MUC1 and metastatic cancer
Expression, function and therapeutic targeting

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Abbreviations: MUC1, mucin 1 (also called CA 15-3, KL-6 and BM7); ECD, extracellular domain; CD, cytoplasmic domain; EGFR, epidermal growth factor receptor; CTC, circulating tumor cell; HIF-1α, hypoxia inducible factor 1α; IFN-γ, interferon γ; IL-6, interleukin 6; AR, androgen receptor; VEGF, vascular endothelial growth factor; PLCγ, phospholipase Cγ; PI3K, phosphoinositide 3 kinase; MMP, matrix metalloproteinase; ECM, extracellular matrix; EMT, epithelial to mesenchymal transition; PDGF-A, platelet-derived growth factor A; PDGFRβ, platelet-derived growth factor receptor β; ERβ, estrogen receptor β; CTGF, connective tissue growth factor; PMIP, PTD4 (protein transduction domain 4) MUC1 inhibitory peptide

MUC1 is a transmembrane mucin that is often overexpressed in metastatic cancers and often used as a diagnostic marker for metastatic progression. The extracellular domain of MUC1 can serve as a ligand for stromal and endothelial cell adhesion receptors, and the cytoplasmic domain engages in several interactions that can result in increased migration and invasion, as well as survival. In this review, we address the role of MUC1 in metastatic progression by assessing clinical studies reporting MUC1 levels at various disease stages, reviewing mouse models utilized to study the role of MUC1 in metastatic progression, discuss mechanisms of MUC1 upregulation, and detail MUC1 protein interactions and signaling events. We review interactions between MUC1 and the extracellular environment, with proteins colocalized in the plasma membrane and/or cytoplasmic proteins, and summarize the role of MUC1 in the nucleus as a transcriptional cofactor. Finally, we review recent publications describing current therapies targeting MUC1 in patients with advanced disease and the stage of these therapies in preclinical development or clinical trials.

Introduction

MUC1, a transmembrane member of the mucin family, has long been associated with metastatic progression, both clinically and experimentally. Progression from a contained tumor to one that can metastasize to a distant organ requires a multitude of steps, including the gaining of invasive capacity, intra- and extravasation, and the ability to colonize and grow at a secondary site (reviewed in Steeg).1 MUC1 is involved in metastatic progression through both its extracellular, O-glycosylated serine/threonine repeat region (the “mucin” domain, MUC1-ECD), as well as through activities of its intracellular domain (MUC1-CD). This role in metastatic progression is highlighted by the frequent observation of MUC1 overexpression in metastatic tissues and circulating tumor cells from patients with advanced adenocarcinoma, and the ability to use anti-MUC1 antibodies as diagnostics for metastatic disease. Mechanistically, MUC1 (both ECD and CD) engages in intercellular and intracellular interactions with other transmembrane proteins, such as ICAM-1 and the epidermal growth factor receptor (EGFR), which have prometastatic capacity themselves. In addition, MUC1 can engage cytoplasmic signaling proteins, such as Src and β-catenin, thereby driving changes in the cytoskeleton and adhesive capacity of the transformed cell. Finally, MUC1 can directly drive transcription of pro-invasive genes, through the proteolytic cleavage and nuclear translocation of MUC1-CD. In this review, we will summarize recent data regarding the expression profile of MUC1 in metastatic cancers and circulating tumor cells, review the direct role of MUC1 in pro-metastatic signal transduction and gene transcription, and discuss the current efforts to target metastatic disease by developing MUC1 targeted therapies. The reader is referred to other excellent reviews regarding the structure, oncogenic properties and clinical utility of MUC1 as a biomarker, including reviews by Baldus et al.,2 Gendler,3 Bafna et al.,4 Kufe5 and Singh et al.6

MUC1 Expression Correlates with Metastasis

In many tumor types, MUC1 expression correlates with aggressive, metastatic disease, poor response to therapy and poor survival. While MUC1 expression is limited to the apical surface of most ductal epithelium, in metastatic disease, MUC1 is overexpressed and becomes localized throughout the cell.7 This has perhaps been most intensively studied in breast cancer, in which MUC1 expression has been evaluated clinically at the level of immunohistochemistry,8,9 RNA,10 shed MUC1 in sera, expression on circulating tumor cells (discussed below) and biochemically,11 and has correlated with poor disease-free and overall survival, as well as axillary node metastases.9 MUC1 expression is seen in all subtypes of breast cancer, including luminal, HER2-
and basal, although in each of these cancer types, expression is highest in those tumors that have metastasized.\textsuperscript{9,12} In other hormonally responsive cancers, including ovarian and prostate, a similar overexpression of MUC1 is observed in advanced disease. In ovarian cancer, patients with metastatic, treatment-resistant disease display elevated levels of MUC1, with greater than 90% of these patients producing antibodies to MUC1.\textsuperscript{13} Additionally, MUC1 expression is high in both primary epithelial ovarian cancers and in metastatic ovarian cancer (> 90%),\textsuperscript{14} with MUC1 cytoplasmic expression correlating with poor overall survival and invasive capacity.\textsuperscript{15} Likewise, in prostate cancer, less than 60% of primary lesions were found to express MUC1 in one study, whereas 90% of lymph node metastases expressed MUC1,\textsuperscript{16} indicating that MUC1 is enriched in metastatic tumors.

In the gastrointestinal system, MUC1 is also strongly correlated with metastatic progression. In gastric cancer, MUC1 is not only expressed in metastatic disease, but also found to be highly expressed in virtually all isolated cancer cells invading throughout the stroma of the primary tumor, indicating it may be promoting initial spread.\textsuperscript{17,18} High MUC1 expression is also associated with invasive intraductal papillary neoplasms of the bile duct,\textsuperscript{19} metastatic liver cancer\textsuperscript{20} and pancreas,\textsuperscript{21,22} as well as lymph node metastasis and vascular invasion in oral squamous cell carcinoma.\textsuperscript{23} As such, MUC1 was found as a useful biomarker to identify occult lymph node metastases in oral squamous cell carcinoma.\textsuperscript{24} Similarly, MUC1 is associated with higher grade tumors and shorter metastasis-free survival in renal cell carcinoma, malignant thyroid cancer, leukemias and lymphomas.\textsuperscript{25-27} Overall, these studies reveal a strong link between MUC1 expression and metastatic progression, inversely correlating MUC1 and disease-free survival due to metastatic spread.

**MUC1 expression on circulating tumor cells and MUC1 serum markers.** Circulating tumor cells (CTCs) are typically identified in approximately 50–80% of patients with defined metastatic breast, colon, or prostate cancer.\textsuperscript{28,29} Whether the remaining patients have CTCs that are either too rare to be captured, lack the surface markers for capture, or may not be present in the bloodstream (e.g., the metastatic cells have remained solely in the tissue or lymphatics) is currently unknown. For those patients with detectable CTCs, the surface antigens currently used clinically to select these cells are epithelial cell adhesion molecule (EpCAM), cytokeratin-19\textsuperscript{30,31} and MUC1.\textsuperscript{32,33} The use of MUC1 as a capture antigen is based on the examples of MUC1 overexpression on CTCs. MUC1 is typically found to be expressed in greater than 60% of captured CTCs from metastatic breast, lung, pancreatic and colon cancer patients, among others.\textsuperscript{34-35} The detection of MUC1 (which is also defined as serum antigens CA 15-3, KL-6 and BM\textsuperscript{76-37}) in patient sera is currently used clinically as a marker of response to therapy and as a prognostic indicator for survival.\textsuperscript{38} In fact, the serum antigen CA 15-3 is currently one of the most widely used serum antigens in breast cancer, with high CA 15-3 levels correlating with higher grade tumors, lymph node involvement, and presence of distant metastases.\textsuperscript{39} Cellular localization of MUC1 is also important, as studies in metastatic breast, colorectal, gall bladder, non-small cell lung and gastric cancer, using KL-6, discovered that MUC1 expression at the circumference of the plasma membrane and/or in the cytoplasm is frequently correlated with deep invasion, lymph node or liver metastasis and decreased five-year survival.\textsuperscript{40-43} These studies demonstrate that MUC1 detection in patient circulation and localization of expression in the tumor are important prognostic factors for metastasis and disease progression.

Mouse models demonstrate MUC1 promotes metastatic progression. MUC1 expression is strongly correlated with metastatic progression in patient samples, both in tissue and circulated tumor cells. In order to further explore this relationship, mouse models of cancer have been employed which demonstrate a role for MUC1 in metastasis, a number of which provide direct evidence for MUC1 in driving metastatic progression. The first model demonstrating a potential role for MUC1 in driving metastatic progression was the MMTV-PyMT mouse model of breast cancer, which was crossed onto a Muc1 mouse (note: in mice, MUC1 is annotated as Muc1) knockout background in a study by Spicer et al.\textsuperscript{44} The MMTV-PyMT transgenic mouse develops multiple tumors in the mammary gland by approximately 10–12 weeks of age, with greater than 90% of these animals displaying lung metastases.\textsuperscript{45} When crossed onto a Muc1 knockout, incidence of lung metastases was found to be lower, although the reduction did not reach statistical significance.\textsuperscript{46} In a separate study, MMTV-Muc1 transgenic mice were created, resulting in late-onset mammary gland tumors that were metastatic to the lung, but only in animals overexpressing full-length Muc1 (Muc1 lacking the cytoplasmic domain was not tumorigenic).\textsuperscript{46} In addition, our laboratory evaluated the role of Muc1 in epidermal growth factor receptor (EGFR)-driven breast cancer by crossing the WAP-TGF\alpha transgenic model onto a Muc1 knockout background. The WAP-TGF\alpha transgenic mouse develops mammary gland carcinoma in 100% of multiparous females, with fewer than 25% of mice presenting lung metastases.\textsuperscript{47} Upon crossing the WAP-TGF\alpha onto a Muc1\textsuperscript{-/-} background, mammary gland carcinoma is reduced to less than 50%, and presentation of lung metastases drops to 0%.\textsuperscript{48} Together, these studies implicate MUC1 as a promoter of metastatic progression in mouse models of breast cancer.

In addition to breast cancer, a role for MUC1 in driving pancreatic and lung cancer metastasis has also been examined. Evaluation of Muc1 during pancreatic cancer progression was performed in the KC or Cre-LSL-KRAS\textsuperscript{G12D} mouse model, a spontaneous mouse model of pancreatic ductal adenocarcinoma that relies on an activating KRAS mutation for tumor progression.\textsuperscript{49} Crossing this mouse onto a Muc1 null background (KC-Muc1-null) resulted in reduced tumor burden and an increase in overall survival as well as a 50% reduction in distant metastasis compared with a KC mouse crossed with a Muc1 transgenic (KC-Muc1), which had Muc1 levels above wild-type. 61% of KC-Muc1 mice exhibited lung metastases, 33% had liver metastases and 23% had peritoneal metastases, whereas only 30% of KC mice with wild-type Muc1 levels had lung metastases, 20% had liver metastases and 10% had peritoneal metastases. The incidence of lung, liver and peritoneal metastases was
further reduced in the KC-Muc1-null mice, only 10% of which developed metastasis in any of the three organs examined, defining a role for MUC1 in pancreatic tumor growth and metastasis in this model.49

The KC, or Cre-LSL-KRASG12D, mouse crossed with a Muc1 transgenic has also been used to look at the effects of MUC1 in lung cancer progression. In this model, Cre-LSL-KRASG12D mice developed lung tumors nine weeks after the administration of a Cre-adenovirus. The addition of transgenic Muc1 (KC-Muc1) induced the formation of twice as many premalignant and malignant masses, and Muc1 positive mouse tumors exhibited many more infiltrating cells.50

Orthotopic models of cancer have also been evaluated for a role of MUC1 in driving metastatic progression. In a model for breast cancer metastasis to the brain, MUC1-expressing bone marrow cells (MA11 cells) were injected into the left ventricle heart chamber of athymic nude mice. The MA11 cell line was derived from a breast cancer which had metastasized to the bone. To isolate the cells, bone marrow was enriched for mucin-expressing cells which were then purified and maintained in culture for a period of time before injection. 87% of mice injected displayed brain metastases, with accompanying neurological symptoms. MUC1 was detected in the serum of 82% of mice showing histological evidence of brain metastasis, demonstrating that MUC1 is prevalent in this cell line and correlating expression with metastatic progression.51 Additionally, an orthotopic lung cancer model was used to assess the significance of Muc1 expression. In an orthotopic model of H358 lung cancer cells, cells which stably express MUC1 siRNA or control siRNA were evaluated for their ability to induce metastatic disease. While 71% of MUC1-expressing tumors progressed to lung metastases, only 14% of mice with non-MUC1-expressing tumors displayed any metastases.52 Similar results were found in an orthotopic pancreatic cancer model wherein S2-013 pancreatic cancer cells were stably transfected with MUC1 siRNA or control siRNA and implanted subcutaneously into mice. Tumors from these mice were excised and a normalized amount of the tumor was then implanted into ceca of recipient mice and metastasis was evaluated. 75% of S2-013-control-siRNA-injected mice displayed local invasion into lymph nodes vs. 29% of S2-013-MUC1-siRNA-injected mice. Furthermore, 64% of S2-013-control-siRNA-injected mice had lung metastases whereas only 32% of S2-013-MUC1-siRNA-injected mice displayed lung metastases.53 Together, these studies demonstrate a role for MUC1 in driving metastatic progression in transgenic, knockout, knock-in and xenograft mouse models of cancer.

**Mechanism of Overexpression**

As we have described, MUC1 expression strongly increases during the clinical progression from normal tissue to metastatic disease. We will next summarize the observations regarding the mechanisms by which MUC1 expression is controlled and any correlations these have to metastatic progression. MUC1 expression is controlled by multiple factors, including alterations in transcription, amplification and post-translational modifications, and many of these regulatory pathways are strongly activated in the invasive, migratory or metastatic state (Fig. 1).

**Transcriptional regulation.** MUC1 is regulated at the transcriptional level by multiple factors, including hypoxia, STATs, hormones and growth factors. In a recent study of clear renal cell carcinoma, MUC1 was found to be a transcriptional target of hypoxia-inducible factor 1 α (HIF1-α), which is upregulated in metastatic cancer, and promotes migration and invasion.54 Hypoxia-enhanced MUC1 transcription was also observed in a human lung adenocarcinoma cell line,55 demonstrating that control of MUC1 levels by hypoxia is not tissue-specific. Furthermore, MUC1 is transcriptionally upregulated by STATs in response to interferon gamma (IFNγ) and interleukin-6 (IL-6) signaling.56 In addition, EGFR can also activate STAT1 and STAT3 in breast cancer tissues57,58 and EGFR activation promotes MUC1 expression.59 Intriguingly, MUC1 and EGFR can interact in cancer cells, resulting in a MUC1-dependent prolongation of EGFR activity.60,61 This may represent an EGFR-STAT-MUC1 positive feedback loop that is a source of MUC1 upregulation in breast cancer, although further studies need to be done in this area.

In prostate cancer cell lines, androgen receptor (AR) was found to regulate MUC1 expression through interaction with a consensus AR-element in the MUC1 promoter, although this result was cell line-specific.62 In this study, AR was found to downregulate MUC1 expression in androgen-dependent, but not androgen-independent cell lines. Interestingly, in another study examining the phenotypic behavior of AR and MUC1, androgen treatment was found to increase expression of MUC1, and increased MUC1 expression correlated with loss of cell-cell adhesion.63 These varied results indicate that there is more work necessary to fully sort out the role of androgen receptor in MUC1 transcriptional regulation.

**Amplification and miRNA regulation of MUC1 expression.** While transcriptional regulation of MUC1 is well-established as a mechanism for MUC1 regulation during cancer progression, amplification was recently also identified as a contributing factor for MUC1 overexpression. Examination of 83 patients for MUC1 gene amplification and protein expression found that 12% of benign lesions and 38% of primary invasive breast carcinoma samples displayed MUC1 genomic amplification.64 Furthermore, meta-analysis of 886 primary invasive breast carcinoma samples from 22 studies demonstrated that 44% had genomic gain of the MUC1 gene. MUC1 gene amplification also correlated significantly with protein expression, indicating that MUC1 gene amplification may be an untapped and important source for MUC1 protein in breast cancer samples, and that amplification of MUC1 may select cells for metastatic progression.64

MUC1 expression and corresponding metastatic phenotypes are also regulated at the post-transcriptional level by microRNAs. Two miRs have been identified to regulate MUC1 translation, including miR-125b, which is upregulated by androgen receptor, and miR-145. The first study to identify miR regulation of MUC1 found that MUC1 translation is repressed by miR-125b, and mir-125b is downregulated in breast cancer cells.65,66 AR also promotes miR-125b expression, resulting in
a suppression of MUC1 translation, and together with AR directly downregulating MUC1 transcription, this may indicate an androgen-dependent MUC1 downregulation program in certain circumstances. Finally, miR-145 was found to suppress MUC1 translation, and reduce MUC1-dependent cell invasion, and miR-145 is frequently found to be lost during colorectal cancer progression.

**Molecular Mechanisms of Metastasis**

The many interaction domains of MUC1 allow for distinct mechanisms by which MUC1 promotes metastasis. These include interactions with proteins in the extracellular matrix, at the cell membrane, in the cytoplasm, and in the nucleus where MUC1 acts as a cofactor for gene transcription (Fig. 1).

**MUC1 extracellular domain drives migration and invasion.** MUC1 is co-translationally processed into two polypeptides that then associate to form the mature transmembrane MUC1 heterodimer. This heterodimer is composed of the extracellular MUC1 (MUC1-ECD), which is non-covalently associated with the transmembrane MUC1-CD via the extracellular stem region. The MUC1-ECD contains many O-glycosyl groups covalently attached to serine/threonine repeats throughout this domain. Tumor-specific MUC1 is underglycosylated, enabling interactions between the MUC1 core protein and many transmembrane receptors and components of the extracellular matrix, such as ICAM-1, an adhesion receptor on the surface of endothelial and peritumoral stromal cells. E-selectin, a receptor present on the endothelial cell surface, also interacts with epithelial cells either via interactions with under-glycosylated MUC1 itself or through the binding of other E-selectin ligands present adjacent to MUC1 on the cell surface. In addition, interactions between MUC1 and E-selectin may promote MUC1 binding to ICAM-1 on the endothelial cell surface. The MUC1-ICAM-1 interaction promotes the migratory capacity of tumor cells through the microenvironment, by facilitating interaction between epithelial and endothelial cells, enabling adhesion of circulating cancer cells to the inner lining of the blood vessel, slowing cell velocity and allowing escape from the blood vessel (reviewed in ref. 72).

In addition to promoting the ability of transformed cells to interact with vascular endothelium, MUC1-ICAM-1 interactions alter the metastatic phenotype of the cancer cell itself. Upon interacting with ICAM-1, Src interacts with the MUC1-CD, an interaction that promotes Src-mediated cytoskeletal rearrangements. The Src family of nonreceptor tyrosine kinases, through their ability to regulate integrin activation and cytoskeletal function, has long been regarded as key mediators of metastatic progression (reviewed in Aleshin). Interactions between MUC1 and Src induce pro-migratory Rac1- and Cdc42-dependent actin reorganization at sites of contact with endothelial cells, thereby promoting an invasive phenotype in the tumor cell. Lamellipodia and filopodia formation as a result of these interactions are induced via Src-CrkL complexes with MUC1-CD, with Src-dependent kinase activity driving cytoskeletal rearrangements. Overall, these studies demonstrate that MUC1 can drive intercellular interactions that promote metastatic spread, as well as intracellular interactions that promote migratory behavior.

In addition to studies focused on interactions of the MUC1 extracellular domain with ICAM-1 and E-selectin, a truncated version of MUC1 spanning only the external stem region, the transmembrane domain, and the juxtamembrane domain was demonstrated to be sufficient to induce cellular EMT, although a precise mechanism was not described. In this study, mouse mammary carcinoma cells expressing this truncated MUC1 showed mesenchymal morphology, decreased E-cadherin expression, increased vimentin expression, and increased invasion through a Matrigel matrix.

**MUC1 expression promotes angiogenesis.** One key aspect of metastatic progression is angiogenesis, which provides an escape route for migratory tumor cells. In addition to promoting the migratory capacity and invasive phenotype of tumor cells, MUC1 can also drive tumor angiogenesis. MUC1 overexpression in non-small cell lung cancer and breast cancer was found to upregulate vascular-endothelial growth factor (VEGF), thereby promoting endothelial migration and tube formation. Though MUC1 is a transcriptional target of HIF1-α as discussed above, MUC1 also interacts with HIF1-α in the cytoplasm and promotes transcriptional upregulation of HIF1-α targets such as leptin, TGFβ3 and VEGF. Furthermore, MUC1 and VEGF expression correlate in human breast cancer cell lines, and MMTV-PyMT mice expressing human MUC1 display more angiogenesis than MMTV-PyMT mice on a Muc1 null background, further supporting a role for MUC1 in the onset of angiogenesis.

**Promotion of pro-metastatic activities of transmembrane receptors.** In polarized epithelium, the MUC1 heterodimer is apically localized. Alternatively, in primary and metastatic tumors, MUC1 is found throughout the plasma membrane, in the cytoplasm and in the nucleus. Membrane-bound MUC1 is constitutively recycled via endocytosis and trafficking through the Golgi, resulting in re-glycosylation of the MUC1-ECD. During cancer progression, when apical and basolateral proteins become co-localized due to a breakdown of tight junctions, MUC1 interacts with a number of previously sequestered transmembrane receptors, including the EGFR. Interactions with EGFR result in the “hijacking” of normal EGFR trafficking, and EGFR becomes preferentially recycled instead of trafficked to the lysosome for degradation, or to other compartments of the cell such as the nucleus or mitochondria. The result is prolonged EGFR signaling, which can drive pro-metastatic interaction and/or regulation of integrins, cadherins, phospholipase Cγ (PLCγ), phospho-inositol 3 kinase (PI3K) and matrix metalloproteinases (MMPs) which contribute to disruption of cell adhesion, induction of cell motility, and degradation of the extracellular matrix (ECM) (reviewed in Haley). In addition, overexpression of MUC1 in primary canine malignant mammary tumors (CMMT) was accompanied by downregulation of galectin-3, which results in upregulation of endogenous MUC1, enabling an auto-feedback loop. Additionally, CMMT cells that have invaded into the vasculature express MUC1 and EGFR at focal adhesions.
Figure 1. MUC1 drives metastatic progression. The protein core of underglycosylated MUC1 interacts with ICAM-1, E-selectin, and Galectin-3 using the extracellular domain. The cytoplasmic domain of MUC1 is phosphorylated by EGFR and Src, among other proteins, and upon Src phosphorylation can induce Rac activity and cytoskeletal change leading to an increase in cell motility. Phosphorylation by EGFR promotes cell motility, and interaction with HIF1-α drives PDGF-A transcription, positively affecting β-catenin transcriptional activity. The cytoplasmic domain of MUC1 interacts with cofactors, such as β-catenin, p120-catenin, and Estrogen Receptor β among other transcription factors, promoting nuclear translocation of these proteins and driving expression of Epithelial to Mesenchymal Transition (EMT) genes. MUC1 expression is upregulated by STAT1/STAT3 binding to the MUC1 promoter, and MUC1 mRNA is downregulated by binding of miR-125b/miR-145.
Recently, our laboratory published evidence that MUC1 and EGFR regulate the expression of c-Met, the hepatocyte growth factor/scatter factor receptor. c-Met is a receptor tyrosine kinase that is often upregulated in metastatic cancers and is involved in metastatic progression (reviewed in Peschard). We found that treatment with a competitive MUC1 inhibitor downregulates c-Met in breast cancer cells, and that MUC1 promotes EGFR- and c-Met-dependent cell motility, scattering and the formation of invasive protrusions. In addition, MUC1 has recently been found to be upregulated in a subset of lung cancers which also have upregulated or constitutively active EGFR and c-Met. In this study, lung cancer cell lines were analyzed for expression of genes associated with tumorigenesis, and MUC1, EGFR and c-Met expression were positively correlated. Cell lines with high expression of all three proteins also displayed high expression of Rho-family GTPases, and SNAIL transcription factor, in addition to other genes involved in EMT and cell motility, suggesting that this subset of lung cancer is very motile and that MUC1, EGFR and c-Met expression may contribute to this motility.

Studies also show that MUC1 interacts with ErbB2, another member of the EGFR family of receptor tyrosine kinases, in breast cancer cells. ErbB2 does not have a ligand-binding domain, but can heterodimerize and phosphorylate EGFR, and ErbB3 and ErbB4, the remaining members of the family. ErbB3 and ErbB4 bind several ligands to become activated, among them Heregulin (reviewed in Citri). The interaction between MUC1 and ErbB2 was found to be driven by Heregulin-binding of ErbB3 or ErbB4 and heterodimerization of either of these proteins with ErbB2. Importantly, this interaction was then observed to promote the nuclear localization of a MUC1-γ-catenin complex. γ-catenin, like β-catenin, is a transcription factor in the Wnt pathway, and can affect genes involved in motility and metastasis. γ-catenin suppresses cell motility and metastasis by downregulating fibronectin, by organizing the actin cytoskeleton through modulation of Rho-family GTPases, and by upregulating Nm23-H1, a known metastasis suppressor. The authors of this study speculate that MUC1 could be sequestering γ-catenin in order to promote cell motility.

Pro-metastatic cytoplasmic interactions. MUC1 has no kinase domain itself, but protein-protein interactions in the cytoplasm allow it to activate signal transduction cascades, many of which have direct roles in driving metastatic progression (reviewed in Singh et al.). Many of these interacting partners have been studied for their roles in affecting transformation directly, including inhibitory interactions with the pro-apoptotic protein Bax and c-abl kinase. MUC1-CD can also directly activate the JAK-1/STAT3 signaling pathway, promoting tumor growth and metastasis in an orthotopic model of lung cancer. Finally, interactions with the tyrosine kinase Src or PCK6 can modulate the interactions between MUC1-CD, GSK3-β and β-catenin. Many of these interactions play a role not only in primary transformation, but in metastatic progression as well.

β-catenin, serving as both an activator of oncogenic transcription and as a suppressor of invasion, has both a protumorigenic and anti-metastatic role to play. As such, its interactions with MUC1 have been shown to promote transformation, primarily through MUC1-β-catenin protein complexes driving transcription of such genes as cyclin D1. In addition, a study by Yuan et al. demonstrated that the downregulation of MUC1 promoted E-cadherin/β-catenin complex formation, reduced nuclear β-catenin, upregulated both E-cadherin and β-catenin expression, and decreased invasive potential in PANC1 pancreatic cancer cells and MCF-7 breast cancer cells. MUC1 can also interact with the SH3 domain of Src via the RXPPXR domain in the MUC1-CD, and the SH2 domain of Grb2 through a phosphorylated tyrosine at the YTPR motif of the MUC1 cytoplasmic domain. These interactions result in an increase in MUC1-dependent metastatic cell behavior including cytoskeletal rearrangement leading to invasive protrusion.

In addition to the interactions described above, MUC1 was also found to induce the expression of platelet-derived growth factor (PDGF-A), thereby promoting cell migration. In a mouse model expressing mutant KRAS in the pancreas to drive pancreatic cancer, MUC1 influences the expression and secretion of PDGF-A through interaction with HIF1-α, a known effector of PDGF-A expression. In this system, MUC1-overexpressing cells are highly dependent on PDGF-A for growth and migration, and PDGF-A and MUC1 are necessary for nuclear translocation of β-catenin. MUC1 is phosphorylated by activated PDGFR-β at two tyrosine residues in the cytoplasmic tail, and PDGF-A expression increases nuclear colocalization of β-catenin and MUC1-CD. Although these interactions do not affect cell proliferation, PDGF-A expression does increase invasion in vitro and tumor growth and metastasis in vivo.
resulting induction of EMT genes, such as vimentin, and N-cadherin. In pancreatic cancer, ChIP-ChIP promoter analysis and microarray demonstrated that MUC1-CD is a cofactor for transcription of genes related to invasion, angiogenesis, and metastasis. Additionally, MUC1-CD promotes expression of connective tissue growth factor (CTGF), a mediator of ECM remodeling and angiogenesis.

### MUC1 Targeted Therapies

The role of MUC1 in both transformation and metastatic progression has led to extensive focus on this protein for the development of targeted therapies to treat metastatic disease (Table 1). A number of groups have developed vaccine-like therapies to target MUC1, largely focusing on the primary tumor, and reviewed in Singh et al. Below, we summarize a number of therapeutic interventions with an emphasis on metastatic disease (Fig. 2).

**Peptide-based therapies.** Upon the identification of protein-protein interactions that drive metastatic progression, we and others began the development of peptide-based therapeutics to block these interactions. In our laboratory, we developed a peptide therapeutic that mimicked the domain of MUC1 that interacts with β-catenin/Src/EGFR. The peptide, PTD-4 MUC1 inhibitory peptide (PMIP), was conjugated to the cell-penetrating peptide sequence PTD-4 to allow for cellular uptake. PMIP blocks interactions by serving as a decoy to endogenous MUC1 binding partners, resulting in an inhibition of both proliferation and invasion in vitro. Importantly, PMIP treatment resulted in a significant decrease in metastatic progression in orthotopic models of breast cancer, demonstrating that inhibition of MUC1 activities can directly inhibit metastasis. Follow-up studies with PMIP further demonstrated the utility of PMIP as a therapeutic intervention for lung cancer, although neither of these studies focused on metastatic disease.

In the Kufe laboratory, the ability of MUC1-CD to form dimers was targeted with a peptide-based therapy termed GO-203. GO-203 is a decoy peptide conjugated to the cell penetrating peptide and targeted to the juxtamembrane region of MUC1, which serves as both the non-canonical nuclear localization signal and the dimerization domain. Treatment of chronic myelogenous leukemia, and non-small cell lung cancer cell lines demonstrated that blocking MUC1 dimerization resulted in cell cycle arrest, an increase in reactive oxygen species and apoptosis. GO-203 treatment of non-small cell lung cancer (NSCLC) cell lines in xenograft tumors caused tumor regression with no evidence of metastasis in treated animals, demonstrating efficacy of this dimerization-blocking drug against MUC1-driven cancer.

**MUC1 antibodies/conjugates.** Anti-MUC1 antibodies have been utilized to directly target MUC1-positive tumors, with several showing potential in a variety of cancer types. GP1.4 antibody promotes the internalization of MUC1, resulting in decreased signaling of EGFR in pancreatic cancer cells, inhibiting ERK phosphorylation and decreasing both proliferation and migration of these cells. Pancreatic cancer cells coated in MUC1-specific HMFG2 antibody conjugated to CpG effectively activate natural killer cells, and intratumoral injection of conjugated MUC1 antibody reduced tumor burden in metastatic mouse models of pancreatic cancer. In addition, in a xenograft mouse model of ovarian cancer, a MUC1 antibody C595 reduced tumor growth, whereas the C595 in combination with docetaxel inhibited tumor growth and metastasis while increasing survival.

Additionally, in a study of 447 patients with epithelial ovarian cancer at a variety of stages and who had already undergone chemotherapy, patients were given either standard treatment or one injection of an yttrium-labeled anti-MUC1 antibody (murine HMFG1 antibody), referred to as Y-muHMFG1. This antibody was designed to bind MUC1 epitopes and kill cancer cells with the radioactive yttrium moiety. Patients who were given the Y-muHMFG1 treatment had higher circulating anti-MUC1 antibodies in response to the treatment, and their CA 15-3 serum assessments were lower, indicating that the levels of circulating MUC1 were being reduced in these patients. Patients with the highest levels of circulating anti-MUC1 antibodies did have higher overall survival (80th percentile of Y-muHMFG1 treated group), though there was no significant difference in survival between the Y-muHMFG1 treated group as a whole and the group who received the standard treatment.

| Table 1. MUC1-dependent metastasis inhibitors |
|----------------------------------------------|
| **Therapy** | **Mechanism** | **Pre-clinical/clinical cancer tested** | **Stage of development** | **References** |
|-------------|----------------|-----------------------------------------|-------------------------|--------------|
| Antibodies  |                |                                         |                         |              |
| C595        | extracellular; targets MUC1 core         | ovarian                   | pre-clinical            | 120          |
| GP1.4       | extracellular; binds MUC1 and leads to EGFR internalization | pancreatic               | pre-clinical            | 118          |
| HMFG1       | extracellular; binds cancer cells and kills with a conjugated yttrium moiety | ovarian                   | phase 3                 | 121          |
| Peptides    |                |                                         |                         |              |
| PMIP        | intracellular; blocks MUC1/EGFR, MUC1/p120-catenin, and MUC1/β-catenin protein interactions | breast, lung             | pre-clinical            | 113 and 114  |
| Vaccines    |                |                                         |                         |              |
| MUC1 cDNA   | vaccine; MUC1 expression on cells activates immune system | melanoma                 | pre-clinical            | 123          |
| M-FP        | vaccine; oxidized mannan conjugated to MUC1-GST fusion protein binds mannose receptor on macrophages | breast                   | phase 3                 | 122          |
| Anti-MUC1  |                |                                         |                         |              |
| C595        | vaccine; oxidized mannan conjugated to MUC1-GST fusion protein binds mannose receptor on macrophages | breast                   | phase 3                 | 123          |

References

1. Singh et al.
2. GO-203.
3. GP1.4.
4. C595.
5. MUC1 cDNA.
6. M-FP.
Vaccination against MUC1. In a separate study, 31 postmenopausal women with stage 2 breast cancer were treated with tamoxifen and either seven injections of oxidized mannan conjugated to a MUC1-GST fusion protein (M-FP), or seven injections of placebo after mastectomy.\textsuperscript{122} While none of the patients treated with M-FP experienced metastases, 20% of
Moreover, after the injection series, patients treated with M-FP had a 45% reduction in the patients treated with placebo presented with metastases. This suggests that the M-FP treatment acted as a vaccine against MUC1.122

Several vaccines have recently shown promise against MUC1-driven cancers.123-126 Evaluation of a MUC1 cDNA as a vaccine has shown promise as a metastasis suppressor.127 In this study, wild-type C57Bl6 mice were given weekly intraarticular injections of a full-length MUC1 cDNA plasmid and assessed against mice injected with empty vector. B16-F10 melanoma cells stably over-expressing MUC1 were then intravenously introduced to the mice and animals were evaluated for the formation of lung metastases. Mice vaccinated with the MUC1 cDNA had significantly fewer lung metastases than mice injected with empty vector, providing evidence that vaccination with MUC1 cDNA suppresses MUC1-dependent lung metastasis development.123

One MUC1 vaccine just finished Phase 2 clinical trials to determine its efficacy against MUC1-driven cancers. The MUC1 vaccine, ImMucin, is composed of a short 21-mer peptide sequence at the N-terminal signal peptide region of MUC1, and was shown in preliminary studies to bind to both human MHC class 1 and 2 immune cell proteins derived from cancer patient tissues. Pre-clinically, mice injected with MUC1-overexpressing DA3 metastatic mouse mammary carcinoma cells experienced longer survival after vaccination with ImMucin than mice injected with the carcinoma cells and treated with vehicle.125

Phase 1 and phase 2 human clinical trials in which multiple myeloma patients were treated with the vaccine have shown promise but have not been formally reported.126

Numerous factors affect the metastatic cascade, including cell motility and invasion, degradation of ECM, neo-vascularization, intra- and extravasation and dormancy and survival at a secondary site.127 Of these, MUC1 has been shown to affect tumor invasion and neo-vascularization, interactions with the vasculature and survival and growth at a secondary site. Clinically, MUC1 expression is highly correlated with advanced disease, poor survival and tumor dissemination. Mechanistically, MUC1 can drive metastatic progression by altering the interaction between tumor cells and their environment, altering the composition of the tumor microenvironment and altering the genetic makeup of the tumor cell itself to produce a pro-metastatic phenotype.128 Due to the prevalence of MUC1 expression in metastatic disease, and the role of metastasis in patient mortality, directly targeting MUC1 would appear to be of paramount importance. While still in the pre-clinical and early clinical stages, MUC1 targeting has now begun in earnest, and appears to hold significant promise for a number of tumor types, including lung, pancreatic and breast.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.
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