a membrane receptor, which undergoes Tyr and Ser phosphorylation and activates cell signaling via Grb2.¹⁷ Other splice variants have been described, but their real function is currently unknown.

II.C. MUC1 and Cell Signaling

Several laboratories have shown that MUC1 is involved in cell signaling.^{18,19} The cytoplasmic tail contains 7 Tyr residues, an SH2 interaction domain, and several

kinase phosphorylation sites (Fig. 2). The MUC1 cytoplasmic tail interacts with proteins involved in neoplasia and cell adhesion, such as c-Src, Lyn and p120ctn,^{20–22} GSK-3 β ,²³ PKC δ ,²⁴ and the four members of the ErbB receptor family. These interactions induce signaling pathways such as “mitogen-activated protein kinase” (MAPK).¹⁸ Therefore, MUC1 governs both cellular differentiation and proliferation. MUC1 is also involved in metastasis because of its role in tumor proliferation²⁵ and its antiadhesive activity.^{26–28} This is due to its ability to bind β -catenin and other family members (γ -catenin but not α -catenin),^{24,29} which are involved in cell interaction and transcriptional regulation.³⁰ A Ser-rich motif (SRM) in the MUC1 cytoplasmic domain is able to bind directly to the β -catenin armadillo repeat³¹ (Fig. 2). This interaction can be regulated by formation of a MUC1-APC (adenomatosis polyposis coli) complex. APC is a tumor suppressor gene, which downregulates β -catenin expression.³² MUC1 can also activate the phosphoinositide 3-kinase (PI3K)/Akt and Bcl-xl antiapoptotic pathways.³³ Neuregulin-1 induces the formation of an ErbB2-MUC1 complex and allows MUC1 association with γ -catenin, inducing its nuclear translocation. Such a complex is only observed in tumor cells, suggesting MUC1 to be a master regulator of the ErbB2 and Wnt pathways.³⁴ The MUC1 cytoplasmic tail (MUC1CT) has the ability to shuttle into the nucleus by associating with nucleoporin Nup62³⁵ and can also interact with transcription factors such as KLF4, p53,³⁶ or receptor FGFR3³⁷ and therefore alter the biological properties of cells. In breast cancer cells, MUC1CT represses p53-mediated transcription by binding to KLF4 and recruiting histone deacetylase1/3 (HDAC1/3).³⁶ FGF1 increases FGFR3-MUC1CT interaction and also induces phosphorylation of MUC1 on the YVEF motif, activating nuclear translocation of MUC1CT and β -catenin (Fig. 2).³⁷ MUC1 expression regulates degradation of ErbB1 by decreasing EGF-stimulated ubiquitination of ErbB1 and by increasing ErbB1 recycling.³⁸ Platelet-derived growth factor (PDGF) catalyzes a Tyr phosphorylation site (HGRYVPP) on MUC1CT and enhances invasion *in vitro* and tumor growth and metastases *in vivo*.³⁹ Recent work has shown that the MUC1 C-terminus (Ct) subunit is glycosylated on Asn-36 and upregulates expression of the lectin galectin-3. In turn, galectin-3 binds to MUC1-Ct at the glycosylated Asn-36 site and forms a bridge between MUC1 and the EGF receptor (Fig. 2). Galectin-3 is essential for EGF-mediated interactions between MUC1 and EGFR.⁴⁰ MUC1 can mediate tumor cell interactions via the ICAM-1 adhesion molecule and its extracellular domain.⁴¹ This strongly indicates an important role for MUC1 in heterotypic adhesion events, suggesting that MUC1 may promote migration and extravasation of metastatic cells via interaction with endothelial cells.

II.D. The Role of MUC1 in Drug Resistance

Recent studies have shown that tumor cells expressing MUC1 exhibit an increased capacity to resist the effects of chemotherapeutic drugs. Ren and colleagues⁴² demonstrated that in colorectal cells treated with the genotoxic agent cisplatin, MUC1 attenuated (1) apoptotic mitochondrial factors release, (2) caspase-3 activation, and

(3) apoptosis induction. Moreover, MUC1 inhibition in mammary and lung tumor cells has been found to increase their sensitivity to genotoxic drugs by decreasing the apoptotic effect following DNA structure modifications. MUC1 can also reduce apoptosis following oxidative stress by regulating intracellular levels of reactive oxygen species (ROS).⁴³ The antitumoral drug indol-3-carbitol inhibits MUC1 expression in mammary cancer cells at a transcriptional level.⁴⁴ In pancreatic tumor cells, mucin O-glycosylation inhibitor benzyl- α -GalNAc promotes the effectiveness of 5-fluorouracil (5-FU).⁴⁵ Thus, intracellular uptake of 5-FU is enhanced in a reduced extracellular matrix environment.⁴⁶



II.E. Muc1 Orthologue

The mouse homologue *Muc1*, located on chromosome 3, exhibits strongly conserved TM and cytoplasmic domains compared with those of human *Muc1*.⁴⁷ The TR contains 16 repeats of 20-21 aa and only shows 34% homology with human MUC1 and the most significant homology rises to 87% for the TM domain and cytoplasmic tail. The 5'-flanking regions also share a high homology with the human sequence (74%). In particular, a region immediately upstream of the TATAA box is highly conserved, displaying close to 100% identity between human and mouse sequences. Globally, human MUC1 peptide sequences shares 70% and 72% homology with mouse and rat Muc1 orthologues, respectively.

Mouse models have provided great insight into the roles of MUC1 *in vivo* and have provided evidence of a direct role for MUC1 in tumor progression. In studies of a mouse model expressing Muc1 mucin cDNA, it was found that the transfectants expressing human mucin showed a reduction in tumor incidence at low inocula and a delay in tumor growth at higher inocula.⁴⁸ This indicated for the first time that Muc1 participates in tumor progression. Mice deficient in Muc1 mucin appeared to develop normally and were healthy and fertile, and these mice showed a significantly slower growth rate of primary breast tumors.⁴⁹

In conclusion, MUC1, with important structural domains conserved throughout evolution, plays key roles in cell signaling, differentiation, and proliferation of epithelial cells. Biological properties of MUC1, its altered expression, and post-translational modifications in cancers also confer an important role to MUC1 in tumor progression, metastasis, and cancer cell chemoresistance.

III. MUC4

III.A. Structure of the *MUC4* Gene

MUC4 was originally identified in our laboratory from a human tracheobronchial mucosa cDNA library using a polyclonal antiserum raised against neutral, sialylated, and sulfated mucin glycoproteins.^{50,51} The *MUC4* gene resides on chromosome 3 at

q29,⁵² is 30.8 kb in size, and contains 26 exons.^{53–55} Exon 1 encodes the 5'-UTR (untranslated) region and the signal peptide, whereas exon 2 contains a long sequence of 951 aa followed by a long TR sequence constituting a central domain. **Twenty-four** exons of 65 to 607 bp are located in the 3'-end, including an alternate exon only present in the sv1 splice variant. To date, 24 cDNAs transcribed from the *MUC4* gene have been identified. These transcripts are the result of a complex alternative splicing mechanism and encode distinct isoforms. Six variants are membrane bound and 18 are secreted. These variants are named *sv0* to *sv21-MUC4*, *MUC4/X*, and *MUC4/Y*. *MUC4/X* and *MUC4/Y* represent two membrane-bound variants lacking a mucin-type central domain.⁸ The *MUC4* complete form *sv0-MUC4* is the main isoform expressed in human tissues. For the other forms, *sv1*–*sv24*, their expression at the protein level remains to be proven.

Transcript sizes vary from 14 to 26 kb, depending on **the** VNTR-type polymorphism.⁵⁵ Moreover, intron 3 contains a mini-satellite polymorphism with a 15-bp motif. Intron 4 contains a 26–32 sequence repeated in tandem, and a 32-bp sequence is repeated in exon 5.⁵⁶

III.B. Structure of the MUC4 Protein

The *MUC4* membrane-bound mucin lacks polymerization domains because its **von Willebrand factor D (VWD)** domain is unusual in that it lacks cysteines (Fig. 1). *MUC4* contains EGF-like domains in its extracellular subunit, which also contains the TR domain.⁵⁷ These EGF domains are conserved in mouse and rat orthologues.^{58,59} The *MUC4* N-terminus (Nt)-deduced aa sequence contains a signal peptide and 27 aa followed by three imperfect repeats of a 126–130 aa motif and a unique sequence of 554 aa. These last two domains are Pro/Thr/Serich (PTS domain). The central domain contains a perfect repeat of 16 aa, which can be repeated between 145 and 395 times. The Ct region is divided into 12 domains (CT1 to CT12): 2 heavily N-glycosylated domains (Nidogen and AMOP domains), a Cys-rich D domain, 3 EGF domains, a 14 aa TM domain and a short 22-aa cytoplasmic domain.^{8,60} **Δ GPDH** putative cleavage site is located between CT4 and CT5. The 930-kDa *MUC4* apomucin is composed of two subunits: *MUC4α* and *MUC4β*. *MUC4* is a mucin with a 2.12-μm-long membrane-bound extracellular domain. Whereas *MUC4α* represents the glycosylated mucin-type subunit, *MUC4β* represents the growth factor-like membrane-bound subunit (Fig. 1). Experimental evidence suggests that the EGF-like domain plays a role in receptor-ligand interactions. The interaction of the *MUC4* EGF-like motif with ErbB2 is the most well characterized, likely serving as a regulator in signaling related to growth, motility, or differentiation properties of the cell.^{30,61–63} It has been speculated that EGF domains located in released extracellular domains of mucins (resulting from cleavage) could also act as growth factors and serve as indicators of alteration of epithelial surfaces. The functions of the other specific domains comprising *MUC4* remain largely unknown. The presence of the AMOP domain in cell adhesion molecules, however, hints toward a role for this domain in adhesion.⁶⁴

The NIDO nidogen domain is an extracellular domain of unknown function found in nidogen and hypothetical proteins.

III.C. The Role of MUC4 in Carcinogenesis

Initial *in vitro* reports indicated that MUC4 might play an important role in the behavior of epithelial tumor cells. Inhibition of MUC4 expression by integration of MUC4 antisense RNA (siRNA) leads to reduced proliferation, motility, and increased cellular aggregation. These cells express a low level of ErbB2 as well as its phosphorylated active form.⁶⁵ Xenografts of cells lacking MUC4 are typically smaller, and metastasis in lymph nodes is reduced. From these studies, it has been hypothesized that MUC4 participates in pancreatic tumor progression by directly altering tumor cell properties, possibly via a modulation of ErbB2 expression (Fig. 3). Nagy and co-workers⁶⁶ showed that MUC4 also plays a role in resistance to the chemotherapeutic drug herceptin. In the herceptin-resistant cell line JIMT-1, ErbB2 is partly masked by MUC4, leading to diminished binding of herceptin to ErbB2, whereas knockdown of MUC4 by RNA interference results in the increased binding of herceptin. Therefore,

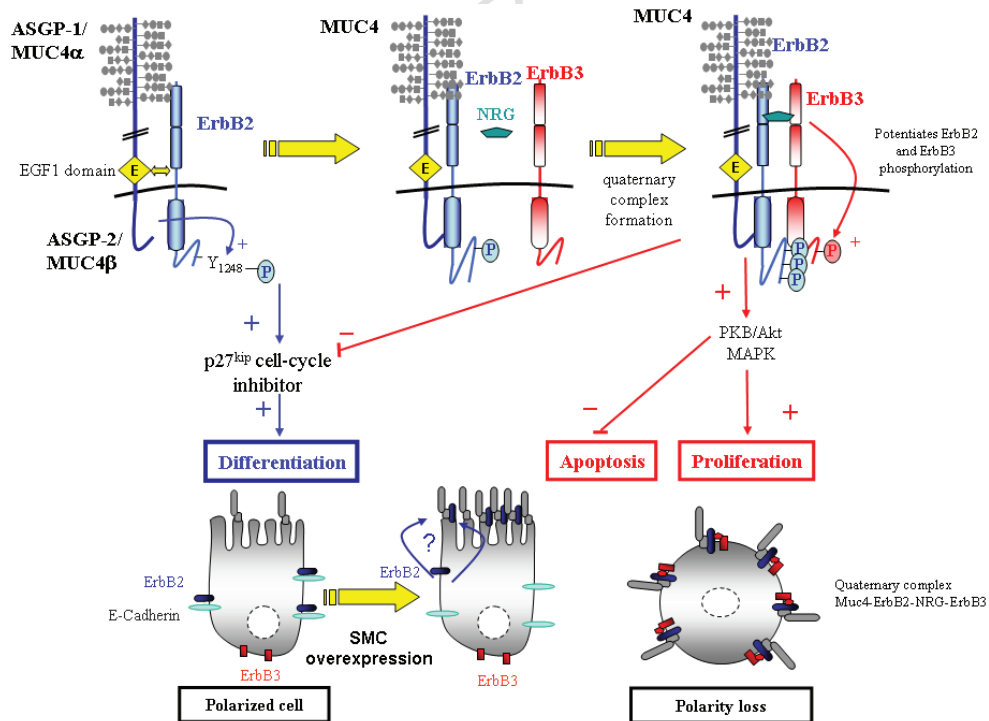


FIGURE 3. MUC4 and cell signaling. MUC4 interacts with ErbB2 and acts as a modulator between proliferation and differentiation via activation of the cell cycle inhibitor p27^{kip} or PKB/ Akt MAPK signaling pathways.

MUC4 might be of potential use as a prognostic indicator of a tumor sensitivity to herceptin. This is of great interest **because** cancer cell resistance to herceptin is common, yet its cause remains unclear.⁶⁶

III.D. Muc4 Orthologues

So far, mouse, rat, and chicken orthologues of *Muc4* have been characterized. Mouse *Muc4* is localized in the region of chromosome 16, which exhibits synteny with human chromosome 3q29 where human *MUC4* is located. *mMuc4* contains a total of 25 exons and an identical exon/intron structure to human *MUC4*. Deduced peptidic sequences indicate that the domain organization has similarities with human *MUC4*. *mMuc4* contains three EGF-like motifs and a mucin-like domain consisting of at least 20 TRs of 124-126 aa.⁵⁸ It is envisaged that a *Muc4*-knockout mouse model will undoubtedly provide important information as to a direct role of *MUC4* in tumorigenesis, especially pancreatic⁶⁷ or esophageal carcinoma.⁶⁸

Rat *Muc4*, also known as SialoMucin Complex (SMC), has been extensively characterized. Ascites SialoGlycoProtein-1 (ASGP1) is the homologue of mucin-like subunit *MUC4* α , and ASGP2 is the homologue of **the** TM subunit *MUC4* β (Fig. 3).⁶⁹ ASGP1 contains the Ser/Thr-rich domain, and ASGP2 contains two EGF-like domains, a Cys-rich stretch, a TM domain, and a short cytoplasmic tail. SMC exists either as a membrane-bound form or as a soluble form, resulting from proteolytic cleavage. Globally, the *MUC4* peptide shares a mild homology with mouse *Muc4* (60%) and rat SMC (61%). The chicken homologue of *Muc4* is located on chromosome 9.⁷⁰ The predicted protein (ENSEMBL: <http://www.ensembl.org>) contains all the domains characteristic of human *MUC4*.

1. SMC and Cell Signaling

The biological roles of SMC have been most extensively studied by Carraway and colleagues.³⁰ Deciphering the role of SMC in cancer progression was extremely important since most biological functions attributed to *MUC4* are based on the structural homology between *MUC4* and SMC. SMC has been shown to have intrinsic properties, such as antiadhesive activity involved in cell-cell or cell-matrix interactions, tumor progression, or metastasis. SMC is also able to interact with the oncogenic receptor ErbB2 and modulates its downstream signaling pathway³⁰ (Fig. 3).

Initial reports showed that the EGF1 domain of ASGP2 binds to ErbB2 before the two components are released at the cell surface. In A375 cells, SMC overexpression induces Tyr1248 phosphorylation of ErbB2.⁶³ Tyr1248 is known to be involved in cell transformation.⁷¹ Moreover, treatment with the ErbB3 ligand neuregulin, without SMC, induces phosphorylation of both ErbB2 and ErbB3. Combined treatment with neuregulin and SMC overexpression potentiates the phosphorylation of ErbB2 and ErbB3 and induces ErbB2-ErbB3 complex formation (Fig. 3). ErbB2-Tyr1248 phosphorylation by SMC is independent of the adhesion state of cells and does not

activate MAPK, PKB/Akt, or PI3K pathways. However, SMC potentiates the PKB/Akt pathway following neuregulin treatment, represses apoptosis, and induces proliferation.⁶³ PKB/Akt represses the cell cycle inhibitor p27kip and stimulates cell cycle progression. Altogether, these observations suggest that SMC acts as a differentiation receptor by inducing ErbB2-limited phosphorylation and as a proliferation modulator when SMC acts synergistically with neuregulin and induces phosphorylation of ErbB2 and ErbB3.

In polarized cells of the colon carcinoma cell line Caco-2, ErbB2 is localized at the basolateral side, whereas SMC is located at the apical side. Transient overexpression of SMC leads to ErbB2 translocation to the apical side, where it interacts with SMC and is phosphorylated on Tyr1248.⁷² Ramsauer et al.⁷² hypothesize that ErbB2 behaves as an polarity sensor of epithelial cells dependent upon SMC-ErbB2 complex formation. Loss of polarity converts the cell from a differentiated state associated with SMC-ErbB2 at the apex to a proliferating state associated with SMC-ErbB2-ErbB3-neuregulin quaternary complex formation (Fig. 3).

2. The Role of SMC in Tumor Progression

SMC overexpression in A375 melanoma cells leads to cell-cell or cell-extracellular matrix interactions disruption in a reversible manner.⁷³ The antiadhesion effect is related to SMC expression and SMC protein size, due to its polymorphism. These interaction inhibitions are due to steric hindrance because of their large glycosylated extracellular domains. ASGP1 (500 nm) could mask cell surface adhesion molecules such as integrin (30 nm) (Fig. 1). SMC overexpression also blocks access to surface antigens and cytotoxic immune “lymphokine associated killer” (LAK) cells and confers resistance properties to antitumor immunity. Moreover, sialyl Lewis-x located on ASGP1 is an E-selectin ligand.⁷⁴ Therefore, SMC might act as an adhesion molecule in the process of extravasation of a primary tumor toward the blood or lymphatic system (Fig. 4).

Initially, SMC was isolated from a highly aggressive mammary adenocarcinoma. SMC is thought to contribute to tumor invasion and metastasis. Solid mammary tumors express a low level of SMC, whereas SMC is highly expressed in aggressive tumor cells extracted from spleen effusion. SMC overexpression increases the metastatic properties of xenografted A375 cells. This increase is due to a reduction of the cleaved caspase product, leading to the reduction of apoptosis.⁷⁵

3. SMC and Drug Resistance

Combined with its proliferative role, SMC plays a role in cancer cell resistance to chemotherapeutic drugs. SMC overexpression reduces the binding of the anti-ErbB2 antibody 4D5 in A375 human melanoma cells and MCF7 breast adenocarcinoma cells. The expression level of ErbB2 is unaltered, which supports the hypothesis of a steric blockage related to SMC-ErbB2 complex formation.⁷⁶ Trastuzumab/Herceptin®

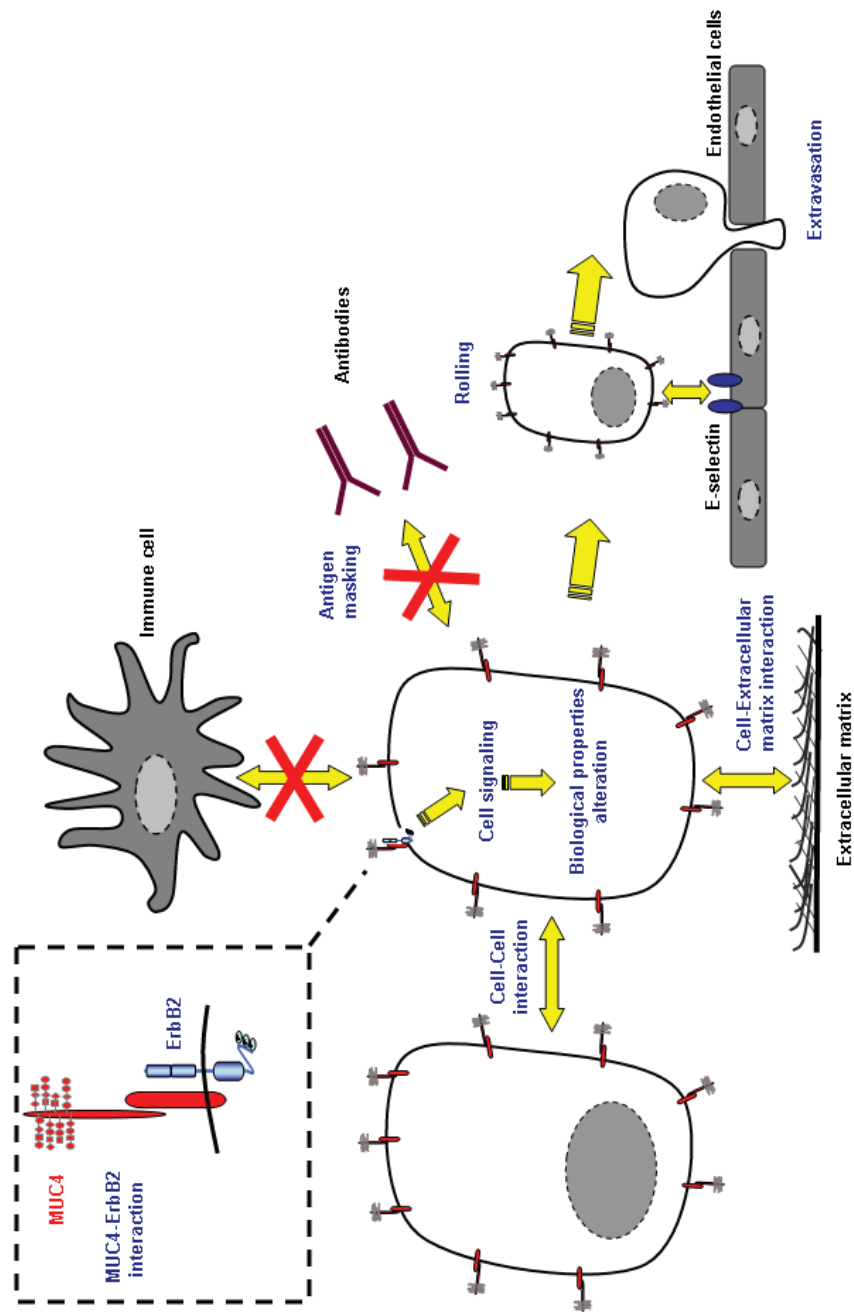


FIGURE 4. The MUC4 mucin and tumor cell behavior. The MUC4 transmembrane mucin plays a role in cell-cell or cell-extracellular matrix interactions. MUC4 interacts with ErbB2 and acts on its downstream signaling pathway, altering the biological properties of the cells. MUC4 alters cell recognition by immune cells or antibodies via a steric hindrance effect. MUC4 may play an important role in the process of metastasis via MUC4 glycan-E-selectin interaction involved during the rolling and extravasation of the tumor cell.

is a monoclonal antibody that acts on ErbB2. Overexpression of SMC by the tumor cell might be a resistance mechanism against herceptin and could therefore be of therapeutic value. It was also shown that SMC provides resistance against chemotherapeutic agents such as taxol, doxorubicine, vinblastin, rhodamine-123, and the 2-deoxyglucose glycolytic inhibitor.⁷⁷ However, SMC-expressing cells maintain a strong resistance to these chemotherapeutic drugs, despite a low level of active antitumor drug transporters, such as glycoprotein P “multidrug resistance” (MDR1) and MDR-related protein (MDRP1). The mechanism responsible for the absence of alteration of sensitivity remains unclear. Reduced apoptosis might be a possibility because SMC inhibits caspase-9 expression.

IV. PERSPECTIVES: MUCs AS THERAPEUTIC TOOLS?

Mucins are thought to play important roles in the biological properties of tumor cells. One could consider mucins as promising therapeutic tools. For this reason, the development of new biological tools targeting mucins for gene therapy and immunotherapeutic approaches has drawn a lot of attention and hope for the future.

IV.A. Immunotherapy

Aberrant expression of the MUC1 core peptide and its glycosylation on human epithelial cancer cells leads to the targeting of tumor-associated epitopes recognized by specific antibodies and cytotoxic T cells. Consequently, these epitopes could be considered as immunotherapeutic targets. Numerous studies are targeting MUC1 in a wide array of cancer models. The MUC1 peptide core or glycopeptide has been used in immunotherapy for immunization. The different strategies showed T-cell and antibody responses and then were challenged for tumor protection. An elegant summary of these strategies can be found in recent reviews by Tang and Alastolopoulos⁷⁸ and Tang et al.⁷⁹ The role of the microenvironment still needs to be deciphered. Thus, naturally occurring MUC1-specific cytotoxic T-cells (CTLs) were isolated and were thought to be capable of preventing or delaying tumor formation in MUC1.Tg transgenic mice, which develop spontaneous tumors of the pancreas.⁸⁰ However, subsequent study showed that these CTLs were nonfunctional within the pancreatic tumor microenvironment, suggesting the use of several immune-evasion mechanisms by tumor cells to evade destruction by CTLs. Alteration of the tumor microenvironment will be necessary for developing an effective immunotherapeutic strategy.⁸¹ Immuno- and radiotherapy can be combined to increase efficiency and tumor response. For example, Mab-PAM4 is an anti-MUC1 antibody that reacts with 85% of pancreatic adenocarcinomas but shows no reactivity against normal pancreas or other tissues. Combined chemotherapy with Gemcitabine and low-dose (131)I-PAM4 radioimmunotherapy may provide an improved alternative for the treatment of pancreatic cancer.⁸² So far, the most advanced clinical trial is a pilot phase III study in early breast cancer using a

MUC1 fusion protein with oxidized mannan and tamoxifen treatment, **which leads to the production of IFN- γ -secreting T cells**. None of the MUC1-injected group showed recurrence, whereas 4 out of 16 recurrences were observed in the placebo group.⁸³

Next to MUC1, the main-targeted mucin in immunotherapy, recent assays have targeted other membrane-bound mucins. One study showed that anti-MUC3 IgG antibody was generated after immunization of naïve peripheral blood B lymphocytes, suggesting possible applications in tumor targeting and immunotherapy.⁸⁴ Toward the goal of making MUC1 a potential target for immunotherapy, DNA vaccines were also developed.⁷⁸ Plasmids encoding human MUC1 and mouse interleukin-18 (pmuIL-18) were generated, and pMUC1/pmuIL-18 vaccinations protected MUC1 transgenic mice from subcutaneous challenge.⁸⁵ A more recent study showed that pMUC1/pmuIL-18 is effective in preventing and treating pulmonary metastases in MUC1.Tg mice, breaks tolerance to MUC1, and induces antigen-specific immunity with protective and therapeutic benefit in MUC1⁺ tumors.⁸⁶ The first studies aimed at using MUC4 in vaccine therapy have been very recently published. A dendritic cell (DC)-based vaccine was developed using cells transduced with the universal DR-restricted Th helper epitope (PADRE) combined with human leukocyte antigen (HLA)-A1- and HLA-A2- restricted epitopes from MUC4 (rAd-pE-DCs). Lymphocytes primed with rAd-pE-DCs generated potent cytotoxic responses, suggesting that the PACRE/MUC4 DNA vaccine could be a potential strategy for immunotherapy of MUC4⁺ tumors.⁸⁷

IV.B. Gene-Based Therapy

Another way to use membrane-bound mucins as therapeutic tools is a gene transfer strategy. In 2004, Aberle and coworkers⁸⁸ monitored the promoter activity of a variety of genes shown to be highly expressed in gastric tumor cells. The *MUC1* promoter was among those showing the highest activity, whereas others, such as the *MUC4* promoter, were moderately expressed when driving cytokine deaminase. The *MUC1* promoter appears to be highly capable of driving a suicide-gene construct in gastric tumors. Further specific studies are required to demonstrate the ability of the *MUC1* promoter to target each tumor model. MUC1 and MUC4 exhibit a cell-specific pattern of expression in epithelial cancers. Subsequently, promoters could be used to drive cytotoxic agents. Adenovirus with *MUC1* promoter driving human somatostatin receptor subtype 2 (hSSTR2) in Panc-1 pancreatic cells demonstrates a significant inhibition of cell proliferation and could be used as a novel specific tool for gene-targeting therapy in MUC1⁺ pancreatic carcinoma.⁸⁹

V. CONCLUSION

Membrane-bound mucins are modular proteins **that** contain conserved domains, such as the epidermal growth factor-like (EGF) domain, potentially involved in important cell signaling pathways. This conserved structure of MUC1 and MUC4 membrane-

bound mucins confers them with important biological roles in extracellular cell-cell and cell-matrix interactions, and in cell signaling, especially involving the ErbB oncogenic receptor family. The structure and known functions of mucins to date implicate a role in driving cancer cells toward carcinogenesis. Thus, membrane-bound mucins offer exciting opportunities as potential therapeutic targets in epithelial cancers. Numerous currently running clinical trials in immunotherapy or DNA vaccine targeting MUC1 in a wide array of cancer models are extremely promising.

MUC4-vaccinated dendritic cells showing cytotoxic responses also allow us to propose MUC4 as a promising therapeutic tool. Using MUC4 as a therapeutic tool is of great interest because of its specific pattern of expression, especially in pancreatic cancer where MUC4 expression is induced during carcinogenesis, whereas it is not expressed in normal pancreas. This is extremely hopeful because pancreatic cancer drastically needs new therapeutic approaches to reduce its disastrous survival rate.

Further clinical trials need to be carried out in early cancer patients to determine the efficiency of using membrane-bound mucin-based vaccines. In the future, it is hoped that increased knowledge will lead to an efficient therapeutic cancer vaccine that can be used not only in cancer patients but also in populations at high risk of developing cancer.

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