Published in final edited form as:

Future Oncol. 2009 December ; 5(10): 1631–1640. doi:10.2217/fon.09.125.

Muc4/MUC4 functions and regulation in cancer

Kermit L Carraway[†],

Department of Cell Biology and Anatomy, University of Miami School of Medicine, Miami, FL 33136, USA, Tel.: +1 305 243 6512, Fax: +1 305 243 4431, KCarrawa@med.miami

George Theodoropoulos,

Department of Cell Biology and Anatomy, University of Miami School of Medicine, Miami, USA

Goldi A Kozloski, and

Department of Cell Biology and Anatomy, University of Miami School of Medicine, Miami, USA

Coralie A Carothers Carraway

Department of Biochemistry and Molecular Biology, University of Miami School of Medicine, Miami, USA

Abstract

The membrane mucin MUC4 (human) is abundantly expressed in many epithelia, where it is proposed to play a protective role, and is overexpressed in some epithelial tumors. Studies on the rat homologue, Muc4, indicate that it acts through anti-adhesive or signaling mechanisms. In particular, Muc4/MUC4 can serve as a ligand/modulator of the receptor tyrosine kinase ErbB2, regulating its phosphorylation and the phosphorylation of its partner ErbB3, with or without the involvement of the ErbB3 ligand neuregulin. Muc4/MUC4 can also modulate cell apoptosis via multiple mechanisms, both ErbB2 dependent and independent. Muc4/MUC4 expression is regulated by multiple mechanisms, ranging from transcriptional to post-translational. The roles of MUC4 in tumors suggest that it may be valuable as a tumor marker or target for therapy.

Keywords

adhesion; apoptosis; ErbB2/ErbB3 regulation; ligand; membrane mucin; metastasis; MUC4; post-translational regulation; proteosomal degradation; transcription

Introduction & implication in cancer

Muc4 is a membrane mucin originally discovered and characterized in a highly metastatic rat mammary adenocarcinoma [1,2]. It was initially called sialomucin complex (SMC) [2] as the mucin nomenclature had not been developed. Human MUC4 was first recognized by cloning a fragment from a tracheal library [3]. Complete sequencing of the rat SMC (Muc4) [4,5] and human MUC4 demonstrated their homology [6]. Rat Muc4/SMC is a heterodimeric glycoprotein composed of a mucin subunit and a transmembrane subunit (Figure 1A) [2]. The

© 2009 Future Medicine Ltd

[†]Author for correspondence: Department of Cell Biology and Anatomy, University of Miami School of Medicine, Miami, FL 33136 USA Tel.: +1 305 243 6512 Fax: +1 305 243 4431 KCarrawa@med.miami.

Financial & competing interests disclosure

Original research was supported by NIH grant CA52498. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. No writing assistance was utilized in the production of this manuscript.

mucin subunit, called ASGP-1 in the rat, contains 11 repeats of approximately 125 amino acids [5] and is highly *O*-glycosylated [7]. The transmembrane subunit (ASGP-2 in the rat; β in the human) contains two EGF domains, a transmembrane sequence and a short cytoplasmic tail and multiple *N*-glycosylation sites [4,8]. The human MUC4 is also heterodimeric, but the mucin subunit (α) contains only three of the 125-amino acid repeats. However, it has extensive (up to 400) polymorphic repeats of 16 amino acids [6]. Thus, it is much larger than the rat Muc4; it is one of the largest of the known membrane mucins. The Muc4 and MUC4 transmembrane subunits are more similar, with 60–70% amino acid identity and conservation of the domain structure [6].

Muc4 is transcribed from a single gene into a 9-kb message and translated into a 300-kDa precursor polypeptide, which is *N*-glycosylated [9]. Cleavage of this precursor into the two subunits occurs before *O*-glycosylation of the mucin subunit, presumably in the endoplasmic reticulum. A second cleavage occurring at approximately the same time can release a soluble form of the mucin containing the ASGP-1 and most of the ASGP-2 [10]. Because of its similar subunit and domain/sequence organization structure, similar processing mechanisms are presumed to occur with human MUC4. Moreover, numerous splice variants of MUC4, both soluble and membrane, have been proposed based on the structure of the gene [11], some of which have been found to be expressed. Thus, the expression of MUC4 isoforms in human tumors can potentially be very complex, but has not been analyzed in detail.

MUC4 has been implicated in multiple human cancers, including pancreatic, lung, breast, gall bladder, salivary gland, prostate, biliary tract and ovarian (Table 1), and may be a useful clinical marker for some of these [12]. Understanding the expression and function of MUC4 in tumors is complicated by the variety of analytical methods used, ranging from transcript analysis to immunohistochemistry, and the difficulty of their application. Thus results obtained from different methods and from different laboratories and on different types of samples may vary widely and even indicate different outcomes of the expression of MUC4. These vagaries make the investigation of the function of MUC4 in tumors very difficult. However, some insights are beginning to emerge. Although the exact role of MUC4 in any cancer remains to be determined, silencing of MUC4 leads to decreased pancreatic tumor growth and metastasis [13]. Thus, a contribution of MUC4 to the aggressiveness of pancreatic cancer appears to be important. Interestingly, a recent study showed the upregulation of MUC4 in breast tumor metastases, but not in primary tumors [CARRAWAY KL 3RD, PERS. COMMUN.]. These results are consistent with a previous study showing the ability of Muc4 to promote tumor metastasis [14]. In contrast, MUC4 appears to be associated with more differentiated and less aggressive head and neck cancers [15]. Thus, the role of MUC4 appears to depend on the particular cancer and cell context.

Muc4 effects on cell adhesion

Because of its large size and extended conformation, Muc4 can block access to the cell surface [16]. In normal epithelial cells this mechanism can protect the epithelium from invasion by microbes [17]. In human pancreatic tumor cells, MUC4 represses cell aggregation [13]. Similarly, tumor cells are protected from killing mechanisms by immune cells [18], and even from antibodies that might bind to specific molecules on the cell surface [19], thus blocking important immune surveillance mechanisms and potential immune-based therapies. With regard to therapy, MUC4 was found to be overexpressed in a human breast cancer resistant to antibody therapy with trastuzumab (Herceptin®) [20]. Silencing of the MUC4 expression restored trastuzumab binding. Moreover, since the action of antibody-based therapies is often based on cell killing by immune cells [21], the ability of Muc4/MUC4 to block cell killing [18] may be even more important than the effects on antibody binding.

An important part of this protection mechanism is the localization of the mucin. In polarized epithelia, Muc4 is located on the apical surfaces [22]. In nonpolarized tumor cells the Muc4 is located circumferentially. Interestingly, Muc4 is able to disrupt cell–cell interactions in tumor cells that can form adhesions [23]. Thus, the Muc4/MUC4 can contribute to dissociation of tumor cells from the primary tumor, facilitating invasion and metastasis. The disruption of adherens junctions and cadherin interactions in tumor cells abrogates contact inhibition in these cells, leading to increased signaling through the Erk pathway that promotes cell proliferation [23]. Therefore, Muc4 can promote tumor cell growth by indirect, anti-adhesive mechanisms, as well as by the more direct signaling mechanisms described below.

Pro-adhesive effects for Muc4 can be envisioned because of its extensive glycosylation of both *N*- and *O*-linked oligosaccharides [7,8], which could interact with cellular lectins. Although such mechanisms have been described for MUC1 [24], no comparable interactions are known for MUC4.

Muc4 signaling via ErbB2

Surprisingly, Muc4 can contribute to both differentiation and proliferation, depending on the cell context. The key to these disparate activities is the ability of Muc4 to act as an intramembrane ligand and modulator for the receptor tyrosine kinase ErbB2 [17,25]. Numerous localization and immunoprecipitation studies in Muc4-transfected cells and in cells naturally expressing Muc4/MUC4 have demonstrated a complex between the mucin and ErbB2 [17]. Since Muc4 interacts with ErbB2 via an EGF domain on the Muc4 [25] and promotes phosphorylation of the ErbB2 [26–28], the mucin has been proposed to be a unique type of ligand, which acts via an intramembrane mechanism (Figure 1) [17]. Interestingly, the Muc4–ErbB2 complex forms shortly after synthesis of the two proteins [27], which has important implications for the function of the complex.

The EGF family of receptor tyrosine kinases plays important roles in development, oncogenesis and apoptosis, including epithelial growth control and wound healing [29]. The family consists of four members, named ErbB1–4. ErbB1 and ErbB4 can be activated by ligands by a classical homodimerization mechanism, in which ligand binding converts an inactive tethered conformation to an active extended conformation. This conformational change frees a loop through which the ErbBs can dimerize, with consequent re-orientation of the kinase domains of the receptor and phosphorylation of the receptor cytoplasmic tails [30]. ErbB2 is proposed to be unable to undergo such changes. It is already in the extended conformation with the loop accessible for dimer formation [31], primed for activation. Notably, ErbB3 has amino acid changes in its kinase domain that repress its kinase activity [32]. ErbB3 thus serves as a docking protein, similar to the insulin receptor substrate, particularly for activation of the phosphatidylinositol 3 (PI3)-kinase/Akt pathway. The ErbB2–ErbB3 pair forms a potent heterodimer that undergoes phosphorylation in the presence of ErbB3 ligand neuregulin [33], and is a major contributor to cell proliferation as well as an inhibitor of apoptosis. However, whether they can form heterodimers depends on the presence and localization of the receptors [27].

In polarized epithelial cells that have no Muc4, ErbB2 is localized to the lateral surfaces of the cells along with ErbB3 (Figure 2A) [27]. However, when Muc4 is present in polarized cells, the Muc4 and ErbB2 form a complex shortly after synthesis. This complex transits to the apical cell surface [27]. These results are consistent with observations of co-localization of ErbB2 and Muc4 at apical surfaces in several epithelial tissues, such as the uterus, mammary gland and airway [17]. This mechanism effectively segregates ErbB2 from ErbB3 (Figure 2B), repressing their ability to form the heterodimers that can drive cell proliferation and motility and inhibit apoptosis. ErbB2 of the Muc4–ErbB2 complex at the apical surface is

phosphorylated on specific tyrosine residues, including 1139 and 1248, and coupled to the activation of the MAP kinase p38 [28], which has been implicated in differentiation [34]. Thus, Muc4 can contribute to maintaining differentiation in polarized epithelia by segregating ErbB2 and ErbB3, and by activating p38 through an ErbB2-dependent mechanism [28].

This segregation mechanism breaks down when barriers establishing cell polarity are lost, as occurs when epithelia are damaged [35] or undergo neoplastic transformation [36] (Figure 2C). ErbB2 and ErbB3 can form complexes even in the absence of ligands [28]. The presence of Muc4 stabilizes the ErbB2–ErbB3 complex and promotes phosphorylation of the ErbB2, but not ErbB3 (Figure 1). The presence of the ErbB3 ligand neuregulin likewise stabilizes the complex and, in addition, promotes phosphorylation of both ErbB2 and ErbB3 [37] (Figure 1). Muc4 can modulate the effect of neuregulin on the receptor phosphorylation by regulating the localization and turnover of the complex [37].

A key issue is which downstream pathways are activated by the action of Muc4 on the ErbB2–ErbB3 complex in tumors. One important element is the potentiation of signaling through the PI3-kinase–Akt pathway [26,37], which is activated by phosphorylated ErbB3 and plays a role in both proliferation and repression of apoptosis. MUC4 silencing in pancreatic tumor cells has been shown to inhibit focal adhesion kinase (FAK) phosphorylation and activation of the Erk pathway [38], the former being involved in cell migration and the latter important to proliferative responses. The involvement of the Muc4 interaction with ErbB2 in cell migration is supported by microarray studies on changes in protein phosphorylation of cellular proteins in tumor cells transfected with a Muc4 derivative that does not show antiadhesive effects. These cells showed both increased FAK phosphorylation and β -catenin levels, suggesting that Muc4 can influence cell migration without directly altering cell adhesion through its action on the ErbB2–ErbB3 complex. MUC4 in ovarian cancer cells can promote FAK phosphorylation, cytoskeletal rearrangements and cell migration [38]. The mechanism (signaling vs anti-adhesion) is unclear, but the implications for ovarian cancer invasiveness and metastasis are likely to be important.

Muc4 & apoptosis

Muc4/MUC4 can promote tumor progression by repressing apoptosis by multiple mechanisms, both ErbB2 dependent and independent [39]. One of these mechanisms is likely to involve activation of the PI3-kinase–Akt pathway [26]. These effects extend to apoptosis induced by differing insults, including loss of adhesion (anoikis), chemotherapeutic drugs [40] and serum starvation [26]. The mechanisms involved in the apoptosis repression vary in different cell lines, but a key element appears to be phosphorylation and inactivation of the pro-apoptotic protein Bad and an increase in the expression of Bcl-xl [39], which promotes survival. The mucin subunit and cytoplasmic domain of Muc4 are not required for these antiapoptotic effects or for ErbB2 signaling.

Transcriptional regulation of Muc4/MUC4

Muc4/MUC4 is regulated by a variety of mechanisms, ranging from transcriptional to post-translational (Table 2). Transcriptional regulation of rat Muc4 has been studied in mammary epithelial cells and 13762 mammary adenocarcinoma cells, from which the membrane mucin was originally isolated [2]. Upregulation of transcription in mammary epithelial cells was observed by treatment with IGF, but not EGF [41]. Inhibition of the Erk pathway blocked the IGF effect. Regulation of Muc4 in the 13762 tumor cells similarly involved the Erk pathway, acting as a potentiator of the transcription factor PEA3 [42]. Muc4 can also be transcriptionally regulated through the JNK pathway. PEA3 binding to the *Muc4* promoter [42] was demonstrated to be specific, acting through one or more of the PEA3 binding sites on the

Muc4 promoter [43,44]. Interestingly, ErbB2 is also regulated by PEA3 [45]. Thus, PEA3 can be involved in the coordination of the regulation of the receptor and its modulator [44].

Transcription of the human *MUC4* gene is complex and regulated by many signaling pathways [46,47]. Extensive studies have been performed on the promoter of human *MUC4* and its transcriptional regulators [48,49], showing four transcriptional start sites, one in a proximal promoter region and three in a distal region. The distal promoter contains a TATA box, but the proximal does not. Multiple binding sites for factors initiating transcription were found in the proximal region, including the glucocorticoid receptor, Sp1, AP-1, PEA3, CACCC box and Med-1. The distal promoter contains many of the same sites and also has a cyclic AMP responsive element. Multiple sites for other signaling pathways, such as protein kinase A, protein kinase C, cytokines and TGF β , have been found. Numerous transcription factors operate on the *MUC4* promoter, including AP2, PEA3, STAT1, SMAD and forkhead box A.

Transcriptional regulation of *MUC4* has been particularly studied in pancreatic cancer cells [12,49]. *MUC4* is poorly expressed, if at all, in the normal pancreas, but is highly expressed in pancreatic cancer [50]. Thus, it has been proposed as a potential target for pancreatic cancer therapy [12]. Targeting mucins is difficult, however, as they have no activities, such as those of enzymes or receptors, that can be easily blocked by small-molecule inhibitors. Therefore, repression of expression of the mucins represents one way of developing targeting strategies. Retinoic acid, interferon- γ and TGF β have all been identified as regulatory factors for *MUC4*. The TGF β has both SMAD-dependent and independent pathways and is negatively regulated by SMAD7 [51]. Regulation by interferon- γ involves STAT-1 [52]. Interferon- γ and retinoic acid synergistically upregulate *MUC4* in pancreatic cancer cells in a process that involves reprogramming of signaling pathways. Other cytokines, including TNF- α , have been implicated in *MUC4* regulation [49]. A synergy between the TNF and interferon- γ activates STATs and NF κ B transcription factors that interact with the *MUC4* promoter to enhance *MUC4* expression [49].

Interleukins, such as IL4 and IL9, have been shown to contribute to *MUC4* regulation in normal tissues, such as the airway, through the JAK-STAT pathway [53]. A similar mechanism has been observed in a lung cancer cell line (Table 2). The STAT pathway has also been observed to be involved in upregulation of *MUC4* in gastric cancer through IL6 (Table 2). In esophageal cancer, bile acids have been implicated in *MUC4* expression through the PI3-kinase signaling pathway and hepatocyte nuclear factor 1 α (Table 2).

Epigenetic regulation of MUC4

Epigenetic regulation of *MUC4* appears to be important in some tumors, and is probably dependent on the presence of two CpG islands in the 5'-flanking region [54]. Five methylation sites were found in pancreatic tumor cells to contribute to this regulation. Moreover, inhibition of histone deacetylation in these cells demonstrated a role for this epigenetic process in the regulation of *MUC4* in these tumors. Breast, lung and colon cancer cell lines have also been shown to regulate *MUC4* by methylation [55]. These studies may provide new approaches for targeting the involvement of *MUC4* in tumor progression [56].

Post-transcriptional regulation of MUC4

MUC4 in human airway epithelial cells is upregulated by transcript stabilization by elastase produced by neutrophils as part of the inflammatory process [57]. In this case similar effects are observed for *MUC4* and *MUC5AC* [58], a gel-forming mucin. Thus, the regulation of airway mucins appears to be complex. It is likely that the regulation of *MUC4* in lung tumors is equally complex.

Post-translational regulation of Muc4

TGF β is a potent repressor of Muc4 expression in rat mammary epithelial cells, but not in rat mammary tumor cells [59]. Transcript and protein analyses indicate that the effect occurs at a post-translational level, involving the protein precursor of the heterodimeric Muc4 [60]. Investigation of the signaling pathways involved demonstrated a role for SMAD2 of the canonical TGF β pathway [61]. Interferon- γ is able to block the TGF β effect by upregulating the inhibitory SMAD7. These results suggested a role for proteosomal degradation in the regulation of Muc4. Blocking proteasome activity increases levels of Muc4 [62]. This block also increases the level of ubiquitinated Muc4 and of Muc4 associated with calnexin and calreticulum. Undegraded Muc4 in cells treated with proteasome inhibitors is shunted to aggresomes [62]. Tumor cells do not show a similar downregulation of Muc4 because the tumors have lost their responsiveness to TGF β [59]. This loss may occur through mutations in the TGF β receptors, the SMAD pathway components or other mechanisms. In the normal mammary gland the loss of TGF β during pregnancy permits the upregulation and secretion of the Muc4 as a protective agent for the epithelia [60]. In contrast, mammary tumors have lost the responsiveness to TGF β , thus they continue to express Muc4, which can promote their survival and proliferation.

Rat Muc4 has been found in squamous epithelia in both the eye [63] and female reproductive tract [64], localized to specific layers of the stratified epithelia. Interestingly, in the eye the localization to the most superficial layers depends on the regulation of the Muc4 by TGF β through the proteosomal degradation mechanism. How Muc4 is regulated in the female reproductive tract squamous epithelia is unknown, but should be investigated as it may provide insight into contributions of this mucin to cancers in these sites.

TGF β has also been shown to downregulate Muc4 in rat uterine epithelial cells, thus contributing to preparing the uterus for embryo implantation [64]. Although the molecular mechanism (transcriptional vs post-translational) is unclear, the effect has been shown to involve a hormone-driven paracrine cellular mechanism [64]. Estrogen and progesterone act on uterine fibroblasts, which produce TGF β . The TGF β then acts on the uterine epithelial cells to block Muc4 expression by inhibiting precursor processing and shunting the Muc4 to the proteasome for degradation. It would be interesting to know whether a similar mechanism for Muc4 regulation is present in hormone-sensitive tumors, such as breast cancer. The multiple effects of TGF β on rat Muc4 indicate the complexity of its functions, operating at different levels (transcriptional vs post-translational) in different tissues. Similarly, TGF β has been shown to upregulate MUC4 in human pancreatic tumors, but downregulate it in rat mammary gland. Thus, the effects of TGF β are very much context dependent.

Conclusion

In spite of great advances in understanding mechanisms of oncogenesis and tumor progression, many aspects are still poorly understood. Recent studies on the mechanisms by which MUC4 may contribute to multiple tumor types indicate that it should be seriously considered not only as a tumor marker, but as a potential target for therapy. In the former case the ability of MUC4 to contribute to resistance mechanisms by blocking immune mechanisms or repressing chemotherapeutic approaches that induce apoptosis suggests that MUC4 expression should be considered in stratifying patients for particular types of therapy. Therapeutically, repression of MUC4 expression might eliminate some of those resistance mechanisms and improve the success rates of some current treatments [65], such as trastuzumab therapy.

Future perspective

The contributions of MUC4 to many types of tumors is still unclear because of inadequate reagents for analysis. New antibodies are needed that eliminate problems with carbohydrate dependence. The mechanisms by which MUC4 represses apoptosis have not been well characterized and need to be investigated at the molecular level. Mechanisms for targeting MUC4 have not received much attention, but the involvement of epigenetic regulation may provide new avenues for blocking MUC4 expression in tumors.

Executive summary

MUC4 functions

- Muc4/MUC4 can block cell and antibody binding to both normal and tumor cells.
- Muc4/MUC4 can repress the effects of trastuzumab on breast tumor cells.
- Muc4 can repress contact inhibition via the Erk signaling pathway.
- Muc4/MUC4 can bind ErbB2 and modulate its phosphorylation and signaling through ErbB3 in the presence and absence of the ErbB3 ligand.
- Muc4/MUC4 can repress apoptosis induced by different mechanisms.
- Muc4/MUC4 can block apoptosis via different mechanisms.

MUC4 regulation

- Muc4/MUC4 can be regulated transcriptionally at multiple promoter sites via different transcription factors and signaling pathways.
- MUC4 transcript stability can contribute to its regulation.
- MUC4 can be regulated epigenetically by DNA methylation or histone acetylation.
- Muc4 can be regulated post-translationally by TGF β through a proteosomal degradation mechanism.

Acknowledgments

We thank Isabelle van Seuning for her comments on the manuscript.

Bibliography

Papers of special note have been highlighted as:

▪ of interest

▪▪ of considerable interest

1. Sherblom AP, Buck RL, Carraway KL. Purification of the major sialoglycoproteins of 13762 MAT-B1 and MAT-C1 rat ascites mammary adenocarcinoma cells by density gradient centrifugation in cesium chloride and guanidine hydrochloride. *J. Biol. Chem* 1980;255:783–790. [PubMed: 7356644]
2. Sherblom AP, Carraway KL, et al. A complex of two cell surface glycoproteins from ascites mammary adenocarcinoma cells. *J. Biol. Chem* 1980;255:12051–12059. [PubMed: 7440586] ▪ First isolation and characterization of rat Muc4, then called sialomucin complex.
3. Porchet N, Nguyen VC, Dufosse J. Molecular cloning and chromosomal localization of a novel human tracheobronchial mucin cDNA containing tandemly repeated sequences of 48 base pairs. *Biochem. Biophys. Res. Commun* 1991;175:414–422. [PubMed: 1673336] ▪▪ First partial clone of human MUC4 and designation as member of the mucin ‘family’

4. Sheng Z, Wu K, Carraway KL, Fregien N. Molecular cloning of the transmembrane component of the 13762 mammary adenocarcinoma sialomucin complex. A new member of the epidermal growth factor superfamily. *J. Biol. Chem* 1992;267:16341–16346. [PubMed: 1379596]
5. Wu K, Fregien N, Carraway KL. Molecular cloning and sequencing of the mucin subunit of a heterodimeric, bifunctional cell surface glycoprotein complex of ascites rat mammary adenocarcinoma cells. *J. Biol. Chem* 1994;269:11950–11955. [PubMed: 8163496]
6. Moniaux N, Nollet S, Porchet N, Degand P, Laine A, Aubert JP. Complete sequence of the human mucin MUC4: a putative cell membrane-associated mucin. *Biochem J* 1999;338:325–333. [PubMed: 10024507]
7. Hull SR, Laine RA, Kaizu T, Rodriguez I, Carraway KL. Structures of the O-linked oligosaccharides of the major cell surface sialoglycoprotein of MAT-B1 and MAT-C1 ascites sublines of the 13762 rat mammary adenocarcinoma. *J. Biol. Chem* 1984;259:4866–4877. [PubMed: 6715325]
8. Hull SR, Sheng Z, Vanderpuye O, David C, Carraway KL. Isolation and partial characterization of ascites sialoglycoprotein-2 of the cell surface sialomucin complex of 13762 rat mammary adenocarcinoma cells. *Biochem J* 1990;265:121–129. [PubMed: 2302161]
9. Sheng ZQ, Hull SR, Carraway KL. Biosynthesis of the cell surface sialomucin complex of ascites 13762 rat mammary adenocarcinoma cells from a high molecular weight precursor. *J. Biol. Chem* 1990;265:8505–8510. [PubMed: 2111320]
10. Komatsu M, Arango ME, Carraway KL. Synthesis and secretion of Muc4/sialomucin complex: implication of intracellular proteolysis. *Biochem J* 2002;368:41–48. [PubMed: 12186632]
11. Escande F, Lemaitre L, Moniaux N, Batra SK, Aubert JP, Buisine MP. Genomic organization of MUC4 mucin gene. Towards the characterization of splice variants. *Eur. J. Biochem* 2002;269:3637–3644. [PubMed: 12153560]
12. Singh AP, Chaturvedi P, Batra SK. Emerging roles of MUC4 in cancer: a novel target for diagnosis and therapy. *Cancer Res* 2007;67:433–436. [PubMed: 17234748]
13. Singh AP, Moniaux N, Chauhan SC, Meza JL, Batra SK. Inhibition of MUC4 expression suppresses pancreatic tumor cell growth and metastasis. *Cancer Res* 2004;64:622–630. [PubMed: 14744777]
14. Komatsu M, Tatum L, Altman NH, Carothers CA, Carraway KL. Potentiation of metastasis by cell surface sialomucin complex (rat MUC4), a multifunctional antiadhesive glycoprotein. *Int. J. Cancer* 2000;87:480–486. [PubMed: 10918186] • Role of Muc4 in promoting metastasis of tumor cells.
15. Weed DT, Gomez-Fernandez C, Yasin M, et al. MUC4 and ErbB2 expression in squamous cell carcinoma of the upper aerodigestive tract: correlation with clinical outcomes. *Laryngoscope* 2004;114:1–32. [PubMed: 15284539]
16. Komatsu M, Carraway CA, Fregien NL, Carraway KL. Reversible disruption of cell-matrix and cell-cell interactions by overexpression of sialomucin complex. *J. Biol. Chem* 1997;272:33245–33254. [PubMed: 9407114] • Effect of Muc4 as anti-adhesive in preventing cellular interactions.
17. Carraway KL, Perez A, Idris N, et al. Muc4/sialomucin complex, the intramembrane ErbB2 ligand, in cancer and epithelia: to protect and to survive. *Prog. Nucleic Acid Res. Mol. Biol* 2002;71:149–185. [PubMed: 12102554]
18. Komatsu M, Yee L, Carraway KL. Overexpression of sialomucin complex, a rat homologue of MUC4, inhibits tumor killing by lymphokine-activated killer cells. *Cancer Res* 1999;59:2229–2236. [PubMed: 10232613]
19. Price-Schiavi SA, Jepson S, Li P, et al. Rat Muc4 (sialomucin complex) reduces binding of anti-ErbB2 antibodies to tumor cell surfaces, a potential mechanism for herceptin resistance. *Int. J. Cancer* 2002;99:783–791. [PubMed: 12115478]
20. Nagy P, Friedländer E, Tanner M, et al. Decreased accessibility and lack of activation of ErbB2 in JIMT-1, a herceptin-resistant, MUC4-expressing breast cancer cell line. *Cancer Res* 2005;65:473–482. [PubMed: 15695389]
21. Clynes RA, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc receptors modulate *in vivo* cytotoxicity against tumor targets. *Nat. Med* 2000;6:443–446. [PubMed: 10742152]
22. McNeer RR, Huang D, Fregien NL, Carraway KL. Sialomucin complex in the rat respiratory tract: a model for its role in epithelial protection. *Biochem J* 1998;330:737–744. [PubMed: 9480884]

23. Pino V, Ramsauer VP, Salas P, Carothers Carraway CA, Carraway KL. Membrane mucin Muc4 induces density-dependent changes in ERK activation in mammary epithelial and tumor cells: role in reversal of contact inhibition. *J. Biol. Chem* 2006;281:29411–29420. [PubMed: 16891313]
24. Hattstrup CL, Gendler SJ. Structure and function of the cell surface (tethered) mucins. *Annu. Rev. Physiol* 2008;70:431–457. [PubMed: 17850209]
25. Carraway KL 3rd, Rossi EA, Komatsu M, et al. An intramembrane modulator of the ErbB2 receptor tyrosine kinase that potentiates neuregulin signaling. *J. Biol. Chem* 1999;274:5263–5266. [PubMed: 10026131] • Report of Muc4 interaction with ErbB2 and modulation of its signaling.
26. Jepson S, Komatsu M, Haq B, et al. Muc4/sialomucin complex, the intramembrane ErbB2 ligand, induces specific phosphorylation of ErbB2 and enhances expression of p27(kip), but does not activate mitogen-activated kinase or protein kinaseB/Akt pathways. *Oncogene* 2002;21:7524–7532. [PubMed: 12386815]
27. Ramsauer VP, Pino V, Farooq A, Carothers Carraway CA, Salas PJ, Carraway KL. Muc4-ErbB2 complex formation and signaling in polarized CACO-2 epithelial cells indicate that Muc4 acts as an unorthodox ligand for ErbB2. *Mol. Biol. Cell* 2006;17:2931–2941. [PubMed: 16624867]
28. Ramsauer VP, Carraway CA, Salas PJ, Carraway KL. Muc4/sialomucin complex, the intramembrane ErbB2 ligand, translocates ErbB2 to the apical surface in polarized epithelial cells. *J. Biol. Chem* 2003;278:30142–30147. [PubMed: 12748185] • Role of Muc4 in localization of ErbB2 in polarized epithelial cells.
29. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. *Nat. Rev. Mol. Cell Biol* 2001;2:127–137. [PubMed: 11252954]
30. Lemmon MA. Ligand-induced ErbB receptor dimerization. *Exp. Cell Res* 2009;315:638–648. [PubMed: 19038249]
31. Garrett TP, McKern NM, Lou M, et al. The crystal structure of a truncated ErbB2 ectodomain reveals an active conformation, poised to interact with other ErbB receptors. *Mol. Cell* 2003;11:495–505. [PubMed: 12620236]
32. Guy PM, Platko JV, Cantley LC, Cerione RA, Carraway KL 3rd. Insect cell-expressed p180erbB3 possesses an impaired tyrosine kinase activity. *Proc. Natl Acad. Sci. USA* 1994;91:8132–8136. [PubMed: 8058768]
33. Carraway KL 3rd, Cantley LC. A new acquaintance for erbB3 and erbB4: a role for receptor heterodimerization in growth signaling. *Cell* 1994;78:5–8. [PubMed: 8033211]
34. Houde M, Laprise P, Jean D, Blais M, Asselin C, Rivard N. Intestinal epithelial cell differentiation involves activation of p38 mitogen activated protein kinase that regulates the homeobox transcription factor CDX2. *J. Biol. Chem* 2001;276:21885–21894. [PubMed: 11283019]
35. Vermeer PD, Einwalter LA, Moninger TO, et al. Segregation of receptor and ligand regulates activation of epithelial growth factor receptor. *Nature* 2003;422:322–326. [PubMed: 12646923]
36. Muthuswamy SK, Li D, Lelievre S, Bissell MJ, Brugge JS. ErbB2, but not ErbB1, reinitiates proliferation and induces luminal repopulation in epithelial acini. *Nat. Cell Biol* 2001;3:785–792. [PubMed: 11533657]
37. Funes M, Miller JK, Lai C, Carraway KL 3rd, Sweeney C. The mucin Muc4 potentiates neuregulin signaling by increasing the cell-surface populations of ErbB2 and ErbB3. *J. Biol. Chem* 2006;281:19310–19319. [PubMed: 16690615]
38. Chaturvedi P, Singh AP, Batra SK. Structure, evolution, and biology of the MUC4 mucin. *FASEB J* 2008;22:966–981. [PubMed: 18024835]
39. Workman HC, Sweeney C, Carraway KL 3rd. The membrane mucin Muc4 inhibits apoptosis induced by multiple insults via ErbB2-dependent and ErbB2-independent mechanisms. *Cancer Res* 69(7): 2845–2852. [PubMed: 19293191] • Multiple effects of Muc4 on apoptosis.
40. Hu YP, Haq B, Carraway KL, Savaraj N, Lampidis TJ. Multidrug resistance correlates with overexpression of Muc4 but inversely with P-glycoprotein and multidrug resistance related protein in transfected human melanoma cells. *Biochem. Pharmacol* 2003;65:1419–1425. [PubMed: 12732353]
41. Zhu X, Price-Schiavi SA, Carraway KL. Extracellular regulated kinase (ERK)-dependent regulation of sialomucin complex/rat Muc4 in mammary epithelial cells. *Oncogene* 2000;19:4354–4361. [PubMed: 10980611]

42. Perez A, Barco R, Fernandez I, Price-Schiavi SA, Carraway KL. PEA3 transactivates the Muc4/sialomucin complex promoter in mammary epithelial and tumor cells. *J. Biol. Chem* 2003;278:36942–36952. [PubMed: 12855694]
43. Price-Schiavi SA, Perez A, Barco R, Carraway KL. Cloning and characterization of the 5' flanking region of the sialomucin complex/rat Muc4 gene: promoter activity in cultured cells. *Biochem J* 2000;349:641–649. [PubMed: 10880365]
44. Fauquette V, Perrais M, Cerulis S, et al. The antagonistic regulation of human MUC4 and *ErbB-2* genes by the Ets protein PEA3 in pancreatic cancer cells: implications for the proliferation/differentiation balance in the cells. *Biochem J* 2005;386:35–45. [PubMed: 15461591]
45. Shepherd TG, Kockeritz L, Szrajber MR, Muller WJ, Hassell JA. The *pea3* subfamily ets genes are required for HER2/Neu-mediated mammary oncogenesis. *Curr. Biol* 2001;11:1739–1748. [PubMed: 11719215]
46. Perrais M, Pigny P, Copin MC, Aubert JP, Van Seuning I. Induction of MUC2 and MUC5AC mucins by factors of the epidermal growth factor (EGF) family is mediated by EGF receptor/Ras/Raf/extracellular signal-regulated kinase cascade and Sp1. *J. Biol. Chem* 2002;277:32258–32267. [PubMed: 12077147]
47. Van Seuning I, Jonckheere N. The membrane-bound mucins: how large O-glycoproteins play key roles in epithelial cancers and hold promise as biological tools for gene-based and immunotherapies. *Crit. Rev. Oncog* 2008;14:177–196. [PubMed: 19409062]
48. Jonckheere, N.; Van Seuning, I. The ever-growing family of membrane-bound mucins. In: Van Seuning, I., editor. *The Epithelial mucins: Structure–function. Roles in cancer and inflammatory diseases*. Kerala, India: Research Signpost; 2008. p. 17-38.
49. Perrais M, Pigny P, Ducourouble MP, et al. Characterization of human mucin gene MUC4 promoter: importance of growth factors and proinflammatory cytokines for its regulation in pancreatic cancer cells. *J. Biol. Chem* 2001;276:30923–30933. [PubMed: 11418607] • Mechanisms of transcriptional regulation of MUC4.
50. Moniaux N, Andrianifahanana M, Brand RE, Batra SK. Multiple roles of mucins in pancreatic cancer, a lethal and challenging malignancy. *Br. J. Cancer* 2004;91:1633–1638. [PubMed: 15494719]
51. Jonckheere N, Perrais M, Mariette C, et al. A role for human *MUC4* mucin gene, the ErbB2 ligand, as a target of TGF- β in pancreatic carcinogenesis. *Oncogene* 2004;23:5729–5738. [PubMed: 15184872]
52. Andrianifahanana M, Agrawal A, Singh AP, et al. Synergistic induction of the *MUC4* mucin gene by interferon- γ and retinoic acid in human pancreatic tumour cells involves a reprogramming of signalling pathways. *Oncogene* 24:6143–6154. [PubMed: 16007204]
53. Damera G, Xia B, Ancha HR, Sachdev GP. IL-9 modulated *MUC4* gene and glycoprotein expression in airway epithelial cells. *Biosci. Rep* 2006;26:55–67. [PubMed: 16779668]
54. Vincent A, Ducourouble MP, Van Seuning I. Epigenetic regulation of the human mucin gene *MUC4* in epithelial cancer cell lines involves both DNA methylation and histone modifications mediated by DNA methyltransferases and histone deacetylases. *FASEB J* 2008;22:3035–3045. [PubMed: 18492726]
55. Yamada N, Nishida Y, Tsutsumida H, et al. Promoter CpG methylation in cancer cells contributes to the regulation of *MUC4*. *Br. J. Cancer* 2009;100:344–351. [PubMed: 19127263]
56. van Seuning I, Vincent A. Mucins: a new family of epigenetic biomarkers in epithelial cancers. *Expert Opin. Med. Diag.* 2009 (In press).
57. Fischer BM, Cuellar JG, Diehl ML, et al. Neutrophil elastase increases MUC4 expression in normal human bronchial epithelial cells. *Am. J. Physiol. Lung Cell. Mol. Physiol* 2003;284:L671–L679. [PubMed: 12495942]
58. Fischer BM, Voynow JA. Neutrophil elastase induces *MUC5AC* gene expression in airway epithelium via a pathway involving reactive oxygen species. *Am. J. Respir. Cell. Mol. Biol* 2002;26:447–452. [PubMed: 11919081]
59. Price-Schiavi SA, Carraway CA, Fregien N, Carraway KL. Post-transcriptional regulation of a milk membrane protein, the sialomucin complex (Ascites sialoglycoprotein (ASGP)-1/ASGP-2, rat muc4), by transforming growth factor β . *J. Biol. Chem* 1998;273:35228–35237. [PubMed: 9857062]

60. Price-Schiavi SA, Zhu X, Aquinin R, Carraway KL. Sialomucin complex (rat Muc4) is regulated by transforming growth factor β in mammary gland by a novel post-translational mechanism. *J. Biol. Chem* 2000;275:17800–17807. [PubMed: 10837499] • Post-translational regulation of Muc4 in rat mammary cells by TGF β
61. Soto P, Price-Schiavi SA, Carraway KL. SMAD2 and SMAD7 involvement in the post-translational regulation of Muc4 via the transforming growth factor- β and interferon- γ pathways in rat mammary epithelial cells. *J. Biol. Chem* 2003;278:20338–20344. [PubMed: 12668667]
62. Lomako WM, Lomako J, Soto P, Carraway CA, Carraway KL. TGF β regulation of membrane mucin Muc4 via proteasome degradation. *J. Cell Biochem* 2009;107:797–802. [PubMed: 19388004]
63. Price-Schiavi SA, Meller D, Jing X, Merritt J, Carvajal ME, Tseng SC, Carraway KL. Sialomucin complex at the rat ocular surface: a new model for ocular surface protection. *Biochem J* 1998;335:457–463. [PubMed: 9761747]
64. Idris N, Carraway KL. Regulation of sialomucin complex/Muc4 expression in rat uterine luminal epithelial cells by transforming growth factor- β : implications for blastocyst implantation. *J. Cell Physiol* 2000;185:310–316. [PubMed: 11025453]
65. Aubert, S.; Van Seuningen, I.; Leroy, X. Mucins in the uro-genital tract. Potential for therapeutic approaches using mucins. In: Van Seuningen, I., editor. *The Epithelial mucins: Structure–function. Roles in cancer and inflammatory diseases*. Kerala, India: Research Signpost; 2008. p. 249-272.
66. Rakha EA, Boyce RW, Abd El-Rehim D, et al. Expression of mucins (MUC1, MUC2, MUC3, MUC4, MUC5AC and MUC6) and their prognostic significance in human breast cancer. *Mod. Pathol* 2005;18:1295–1304. [PubMed: 15976813]
67. Llinares K, Escande F, Aubert S, et al. Diagnostic value of MUC4 immunostaining in distinguishing epithelial mesothelioma and lung adenocarcinoma. *Mod. Pathol* 2004;17:150–157. [PubMed: 14657954]
68. Hanaoka J, Kontani K, Sawai S, et al. Analysis of MUC4 mucin expression in lung carcinoma cells and its immunogenicity. *Cancer* 2001;92:2148–2157. [PubMed: 11596032]
69. Kwon KY, Ro JY, Singhal N, et al. MUC4 expression in nonsmall cell lung carcinomas: relationship to tumor histology and patient survival. *Arch. Pathol. Lab. Med* 2007;131:593–598. [PubMed: 17425390]
70. Tsutsumida H, Goto M, Kitajima S, et al. MUC4 expression correlates with poor prognosis in small-sized lung adenocarcinoma. *Lung Cancer* 2007;55:195–203. [PubMed: 17126950]
71. Saitou M, Goto M, Horinouchi M, et al. MUC4 expression is a novel prognostic factor in patients with invasive ductal carcinoma of the pancreas. *J. Clin. Pathol* 2005;58:845–852. [PubMed: 16049287]
72. Swartz MJ, Batra SK, Varshney GC, et al. MUC4 expression increases progressively in pancreatic intraepithelial neoplasia. *Am. J. Clin. Pathol* 2002;117:791–796. [PubMed: 12090430]
73. Tamada S, Shibahara H, Higashi M, et al. MUC4 is a novel prognostic factor of extrahepatic bile duct carcinoma. *Clin. Cancer Res* 2006;12:4257–4264. [PubMed: 16857800]
74. Shibahara H, Tamada S, Higashi M, et al. MUC4 is a novel prognostic factor of intrahepatic cholangiocarcinoma-mass forming type. *Hepatology* 2004;39:220–229. [PubMed: 14752841]
75. Zhang S, Zhang HS, Cordon-Cardo C, Ragupathi G, Livingston PO. Selection of tumor antigens as targets for immune attack using immunohistochemistry: protein antigens. *Clin. Cancer Res* 1998;4:2669–2676. [PubMed: 9829729]
76. Chauhan SC, Singh AP, Ruiz F, et al. Aberrant expression of MUC4 in ovarian carcinoma: diagnostic significance alone and in combination with MUC1 and MUC16 (CA125). *Mod. Pathol* 2006;19:1386–1394. [PubMed: 16880776]
77. Bhattacharyya SN, Dubick MA, Yantis LD, et al. *In vivo* effect of wood smoke on the expression of two mucin genes in rat airways. *Inflammation* 2004;28:67–76. [PubMed: 15379212]
78. Liévin-Le Moal V, Huet G, Aubert JP, et al. Activation of mucin exocytosis and upregulation of *MUC* genes in polarized human intestinal mucin-secreting cells by the thiol-activated exotoxin listeriolysin O. *Cell Microbiol* 2002;4:515–529. [PubMed: 12174086]
79. Damera G, Xia B, Sachdev GP. IL-4 induced MUC4 enhancement in respiratory epithelial cells *in vitro* is mediated through JAK-3 selective signaling. *Respir. Res* 2006;7:39. [PubMed: 16551361]

80. Mejías-Luque R, Peiró S, Vincent A, Van Seuning I, de Bolós C. IL-6 induces *MUC4* expression through gp130/STAT3 pathway in gastric cancer cell lines. *Biochim. Biophys. Acta* 2008;1783:1728–1736. [PubMed: 18573283]
81. Choudhury A, Singh RK, Moniaux N, El-Metwally TH, Aubert JP, Batra SK. Retinoic acid-dependent transforming growth factor- β 2-mediated induction of MUC4 mucin expression in human pancreatic tumor cells follows retinoic acid receptor- α signaling pathway. *J. Biol. Chem* 2000;275:33929–33936. [PubMed: 10938282]
82. Andrianifahanana M, Agrawal A, Singh AP, et al. Synergistic induction of the *MUC4* mucin gene by interferon- γ and retinoic acid in human pancreatic tumour cells involves a reprogramming of signaling pathways. *Oncogene* 2005;24:6143–6154. [PubMed: 16007204]
83. Singh AP, Chauhan SC, Andrianifahanana M, et al. *MUC4* expression is regulated by cystic fibrosis transmembrane conductance regulator in pancreatic adenocarcinoma cells via transcriptional and post-translational mechanisms. *Oncogene* 2007;26:30–41. [PubMed: 16799633]
84. Mariette C, Perrais M, Leteurtre E, et al. Transcriptional regulation of human mucin MUC4 by bile acids in oesophageal cancer cells is promoter-dependent and involves activation of the phosphatidylinositol 3-kinase signalling pathway. *Biochem J* 2004;377:701–708. [PubMed: 14583090]
85. Piessen G, Jonckheere N, Vincent A, et al. Regulation of the human mucin MUC4 by taurodeoxycholic and taurochenodeoxycholic bile acids in oesophageal cancer cells is mediated by hepatocyte nuclear factor 1 α . *Biochem J* 2007;402:81–91. [PubMed: 17037983]
86. Jonckheere N, Vincent A, Perrais M, et al. The human mucin MUC4 is transcriptionally regulated by caudal-related homeobox, hepatocyte nuclear factors, forkhead box A, and GATA endodermal transcription factors in epithelial cancer cells. *J. Biol. Chem* 2007;282:22638–22650. [PubMed: 17553805]

Figure 1. Conformational model for the role of Muc4/MUC4 in ErbB2 and ErbB3 modulation
Muc4/MUC4 (A) binds ErbB2 and can activate phosphorylation of the tyrosines of the tail of an ErbB2-ErbB3 complex (red circles, [B]) in the absence of the ErbB3 ligand neuregulin. The neuregulin is required for the phosphorylation of the ErbB3 (C) and full downstream signaling effects from the ErbB2-ErbB3 complex. The major extracellular domains of the ErbB2 are numbered 1-4 N-terminal to C-terminal.
C: C-terminal region of kinase domain of ErbB2 and ErbB3; L: Ligand for ErbB3, neuregulin;
N: N-terminal region of kinase domain of ErbB2 and ErbB3.

Figure 2. Muc4/MUC4 contribution to segregation of ErbB2 and ErbB3 in polarized epithelial cells
Muc4/MUC4 interacts with ErbB2 soon after the two are synthesized in the cells and facilitates trafficking of the ErbB2 to the apical surface, where it is effectively segregated from ErbB3, still at the lateral surface, (A) vs (B). Loss of polarity, as in damaged epithelia or tumor cells, breaks the segregation barrier and allows the formation of the ErbB2–ErbB3 complex (C), which is a key element of downstream signaling. Cells that do not contain Muc4/MUC4 have ErbB2 localized to their lateral surfaces along with the ErbB3 (A). Whether they are associated is unclear, but they are not phosphorylated and do not participate in signaling from the lateral surface.

Table 1

Examples of expression of MUC4 in tumors.

Site	Type	Analysis	Outcome/prognosis	Ref.
Breast	Invasive	IHC, microarrays	No correlation	[66]
Lung	Adenocarcinomas	IHC, transcript	Lung vs mesothelioma	[67]
	Carcinoma	Serum Ab, IHC	Not specified	[68]
	NSCLC	IHC, microarray	Favorable	[69]
	Adenocarcinoma	IHC	Poor	[70]
Pancreas	IDC	IHC	Poor	[71]
	PDAC	IHC	Poor	[72]
Bile duct	Extrahepatic	IHC	Poor	[73]
	Intrahepatic	IHC	Poor	[74]
Colon	Not specified	IHC	Not specified	[75]
Ovarian	Various	IHC	No correlation	[76]

Ab: Antibody; IDC: Invasive ductal carcinoma; IHC: Immunohistochemistry; NSCLC: Non-small-cell lung carcinoma; PDAC: Pancreatic ductal adenocarcinoma.

Table 2

Regulatory mechanisms for Muc4/MUC4.

Tissue	Signaling pathway	Mechanism	Ref.
Airway	Not known	Transcript stability	[57]
Airway	Not known	Transcription	[77]
Intestinal	Not known	Transcription	[78]
Lung	IL4/JAK3	Transcription	[79]
Gastric mucosa	IL6/STAT3	Transcription	[80]
Pancreas	TGF β 2/RAR	Transcription	[81]
	STAT1/RAR	Transcription	[82]
	Not known	Transcript/post-transcript	[83]
	AP2 α	Transcription	[44]
	TGF β /SMAD4	Transcription	[51]
	CFTR	Transcript/post-transcript	[12]
Rat mammary	IGF/Erk	Transcription	[41]
	TGF β 1/SMAD2	Post-translational	[58–60]
Esophageal	Bile acid/PI3K	Transcription?	[84]
	Bile acid/HNF1 α	Transcription	[85]
Rat uterus	TGF β	Transcription	[63]
Numerous	Multiple	Transcription	[86]
Numerous	DNA methylation	Epigenetic	[54,55]

CFTR: Cystic fibrosis transmembrane conductance regulator; HNF: Hepatocyte nuclear factor; IL: Interleukin; PI3K: Phosphatidylinositol-3-kinase; RAR: Retinoic acid receptor; STAT: Signal transducer and activator of transcription.