REVIEW

MUC1-C oncoprotein as a target in breast cancer: activation of signaling pathways and therapeutic approaches

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Mucin 1 (MUC1) is a heterodimeric protein formed by two subunits that is aberrantly overexpressed in human breast cancer and other cancers. Historically, much of the early work on MUC1 focused on the shed mucin subunit. However, more recent studies have been directed at the transmembrane MUC1-C-terminal subunit (MUC1-C) that functions as an oncoprotein. MUC1-C interacts with EGFR (epidermal growth factor receptor), ErbB2 and other receptor tyrosine kinases at the cell membrane and contributes to activation of the PI3K→AKT and mitogen-activated protein kinase kinase (MEK)→extracellular signal-regulated kinase (ERK) pathways. MUC1-C also localizes to the nucleus where it activates the Wnt/β-catenin, signal transducer and activator of transcription (STAT) and NF (nuclear factor)-κB RelA pathways. These findings and the demonstration that MUC1-C is a druggable target have provided the experimental basis for designing agents that block MUC1-C function. Notably, inhibitors of the MUC1-C subunit have been developed that directly block its oncogenic function and induce death of breast cancer cells *in vitro* and in xenograft models. On the basis of these findings, a first-in-class MUC1-C inhibitor has entered phase I evaluation as a potential agent for the treatment of patients with breast cancers who express this oncoprotein.

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INTRODUCTION

The mucin (MUC) family of high-molecular-weight glycoproteins evolved in metazoans to provide protection for epithelial cell layers that are exposed to the external environment. The human MUC family (MUC1 to MUC21) includes secreted and transmembrane MUCs that afford protection of the epithelia lining the respiratory and gastrointestinal tracts, and lining ducts in organs such as the mammary gland, liver, pancreas and kidneys. The secreted MUCs form a mucous gel that functions as a physical barrier. The transmembrane MUCs contribute to this protective barrier through ectodomains that contain O-glycosylated tandem repeat structures. The transmembrane MUCs also contain single membrane-spanning regions and cytoplasmic domains (CDs) that transduce stress signals to promote growth and survival responses for repair of the epithelial layer.¹

MUC1 is a transmembrane MUC that was identified by its marked overexpression in human breast cancers as compared with that in normal ductal breast epithelial cells.² MUC1 is translated as a single polypeptide that undergoes autocleavage into two subunits that form a stable non-covalent heterodimeric complex at the cell membrane (Figure 1a).^{3–5} The MUC1-N-terminal subunit (MUC1-N) is the MUC component of the heterodimer containing variable numbers of 20-amino-acid (aa) tandem repeats that are glycosylated on serine and threonine residues (Figure 1a).^{6,7} The MUC1-C includes a 58-aa extracellular domain, a 28-aa transmembrane domain and a 72-aa CD (Figure 1b).

MUC1-N is tethered to the transmembrane MUC1-C subunit as a complex along the apical borders of normal polarized breast epithelia (Figure 2a). Release of MUC1-N from the cell surface leaves MUC1-C to function as a putative receptor to signal stress to the interior of the cell (Figure 2a).¹

In breast tumor cells with loss of apical–basal polarity, the MUC1-N/MUC1-C complex is found over the entire cell membrane.² MUC1-N is shed from the surface of carcinoma cells.⁸ In addition, as detected by the CA15-3 assay, MUC1-N is found at increased levels in the plasma of patients with breast cancer.⁹ Loss of apical–basal polarity in breast cancer cells is also associated with interactions between MUC1-C and receptor tyrosine kinases (RTKs), such as EGFR and ErbB2, that are normally positioned at the basal–lateral borders (Figure 2b). The repositioning of MUC1 that occurs with transformation is also associated with substantial upregulation of MUC1 levels, supporting the ‘aberrant overexpression’ of MUC1 in breast carcinomas (Figure 2c).

Overexpression of MUC1 in breast cancer cells

MUC1 is aberrantly overexpressed in over 90% of breast tumors.^{10–12} The overexpression of MUC1 in breast carcinomas is the result, in part, of genetic alterations and dysregulation of transcription. The *MUC1* gene is located at chromosome 1q21, a region frequently altered in breast cancer cells.¹³ Amplification of the *MUC1* gene has been found in about 40% of breast cancers and significantly correlates with increases in MUC1 messenger RNA and protein levels.^{14,15} The *MUC1* promoter contains binding sites for diverse transcription factors and is activated in breast cancer cells.^{16–18} As described in more detail below, the MUC1-C subunit functions in auto-inductive loops with the STAT1/3 and NF (nuclear factor)-κB RelA transcription factors that confer activation of the *MUC1* gene and its overexpression in breast cancer cells.^{19–21}

Other studies have demonstrated that MUC1 overexpression is regulated by micro RNAs. miR-125b, which is downregulated in breast cancer cells, interacts with the MUC1 3'UTR and suppresses

Figure 1. Structure of the MUC1 heterodimer. **(a)** MUC1 undergoes autoproteolysis at a SEA (sea-urchin sperm protein, enterokinase and agrin) domain to generate two subunits that, in turn, form a stable non-covalent heterodimer. The MUC1-N and MUC1-C nomenclature is used to designate positioning of the subunits after cleavage and to distinguish them from genetic isoforms that are subclassified with Greek characters; for example, ER α and ER β , the protein kinase C isoenzymes, PDGF receptors and others. **(b)** The MUC1-C subunit contains a 58-aa extracellular domain that is glycosylated on asparagine at a N³⁶LT site. The MUC1-C 72-aa CD interacts with multiple effectors as described below and is sufficient to induce transformation.

MUC1 protein, but not messenger RNA, levels.²² miR-145 is also downregulated in breast cancer cells, binds to the MUC1 3'UTR and suppresses MUC1 expression.²³ In addition, miR-1226, which has no other known targets, binds to the MUC1 3'UTR and downregulates MUC1 protein levels.²⁴ However, it is not known whether miR-1226 levels are decreased in breast cancer cells as compared with normal mammary epithelial cells.

Other work has further demonstrated that MUC1 translation is markedly upregulated in nonmalignant breast epithelial cells in response to EGF or heregulin stimulation and activation of PI3K→AKT signaling. In breast cancer cells, MUC1 translation is constitutively upregulated by the PI3K→AKT→mTORC1 pathway and the eIF4A RNA helicase. These findings have supported an auto-inductive loop in which PI3K→AKT signaling increases translation of the MUC1-C protein and, in turn, MUC1-C contributes to the activation of the PI3K→AKT pathway by mechanisms that are described below. In contrast to activating mutations of the PI3K pathway in breast cancer,²⁵ there is no evidence that mutants are responsible for the MUC1-C oncogenic function. Indeed, certain mutants of the MUC1-C CD act as dominant negatives of the malignant phenotype when expressed in carcinoma cells.²⁶

A model of transformation induced by overexpression of the MUC1-C subunit was developed, which identified activation of gene families involved in oncogenesis and metabolism.²⁷ A set of experimentally derived MUC1-C-induced genes associated with tumorigenesis was applied to the analysis of a primary breast cancer database ($n = 295$). A 35-gene MUC1-C-induced signature was found to predict highly significant decreases in both disease-free and overall survival.²⁷ A set of 38 MUC1-C-induced genes associated with lipid metabolism was also applied to the analysis of ER+ breast cancer patients treated with tamoxifen.²⁸ The results from two individual databases showed that patients with tumors overexpressing MUC1 and the lipid metabolic pathways are at significantly higher risk for recurrence and death.²⁸ These findings indicate that overexpression of MUC1-C contributes to

Figure 2. Aberrant localization and overexpression of MUC1-C in breast cancer cells. **(a)** The MUC1-N/MUC1-C heterodimer is expressed at the apical border of normal mammary epithelial cells. MUC1-N extends beyond the glycocalyx of the epithelial cells and contributes to the protective mucous barrier. With activation of the epithelial stress response and release of MUC1-N from the cell surface into the mucous gel, MUC1-C functions as a second line of defense to signal stress to the interior of the cell and thereby protects against loss of integrity of the epithelial layer. Figure modified from Kufe.¹ **(b)** In normal epithelial cells, apical MUC1-C is sequestered from RTKs that localize to the basal-lateral borders. However, with transformation and loss of polarity, MUC1-C is repositioned over the entire cell membrane and forms complexes with RTKs that promote RTK signaling and upregulation of MUC1-C expression. Figure modified from Kufe.¹ **(c)** Tissue section containing foci of breast carcinoma cells surrounding normal ducts, which was stained with an anti-MUC1 antibody. Expression of MUC1 along the apical borders of normal ductal epithelial cells (arrow) is in contrast to the increased expression of MUC1 over the entire cell membrane and in the cytoplasm of breast cancer cells. Figure originally published in Gaemers *et al.*⁸⁶

the regulation of genes that are highly predictive of clinical outcome in breast cancer.

MUC1-C SIGNALING AT THE CELL MEMBRANE

MUC1-C associates with EGFR

The MUC1-N/MUC1-C complex is expressed at the apical membrane of polarized epithelial cells.² Conversely, the EGFR localizes to the basolateral membrane in normal polarized epithelia.²⁹ With loss of polarity associated with the epithelial stress response³⁰ or transformation, MUC1-C and EGFR are expressed over the entire cell membrane and are repositioned to form complexes. The MUC1-C extracellular domain is glycosylated on Asp-36, which functions as a binding site for galectin-3, and the formation of galectin-3 bridges that position MUC1-C with EGFR at the cell membrane (Figure 3a).³¹ The MUC1-

Figure 3. Interaction between MUC1-C and EGFR at the cell membrane. **(a)** The MUC1-C subunit forms complexes with EGFR at the cell membrane that are mediated extracellularly by galectin-3 bridges.³¹ The MUC1-C CD is phosphorylated by EGFR and other RTKs. In turn, the MUC1-C CD functions as an adaptor for binding of the PI3K SH2 domains and activation of the PI3K→AKT pathway.^{46,38} **(b)** The 72-aa MUC1-C CD is phosphorylated by diverse RTKs and non-RTKs, providing binding sites for SH2 domains in effectors that, in addition to PI3K,^{46,38} include SRC³³ and GRB2.⁵²

C CD also functions as a substrate for EGFR phosphorylation on the Y⁴⁶EKV motif.³² In turn, pYEKV functions as a binding site for the SRC homology 2 (SH2) domain.^{32,33}

In transgenic mouse models, overexpression of MUC1 is associated with binding to EGFR in mammary glands and the induction of breast tumors.^{34,35} In addition, in an EGFR-dependent whey-acidic protein-TGF α (transforming growth factor) transgenic model, depletion of MUC1 decreases cyclin D1 expression and delays the onset of breast tumors, indicating that MUC1 contributes to EGFR-mediated tumorigenesis.³⁶ The interaction between MUC1 and EGFR at the cell membrane has been shown to increase EGFR internalization and recycling.^{36,37} Other work has demonstrated that MUC1-C promotes EGFR-dependent activation of the PI3K→AKT pathway³⁸ and localization of EGFR to the nucleus.³⁹ These findings have established a role for MUC1-C in EGFR turnover at the cell membrane and in EGFR signaling in the cytoplasm and nucleus (Figure 3a).

Interaction between MUC1-C and other RTKs

With regard to other ErbB family members, studies in breast cancer cells have shown that MUC1 associates with ErbB2/HER2 and that this interaction is increased by heregulin stimulation.⁴⁰ MUC1 is also detectable in complexes with ErbB3 and ErbB4 in breast cancer cells⁴⁰ and in mouse mammary glands.³⁴ Other studies have shown that MUC1-C associates with fibroblast growth factor receptor 3 (FGFR3), is phosphorylated on Tyr-46 in response to FGFR3 activation and contributes to FGFR3 and SRC signaling (Figure 3b).⁴¹ MUC1-C also associates with hepatocyte growth factor receptor and is subject to hepatocyte growth factor receptor phosphorylation on Tyr-20 (Figure 3b).⁴² Phosphorylation of the Y²⁰HPM motif confers the interaction between MUC1-C and PI3K

p85 (Figures 3a and b).³⁸ Another example is supported by the demonstration that MUC1-C associates with the platelet-derived growth factor receptor β , which phosphorylates the MUC1-C CD on Tyr-20 and Tyr-35, and enhances MUC1-mediated invasion (Figure 3b).⁴³

These findings have collectively supported a role for MUC1-C in interactions with diverse RTKs at the cell membrane and in their downstream signaling pathways.

MUC1-C activates the PI3K→AKT pathway

The PI3K pathway is of importance to the growth, survival and metabolism of cancer cells.⁴⁴ PI3Ks transduce signals from RTKs and G-protein coupled receptors by generating phospholipids that confer activation of the serine–threonine protein kinase AKT and other effectors.⁴⁵ The PI3K p85 regulatory subunit contains SH2 domains that interact with pYXXM sequences on activated RTKs. In turn, PI3K p85-mediated inhibition of the PI3K p110 catalytic subunit is repressed and p110 generates phosphatidylinositol-3,4,5-triphosphate necessary for recruitment and activation of AKT at the cell membrane.

MUC1-C functions as a docking site for activation of PI3K→AKT signaling (Figure 3b). Initial studies demonstrated that MUC1-induced transformation of rat fibroblasts is associated with increases in p-AKT and that this response is blocked by the PI3K inhibitor LY294002.⁴⁶ Overexpression of MUC1 in DA3 mouse breast cancer cells is also associated with a PI3K-dependent enhancement of growth.⁴⁷ The MUC1-C CD contains a Y²⁰HPM motif that, when phosphorylated on tyrosine, functions as a binding site for the PI3K p85 SH2 domains (Figure 3b). In this context, PI3K p85 associates with the MUC1-C CD *in vitro* and in cells.^{48,38} In addition, mutation of the MUC1-C CD at Tyr-20 blocks binding of PI3K p85 and attenuates MUC1-C-induced activation of the PI3K→AKT pathway.^{48,38}

The significance of MUC1-C-induced activation of the PI3K→AKT pathway is at an early stage of interrogation. One study has shown that overexpression of MUC1-C in breast cancer cells is associated with AKT-mediated increases in glucose uptake and lactate production, indicating that MUC1-C stimulates glycolysis through PI3K→AKT signaling.⁴⁹ Overexpression of MUC1 in NSCLC cells resulted in a marked increase in p-AKT, which was associated with an increase in vascular endothelial growth factor production and proangiogenic activity.⁵⁰ Similar results were obtained in breast cancer cells, supporting a role for MUC1 in promoting vascular endothelial growth factor-mediated angiogenesis by activation of the PI3K→AKT pathway.⁵¹ A significant correlation between MUC1 and vascular endothelial growth factor expression was also identified in human breast carcinomas.⁵¹ The PI3K→AKT pathway functions in the activation of diverse effectors that promote growth and survival,^{44,45} thus, overexpression of MUC1-C and the upregulation of PI3K→AKT signaling in breast cancer cells represents one mechanism by which MUC1-C functions as an oncoprotein.

MUC1-C contributes to activation of the MEK→ERK pathway

The MUC1-C CD contains a Y⁶⁰TNP motif that, when phosphorylated on tyrosine, functions as a binding site for the GRB2 SH2 domain, linking MUC1-C to son-of-sevenless (SOS) and thereby RAS signaling (Figure 3b).⁵² Involvement of MUC1-C in activation of the RAS→RAF→MEK→ERK pathway was supported in part by the demonstration that ERK1/2 is activated in the mammary glands from MUC1 transgenic mice.³⁴ In addition, activation of the MUC1-C CD results in the induction of MEK→ERK by a mechanism that is blocked by a dominant-negative RAS mutant and by a MEK inhibitor.⁵³ Other studies have shown that overexpression of MUC1 in mouse breast cancer cells⁴⁷ and in human lung cancer cells⁵⁰ results in ERK activation, thus providing experimental models for defining more precisely how MUC1-C contributes to RAS→RAF→MEK→ERK signaling (Figure 3b).

INTRACELLULAR LOCALIZATION OF THE MUC1-C SUBUNIT

MUC1-C is internalized from the cell membrane by clathrin-mediated endocytosis by a mechanism involving binding of AP-2 to Y²⁰HPM and GRB2 to pY⁶⁰TNP sites in the MUC1-C CD (Figure 4).⁵⁴ Recycling of MUC1-C from endosomes back to the cell membrane is conferred by palmitoylation of the MUC1-C CD on cysteines in the cysteine-glutamine-cysteine (CQC) motif.⁵⁵ The overexpression of MUC1-C as found in breast cancer cells is associated with accumulation of MUC1-C in the cytoplasm, probably as a result of retrograde trafficking during endocytosis and return to the endoplasmic reticulum (Figure 4).¹ In this context, EGFR is a binding partner of MUC1-C at the cell membrane, which is released from the endoplasmic reticulum into the cytosol and then associates with importin- β for transport into the nucleus.⁵⁶

Similar to EGFR, MUC1-C interacts with importin- β in the cytoplasm and is transported to the nucleus (Figure 4).⁵⁷ Binding of MUC1-C to importin- β is dependent on the formation of MUC1-C homodimers.⁵⁷ The MUC1-C CD CQC motif cysteines are necessary and sufficient for MUC1-C dimerization in the cytoplasm of breast cancer cells (Figure 3b).⁵⁸ The MUC1-C CQC motif cysteines also represent a target for blocking nuclear localization and function of this oncogenic subunit.^{57,58}

Other studies have shown that cytosolic MUC1-C localizes to the mitochondrial outer membrane, where it blocks apoptosis in response to genotoxic stress (Figure 4).⁵⁹ Stimulation of breast cancer cells with heregulin or fibroblast growth factor is associated with increased localization of MUC1-C to mitochondria by a mechanism involving activation of SRC and transport of MUC1-C by the molecular chaperone HSP70/HSP90 complex.^{60,41} Localization of MUC1-C to mitochondria is also dependent on the formation of MUC1-C homodimers through the CQC motif cysteines in the CD. How MUC1-C blocks activation of the intrinsic apoptotic pathway is a focus of active investigation.

Figure 4. Intracellular cycling of MUC1-C to the nucleus and mitochondria. MUC1-C cycles from the cell membrane to endosomes and then back to the cell membrane.^{54,55} Alternatively, with overexpression in breast cancer cells, MUC1-C accumulates in the cytoplasm, where it forms homodimers that are mediated by the CQC motif in the MUC1-C CD.⁵⁸ MUC1-C dimerization is necessary for transport into the nucleus by importin- β .⁵⁷ In the nucleus, MUC1-C associates with transcription factors, such as STAT1/3, NF- κ B RelA and ER α , and contributes to transactivation of their target genes.¹ MUC1-C is also transported to the mitochondrial outer membrane where it blocks activation of the intrinsic apoptotic pathway.⁵⁹

INVOLVEMENT OF MUC1-C IN NUCLEAR SIGNALING

MUC1-C contributes to activation of the Wnt/ β -catenin/TCF (T-cell factor)/ β -catenin pathway and cyclin D1 expression

The canonical Wnt signaling pathway is of importance to developmental cell fate and cancer.⁶¹ The Wnt pathway controls the cytoplasmic abundance of β -catenin, a key component of the adherens junction of epithelial cells that links cadherin adhesion molecules at the cell surface to the actin cytoskeleton. Cytoplasmic levels of β -catenin are regulated by glycogen synthase kinase 3 β (GSK3 β) in a complex with the adenomatous polyposis coli protein and axin. Activation of the Wnt pathway inhibits GSK3 β and thereby results in the stabilization of β -catenin.⁶¹ In turn, the accumulation of β -catenin promotes formation of complexes with members of the T-cell factor/leukocyte-enhancing factor 1 family of transcription factors and the activation of Wnt target genes.

E-cadherin and adenomatous polyposis coli contain SXXXXSSL sites that are responsible for β -catenin binding. The observation that the MUC1-C CD also contains a SXXXXSSL motif led to the demonstration that β -catenin binds directly to this site (Figure 5, insert),⁶² and thus established the first link between MUC1 and the Wnt pathway. A subsequent study extended these findings by demonstrating that GSK3 β binds directly to a STDRSPYEKV site in the MUC1-C CD and phosphorylates the serine residue adjacent to proline (Figure 5, insert).⁶³ Phosphorylation of the MUC1-C CD by GSK3 β decreased the interaction with β -catenin and thereby increased the formation of E-cadherin/ β -catenin complexes.⁶³ Further regulation of the interaction between MUC1-C and β -catenin was established from other studies demonstrating that phosphorylation of the MUC1-C CD by EGFR and SRC at the YEKV site and by protein kinase C δ at the TDR site increases β -catenin binding (Figure 5, insert).^{32,33,64}

Figure 5. MUC1-C activates the Wnt/ β -catenin pathway and confers activation of cyclin D1 expression. The MUC1-C CD contains a SAGNGGSSLS motif that binds directly to the β -catenin Armadillo repeats (insert).^{62,69} GSK3 β , an effector of the Wnt pathway, binds to the upstream STDRSPYEKV site, phosphorylates Ser-44 and decreases binding of MUC1-C to β -catenin.^{63,69} Phosphorylation of (i) Tyr-46 by EGFR and SRC, and (ii) Thr-41 by protein kinase C δ , increases the interaction between MUC1-C and β -catenin (insert).^{33,32,64} Cytoplasmic MUC1-C homodimers bind to β -catenin and stabilize β -catenin by blocking GSK3 β -mediated phosphorylation and degradation of β -catenin. In the nucleus, MUC1-C forms a complex with β -catenin and TCF7L2 on the cyclin D1 promoter and coactivates cyclin D1 expression.⁷³

Subsequent studies in mouse mammary tumor virus (MMTV)-Wnt-1 transgenic mice showed that mouse Muc1 forms complexes with β -catenin at the cell membrane and in the cytoplasm of mammary tumor cells (Figure 5b).⁶⁵ In addition, MUC1-C/ β -catenin complexes were detected in the nucleus of MUC1-transformed rat fibroblasts⁶⁶ and MUC1-transfected human pancreatic cancer cell lines.⁶⁷ The functional significance of the nuclear MUC1-C/ β -catenin interaction is supported by the finding that the MUC1-C CD activates β -catenin/TCF-mediated transcription and that this effect is abrogated by mutating the EGFR/SRC phosphorylation site that promotes binding of MUC1-C to β -catenin.⁶⁸ Additional insights were derived from the demonstration that the MUC1-C CD increases β -catenin abundance in the nucleus by binding to the β -catenin Armadillo repeats and blocking GSK3 β -mediated phosphorylation and degradation of β -catenin.⁶⁹

Disruption of the MUC1-C/ β -catenin interaction by mutating the SXXXXSSL site in the MUC1-C CD attenuates MUC1-C-induced anchorage-independent growth and tumorigenicity, indicating that MUC1-C induces transformation, at least in part, by stabilizing β -catenin and promoting the activation of Wnt target genes.⁶⁹ In support of this model, expression of the MUC1-C CD in the mammary glands of transgenic mice results in marked hyperplasia, increases abundance of β -catenin in the nucleus and induces activation of the Wnt target genes encoding cyclin D1 and c-Myc.⁷⁰

The work reviewed above established the importance of MUC1-C-induced stabilization of β -catenin in aberrant activation of the canonical Wnt pathway. Nevertheless, a missing piece in the model was how MUC1-C mechanistically activates TCF-mediated transcription. TCF family members bind to Wnt-responsive elements and suppress transcription through interactions with the Groucho repressor.^{71,72} Stabilization of β -catenin promotes its binding to TCFs and in turn the displacement of Groucho. Thus, MUC1-C-induced stabilization of β -catenin could be sufficient to activate TCFs and induce transcription. However, recent studies have shown that the MUC1-C CD (i) directly associates with the TCF4/TCF7L2 transcription factor; (ii) blocks the interaction between TCF7L2 and the C-terminal-binding protein, a repressor of TCF7L2-mediated transcription; and (iii) promotes the recruitment of β -catenin and the coactivator p300 on the cyclin D1 promoter.⁷³ These findings have collectively provided support for a pathway in which MUC1-C activates the canonical Wnt pathway by stabilizing β -catenin and by activating TCF4/TCF7L2-mediated transcription of Wnt target genes (Figure 5).

Cyclin D1 is a target gene of the Wnt/ β -catenin pathway, which is of importance to mammary tumorigenesis.⁷⁴ Cyclin D1-deficient mice are resistant to induction of mammary cancers induced by *erbB2* and *ras*.⁷⁵ Mice expressing a mutant cyclin D1 ineffective in activating CDK4/6 are also resistant to *ErbB2*-induced breast cancers.⁷⁶ The *cyclin D1* gene is amplified in about 20% of human breast cancers and cyclin D1 is overexpressed in about 50%.^{77–80} Interestingly, overexpression of MUC1 and cyclin D1 is significantly linked in diverse human tumors.⁸¹ *In vitro* studies have also demonstrated that the MUC1-C CD contributes to the induction of cyclin D1 expression.^{68,82} Activation of cyclin D1 expression is significantly decreased in whey-acidic protein-TGF α (transforming growth factor)/Muc1(–/–) mice compared with those expressing Muc1.³⁶ In addition, expression of the MUC1-C CD in the mammary gland of transgenic mice is associated with pronounced hyperplasia, localization of β -catenin to the nucleus and induction of cyclin D1 expression.⁷⁰ Other studies have shown that MUC1-C occupies the *cyclin D1* promoter with the β -catenin/TCF7L2 complex and promotes *cyclin D1* transcription.⁷³ In concert with these results, silencing MUC1-C in breast cancer cells decreases activation of the *cyclin D1* promoter and downregulates cyclin D1 expression.⁷³ Taken together, these findings have provided compelling support for MUC1-C-induced cyclin D1 expression through activation of the β -catenin/TCF7L2 pathway in breast cancer cells.

MUC1-C activates STAT1/3 in an auto-inductive loop

STAT family members have been implicated in the link between inflammation and cancer.⁸³ STAT1 is activated in the inflammatory response to interferons and is constitutively upregulated in breast and other carcinomas.⁸⁴ The STAT3 transcription factor is an effector of the interleukin-6 (IL-6) inflammatory response.⁸³ Activated STAT3 induces transformation and has been detected in various carcinomas.⁸³

MUC1 expression in normal breast epithelial cells and breast cancer cells is induced by proinflammatory cytokines, such as interferon- γ , and activation of STAT1.⁸⁵ Analysis of genes differentially expressed in cells transformed by MUC1 expression further linked MUC1 and STAT1 to a network associated with growth and inflammation.¹⁹ MUC1-C associates with STAT1 in cells through direct binding of the MUC1-C CD to the STAT1 DNA-binding domain.¹⁹ The interaction between MUC1-C and STAT1 is induced by interferon stimulation of nonmalignant mammary epithelial cells and is constitutively present in breast cancer cells. The MUC1-C/STAT1 interaction promotes the activation of STAT1 target genes, including *MUC1* itself (Figure 6a).¹⁹ In this regard, the *MUC1* promoter contains a STAT-responsive element that contributes to MUC1 overexpression in carcinoma cells.⁸⁶ Of relevance to breast cancer, coexpression of MUC1 and the STAT1 pathway in primary breast tumors is associated with decreased recurrence-free and overall survival.¹⁹

Other studies have shown that MUC1-C associates with the JAK1/STAT3 complex in breast cancer cells.²¹ The MUC1-C CD interacts directly with JAK1 and the STAT3 DNA-binding domain, supporting a role for MUC1-C in promoting JAK1/STAT3 complexes and JAK1-mediated phosphorylation of STAT3.²¹ In addition, activation of STAT3 in nonmalignant breast epithelial cells by IL-6, IL-10 or IL-22 is dependent on MUC1-C.²¹ As found with STAT1, complexes of MUC1-C/STAT3 are detectable on the promoters of STAT3 target genes, such as *cyclin D1* and *STAT3*. Moreover, MUC1-C and STAT3 are detectable in a complex on the STAT-responsive element in the *MUC1* promoter in breast cancer cells.²¹ Thus, MUC1-C and STAT3 function in an auto-inductive loop that may have a role in linking the IL-6-induced inflammatory response to cancer cell survival (Figure 6a).

Further evidence for involvement of MUC1 in inflammatory STAT signaling is provided in an interesting model of Epstein–Barr virus-induced transformation and cellular invasion.⁸⁷ The major Epstein–Barr virus oncoprotein, latent protein 1 (LMP1), induces MUC1 expression through binding of STAT1 and STAT3 to the *MUC1* promoter.⁸⁷ In addition, LMP1-induced cell invasiveness is suppressed by silencing MUC1, indicating that the increases in MUC1 expression contribute to the metastasis of Epstein–Barr virus-infected tumor cells.⁸⁷

MUC1-C participates in I κ B kinase (IKK)/NF- κ B RelA signaling

The NF- κ B transcription factor RelA/p65 localizes to the cytoplasm with members of the I κ B family of inhibitor proteins.⁸⁸ Phosphorylation of I κ B α by the I κ B kinase (IKK α , IKK β , IKK γ) complex induces I κ B α degradation and thereby the release of RelA for nuclear translocation. For example, activation of the canonical NF- κ B pathway in response to tumor necrosis factor- α (TNF α) induces IKK β -mediated phosphorylation of I κ B α with increased localization of RelA to the nucleus. As a result, activation of RelA target genes promotes tumor development through induction of inflammatory responses, growth and survival.⁸⁸

Stimulation of normal human mammary epithelial cells with TNF α has been shown to be associated with binding of RelA to the *MUC1* promoter and induction of MUC1 expression.⁸⁵ In addition, mutation of the seven tyrosines in the MUC1-C CD decreases nuclear localization of NF- κ B,⁸⁹ supporting the potential for a MUC1-C/RelA auto-inductive loop.

Figure 6. MUC1-C forms complexes with the STAT1/3 and NF-κB RelA transcription factors that in turn activate the *MUC1* gene in auto-inductive loops. (a) Schema for a MUC1-C/STAT1/3 auto-inductive loop involving activation of *MUC1* and other STAT1/3-dependent genes. Figure modified from Khodarev *et al.*, 2010.¹⁹ (b) Model for the proposed effects of MUC1-C on activation of the NF-κB pathway through interactions with IKKβ-IKKγ and RelA in an auto-inductive loop. Figure modified from Ahmad *et al.*, 2009.²⁰

Studies in breast cancer cells further demonstrated that MUC1-C constitutively associates with the IKK complex through a direct interaction between the MUC1-C CD and IKKβ-IKKγ.⁹⁰ In nonmalignant mammary epithelial cells stimulated with TNFα, MUC1-C is recruited to the TNF-receptor 1 complex by a TRADD/TRAF2-dependent mechanism and interacts with IKKβ-IKKγ (Figure 6b).⁹⁰ The interaction of MUC1-C with IKKβ-IKKγ promotes IKKβ activation, which results in the phosphorylation and degradation of IκBα. These findings have provided support for a model in which MUC1-C is of importance for the activation of IKKβ and have indicated that overexpression of MUC1-C in breast and other carcinomas promotes sustained induction of the IKKβ-RelA pathway (Figure 6b).

Subsequent studies extended the above findings by demonstrating that MUC1-C also associates with RelA.²⁰ The MUC1-C/

RelA interaction was found to be constitutive in breast cancer cells and inducible in TNFα-stimulated nonmalignant breast epithelial cells. The MUC1-C CD interacts directly with RelA at the Rel homology domain and, notably, blocks binding of RelA to its inhibitor IκBα.²⁰ The MUC1-C/RelA complex localizes to the nucleus and occupies the promoters of RelA target genes, such as *Bcl-xL*. The MUC1-C/RelA complex was also found to occupy the *MUC1* promoter and contribute to RelA-mediated induction of the *MUC1* gene.²⁰ These results thus provided evidence for an auto-inductive loop in which binding of MUC1-C to RelA activates RelA and thereby induction of MUC1-C expression (Figure 6b).

Interaction between MUC1-C and estrogen receptor α (ERα)

The *MUC1* promoter contains estrogen-responsive elements.⁹¹ Studies in ER+ breast cancer cells have shown that ERα occupies estrogen-responsive elements in the *MUC1* promoter and activates *MUC1* transcription.⁹¹ Other work has shown that MUC1-C associates with ERα in breast cancer cells and that this interaction is stimulated by 17β-estradiol (E2).⁹² The MUC1-C CD binds directly to the ERα DNA-binding domain. The functional significance of the MUC1-C/ERα interaction is supported by the demonstration that MUC1-C stabilizes ERα by blocking its ubiquitination and degradation. MUC1-C/ERα complexes are detectable on the promoters of estrogen-responsive genes, such as *p52* and *cathepsin D*, and increase recruitment of p160 coactivators.⁹² MUC1-C also stimulates ERα-mediated transcription, antagonizes the inhibitory effects of tamoxifen and contributes to E2-induced growth and survival of breast cancer cells. These findings lend support for the contention that MUC1-C is of importance to the activation of ERα function in breast cancers and provide the basis for further studies on the role of MUC1-C in tamoxifen resistance.

TARGETING MUC1 AS A THERAPEUTIC APPROACH

Monoclonal antibodies (MAbs)

The MUC1-N tandem repeats are highly immunogenic in mice. As such, a number of MAbs have been generated against this subunit and several of these have been developed for clinical evaluation. As a recent example, AS1402 is a humanized immunoglobulin (IgG1) MAb that binds to the MUC1-N tandem repeats and induces antibody-dependent cellular cytotoxicity against MUC1-positive breast cancer cells.⁹³ In a Phase I trial, AS1402 levels were achieved that conferred maximal antibody-dependent cellular cytotoxicity activity *in vitro*.⁹³ On the basis of these findings, a Phase II trial of AS1402 in combination with letrozole was performed in patients with advanced or metastatic ER+ breast cancer.⁹⁴ The results showed that adding AS1402 to letrozole has no effect on efficacy compared with that obtained with letrozole alone.⁹⁴

As noted above, MUC1-N is shed from the surface of breast cancer cells and is found at increased levels in the plasma of women with metastatic breast cancer. Extracellular pools of MUC1-N thus represent a barrier for the delivery of a MAb to the surface of breast tumor cells for induction of antibody-dependent cellular cytotoxicity. The same argument applies to anti-MUC1-N MAbs developed to deliver a toxin or a radionuclide; the circulating pool of MUC1-N would have to be overcome to target the surface of MUC1-expressing breast tumor cells. In contrast, MUC1-C is not subject to release from the cell surface and is undetectable in plasma. Thus, the 58-aa MUC1-C extracellular domain represents a potential target for the development of MAbs that could more effectively reach the surface of breast tumor cells.

Anti-MUC1 vaccines

Unlike antibodies, the circulating MUC1-N pool should not pose a barrier for vaccines that induce T-cell responses against MUC1

peptides that are presented by MHC class I/II molecules on the breast cancer cell surface. Few anti-MUC1 vaccines, however, have advanced to later-stage clinical trials for the treatment of breast cancer. L-BLP25 (Stimuvax; EMD Serono) is a liposome-based vaccine that incorporates a MUC1-N tandem repeat peptide. Stimuvax had been under development for both breast and lung cancer, and is now in a phase III trial for unresectable stage III NSCLC. PANVAC-V (NSC #727026) is a recombinant vaccinia virus-based vaccine that contains transgenes for MUC1, CEA and three T-cell costimulatory molecules (B7.1, LFA-3 and ICAM-1).⁹⁵ PANVAC-F (NSC #727027) is a similar vaccine constructed by inserting the same transgenes into a replication-defective fowlpox virus.⁹⁵ In a recently reported study, 12 patients with breast cancer were vaccinated with a subcutaneous priming dose of PANVAC-V followed by boosting doses of PANVAC-F.⁹⁶ Four patients had stable disease and one patient achieved a complete response by RECIST, which has continued for 37 months. On the basis of findings that combining PANVAC with docetaxel has no effect on antigen-specific T-cell responses, a trial of docetaxel without and with PANVAC is being conducted for patients with metastatic breast cancer.^{97,98} Of the 21 patients enrolled to date, the time to progression in the docetaxel-alone arm is 2.9 months (similar to previous studies) as compared with 8.0 months in the docetaxel + PANVAC arm.⁹⁹ This promising trial is currently underway at the National Cancer Institute and M.D. Anderson Cancer Center.

Agents that directly target the MUC1-C CD

As noted above, the MUC1-C CD contains a CQC motif that is necessary for its dimerization and oncogenic function.⁵⁷ Accordingly, cell-penetrating peptides and small molecules have been developed that directly target the CQC motif and block MUC1-C dimerization in breast cancer cells.^{100,101} The first-in-class cell-penetrating MUC1-C peptide inhibitor, GO-201, binds directly to the MUC1-C CD at the CQC motif and attenuates localization of MUC1-C to the nucleus.¹⁰⁰ GO-201 treatment was also associated with arrest of growth and induction of late apoptotic/necrotic cell death. Inhibition of MUC1-C dimerization affects interactions between MUC1-C and multiple effectors at the cell membrane and in the cytoplasm and nucleus. For example, GO-201 suppresses activation of the Wnt/ β -catenin pathway in breast cancer cells by blocking the interaction between MUC1-C and TCF7L2.⁷³ Inhibition of MUC1-C also blocks its interaction with STAT3 and suppresses activation of the STAT3 pathway.²¹ Other studies have shown that GO-201 disrupts the interaction between MUC1-C and NF- κ B RelA and decreases the expression of NF- κ B target genes in breast cancer cells.²⁰ Administration of GO-201 to immunocompromised mice bearing MCF-7 (ER+), ZR-75-1 (ER+) and MDA-MB-231 (triple negative) breast tumor xenografts resulted in prolonged regressions,¹⁰⁰ indicating that targeting MUC1-C could be a potential therapeutic approach in the clinic. On the basis of these findings and activity in other carcinoma models,^{102,38} a second-generation MUC1-C inhibitor, designated GO-203, has entered Phase I evaluation for patients with refractory solid tumors.

CONCLUSIONS

In summary, the overexpression of MUC1-C in breast cancers has been linked to the activation of several pathways that are known to be of importance to the development of breast cancer. The oncogenic function of MUC1-C is dependent, at least in large part, on its dimerization, which is mediated by the CQC motif in the CD. The development of agents that block MUC1-C dimerization has further demonstrated that MUC1-C is a druggable target. These findings have provided the experimental basis for targeting MUC1-C in patients with breast cancers who express this

oncoprotein. Notably, in addition to breast cancer, MUC1-C is aberrantly overexpressed in diverse carcinomas, such as those derived from lung,³⁸ prostate¹⁰³ and other epithelial tissues.¹ On the basis of the importance of MUC1-C to breast cancer as reviewed here, this oncoprotein may also contribute to the development of these other carcinomas and represent a target for their treatment.

CONFLICT OF INTEREST

Dr Kufe has ownership interest in Genus Oncology and is a consultant to the company.

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