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Signaling Mucins: The New Kids on the MAPK Block

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ABSTRACT: Signaling mucins are an emerging group of cell adhesion receptors that activate mitogen-activated protein kinase (MAPK) pathways at the level of RAS/RHO. Recent discoveries on several fronts, including in the model eukaryote budding yeast, have broadened our understanding of this family of signaling molecules. Progress in characterizing three signaling mucins, MUC1, Muc4, and Msb2, points to a surprising degree of functional overlap in the regulation and mechanism-of-activation of these molecules. The prevailing new insight is one of receptor activation by proteolytic cleavage that closely mirrors the developmental signaling factor, Notch. The unexpected parallels between signaling mucins and Notch spark new questions about mucin activation and provoke a double take at this fledgling class of signaling adhesion molecule.

KEY WORDS: signal transduction, glycosylation, RAS, Cdc42, protease, Notch

I. INTRODUCTION

A. Signaling Mucins as MAPK Regulatory Proteins

Although mucins have been extensively studied for more than 70 years, only in the last decade have clues to their signaling nature come to light. Signaling mucins constitute a subset of the mucin family of proteins, which are glycosylated cell adhesion molecules (CAMs) distinguished by a variable number of O-glycosylated tandem repeats.[2,3] Signaling mucins are distinguished from other mucin members by their cytoplasmic domain, which is connected to the external portion of the molecule by an integral-membrane motif, and which interfaces with cytosolic signal transduction machinery. Signaling mucins are therefore multifaceted molecules that not only provide the cell with adhesive functions but also connect to intracellular signaling and polarity machinery to provide the cell with information about —and a responsiveness to—its extracellular contacts.

Signaling mucins activate signal transduction pathways at the level of small GTPases.[4–8] Small GTPases cycle between their active GTP-bound state and an inactive GDP-bound state. To facilitate this transition are activating guanine nucleotide exchange factors (GEFs)[9,10] and inactivating GTPase-activating proteins (GAPs).[11] RAS is the prototypical member of the small GTPase family that initiates a global cellular response by activation of multiple signaling cascades.[12] Like RAS, RAS homology (RHO) GTPases govern multiple aspects of cellular behavior.[13] A well-studied member of the RHO GTPase family is Cdc42, which is a regulator of cell polarity[14] and signal transduction.[15,16] Whereas RAS connects directly to adenylate cyclase to induce production of the second messenger cyclic adenosine monophosphate

ABBREVIATIONS

CAM, cell adhesion molecule; **EGF,** epidermal growth factor; **ERα,** estrogen receptor alpha; **ErbB,** epidermal growth factor receptor B; **ERK,** extracellular signal-regulated kinase; **GSK3-β,** glycogen synthase kinase 3-beta; **MAPK,** mitogen-activated protein kinase; **PAK,** p21-activated kinase; **RHO,** RAS homology; **SEA,** sperm protein, enterokinase, and agrin; **SH2,** Src-Homology 2; **SH3,** Src-Homology 3.

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(cAMP),[17] Cdc42 associates with p21-activated kinases (PAKs) to induce kinase activation.[18–20]

Both RAS and RHO typically connect to canonical mitogen-activated protein kinase (MAPK) cascades.[21] MAPK cascades act in three-tiered phosphorelay systems—MAP kinase kinase kinases ($MAPK_3$s) phosphorylate and activate MAP kinase kinases ($MAPK_2$s), which in turn activate MAPKs. MAPK activation triggers phosphorylation and activation of transcription factors that initiate a transcriptional response.[22] In addition, MAPK cascade components influence cell polarity and cell-cycle progression by phosphorylation of specific target proteins. In this way, signaling mucins are capable of initiating a broad-based cellular response.

Several recent reviews discuss signaling mucins.[7,8,23] In this review, we compare signaling mucin function of the two well-characterized mammalian mucins (MUC1 and Muc4) to a newly characterized signaling mucin from the versatile model eukaryote budding yeast *Saccharomyces cerevisiae* (Msb2). Recent discoveries demonstrate that signaling mucins are activated by multiple cleavage events, likening their activation to that of the Notch receptor and other protease-activated receptors. Further exploration of signaling mucin regulation and function using new approaches and model systems will synergize progress toward understanding this important class of signaling CAM.

B. The Quandary of Specificity in MAPK Pathways

To fully appreciate the functional role of signaling mucins, it is necessary to discuss the issue of specificity in reference to MAPK pathways. Recent advances in the frontiers of genomics and proteomics indicate that signaling molecules, rather than acting in strictly linear pathways, function in web-like protein interaction networks.[14,24] Many signaling proteins, including RAS and MAPK components, are in fact general factors that function in multiple pathways in the same cell. Depending on the occasion, RAS can activate one MAPK pathway in a given setting and a second MAPK pathway in a different setting.[25] How a cell keeps the signals straight is a remarkable achievement that is poorly understood.[26] This puzzle manifests itself in human disease because inappropriate signaling between pathways, commonly referred to as cross talk, is responsible for a host of diseases. Many human cancers, for example, can be attributed to unregulated MAPK activity originating at the level of RAS/RHO.[27]

Issues related to MAPK specificity are brought into sharp focus by examining the MAPK pathways in yeast. Of the five MAPK pathways in yeast, three exhibit extensive sharing of components (Fig. 1). For example, Cdc42, its PAK Ste20, and the $MAPK_3$ Ste11 are required to activate all three MAPK pathways (Fig. 1). Each of the pathways senses a different stimulus and induces a distinct response, resulting in the transcriptional induction of nonoverlapping target genes that leads to formation of a distinct cell type (Fig. 1). One of the ways a specific response is achieved is through scaffolding proteins. Scaffolding molecules bind to and recruit general factors to function with pathway-specific activators.[25] In yeast, two scaffolding proteins at the level of the MAPK have been identified. The scaffold for the mating pathway, Ste5, directs the general $MAPK_3$ Ste11 and $MAPK_2$ Ste7 to differentially activate the mating pathway MAPK Fus3 (Fig. 1).[28] The scaffolding protein for the high osmolarity glycerol response (HOG) pathway, Pbs2, is also the $MAPK_2$ for the HOG pathway. Pbs2 induces Ste11 to function with the HOG pathway MAPK Hog1 (Fig. 1).[29] Similar regulation presumably occurs at other points along the signaling cascade, such as at the level of Cdc42 (Fig. 1). Issues related to MAPK specificity are undoubtedly equally convoluted in mammalian systems, wherein a plethora of additional factors regulate spatial, temporal, and developmental aspects of cellular behavior. Because the signaling molecules that constitute these pathways are highly conserved throughout eukaryotes,[30–32] insights into pathway regulation in yeast are likely to pertain to mammalian signaling pathways as well.

II. MEMBERS OF THE SIGNALING MUCIN FAMILY

A. The Prototypical Signaling Mucin MUC1

Much of what we know about signaling mucins comes from extensive studies on the MUC1 pro-

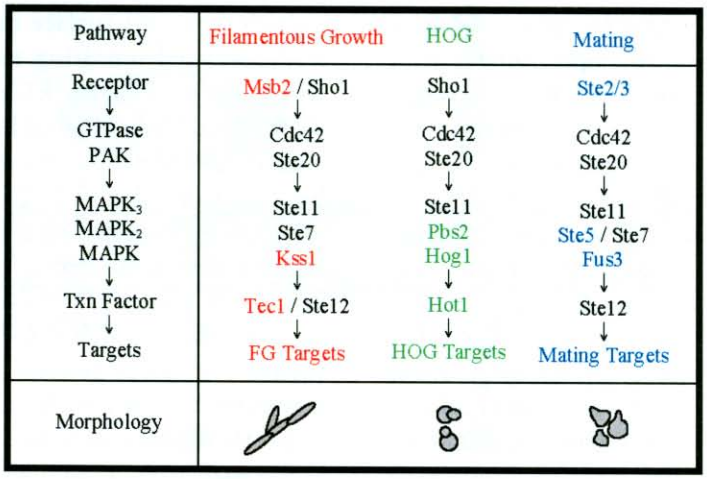

FIGURE 1. Signaling pathway specificity in yeast. At left is a reference pathway composed of a receptor, GTPase, and mitogen-activated protein kinase (MAPK) cascade. Such signaling modules are functionally conserved throughout eukaryotes. At right are three MAPK pathways that use overlapping and pathway-specific components. Most of the proteins (in black) are required in multiple pathways. Proteins specific for the filamentous growth pathway (red), the high osmolarity glycerol response (HOG) pathway (green), and the mating pathway (blue) are also shown. Pathway-specific proteins recruit general factors such that the appropriate target genes are expressed (Targets), and the correct morphology is achieved (sketches of cells). Characterizing mechanisms related to "sharing" components in a model organism provides a framework for understanding specificity.

tein. Before the signaling nature of MUC1 was uncovered, its central role as a diagnostic cancer marker was established, which led to cloning and characterization of the adhesive properties of the protein.[33] Initial results, suggesting that MUC1 might also function as a signaling molecule,[34] were corroborated when the cytoplasmic tail of MUC1, which shows sequence similarity with cytokine receptors, was shown to be phosphorylated on tyrosine residues.[35] On the heels of this discovery was the fact that phosphorylated MUC1 associates with the adaptor protein Grb2, and that MUC1-Grb2 and Sos, the guanine nucleotide exchange factor for RAS, form a complex (Fig. 2).[36] MUC1 also associates with the epidermal growth factor receptor (ErbB) further connecting MUC1 to the RAS pathway.[37,38] As RAS initiates signaling of the MEK-ERK MAPK pathway, as well as other signaling pathways, these findings firmly place MUC1 at the head of a global signaling cascade.[39]

These pioneering advances led to an explosion of findings that connect MUC1 to multiple intracellular signaling pathways (Fig. 2). Primarily, a connection between MUC1 has been established with components of the Wnt pathway, a developmental signaling pathway in metazoans.[40] MUC1 connects to the Wnt pathway through interactions with β-catenin,[41] a molecule typically found associated with the cytoplasmic domains of the cadherin family of CAMs in intercellular junctions[42] that also serves as a potent activator of Wnt[43] (Fig. 2). The cytoplasmic domain of MUC1 also interacts with another member of the Wnt pathway, glycogen synthase kinase 3-β (GSK3-β).[44] Competition for MUC1 by GSK3-β and β-catenin is regulated by the kinase c-Src. Src phosphorylates the cytoplasmic domain of MUC1 to inhibit GSK3-β interaction and promote the β-catenin interaction.[45] The range of pathways influenced by MUC1 is presumably greater than its role in the RAS and Wnt pathways: MUC1 has recently been connected to the NF-κB pathway[46] and to the tumor suppressor p53.[47] Paradoxically, MUC1 mutants lacking Tyrs in the cytoplasmic tail show enhanced signaling in the ERK MAPK pathway but are defective for NF-κB pathway activity.[46] This inexplicable result underscores the complexities of MUC1 regulation.

The connection between MUC1 and Wnt led to the mechanistic breakthrough that the cytoplasmic domain of MUC1 functions in the nucleus

of the cell.[48–50] MUC1's cytoplasmic tail localizes to the nucleus and co-immunoprecipitates with β-catenin[41,51,52] to mediate Wnt pathway target gene expression.[53] MUC1 also binds to the transcription factor estrogen receptor alpha (ERα).[54] The cytoplasmic tail of MUC1 stabilizes ERα by blocking its ubiquitination. Chromatin immunoprecipitation assays further demonstrate that MUC1 activates ERα by promoting promoter occupancy and stimulating recruitment of ERα coactivators[54] (Fig. 2). Moreover, the cytoplasmic tail of MUC1 has been identified in other cellular locales. MUC1 influences the expression of genes that inhibit apoptosis,[4,55,56] and consistent with this role, the cytoplasmic portion of MUC1 is found in the mitochondria.[57]

The exciting discovery that the cytoplasmic domain of MUC1 is released from the plasma membrane mechanistically connects signaling mucins with other receptors that undergo regulated cleavage, like Notch. Notch controls cell fate during development.[58] The Notch protein is a single-pass cell-surface molecule that contains a glycosylated repeating motif of multiple epidermal growth factor (EGF)-like domains (Fig. 3).[59,60] Notch is processed by cleavage at multiple sites in the extracellular domain, including upon binding to its ligand Delta. Activation leads to additional processing of the cytoplasmic tail, where it dissociates from the plasma membrane, enters the nucleus, and collaborates with transcription factors such as CSL to induce developmental regulatory genes[61] (Fig. 3). Side-by-side comparison between Notch and MUC1 highlights their common topology, glycosylation, and repeat structure, as well as the common mechanism of cleavage in the extracellular and cytoplasmic potions of the proteins (Fig. 3). Unlike Notch, MUC1 may not play a primary role in development, as MUC1 knockout mice are viable.[37] Nonetheless, the parallels between these signaling systems are quite striking and suggest a common theme in the mechanism of activation between these receptors.

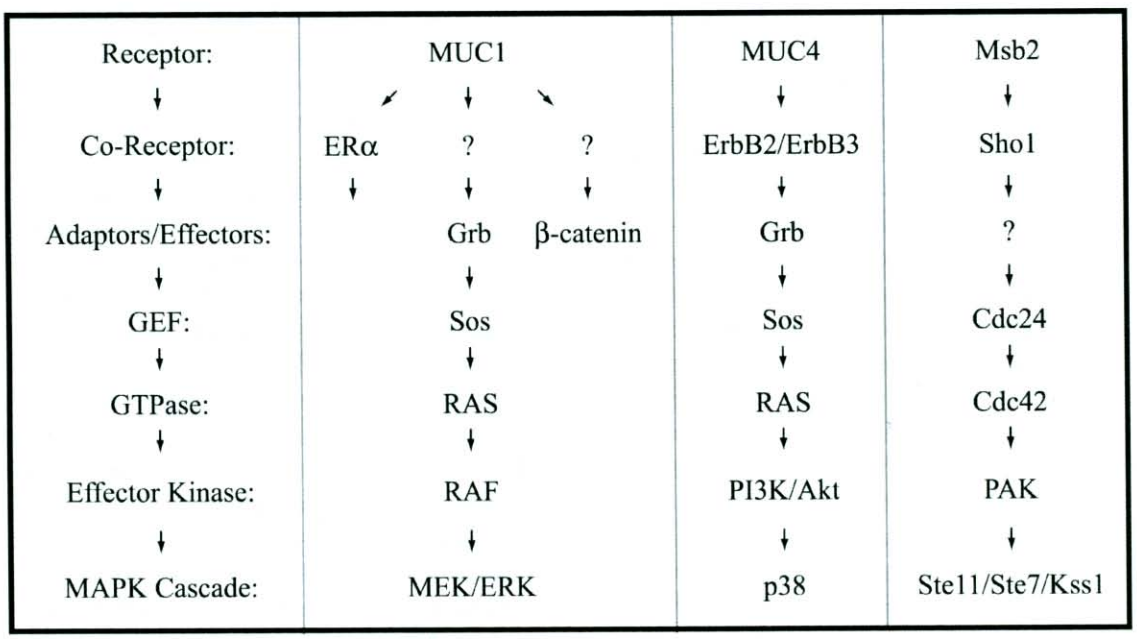

FIGURE 2. Comparison of the three established mucin-dependent signaling pathways. The three characterized signaling mucins in the context of their cognate pathways are shown. At left is a reference pathway. Multiple arrows refer to the RAS-independent functions of MUC1, which has been identified. It is unclear whether Muc4 directly associates with Grb2, or whether activation proceeds through the ErbB2-Grb interaction. Several MUC1 (ErbB, NF-κB, c-Src) and Muc4 (neuregluin-β) effectors and interacting proteins are not shown, and the direct connections between Muc4 and RAF and Msb2 and Cdc42 are not shown.

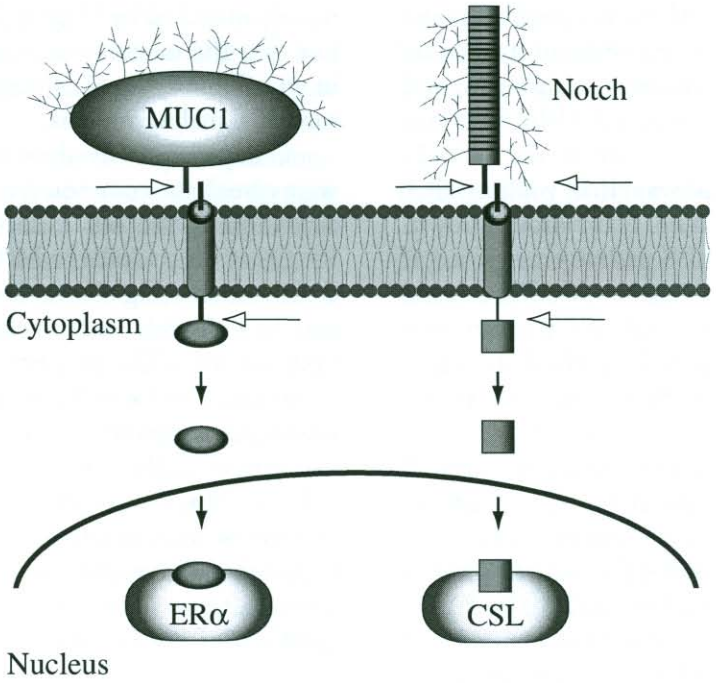

FIGURE 3. Comparison of Notch and MUC1 activation. Both Notch and MUC1 are cell-surface transmembrane glycoproteins containing tandem repeat motifs. Both proteins are processed in the extracellular and cytoplasmic domains (arrows). The cytoplasmic domains of both proteins are shown entering the nucleus, where they associate with transcription factors to induce a cellular response. Notch is processed at multiple sites in the extracellular domain, as denoted by multiple arrows.

B. A Portrait in Contrast: Muc4

Insights into the signaling role of Muc4 have benefited largely from characterization of its rat homolog (sialomucin complex [SMC] or ASGP-1 and ASGP-2).[62,63] Muc4 is a transmembrane mucin implicated in the protection of epithelia by anti-adhesive functions and tumor metastasis in a variety of cancers. Muc4 is also a signaling molecule. Muc4 serves as an intramembrane ligand for the receptor tyrosine kinase ErbB2 (ErbB2/HER2/Neu) (Fig. 2). Muc4 promotes phosphorylation of ErbB2, and induces a differentiated state by activation of the cell-cycle inhibitor, p27 (kip).[64] Like MUC1, Muc4 activation induces recruitment of Grb2, in this case through ErbB2, which leads to activation of RAS and a p38-dependent MAPK pathway[65] (Fig. 2). More recently, Muc4 has been shown to bypass RAS by directly stimulating the activity of the Raf-1 kinase, a RAS pathway effector,[66] resulting in activation of the ERK pathway (Fig. 2).

Additional progress has been made in the relationship between Muc4 and the extracellular growth factor neuregluin-β. Muc4 promotes neuregluin-β-dependent Tyr phosphorylation of ErbB2.[67] How this is accomplished is not entirely clear, although it appears to function through the phosphatidylinositol 3-kinase (PI3 kinase) pathway.[67] Indeed, multiple mechanisms may be at play in this context, as Muc4 likely influences ErbB2 localization (see below), ErbB2 retention at the plasma membrane, and neuregluin binding at the cell surface.[67] Therefore, Muc4 may influence cellular signaling by a multiplicity of functions, a subset of which connects like MUC1 to the RAS pathway.

C. Msb2: The Yeast Signaling Mucin

The discovery and characterization of a mucin member in budding yeast has expanded our understanding of this class of signaling molecule.

The yeast mucin, called Msb2, is a member of the mucin family of proteins, by virtue of its tandem repeats, its highly glycosylated ectodomain, and the presence of a cytoplasmic tail. Msb2 functions not in the RAS pathway, but in the Cdc42-dependent MAPK pathway that induces filamentous growth.[68–71] The filamentous growth pathway is a typical MAPK pathway composed of Cdc42, PAK Ste20,[19,20] and the MAPK cascade that controls the activity of two transcription factors (Fig. 2).[69,72] Cells lacking Msb2 are defective for MAPK activity and defective for filamentous growth.

Msb2 connects to two proteins at the head of the filamentous growth pathway (Fig. 2). One of these is called Sho1,[73] which operates at the head of two MAPK pathways: the filamentous growth and the HOG pathway (Fig. 1).[74,75] The HOG pathway controls the response to salt or osmotic stress, and Sho1 was identified initially as an osmosensor.[76–78] Sho1 contains four transmembrane domains and is a member of the tetraspan family of signaling molecules, which includes the dystroglycan[79,80] and immunological CD82[81,82] receptors. Sho1 also contains a cytoplasmic Src-Homology 3 (SH3) domain[83,84] that directs the protein to the HOG pathway by binding to the $MAPK_2$ Pbs2.[73,85] In addition to Sho1, Msb2 also interacts with Cdc42 (Fig. 2). Msb2 does not appear to have a function in the Mating pathway[86] and plays only a minor role in the HOG pathway.[87] Therefore, it is likely that Msb2 promotes Sho1 and Cdc42 function in the filamentous growth pathway and may serve as a cell-surface scaffold.

A surprising finding that has come out of Msb2 characterization is that deletion of the mucin domain of the protein ($Msb2^{\Delta mucin}$) results in MAPK pathway activation. This provocative result suggests that mucin repeats of Msb2 function in an inhibitory manner. Several other findings corroborate this result. First, Msb2 lacking its mucin domain induces phenotypes consistent with an activated MAPK pathway, particularly induction of pathway-specific transcriptional reporters and hyperpolarized growth. Second, deletions of progressively larger regions that include the mucin domain also induce hyperactivity. Finally, defects in glycosylation of Msb2 activate the protein. Notably, mucin repeat regions are known to be heavily modified by O-linked glycosylation.[3] The fact that the mucin homology domain functions in an inhibitory capacity might be a general feature of signaling mucins.

Comparison of the three signaling mucin pathways reveals the common theme of small GTPase activation at the cell surface (Fig. 2). RAS or RHO activation leads to MAPK activation in all three systems (Fig. 2). At least for MUC1, cell-surface activation leads to the activation of multiple pathways. This property is likely to pertain to Muc4 and Msb2 as well. In the following sections, we discuss regulatory commonalities between signaling mucins. By comparing the three proteins—MUC1, Muc4, and Msb2—a common picture emerges of how mucin receptors become activated. Examining the molecules in direct contrast also points out gaps in our understanding of particular members of the family.

III. COMMON PROPERTIES OF THE SIGNALING MUCIN FAMILY

A. Posttranslational Processing

Mammalian signaling mucins undergo posttranslational processing in the endoplasmic reticulum that results in shedding of the glycosylated ectodomain of the protein at the cell surface.[88,89] The protease responsible for cleavage, and the consequences of cleavage on mucin function have until recently remained elusive. A recent advance has been the identification of an autocleavage domain in MUC1 and other heavily O-glycosylated membrane-spanning proteins.[90–92] The autocleavage domain or Sperm protein, Enterokinase, and Agrin (SEA) module is a highly conserved domain based on its secondary structure (β-α-β-β-α)[91] and limited sequence homology.[93] Specifically, MUC1 undergoes autocleavage at a conserved GSVVV motif.[90,91] Although the discovery of a SEA domain answers important questions related to mucin processing, posttranslational modification of mucins must occur through multiple mechanisms. For example, processing of MUC1 must also occur at the cytoplasmic-transmembrane domain interface because the cytoplasmic tail of MUC1 also functions as a cytosolic signaling molecule.[54] In addition, Muc4 does not appear to

undergo autocleavage but rather an enzyme-dependent cleavage at low pH at a GD-PH sequence.[94] The enzyme that cleaves Muc4 has not been identified.

Recent work in our laboratory has demonstrated that the glycosylated extracellular domain of Msb2 is secreted from the cell (Vadaie and Cullen, unpublished work). This result indicates that Msb2, like mammalian mucins, is modified by cleavage. Msb2 does not have the characteristic secondary structure of SEA domains[95] assessed by the programs PROF and NORS,[96] nor does it show amino acid sequence similarity to SEA domains from human MUC1.[97] Msb2 also does not contain a GD-PH sequence. To identify the protease required for cleavage of Msb2, we undertook a proteomics approach. A collection of yeast mutants, each defective for a specific protease, was examined from a complete collection of 4800 nonessential yeast mutants.[98] Using this approach, we identified a family of aspartyl proteases required for processing of Msb2 (Vadaie and Cullen, unpublished work). These proteases, called yapsins,[99] are GPI-anchored proteases that cleave at monobasic residues. At this point, it is not clear whether cleavage of Msb2 occurs in the endoplasmic reticulum or at the cell surface, as yapsins function at both cellular locations.

Might secreted aspartyl proteases cleave mammalian mucins? Secreted aspartyl proteases are found in mammalian cells.[100] Like signaling mucins, aspartyl proteases are transcriptional targets of MAPK pathways[101] and diagnostic markers for several tumor types.[102–104] For example, Cathepsin D is overexpressed and hypersecreted in breast cancer cells[105] and serves as a marker for poor prognosis in breast cancer[105] and glioma patients.[106] The functional connections between signaling mucins and aspartyl proteases indicate the possibility that aspartyl proteases might process mammalian mucins.

The discovery that Msb2 is cleaved by yapsins introduces another insight into mucin regulation. As the glycosylated ectodomain of Msb2 is inhibitory to MAPK activation,[86] cleavage of Msb2 may induce the MAPK activation function of the protein. This finding strengthens ties to protease-dependent activation of receptors Notch[107,108] and other similarly regulated receptors, like protease-activated receptors (PARs).[109] Cleavage-dependent activation of mammalian mucins that are proteolytically processed[110] may be a common mechanism shared by signaling mucins.

B. Signaling Mucins and Cell Polarity

Signaling mucins are secreted proteins typically found on the apical face of epithelial cells. At these sites are also concentrated signaling proteins including small GTPases and their regulators.[111] MUC1, which is secreted from glandular cells, is highly polarized on several types of epithelial cells.[112–114] In addition, MUC1 becomes depolarized in cancer cells.[115,116] The apical distribution of MUC1 is not controlled by tight junctions between cells but occurs as a result of cytoplasmic cytoskeletal organization.[113] Paradoxically, it has been postulated that MUC1's cytoplasmic tail is not required for apical targeting.[114] Consistent with this finding, targeting sequences in the MUC1 extracellular domain have been identified that specify the protein to apical surfaces.[117] Whether MUC1's polarized location along these surfaces contributes to its signaling function remains unclear.

Muc4 is also localized to polarized sites. In a series of elegant studies, a function for Muc4 has been elucidated that connects polarized growth to pathway specification.[65,67,118] Primarily, Muc4 expression induces the relocalization of ErbB2 to the apical membrane from its resting location at adherens junctions. Muc4 also promotes retention of ErbB2 at the cell surface.[67] Intriguingly, Muc4 does not alter the localization of ErbB2's heterodimerization partner, ErbB3. This interesting result suggests a mechanism whereby the two receptors are functionally separated. Polarized ErbB2 leads to differential activation of the p38/Akt MAPK pathway, but not the Erk or JNK pathways. The ability of Muc4 to segregate ErbB receptors to alter downstream signaling cascades in polarized epithelial cells suggests a mechanism of MAPK specification.[65]

Like MUC1 and Muc4, Msb2 is a polarized molecule. Msb2 activation of the filamentous growth pathway results in the reorganization of polarity to promote a distal-pole mode of budding that results in the formation of a filament. Msb2, Cdc42, and the MAPK pathway are required for

establishing the distal-pole budding pattern.[19,20,70,86] Although the mechanism by which this is accomplished is largely a mystery, bud-site-selection proteins[119] have been identified that are required for the change in polarity, including the distal-pole landmark, Bud8, which is required for the MAPK-dependent change in cell polarity during filamentous growth.[120]

We have recently shown that Msb2, which is itself localized to polarized sites,[86] induces the polarization of Sho1 and the GEF for Cdc42 to the distal pole (Vadaie and Cullen, unpublished data). We also found that Sho1 associates with the Cdc42 GEF and Bud8 (Vadaie and Cullen, unpublished data). Polarized localization of the GEF results in localized activation of Cdc42,[121] providing an explanation for the reorganization of cell polarity in this system.

C. Signaling Mucin Gene Expression and Positive-Feedback Loops

Signaling mucin genes share a common mode of regulation in that their expression is induced by positive-feedback loops. Such autofeedback is particularly striking in cancer cells, wherein MUC1 and Muc4 are highly expressed and serve as diagnostic surface markers. In the case of MUC1, the MUC1 gene is induced by ERα.[122–128] As the MUC1 cytoplasmic tail binds to and stabilizes ERα,[54] MUC1 has the capacity to directly induce its own expression. Likewise, the MUC1 gene is induced by a number of other transcription factors that function as targets of signaling cascades, including MZF-1, DbpA,[129] Sp1,[130] and GATA3.[131] Of course, the MUC1 promoter is complex and is subject to negative regulation, for example, by ErbB2 activation.[132] MUC1 expression, therefore, is autoinducible and controlled by multiple positive (and negative) feedback loops.

Muc4 is an activator of ERK, and the Muc4 gene is induced by the ERK pathway, initiating a positive-feedback loop.[133] The Muc4 gene is specifically induced by the Ets family member of transcription factors, PEA3,[134,135] which acts in synergy with c-Jun and Sp1 to transactivate the proximal region of the Muc4 promoter.[134] As PEA3 is a target of the RAS and MEK1 pathway, it is likely that Muc4 influences its own expression by this mechanism.[135] PEA2 also attenuates ErbB2 expression, and because Muc4 and ErbB2 are interacting partners, in this instance, such regulation may lead to homeostasis.[134] The Muc4 gene is upregulated by NF-κB,[136] although Muc4 has not been shown to function in this pathway. Muc4 expression is also influenced by GATA5,[137] which may point to a connection between MUC1 and Muc4 expression by members of the GATA family of transactivator proteins, which have the consensus sequence (A/T)GATA(A/G) and share a steroid hormone-receptor superfamily C4 zinc-finger DNA-binding motif.[138] Likewise, both genes are induced by Sp1.[130,134]

Like its mammalian counterparts, the *MSB2* gene is autoinduced. The *MSB2* gene is a transcriptional target of the MAPK pathway that induces filamentous growth. Indeed, Msb2 was discovered as a component of the filamentous growth pathway by DNA microarray analysis, in which *MSB2* was identified as a prominently induced target gene.[86] The *MSB2* gene has two consensus sites for the transcription factor Ste12.[86] Consensus sites were also identified for the transcription factor Tec1,[139] a member of the TEA/ATTS family of transcriptional regulators.[72] Both of these transcription factors are components of the filamentous growth pathway.[140] Confirmation that expression of *MSB2* is induced by the MAPK pathway was assessed by a transcriptional reporter.[86] Positive-feedback loops constitute an elemental mechanism of signal amplification. The fact that signaling mucins are transcriptional targets of the pathways in which they operate dictates a central role for these molecules in pathway regulation.

D. Signaling Mucins and Disease

Signaling mucins in mammalian cells contribute to cancer through multiple mechanisms.[141] At one level their antiadhesion properties alter the cell surface of tumor cells to promote metastasis.[66] At another level, mucin-dependent MAPK activation stimulates cell proliferation and unregulated cellular behaviors. Other mechanisms have also been attributed to the augmentation of cancers by signaling mucins. For example, the ectodomain of MUC1 acts as a ligand for T cells that suppresses

their proliferation and the immune response against cancer cells.[142,143]

Mucin genes, MUC1 and Muc4, are transcriptionally upregulated in many tumors (eg, ovarian, prostate, breast, and pancreatic), making these molecules diagnostic markers for cancer cells. Indeed, MUC1 expression is induced by Epstein-Barr virus, which commonly leads to tumor formation.[144] As such, a number of therapies, especially immunotherapies, have been designed with signaling mucins in mind.[145–147] A compelling series of findings have recently shown that downregulation of MUC1 by RNA interference leads to inhibition of cell proliferation and colony formation,[148,149] as well as a decrease in the level of the EGF receptor, itself a MAPK regulatory protein and cancer marker.[149] A recent approach on the drawing board is to target the cytoplasmic tail of MUC1 directly.[150] The global and complex functioning of signaling mucins should be a consideration in developing strategies for cancer therapy. For example, it remains unclear if targeting MUC1 does not stimulate its signaling function. Moreover, as signaling mucins are paradoxical molecules with respect to their cell adhesion and signaling functions,[151] a clear understanding of the effects of targeting these proteins is necessary for therapeutic approaches to be successful.

Signaling mucins also function at the host-pathogen boundary. MUC1 serves as a point of entry for the bacterial pathogen *Pseudomonas aeruginosa*.[152] In addition, MUC1 activates its cognate MAPK pathway in response to *P. aeruginosa* infection.[153] Moreover, MUC1 appears to antagonize clearance of *P. aeruginosa*, based on MUC1 knockout mouse data.[154] The discovery of signaling mucins in fungi suggests that fungal signaling mucins also play a role in pathogenesis. Msb2 has orthologs in pathogenic fungal species, including the fungal pathogen *Candida albicans*. The *C. albicans* Msb2 homolog is likely a cell-surface molecule, which may directly influence the early stages of virulence, including attachment and penetration into host tissue. Moreover, if the *C. albicans* Msb2 homolog functions in a signaling capacity, then morphogenetic alterations in cell signaling and polarity, which are dependent on Cdc42 and the filamentation MAPK pathway,[155,156] will also be governed by mucin function.

IV. CONCLUSIONS AND FUTURE DIRECTIONS

The three characterized signaling mucins (MUC1, Muc4, and Msb2) share a common blueprint for function (Fig. 4). In particular, they are single pass cell-surface glycoproteins with a cytoplasmic domain that connects to regulators of the small GTPase family of proteins. In addition, they are modified by posttranslational processing into integral-membrane and secreted forms. Moreover, the processed cytoplasmic form represents the activated version of the signaling molecule (Fig. 4). This blueprint of a signaling mucin suggests several areas for future exploration.

First, understanding how mucins contribute to specificity in terms of signaling pathway function is a chief issue related to human health. Cross talk between MAPK pathways induces pathway activation that in mammalian cells causes cancer.[26] If mammalian mucins, like Msb2, contribute to pathway specification or despecification in cancer cells, then understanding the mechanistic underpinnings of such regulation is crucial to determining the role these molecules actually play in signaling pathway regulation. A particularly pressing question, for example, is whether Muc4 and Msb2, like MUC1, have cytoplasmic tails that act as transcriptional facilitators in the nucleus, and if so, which factors serve as their interacting partners. If this proves to be the case, then specification at the level of individual transcription factors might provide a general mode for pathway specification by signaling mucins.

Second, it is relevant to determine if the mucin domains of MUC1 and Muc4 have inhibitory roles in signaling. If so, mutation of mucin domains may cause pathway activation and contribute to cancer progression in mammalian cells. The sequence similarity of mucin tandem repeats makes them highly susceptible to recombination-mediated deletion, as can occur for Msb2.[86] Moreover, if as for Msb2,[86] the hyperactivity is dominant, then unregulated pathway activation in mucin-deleted receptors may be prevalent among human cancers. Variability in the number of tandem repeats has functional consequences on mucin function,[157] contributing to diseases such as asthma,[158] human infertility,[159] and cancer.[160] Individual differences in response to mucin target-

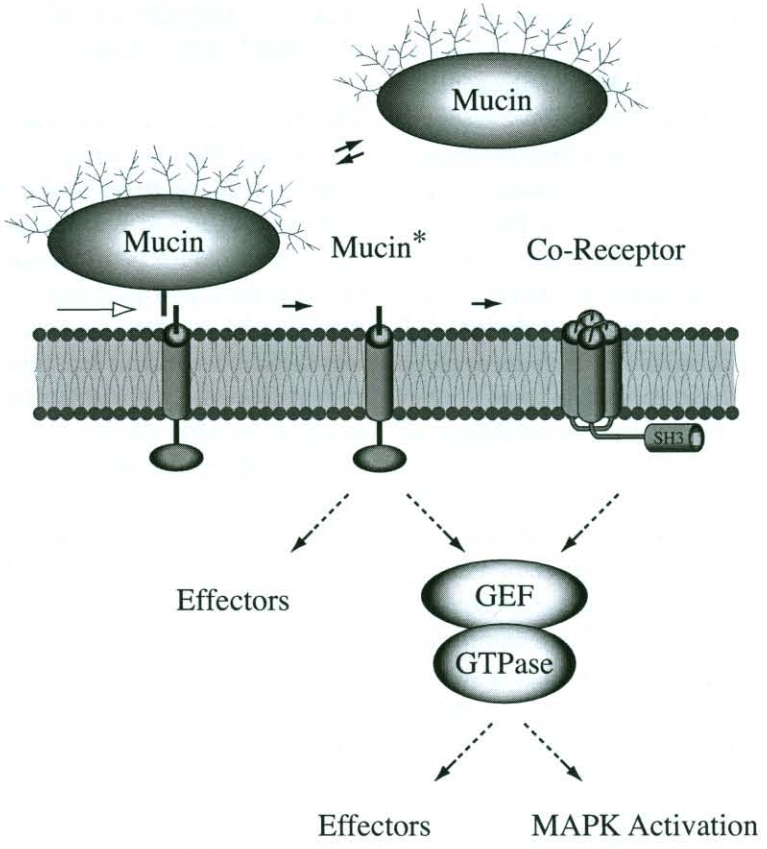

FIGURE 4. Generalized model for signaling mucin activation. The ectodomain of the mucin is cleaved (open arrow) and secreted from the cell. The cleaved polypeptide (Mucin*) is the active form of the receptor that activates other cell-surface molecules (Co-Receptor). A 4-pass co-receptor is shown (like Sho1); ErbB2 is a single-pass receptor. The cytoplasmic tail of Mucin* also activates effector proteins and guanine nucleotide exchange factors (GEFs) to initiate RAS/RHO activation. These signaling events culminate in mitogen-activated protein kinase (MAPK) pathway activation, as well as activation of multiple effector pathways.

ing for immunotherapy has also been attributed to variability within the repeat region.[161] Indeed, underglycosylated MUC1 has been identified in association with human tumors,[143] and versions of Muc4 containing different numbers of repeats in the mucin homology domain induce differences in cell-contact dependent behavior.[66] Whether variability in the repeat region influences cell adhesion or cellular behavior as a consequence of altered MAPK activity is an open question.

Finally, the ligands that mucin ectodomains sense remains poorly understood. MUC1 has been shown to interact with the intercellular adhesion molecule,[1] and this interaction results in the initiation of an internal calcium signal.[5] It has also been postulated that MUC1 senses mechanical shear at the plasma membrane, possibly by contact with other cells or the extracellular matrix.[91] Muc4 has been reported to interface with neuregluin-β and potentiate ErbB2-ErbB3 dimerization and activation.[67] More recently, Muc4 has been shown to function in a reversal of contact inhibition,[66] which may contribute to its ability to promote invasiveness. Precisely how Muc4 senses other cells to mediate this response remains to be determined. Msb2 activates a signaling pathway that is controlled by nutrient limitation,[68] although it is not clear that Msb2 is a nutrient sensor.

Determining the role of signaling mucins in other genetically tractable model organisms will be a fertile area of future investigations. For example, a MUC1 homolog has been identified in the nematode *Caenorhabditis elegans*, which contains a SEA domain as well as a tyrosine-rich

cytoplasmic tail.[97] Although a function is yet to be ascribed to this molecule, it will be instructive to determine whether this ortholog is secreted, and if the cytoplasmic domain connects to RAS/RHO-driven MAPK pathways.

A. Concluding Remarks

Although it is clear that mucins are receptors that operate at the head of RAS/RHO MAPK pathways, the mechanisms by which these proteins function has only recently come to light. A possible explanation for why these molecules have been underrepresented may be perception; the slippery reputation of molecules that contribute to mucus formation may be hard to shake. Another is that mucins have widely different functional roles in cell adhesion, making generalizations about their adhesive or antiadhesive functions appear paradoxical. Nonetheless, their central roles in cell adhesion, signaling, and disease, coupled with new mechanistic insights coming in part from model systems, makes signaling mucins a heavyweight contender in the arena of receptor signaling.

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REFERENCES

1. Anderson RK, Fogelson SJ. The secretion of gastric mucin in man. A comparative study in the normal subject and in the patient with peptic ulcer in response to an alcohol test meal. J Clin Invest. 1936; 15(2):169–72.
2. Gendler S, Taylor-Papadimitriou J, Duhig T, Rothbard J, Burchell J. A highly immunogenic region of a human polymorphic epithelial mucin expressed by carcinomas is made up of tandem repeats. J Biol Chem. 1988;263(26):12820–3.
3. Silverman HS, Sutton-Smith M, McDermott K, Heal P, Leir SH, Morris HR, Hollingsworth MA, Dell A, Harris A. The contribution of tandem repeat number to the O-glycosylation of mucins. Glycobiology. 2003; 13(4):265–77.
4. Raina D, Kharbanda S, Kufe D. The MUC1 oncoprotein activates the anti-apoptotic phosphoinositide 3-kinase/Akt and Bcl-xL pathways in rat 3Y1 fibroblasts. J Biol Chem. 2004;279(20): 20607–12.
5. Rahn JJ, Shen Q, Mah BK, Hugh JC. MUC1 initiates a calcium signal after ligation by intercellular adhesion molecule-1. J Biol Chem. 2004;279(28): 29386–90.
6. Mukherjee P, Tinder TL, Basu GD, Gendler SJ. MUC1 (CD227) interacts with lck tyrosine kinase in Jurkat lymphoma cells and normal T cells. J Leukoc Biol. 2005;77(1):90–9.
7. Carraway KL, Ramsauer VP, Haq B, Carothers Carraway CA. Cell signaling through membrane mucins. Bioessays. 2003;25(1):66–71.
8. Clevers H. Signaling mucins in the (S)limelight. Dev Cell. 2004;7(2):150–1.
9. Butty AC, Perrinjaquet N, Petit A, Jaquenoud M, Segall JE, Hofmann K, Zwahlen C, Peter M. A positive feedback loop stabilizes the guanine-nucleotide exchange factor Cdc24 at sites of polarization. EMBO J. 2002;21(7):1565–76.
10. Karnoub AE, Worthylake DK, Rossman KL, Pruitt WM, Campbell SL, Sondek J, Der CJ. Molecular basis for Rac1 recognition by guanine nucleotide exchange factors. Nat Struct Biol. 2001;8(12):1037–41.
11. Rittinger K, Walker PA, Eccleston JF, Nurmahomed K, Owen D, Laue E, Gamblin SJ, Smerdon SJ. Crystal structure of a small G protein in complex with the GTPase-activating protein rhoGAP. Nature. 1997; 388(6643):693–7.
12. Rodriguez-Viciana P, Tetsu O, Oda K, Okada J, Rauen K, McCormick F. Cancer targets in the Ras pathway. Cold Spring Harb Symp Quant Biol. 2005; 70:461–7.
13. Hall A. Rho GTPases and the control of cell behaviour. Biochem Soc Trans. 2005;33(Pt 5):891–5.
14. Drees BL, Sundin B, Brazeau E, Caviston JP, Chen GC, Guo W, Kozminski KG, Lau MW, Moskow JJ, Tong A, Schenkman LR, McKenzie A III, Brennwald P, Longtine M, Bi E, Chan C, Novick P, Boone C, Pringle JR, Davis TN, Fields S, Drubin DG. A protein interaction map for cell polarity development. J Cell Biol. 2001;154(3):549–71.
15. Etienne-Manneville S. Cdc42—the centre of polarity. J Cell Sci. 2004;117(Pt 8):1291–300.
16. Johnson DI. Cdc42: An essential Rho-type GTPase controlling eukaryotic cell polarity. Microbiol Mol Biol Rev. 1999;63(1):54–105.
17. Carter GW, Rupp S, Fink GR, Galitski T. Disentan-

gling information flow in the Ras-cAMP signaling network. Genome Res. 2006;16(4):520–6.

18. Simon MN, De Virgilio C, Souza B, Pringle JR, Abo A, Reed SI. Role for the Rho-family GTPase Cdc42 in yeast mating-pheromone signal pathway. Nature. 1995;376(6542):702–5.
19. Peter M, Neiman AM, Park HO, van Lohuizen M, Herskowitz I. Functional analysis of the interaction between the small GTP binding protein Cdc42 and the Ste20 protein kinase in yeast. EMBO J. 1996; 15(24):7046–59.
20. Leberer E, Wu C, Leeuw T, Fourest-Lieuvin A, Segall JE, Thomas DY. Functional characterization of the Cdc42p binding domain of yeast Ste20p protein kinase. EMBO J. 1997;16(1):83–97.
21. Breitkreutz A, Tyers M. MAPK signaling specificity: it takes two to tango. Trends Cell Biol. 2002;12(6): 254–7.
22. Schwartz MA, Madhani HD. Principles of map kinase signaling specificity in Saccharomyces cerevisiae. Annu Rev Genet. 2004;38:725–48.
23. Singh PK, Hollingsworth MA. Cell surface-associated mucins in signal transduction. Trends Cell Biol. 2006; 16(9):467–76.
24. Ideker T. A systems approach to discovering signaling and regulatory pathways—or, how to digest large interaction networks into relevant pieces. Adv Exp Med Biol. 2004;547:21–30.
25. Barsyte-Lovejoy D, Galanis A, Sharrocks AD. Specificity determinants in MAPK signaling to transcription factors. J Biol Chem. 2002;277(12):9896–903.
26. Endy D, Yaffe MB. Signal transduction: molecular monogamy. Nature. 2003;426(6967):614–5.
27. Scaltriti M, Baselga J. The epidermal growth factor receptor pathway: a model for targeted therapy. Clin Cancer Res. 2006;12(18):5268–72.
28. Printen JA, Sprague GF Jr. Protein-protein interactions in the yeast pheromone response pathway: Ste5p interacts with all members of the MAP kinase cascade. Genetics. 1994;138(3):609–19.
29. Posas F, Saito H. Osmotic activation of the HOG MAPK pathway via Ste11p MAPKKK: scaffold role of Pbs2p MAPKK. Science. 1997;276(5319): 1702–5.
30. Dan I, Watanabe NM, Kusumi A. The Ste20 group kinases as regulators of MAP kinase cascades. Trends Cell Biol. 2001;11(5):220–30.
31. Manning G, Plowman GD, Hunter T, Sudarsanam S. Evolution of protein kinase signaling from yeast to man. Trends Biochem Sci. 2002;27(10):514–20.
32. Jonak C, Heberle-Bors E, Hirt H. MAP kinases: universal multi-purpose signaling tools. Plant Mol Biol. 1994;24(3):407–16.
33. Siddiqui J, Abe M, Hayes D, Shani E, Yunis E, Kufe D. Isolation and sequencing of a cDNA coding for the human DF3 breast carcinoma-associated antigen. Proc Natl Acad Sci U S A. 1988;85(7):2320–3.
34. Wreschner DH, Zrihan-Licht S, Baruch A, Sagiv D, Hartman ML, Smorodinsky N, Keydar I. Does a novel form of the breast cancer marker protein, MUC1, act as a receptor molecule that modulates signal transduction? Adv Exp Med Biol. 1994;353:17–26.
35. Zrihan-Licht S, Baruch A, Elroy-Stein O, Keydar I, Wreschner DH. Tyrosine phosphorylation of the MUC1 breast cancer membrane proteins. Cytokine receptor-like molecules. FEBS Lett. 1994;356(1):130–6.
36. Pandey P, Kharbanda S, Kufe D. Association of the DF3/MUC1 breast cancer antigen with Grb2 and the Sos/Ras exchange protein. Cancer Res. 1995;55(18): 4000–3.
37. Schroeder JA, Thompson MC, Gardner MM, Gendler SJ. Transgenic MUC1 interacts with epidermal growth factor receptor and correlates with mitogen-activated protein kinase activation in the mouse mammary gland. J Biol Chem. 2001;276(16):13057–64.
38. Li Y, Ren J, Yu W, Li Q, Kuwahara H, Yin L, Carraway KL III, Kufe D. The epidermal growth factor receptor regulates interaction of the human DF3/MUC1 carcinoma antigen with c-Src and beta-catenin. J Biol Chem. 2001;276(38):35239–42.
39. Meerzaman D, Shapiro PS, Kim KC. Involvement of the MAP kinase ERK2 in MUC1 mucin signaling. Am J Physiol Lung Cell Mol Physiol. 2001;281(1): L86–91.
40. Kikuchi A, Yamamoto H, Kishida S. Multiplicity of the interactions of Wnt proteins and their receptors. Cell Signal. 2007;19(4):659–71.
41. Yamamoto M, Bharti A, Li Y, Kufe D. Interaction of the DF3/MUC1 breast carcinoma-associated antigen and beta-catenin in cell adhesion. J Biol Chem. 1997; 272(19):12492–4.
42. Peifer M, McCrea PD, Green KJ, Wieschaus E, Gumbiner BM. The vertebrate adhesive junction proteins beta-catenin and plakoglobin and the Drosophila segment polarity gene armadillo form a multigene family with similar properties. J Cell Biol. 1992;118(3): 681–91.
43. Guger KA, Gumbiner BM. beta-Catenin has Wnt-like activity and mimics the Nieuwkoop signaling center in Xenopus dorsal-ventral patterning. Dev Biol. 1995;172(1):115–25.
44. Li Y, Bharti A, Chen D, Gong J, Kufe D. Interaction of glycogen synthase kinase 3beta with the DF3/MUC1 carcinoma-associated antigen and beta-catenin. Mol Cell Biol. 1998;18(12):7216–24.
45. Li Y, Kuwahara H, Ren J, Wen G, Kufe D. The c-Src tyrosine kinase regulates signaling of the human DF3/MUC1 carcinoma-associated antigen with GSK3 beta and beta-catenin. J Biol Chem. 2001;276(9):6061–4.

46. Thompson EJ, Shanmugam K, Hattrup CL, Kotlarczyk KL, Gutierrez A, Bradley JM, Mukherjee P, Gendler SJ. Tyrosines in the MUC1 cytoplasmic tail modulate transcription via the extracellular signal-regulated kinase 1/2 and nuclear factor-kappaB pathways. Mol Cancer Res. 2006;4(7):489–97.

47. Wei X, Xu H, Kufe D. Human MUC1 oncoprotein regulates p53-responsive gene transcription in the genotoxic stress response. Cancer Cell. 2005;7(2): 167–78.

48. Li Y, Yu WH, Ren J, Chen W, Huang L, Kharbanda S, Loda M, Kufe D. Heregulin targets gamma-catenin to the nucleolus by a mechanism dependent on the DF3/MUC1 oncoprotein. Mol Cancer Res. 2003;1 (10):765–75.

49. Li Y, Liu D, Chen D, Kharbanda S, Kufe D. Human DF3/MUC1 carcinoma-associated protein functions as an oncogene. Oncogene. 2003;22(38):6107–10.

50. Li Y, Chen W, Ren J, Yu WH, Li Q, Yoshida K, Kufe D. DF3/MUC1 signaling in multiple myeloma cells is regulated by interleukin-7. Cancer Biol Ther. 2003;2(2):187–93.

51. Wen Y, Caffrey TC, Wheelock MJ, Johnson KR, Hollingsworth MA. Nuclear association of the cytoplasmic tail of MUC1 and beta-catenin. J Biol Chem. 2003;278(39):38029–39.

52. Li Y, Kufe D. The Human DF3/MUC1 carcinoma-associated antigen signals nuclear localization of the catenin p120(ctn). Biochem Biophys Res Commun. 2001;281(2):440–3.

53. Huang L, Ren J, Chen D, Li Y, Kharbanda S, Kufe D. MUC1 cytoplasmic domain coactivates Wnt target gene transcription and confers transformation. Cancer Biol Ther. 2003;2(6):702–6.

54. Wei X, Xu H, Kufe D. MUC1 oncoprotein stabilizes and activates estrogen receptor alpha. Mol Cell. 2006;21(2):295–305.

55. Yin L, Huang L, Kufe D. MUC1 oncoprotein activates the FOXO3a transcription factor in a survival response to oxidative stress. J Biol Chem. 2004;279 (44):45721–7.

56. Raina D, Ahmad R, Kumar S, Ren J, Yoshida K, Kharbanda S, Kufe D. MUC1 oncoprotein blocks nuclear targeting of c-Abl in the apoptotic response to DNA damage. EMBO J. 2006;25(16):3774–83.

57. Ren J, Bharti A, Raina D, Chen W, Ahmad R, Kufe D. MUC1 oncoprotein is targeted to mitochondria by heregulin-induced activation of c-Src and the molecular chaperone HSP90. Oncogene. 2006;25(1):20–31.

58. Bray SJ. Notch signalling: a simple pathway becomes complex. Nat Rev Mol Cell Biol. 2006;7(9):678–89.

59. Rand MD, Lindblom A, Carlson J, Villoutreix BO, Stenflo J. Calcium binding to tandem repeats of EGF-like modules. Expression and characterization of the EGF-like modules of human Notch-1 implicated in receptor-ligand interactions. Protein Sci. 1997;6(10): 2059–71.

60. Luo Y, Haltiwanger RS. O-fucosylation of notch occurs in the endoplasmic reticulum. J Biol Chem. 2005;280(12):11289–94.

61. Bray S. Notch. Curr Biol. 2000;10(12):R433–5.

62. Sherblom AP, Carraway KL. A complex of two cell surface glycoproteins from ascites mammary adenocarcinoma cells. J Biol Chem. 1980;255(24):12051–9.

63. 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(2):783–90.

64. Jepson S, Komatsu M, Haq B, Arango ME, Huang D, Carraway CA, Carraway KL. 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(49):7524–32.

65. 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(7): 2931–41.

66. Pino V, Ramsauer VP, Salas P, Carothers Carraway CA, Carraway KL. Membrane mucin Muc4 induces density-dependent changes in ERK activation in mammary epithelial and tumor cells: role in reversal of contact inhibition. J Biol Chem. 2006;281(39): 29411–20.

67. Funes M, Miller JK, Lai C, Carraway KL III, Sweeney C. The mucin Muc4 potentiates neuregulin signaling by increasing the cell-surface populations of ErbB2 and ErbB3. J Biol Chem. 2006;281(28):19310–9.

68. Gimeno CJ, Ljungdahl PO, Styles CA, Fink GR. Unipolar cell divisions in the yeast S. cerevisiae lead to filamentous growth: regulation by starvation and RAS. Cell. 1992;68(6):1077–90.

69. Liu H, Styles CA, Fink GR. Elements of the yeast pheromone response pathway required for filamentous growth of diploids. Science. 1993;262(5140):1741–4.

70. Roberts RL, Fink GR. Elements of a single MAP kinase cascade in Saccharomyces cerevisiae mediate two developmental programs in the same cell type: mating and invasive growth. Genes Dev. 1994;8(24):2974–85.

71. Cullen PJ, Sprague GF Jr. Glucose depletion causes haploid invasive growth in yeast. Proc Natl Acad Sci U S A. 2000;97(25):13619–24.

72. Madhani HD, Fink GR. Combinatorial control required for the specificity of yeast MAPK signaling. Science. 1997;275(5304):1314–7.

73. Maeda T, Takekawa M, Saito H. Activation of yeast PBS2 MAPKK by MAPKKKs or by binding of an SH3-containing osmosensor. Science. 1995;269 (5223):554–8.

74. Brewster JL, de Valoir T, Dwyer ND, Winter E, Gustin MC. An osmosensing signal transduction pathway in yeast. Science. 1993;259(5102):1760–3.

75. Brewster JL, Gustin MC. Positioning of cell growth and division after osmotic stress requires a MAP kinase pathway. Yeast. 1994;10(4):425–39.

76. O'Rourke SM, Herskowitz I. The Hog1 MAPK prevents cross talk between the HOG and pheromone response MAPK pathways in Saccharomyces cerevisiae. Genes Dev. 1998;12(18):2874–86.

77. Cullen PJ, Schultz J, Horecka J, Stevenson BJ, Jigami Y, Sprague GF Jr. Defects in protein glycosylation cause SHO1-dependent activation of a STE12 signaling pathway in yeast. Genetics. 2000;155(3):1005–18.

78. Lee BN, Elion EA. The MAPKKK Ste11 regulates vegetative growth through a kinase cascade of shared signaling components. Proc Natl Acad Sci U S A. 1999;96(22):12679–84.

79. Michele DE, Campbell KP. Dystrophin-glycoprotein complex: post-translational processing and dystroglycan function. J Biol Chem. 2003;278(18):15457–60.

80. Durbeej M, Campbell KP. Muscular dystrophies involving the dystrophin-glycoprotein complex: an overview of current mouse models. Curr Opin Genet Dev. 2002;12(3):349–61.

81. Lebel-Binay S, Gil ML, Lagaudriere C, Miloux B, Marchiol-Fournigault C, Quillet-Mary A, Lopez M, Fradelizi D, Conjeaud H. Further characterization of CD82/IA4 antigen (type III surface protein): an activation/differentiation marker of mononuclear cells. Cell Immunol. 1994;154(1):468–83.

82. Lebel-Binay S, Lagaudriere C, Fradelizi D, Conjeaud H. CD82, member of the tetra-span-transmembrane protein family, is a costimulatory protein for T cell activation. J Immunol. 1995;155(1):101–10.

83. Musacchio A, Gibson T, Lehto VP, Saraste M. SH3—an abundant protein domain in search of a function. FEBS Lett. 1992;307(1):55–61.

84. Musacchio A, Noble M, Pauptit R, Wierenga R, Saraste M. Crystal structure of a Src-homology 3 (SH3) domain. Nature. 1992;359(6398):851–5.

85. Zarrinpar A, Park SH, Lim WA. Optimization of specificity in a cellular protein interaction network by negative selection. Nature. 2003;426(6967):676–80.

86. Cullen PJ, Sabbagh W Jr., Graham E, Irick MM, van Olden EK, Neal C, Delrow J, Bardwell L, Sprague GF Jr. A signaling mucin at the head of the Cdc42- and MAPK-dependent filamentous growth pathway in yeast. Genes Dev. 2004;18(14):1695–708.

87. O'Rourke SM, Herskowitz I. A third osmosensing branch in Saccharomyces cerevisiae requires the Msb2 protein and functions in parallel with the Sho1 branch. Mol Cell Biol. 2002;22(13):4739–49.

88. Komatsu M, Arango ME, Carraway KL. Synthesis and secretion of Muc4/sialomucin complex: implication of intracellular proteolysis. Biochem J. 2002; 368(Pt 1):41–8.

89. Ligtenberg MJ, Kruijshaar L, Buijs F, van Meijer M, Litvinov SV, Hilkens J. Cell-associated episialin is a complex containing two proteins derived from a common precursor. J Biol Chem. 1992;267(9):6171–7.

90. Levitin F, Stern O, Weiss M, Gil-Henn C, Ziv R, Prokocimer Z, Smorodinsky NI, Rubinstein DB, Wreschner DH. The MUC1 SEA module is a self-cleaving domain. J Biol Chem. 2005;280(39): 33374–86.

91. Macao B, Johansson DG, Hansson GC, Hard T. Autoproteolysis coupled to protein folding in the SEA domain of the membrane-bound MUC1 mucin. Nat Struct Mol Biol. 2006;13(1):71–6.

92. Palmai-Pallag T, Khodabukus N, Kinarsky L, Leir SH, Sherman S, Hollingsworth MA, Harris A. The role of the SEA (sea urchin sperm protein, enterokinase and agrin) module in cleavage of membrane-tethered mucins. Febs J. 2005;272(11):2901–11.

93. Bork P, Patthy L. The SEA module: a new extracellular domain associated with O-glycosylation. Protein Sci. 1995;4(7):1421–5.

94. Soto P, Zhang J, Carraway KL. Enzymatic cleavage as a processing step in the maturation of Muc4/sialomucin complex. J Cell Biochem. 2006;97(6):1267–74.

95. Schlessinger A, Yachdav G, Rost B. PROFbval: predict flexible and rigid residues in proteins. Bioinformatics. 2006;22(7):891–3.

96. Rost B, Yachdav G, Liu J. The PredictProtein server. Nucleic Acids Res. 2004;32(Web Server issue): W321–6.

97. Wreschner DH, McGuckin MA, Williams SJ, Baruch A, Yoeli M, Ziv R, Okun L, Zaretsky J, Smorodinsky N, Keydar I, Neophytou P, Stacey M, Lin HH, Gordon S. Generation of ligand-receptor alliances by "SEA" module-mediated cleavage of membrane-associated mucin proteins. Protein Sci. 2002;11(3):698–706.

98. Giaever G, Chu AM, Ni L, Connelly C, Riles L, Veronneau S, Dow S, Lucau-Danila A, Anderson K, Andre B, Arkin AP, Astromoff A, El-Bakkoury M, Bangham R, Benito R, Brachat S, Campanaro S, Curtiss M, Davis K, Deutschbauer A, Entian KD, Flaherty P, Foury F, Garfinkel DJ, Gerstein M, Gotte D, Guldener U, Hegemann JH, Hempel S, Herman Z, Jaramillo DF, Kelly DE, Kelly SL, Kotter P, LaBonte D, Lamb DC, Lan N, Liang H, Liao H, Liu L, Luo C, Lussier M, Mao R, Menard P, Ooi SL, Revuelta JL, Roberts CJ, Rose M, Ross-Macdonald P, Scherens B, Schimmack G, Shafer B, Shoemaker DD, Sookhai-Mahadeo S, Storms RK, Strathern JN, Valle G, Voet M, Volckaert G, Wang CY, Ward TR,

Wilhelmy J, Winzeler EA, Yang Y, Yen G, Youngman E, Yu K, Bussey H, Boeke JD, Snyder M, Philippsen P, Davis RW, Johnston M. Functional profiling of the Saccharomyces cerevisiae genome. Nature. 2002;418 (6896):387–91.

99. Krysan DJ, Ting EL, Abeijon C, Kroos L, Fuller RS. Yapsins are a family of aspartyl proteases required for cell wall integrity in Saccharomyces cerevisiae. Eukaryot Cell. 2005;4(8):1364–74.

100. Dash C, Kulkarni A, Dunn B, Rao M. Aspartic peptidase inhibitors: implications in drug development. Crit Rev Biochem Mol Biol. 2003;38(2):89–119.

101. Tamagno E, Parola M, Bardini P, Piccini A, Borghi R, Guglielmotto M, Santoro G, Davit A, Danni O, Smith MA, Perry G, Tabaton M. Beta-site APP cleaving enzyme up-regulation induced by 4-hydroxynonenal is mediated by stress-activated protein kinases pathways. J Neurochem. 2005;92(3):628–36.

102. Duffy MJ, Reilly D, Brouillet JP, McDermott EW, Faul C, O'Higgins N, Fennelly JJ, Maudelonde T, Rochefort H. Cathepsin D concentration in breast cancer cytosols: correlation with disease-free interval and overall survival. Clin Chem. 1992;38(10):2114–6.

103. Rochefort H. Biological and clinical significance of cathepsin D in breast cancer. Acta Oncol. 1992;31(2): 125–30.

104. Diaz M, Rodriguez JC, Sanchez J, Sanchez MT, Martin A, Merino AM, Vizoso F. Clinical significance of pepsinogen C tumor expression in patients with stage D2 prostate carcinoma. Int J Biol Markers. 2002;17(2):125–9.

105. Liaudet-Coopman E, Beaujouin M, Derocq D, Garcia M, Glondu-Lassis M, Laurent-Matha V, Prebois C, Rochefort H, Vignon F. Cathepsin D: newly discovered functions of a long-standing aspartic protease in cancer and apoptosis. Cancer Lett. 2006;237(2):167–79.

106. Fukuda ME, Iwadate Y, Machida T, Hiwasa T, Nimura Y, Nagai Y, Takiguchi M, Tanzawa H, Yamaura A, Seki N. Cathepsin D is a potential serum marker for poor prognosis in glioma patients. Cancer Res. 2005;65(12):5190–4.

107. Bland CE, Kimberly P, Rand MD. Notch-induced proteolysis and nuclear localization of the Delta ligand. J Biol Chem. 2003;278(16):13607–10.

108. Yang X, Klein R, Tian X, Cheng HT, Kopan R, Shen J. Notch activation induces apoptosis in neural progenitor cells through a p53-dependent pathway. Dev Biol. 2004;269(1):81–94.

109. Barry GD, Le GT, Fairlie DP. Agonists and antagonists of protease activated receptors (PARs). Curr Med Chem. 2006;13(3):243–65.

110. Brayman M, Thathiah A, Carson DD. MUC1: a multifunctional cell surface component of reproductive tissue epithelia. Reprod Biol Endocrinol. 2004; 2(1):4.

111. Ten Klooster JP, Hordijk PL. Targeting and localized signalling by small GTPases. Biol Cell. 2007; 99(1):1–12.

112. Mather IH, Jack LJ, Madara PJ, Johnson VG. The distribution of MUC1, an apical membrane glycoprotein, in mammary epithelial cells at the resolution of the electron microscope: implications for the mechanism of milk secretion. Cell Tissue Res. 2001;304(1): 91–101.

113. Parry G, Beck JC, Moss L, Bartley J, Ojakian GK. Determination of apical membrane polarity in mammary epithelial cell cultures: the role of cell-cell, cell-substratum, and membrane-cytoskeleton interactions. Exp Cell Res. 1990;188(2):302–11.

114. Hey NA, Meseguer M, Simon C, Smorodinsky NI, Wreschner DH, Ortiz ME, Aplin JD. Transmembrane and truncated (SEC) isoforms of MUC1 in the human endometrium and Fallopian tube. Reprod Biol Endocrinol. 2003;1:2.

115. Kufe D, Inghirami G, Abe M, Hayes D, Justi-Wheeler H, Schlom J. Differential reactivity of a novel monoclonal antibody (DF3) with human malignant versus benign breast tumors. Hybridoma. 1984;3(3):223–32.

116. Perey L, Hayes DF, Maimonis P, Abe M, O'Hara C, Kufe DW. Tumor selective reactivity of a monoclonal antibody prepared against a recombinant peptide derived from the DF3 human breast carcinoma-associated antigen. Cancer Res. 1992;52(9):2563–8.

117. Pemberton LF, Rughetti A, Taylor-Papadimitriou J, Gendler SJ. The epithelial mucin MUC1 contains at least two discrete signals specifying membrane localization in cells. J Biol Chem. 1996;271(4):2332–40.

118. 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(32): 30142–7.

119. Chant J, Pringle JR. Patterns of bud-site selection in the yeast Saccharomyces cerevisiae. J Cell Biol. 1995; 129(3):751–65.

120. Cullen PJ, Sprague GF Jr. The roles of bud-site-selection proteins during haploid invasive growth in yeast. Mol Biol Cell. 2002;13(9):2990–3004.

121. Shimada Y, Gulli MP, Peter M. Nuclear sequestration of the exchange factor Cdc24 by Far1 regulates cell polarity during yeast mating. Nat Cell Biol. 2000; 2(2):117–24.

122. Zaretsky JZ, Barnea I, Aylon Y, Gorivodsky M, Wreschner DH, Keydar I. MUC1 gene overexpressed in breast cancer: structure and transcriptional activity of the MUC1 promoter and role of estrogen receptor alpha (ERalpha) in regulation of the MUC1 gene expression. Mol Cancer. 2006;5:57.

123. Abba MC, Hu Y, Sun H, Drake JA, Gaddis S, Baggerly K, Sahin A, Aldaz CM. Gene expression

signature of estrogen receptor alpha status in breast cancer. BMC Genomics. 2005;6(1):37.

124. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lonning PE, Borresen-Dale AL, Brown PO, Botstein D. Molecular portraits of human breast tumours. Nature. 2000;406(6797):747–52.

125. Gruvberger S, Ringner M, Chen Y, Panavally S, Saal LH, Borg A, Ferno M, Peterson C, Meltzer PS. Estrogen receptor status in breast cancer is associated with remarkably distinct gene expression patterns. Cancer Res. 2001;61(16):5979–84.

126. West M, Blanchette C, Dressman H, Huang E, Ishida S, Spang R, Zuzan H, Olson JA Jr., Marks JR, Nevins JR. Predicting the clinical status of human breast cancer by using gene expression profiles. Proc Natl Acad Sci U S A. 2001;98(20):11462–7.

127. van de Vijver MJ, He YD, van't Veer LJ, Dai H, Hart AA, Voskuil DW, Schreiber GJ, Peterse JL, Roberts C, Marton MJ, Parrish M, Atsma D, Witteveen A, Glas A, Delahaye L, van der Velde T, Bartelink H, Rodenhuis S, Rutgers ET, Friend SH, Bernards R. A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med. 2002;347(25):1999–2009.

128. Sotiriou C, Neo SY, McShane LM, Korn EL, Long PM, Jazaeri A, Martiat P, Fox SB, Harris AL, Liu ET. Breast cancer classification and prognosis based on gene expression profiles from a population-based study. Proc Natl Acad Sci U S A. 2003;100(18): 10393–8.

129. Shiraga T, Smith D, Nuthall HN, Hollingsworth MA, Harris A. Identification of two novel elements involved in human MUCI gene expression in vivo. Mol Med. 2002;8(1):33–41.

130. Morris JR, Taylor-Papadimitriou J. The Sp1 transcription factor regulates cell type-specific transcription of MUC1. DNA Cell Biol. 2001;20(3):133–9.

131. Abba MC, Nunez MI, Colussi AG, Croce MV, Segal-Eiras A, Aldaz CM. GATA3 protein as a MUC1 transcriptional regulator in breast cancer cells. Breast Cancer Res. 2006;8(6):R64.

132. Canbay E. Erb-B2 homodimerization inhibits MUC1 transcription in cultured human mammary epithelial cells. Cell Biol Int. 2003;27(6):477–81.

133. 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(38):4354–61.

134. Fauquette V, Perrais M, Cerulis S, Jonckheere N, Ducourouble MP, Aubert JP, Pigny P, Van Seuningen I. 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(Pt 1):35–45.

135. 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(38): 36942–52.

136. Jonckheere N, Perrais M, Mariette C, Batra SK, Aubert JP, Pigny P, Van Seuningen I. A role for human MUC4 mucin gene, the ErbB2 ligand, as a target of TGF-beta in pancreatic carcinogenesis. Oncogene. 2004;23(34):5729–38.

137. Ren CY, Akiyama Y, Miyake S, Yuasa Y. Transcription factor GATA-5 selectively up-regulates mucin gene expression. J Cancer Res Clin Oncol. 2004;130-(5):245–52.

138. Merika M, Orkin SH. DNA-binding specificity of GATA family transcription factors. Mol Cell Biol. 1993;13(7):3999–4010.

139. Zeitlinger J, Simon I, Harbison CT, Hannett NM, Volkert TL, Fink GR, Young RA. Program-specific distribution of a transcription factor dependent on partner transcription factor and MAPK signaling. Cell. 2003;113(3):395–404.

140. Madhani HD, Fink GR. The riddle of MAP kinase signaling specificity. Trends Genet. 1998;14(4): 151–5.

141. Carraway KL, Ramsauer VP, Carraway CA. Glycoprotein contributions to mammary gland and mammary tumor structure and function: roles of adherens junctions, ErbBs and membrane MUCs. J Cell Biochem. 2005;96(5):914–26.

142. Agrawal B, Krantz MJ, Reddish MA, Longenecker BM. Cancer-associated MUC1 mucin inhibits human T-cell proliferation, which is reversible by IL-2. Nat Med. 1998;4(1):43–9.

143. Agrawal B, Longenecker BM. MUC1 mucin-mediated regulation of human T cells. Int Immunol. 2005;17(4): 391–9.

144. Kondo S, Yoshizaki T, Wakisaka N, Horikawa T, Murono S, Jang KL, Joab I, Furukawa M, Pagano JS. MUC1 induced by Epstein-Barr virus latent membrane protein 1 causes dissociation of cell-matrix interaction and cellular invasiveness via STAT signaling. J Virol. 2007;81(4):1554–62.

145. Doi M, Yokoyama A, Kondo K, Ohnishi H, Ishikawa N, Hattori N, Kohno N. Anti-tumor effect of the anti-KL-6/MUC1 monoclonal antibody through exposure of surface molecules by MUC1 capping. Cancer Sci. 2006;97(5):420–9.

146. Snyder LA, Goletz TJ, Gunn GR, Shi FF, Harris MC, Cochlin K, McCauley C, McCarthy SG, Branigan PJ, Knight DM. A MUC1/IL-18 DNA vaccine induces anti-tumor immunity and increased survival in MUC1 transgenic mice. Vaccine. 2006; 24(16):3340–52.

147. Danielczyk A, Stahn R, Faulstich D, Loffler A, Mar-

ten A, Karsten U, Goletz S. PankoMab: a potent new generation anti-tumour MUC1 antibody. Cancer Immunol Immunother. 2006;55(11):1337–47.

148. Tsutsumida H, Swanson BJ, Singh PK, Caffrey TC, Kitajima S, Goto M, Yonezawa S, Hollingsworth MA. RNA interference suppression of MUC1 reduces the growth rate and metastatic phenotype of human pancreatic cancer cells. Clin Cancer Res. 2006; 12(10):2976–87.
149. Li X, Wang L, Nunes DP, Troxler RF, Offner GD. Suppression of MUC1 synthesis downregulates expression of the epidermal growth factor receptor. Cancer Biol Ther. 2005;4(9):968–73.
150. Hu XF, Yang E, Li J, Xing PX. MUC1 cytoplasmic tail: a potential therapeutic target for ovarian carcinoma. Expert Rev Anticancer Ther. 2006;6(8):1261–71.
151. Agrawal B, Gendler SJ, Longenecker BM. The biological role of mucins in cellular interactions and immune regulation: prospects for cancer immunotherapy. Mol Med Today. 1998;4(9):397–403.
152. Lillehoj EP, Hyun SW, Kim BT, Zhang XG, Lee DI, Rowland S, Kim KC. Muc1 mucins on the cell surface are adhesion sites for Pseudomonas aeruginosa. Am J Physiol Lung Cell Mol Physiol. 2001;280(1): L181–7.
153. Lillehoj EP, Kim H, Chun EY, Kim KC. Pseudomonas aeruginosa stimulates phosphorylation of the airway epithelial membrane glycoprotein Muc1 and activates MAP kinase. Am J Physiol Lung Cell Mol Physiol. 2004;287(4):L809–15.
154. Lu W, Hisatsune A, Koga T, Kato K, Kuwahara I, Lillehoj EP, Chen W, Cross AS, Gendler SJ, Gewirtz AT, Kim KC. Cutting edge: enhanced pulmonary clearance of Pseudomonas aeruginosa by Muc1 knockout mice. J Immunol. 2006;176(7):3890–4.
155. Bassilana M, Hopkins J, Arkowitz RA. Regulation of the Cdc42/Cdc24 GTPase module during Candida albicans hyphal growth. Eukaryot Cell. 2005;4(3): 588–603.
156. Ernst JF. Regulation of dimorphism in Candida albicans. Contrib Microbiol. 2000;5:98–111.
157. aquette Y, Merlen Y, Malette B, Bleau G. Allelic polymorphism in the hamster oviductin gene is due to a variable number of mucin-like tandem repeats. Mol Reprod Dev. 1995;42(4):388–96.
158. Kirkbride HJ, Bolscher JG, Nazmi K, Vinall LE, Nash MW, Moss FM, Mitchell DM, Swallow DM. Genetic polymorphism of MUC7: allele frequencies and association with asthma. Eur J Hum Genet. 2001;9(5):347–54.
159. Goulart LR, Vieira GS, Martelli L, Inacio J, Goulart IM, Franco JG Jr. Is MUC1 polymorphism associated with female infertility? Reprod Biomed Online. 2004; 8(4):477–82.
160. Choudhury A, Moniaux N, Winpenny JP, Hollingsworth MA, Aubert JP, Batra SK. Human MUC4 mucin cDNA and its variants in pancreatic carcinoma. J Biochem (Tokyo). 2000;128(2):233–43.
161. Fowler JC, Teixeira AS, Vinall LE, Swallow DM. Hypervariability of the membrane-associated mucin and cancer marker MUC1. Hum Genet. 2003;113(6): 473–9.