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Invited Mini Review

Mucin in cancer: a stealth cloak for cancer cells

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Mucins are high molecular-weight epithelial glycoproteins and are implicated in many physiological processes, including epithelial cell protection, signaling transduction, and tissue homeostasis. Abnormality of mucus expression and structure contributes to biological properties related to human cancer progression. Tumor growth sites induce inhospitable conditions. Many kinds of research suggest that mucins provide a microenvironment to avoid hypoxia, acidic, and other biological conditions that promote cancer progression. Given that the mucus layer captures growth factors or cytokines, we propose that mucin helps to ameliorate inhospitable conditions in tumor-growing sites. Additionally, the composition and structure of mucins enable them to mimic the surface of normal epithelial cells, allowing tumor cells to escape from immune surveillance. Indeed, human cancers such as mucinous carcinoma, show a higher incidence of invasion to adjacent organs and lymph node metastasis than do non-mucinous carcinoma. In this mini-review, we discuss how mucin provides a tumor-friendly environment and contributes to increased cancer malignancy in mucinous carcinoma. [BMB Reports 2021; 54(7): 344-355]

INTRODUCTION

The mucosal surfaces of the body protect against various external environments. The intestinal tract is the guardian of the innate host defense because of the secretory factors of intestinal goblet cells (1). Colonization by intestinal bacteria is limited to an outer mucus layer and interacts with mucin glycoproteins, whereas an inner mucus layer is entirely devoid of bacteria (2). Thus, the defection of mucus layers increases bacterial interaction with the surface epithelium. Additionally, abnormality of mucins and mucin structure has occurred in

mucinous colorectal carcinoma (MCC) (3). Since tumor growth sites induce inhospitable conditions for them to survive, mucins are suggested as an oncogenic microenvironment that avoids hypoxia, acidic, and other biological hurdles. The composition and structure of mucins enable them to mimic the surface of tumor cells like the surface of normal epithelial cells (4). Additionally, the mucus layer captures growth factors or cytokines, contributing to cell growth of the tumor. Alternatively, these properties interfere with the interaction between the immune system and tumor cells. Indeed, a high concentration of soluble mucins downregulates the motility and activation status of leukocytes (5). It was also reported that cell surface mucin contributes to cell proliferation and differentiation (6).

MCC shows a higher incidence of invasion to adjacent organs and lymph node metastasis than does non-mucinous colorectal carcinoma (7). Also, MCC is characterized by a large amount of extracellular mucin, and mucin pools contain malignant epithelium (4, 7). However, the function of mucin especially in MCC pathology is not completely understood. Thus, the unveiling of mucin's role and molecular mechanisms for MCC tumorigenesis and understanding MCC mouse models is required for MCC study. In this mini-review, we briefly discuss major MCC-related mucins and their roles in MCC development. Further, we introduce the currently known MCC therapeutic drugs and mouse models proposed for MCC study.

GEL-FORMING AND TRNASMEMEBRANE MUCINS

The major constituent of mucus layers is mucins which are high-molecular-weight epithelial O-glycosylated glycoprotein (8) and are implicated in pathogenesis in cancer, especially mucinous adenocarcinomas. Currently, 21 mucin genes are known in humans. The mucins are classified into two groups based on their structure and functions: (i) secreted gel-forming mucins and (ii) transmembrane mucins. Gel-forming mucins including MUC2, MUC5AC, MUC5B, MUC6, and MUC19, cover epithelial cells in various organs (Table 1). Gel-forming mucins are secreted oligomeric mucin and might be responsible for the properties of mucus. Transmembrane mucins such as MUC1, MUC3, MUC4, MUC12, MUC13, MUC15, MUC16, MUC17, MUC21, and MUC22, exhibit monomeric structural characteristics and mainly located on the cell surface, and

Table 1. Mucin expression in human organs

Organs		Gel-forming mucins	Transmembrane mucins
Esophagus		MUC5B	MUC1, MUC4, MUC20
Stomach		MUC5AC, MUC6	MUC1, MUC3, MUC13, MUC20
Liver		MUC2, MUC5AC, MUC5B, MUC6	MUC1, MUC3
Pancreas		MUC5AC, MUC5B, MUC6	MUC1, MUC11, MUC12, MUC20
Lung		MUC2, MUC5AC, MUC5B	MUC1, MUC3, MUC4, MUC11, MUC13, MUC20
Reproductive tract	Male		MUC1
	Female	MUC5AC, MUC5B, MUC6	MUC1, MUC4, MUC12
Intestine	Duodenum	MUC2, MUC6	MUC1, MUC3, MUC17, MUC20
	Small intestine	MUC2	MUC1, MUC3, MUC17, MUC20
	Colorectum	MUC2	MUC1, MUC3, MUC4, MUC11, MUC12, MUC13, MUC17, MUC20

might play a role in separating cells from the external environment (4). Mucins contain oligosaccharides, having excellent structural diversity (9), but their function is still unclear. However, polypeptide-attached oligosaccharide expands the volume of polypeptides through structural modification. Thus, mucins fill the space and raises its gel-forming character. Subsequently, it may lead to various physiological effects by increasing the chance of binding with a growth factor and cytokine for the survival of tumor cells (Fig. 1).

Gel-forming mucins in cancer
MUCIN2 (MUC2): Secreted protein MUC2 is a major component of the mucus layer in the small and large intestines. Decreased MUC2 is highly involved in the early stage of colorectal cancer (CRC) development, which is caused by methylation of the *MUC2* promoter (10). Given that the *Muc2*-deficient mouse developments colitis (11), methylation of the *MUC2* promoter might contribute to CRC initiation. However, the results of *MUC2* gene promoter methylation analysis of MCC and non-MCC by narrowing the scope in the entire CRC analysis shows demethylation in the *MUC2* promoter of MCC (10). MCC (10-15% of human CRC) is metastatic and therapeutically resistant (12) and shows accumulated genetic mutations including *K-Ras*, *TOP-1*, *MAPK*, and *BRAF* (12-18). However, MCC shows a low frequency of *TP53* mutation and protein expression. Also, TP53 and p21 positively control *MUC2* via transcriptional activation (19). These studies imply that MCC may show a genetic background difference from CRC for amplifying MUC2, at least for *TP53* mutation or expression. Indeed, the *Adenomatous polyposis coli (APC)* mutation, with the highest frequency in CRC, is relatively low in MCC. In summary, CRC (non-MCC) downregulates MUC2 to foster an oncogenic environment by inflammation or colitis, whereas MCC might try to employ its unique genetic background for generating the mucinous environment including MUC2 secretion.
MUCIN5AC (MUC5AC): In normal physiological conditions, MUC5AC is barely secreted in intestinal mucus (20). But, similar to MUC2, MUC5AC is expressed at a high level in

MCC and by microsatellite instability (MSI)-high tumors (21, 22). Cancer patient tissues show MUC5AC-positive tumor cells (35-100%), which depend on the tumor type (Adenocarcinoma: 147/420 [35%], Adenocarcinoma 1-49% mucinous component: 119/167 [71%], Mucinous > 50%: 46/49 [94%], Signet-ring cell carcinoma: 8/8 [100%]) (23). Signet-ring cells produce aberrant mucin and demonstrate high levels of MSI. The mechanism of MSI generation is involved in the dysfunction of the DNA mismatch repair protein. In normal tissue, DNA mismatch repair proteins correct errors during DNA replication. However, impaired DNA mismatch repair proteins in tumor cells trigger the possibility of MSI generation, subsequently resulting in chromosomal instability (CIN). Hypomethylation of the *MUC5AC* promoter is a predictor of MSI (24). Furthermore, elevated MUC5AC is associated with mismatch repair-deficiency (25) and downregulation of TP53 and its target gene p21 (26), which are strongly related to the maintenance of chromosomal stability. Since β-catenin has been reported to induce impaired DNA damage repair via LIG4 (27), MUC5AC-induced β-catenin (26) might be involved in MSI via radioresistance. Although Wnt/β-catenin signaling contributes to radioresistance, MSI does not happen under Wnt/β-catenin signaling hyperactivated conditions that do not show an increase in mucins (28-30). Moreover, MUC5AC shows a negative correlation with MLH1, a protein for DNA damage repair, in MCC (22). Thus, it is likely that MUC5AC is a key cooperator for MSI, but veiled a detailed molecular mechanism. Recently, it was reported that MUC5AC increases tumor heterogeneity (31), which may result from MSI. Increased tumor heterogeneity provides an advantage for escape from immune surveillance.
MUCIN5B (MUC5B): Consistent with MUC2 and MUC5AC, hypermethylation of the *MUC5B* promoter is a major regulatory mechanism for silencing it (32). Intriguingly, intronic regions regulate MUC5B (33-35). A 256bp segment of the first intron of the *MUC5B* contains eight tandem repeat GA boxes located in the GAGGG box, which interact with transcription factors such as Sp1, GATA-1, and AP-2 (35). These transcrip-
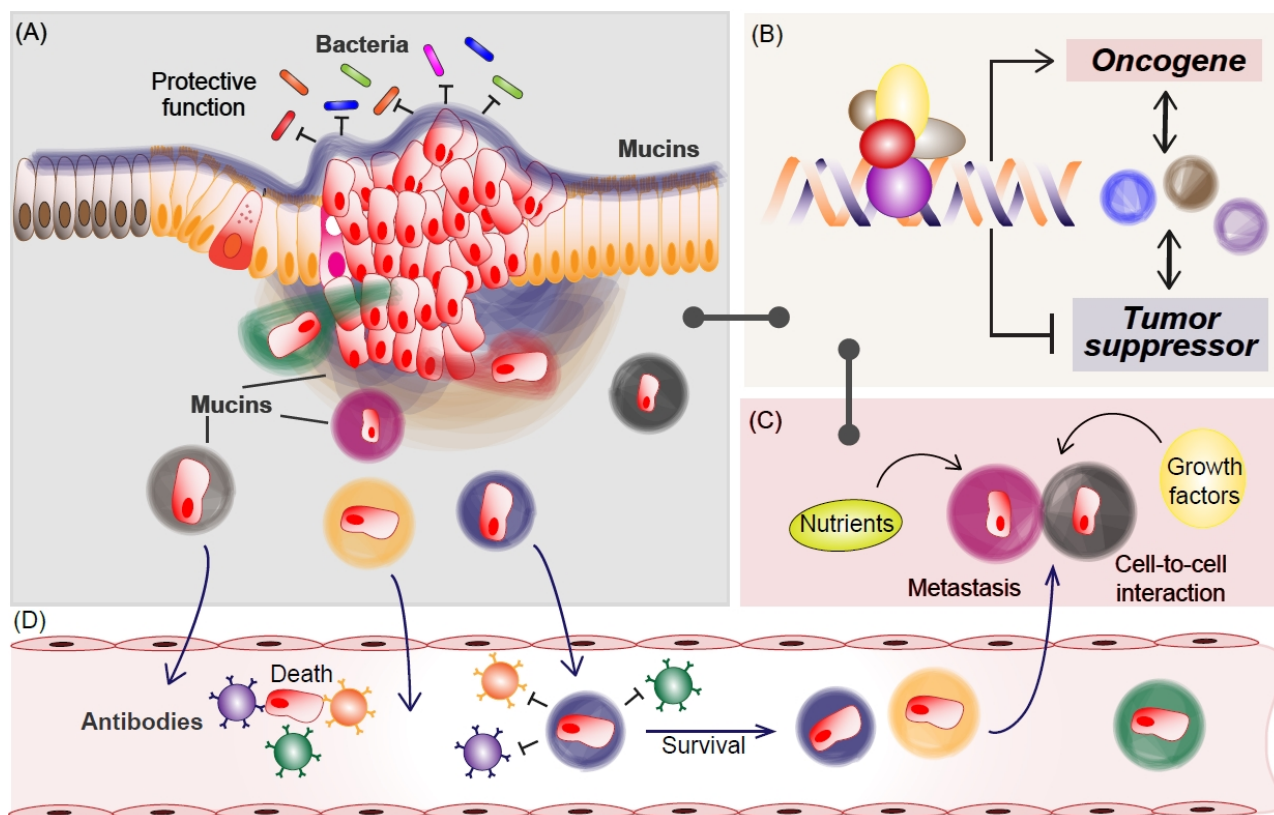
Fig. 1. The role of mucins in MCC progression. In normal conditions, mucins protect epithelial cells from external environments such as bacteria (A). In MCC, various mucins transcriptionally increase oncogenic factors while inhibiting tumor suppressor (B), which might be mediated by mucin-captured nutrients or growth factors (C). Additionally, mucins protect MCC from immune surveillance via mimic epithelial surface during metastasis (A and D). For the journey to secondary tumor formation, many cancer cells are killed by the immune system in the circulating system (D). Metastasized mucin-cloaked cancer cells take advantage of including transcription, nutrients, and growth factors (C), for secondary MCC development, which would be shared via cell-to-cell interaction.

tion factors suggest a role of MUC5B in cell differentiation during development. Moreover, the 37th intron of *MUC5B* interacts with Sp1 and NF1-MUC5B via the GC-rich region (36). These involvements of the intronic region in the regulation of the *MUC* gene are not found in other *MUCs* except to *MUC5B*. Long non-coding RNA also upregulates MUC5B and promotes cancer mobility and metastasis (37). The abnormal expression of the MUB5B on the biological behavior led to aggressive tumorigenesis including chemotherapeutic resistance and impairment of antitumor immune response (38). Although several studies reported that MUC5B knockdown reduces chemoresistance and decreases in Wnt/β-catenin signaling-induced cell proliferation and migration, the detailed molecular mechanism of how MUC5B contributes to MCC malignancy is still unclear. However, given that MUC5B-induced Wnt/β-catenin might contribute to cell migration, invasion, and chemoresistance (27, 39, 40), it is plausible that the interaction of Wnt/β-catenin signaling with MUC5AC and MUC5B may orchestrate the metastatic environment in MCC progression.

MUCIN6 (MUC6): MUC5AC and MUC6 are the major gastric mucins. Whereas MUC5AC is located on the epithelial cell surface, MUC6 is expressed in glandular structures. Staining in a subset of CRC displays exclusively cytoplasmic normal tissue (23). Thus, the expression of tumor cells might result from de novo synthesis by oncogenic signaling. The regulatory mechanism of MUC6 expression is related to the status of promoter methylation, BRAF-V600E mutation, a marker of MCC, as well as *MLH1* methylation. Additionally, p53 overexpression shows an inverse association with MUC6 (23). These studies demonstrate a similar regulatory mechanism of gel-forming mucin. Investigation of transcriptional regulatory machinery reported that the Notch signaling pathway increases mRNA levels of the *MUC6* and *MUC5AC* via Hath1, a crucial transcription factor for the Notch signaling pathway, in cancer cell lines (41). MUC6 is frequently associated with the nuclear β-catenin in gastric cancer and younger patients (less than or equal to 40 years old) are more likely to secrete MUC6 (42, 43). Interestingly, similar to MUC5, positive regulation of MUC6 is

observed at the early stage of tumor development, but is diminished at late tumorigenesis, suggesting that MUC6 inhibits tumor cell migration (44). Indeed, MUC6 deficient patients showed short progression-free survival and cancer-specific survival, especially in stage II and III CRC patients (45), implying the involvement of MUC6 in inhibiting tumorigenesis. Conversely, a high level of MUC5AC increased progression-free survival in stage II and III CRC patients.

Together, gel-forming mucins are clustered in chromosome 11p15.5 and share similar regulatory machinery. However, their expression pattern leads to difficulty in targeting an MCC-specific therapeutic strategy. Additionally, they orchestrate diverse physiological events in MCC by communicating either with other signaling pathways or among mucins. Therefore, to resolve these scientific and translational medicine issues, the preclinical animal model of MCC mimicking is likely required.

Transmembrane mucins in cancer

MUCIN1 (MUC1): MUC1 is a single-pass transmembrane protein with a glycosylated extracellular domain. As a metabolic master regulator (46), MUC1 is mainly expressed in the epithelial cells of the stomach, intestine, and lungs. MUC1 competes with E-cadherin in the cytosol to bind β-catenin, by receptor tyrosine kinases (RTKs)-mediated MUC1 phosphorylation (47). Also, Protein Kinase C-δ (PKC-δ) promotes interaction between MUC1 and β-catenin (48, 49). As a negative regulator of β-catenin, GSK3β mediates the phosphorylation of β-catenin for proteasomal β-catenin degradation. However, since MUC1 blocks GSK3β-mediated β-catenin phosphorylation (50), the MUC1-β-catenin complex stably activates Wnt/β-catenin signaling. Besides, MUC1 binds to ErbB1 and increases p-ERK1/2 through the MAPK pathway (51). Anti-cancer immunity is frequently suppressed by oncogenic signals. MUC1 causes immunosuppression in CRC (52) and induces T-cell tolerance in vivo (53). Additionally, apoptosis activating pathway defects such as masking of death receptor (54) and attenuated cytochrome c releasing and caspase-3 activation (55) are led by MUC1. Also, MUC1 increases the survival rate response to cytotoxic or oxidative stress by activating phosphoinositol-3 kinase (PI3K) and the Akt pathway (55-57). These studies show that MUC1 might be a multifaced oncoprotein in MCC.

Epigenetic regulation of *MUC1* shows the CpG island in the *MUC1* promoter and methylation of histone H3K9, recovered by acetylation of histone H3K9 (47, 58). Thus, cancer cells increase MUC1 by demethylation of promoter and histone H3K9 and acetylation of H3K9. Further, the *MUC1* promoter contains transcription factor binding sites for Sp1, AP1-4, NF-1, NF-κB, PPAR, and estrogen receptor (59). It was reported that proinflammatory cytokines such as TNF-α and IFN-γ, elevate MUC1 independently from NF-κB and STAT1α (60).

MUC1 is a single polypeptide chain that harbors three domains an extracellular domain, a transmembrane domain, and a Sea urchin sperm protein enterokinase and agrin (SEA) domain (47). MUC1 is autoproteolytically cleaved after trans-

lation at the GSVVV motif in the SEA domain, located within an extracellular domain, because of conformational stress (61). Cleaved MUC1 in GSVVV motif generates two peptide fragments, N-terminal subunit (MUC1-N) and C-terminal subunit (MUC1-C) (61). *MUC1* exhibits seven exons that encode MUC1-N (exons 1-4) and MUC1-C (exons 4-7) (47). Alternative splicing of *MUC1* produces about 80 isoforms of MUC1 with exon skipping and intron retention (62), the most common isoforms being MUC1-A, MUC1-B, MUC1-C, MUC1-D, MUC1-X, MUC1-Y, and MUC1-ZD (62). Especially, MUC1-C is associated with oncogenic effects through interaction with various transcription factors such as STAT3, NF-κB, p53, and β-catenin (63-66). Glycosylated MUC1-C provides a bridge between ErbB1 and epidermal growth factor receptor (EGFR), which stabilizes and improves the EGFR-associated signaling pathway for cell proliferation (51). Intriguingly, MUC1-C itself translocates into the nucleus and controls various physiological effects through β-catenin, FOXO3a, p53, and ER-α (64, 67-69). In summary, MUC1 plays a crucial role via various isoforms in constructing 'a great wall' to avoid anti-cancer therapeutic strategies.

MUCIN4 (MUC4): Like other transmembrane mucins, MUC4 also plays a role in protecting the epithelial surface (70). CRC patients show low expression of MUC4 (99/132 [75%]) (71), which might be mediated by Wnt/β-catenin signaling. Nuclear β-catenin promotes HES1, an antagonist of HATH1 (72). Given the increase of MUC4 by HATH1, CRC might display a low level of MUC4 by Wnt signaling. MUC4 controls cell proliferation, differentiation, apoptosis, and tumor progression via three EGF-like domains, which play as an intramembrane ligand to activate ErbB2 (73). The interaction between MUC4 and ErbB2 activates the downstream pathway of EGF signaling such as PI3K-Akt pathway, which related to proliferation and apoptosis in tumorigenesis. MCC might employ MUC4 for tumor progression instead of Wnt/β-catenin signaling activation.

MUCIN16 (MUC16): In normal tissue, MUC16, previously known as CA125, expresses in the epithelial lining of several organs (74, 75). It functions as a barrier against the external environment and supports the maintenance of the mucus layer (76). Given high expression in various cancer, MUC16 is extensively employed as a biomarker (77). It is one of the frequently mutated genes, resulting in increased tumor growth and malignancy (78-80). CA125 is a tandem repeated peptide (60+ repeats of 156 amino acids) of the MUC16, which promotes cancer cell proliferation and resistance to immune surveillance (81). MUC16 inhibits the function of Natural Killer (NK) cells via direct binding to the Siglec-9 receptor of NK cells, resulting in evasion of the innate immune response (82, 83). Also, the interaction may inhibit intimate interactions between NK and cancer cells (84). The interaction between MUC16 and mesothelin, a protein located in the mesothelial lining of the peritoneal cavity, triggers cancer metastasis (85) by facilitating attachment of cancer cells to the mesothelial lining (86, 87).

Because of MUC16-induced JAK-STAT, knockdown of MUC16

displays decreased in vitro and in vivo proliferation of cancer cells (88, 89). Furthermore, knockdown studies of MUC16 demonstrated that MUC16 affects caspase-dependent/-independent apoptosis, colony-forming ability, adhesion, migration, invasive ability, epithelial-mesenchymal transition (EMT), and chemoresistance of cancer cells (90-92), which suggest an increased cancer stage by MUC16 overexpression. Especially, the MUC16 C-terminal fragment (CT) leads to upregulation of the stem cell-related genes via interaction with JAK2. It may lead to the dedifferentiation of cancer cells to obtain malignant properties. Also, MUC16 CT disrupts deregulation of β-catenin and E-cadherin at the junctional complex, leading to EMT in cancer cells (93). Thus, MUC16 is a strong target for the development of anti-cancer therapy.

Together, transmembrane mucins such as MUC1 and MUC16 protect internal cells from the external environment. Moreover, it has the ability to play a crucial role in signaling pathway transduction while having various isoforms. Despite these indispensable roles in normal tissues, mucins are highly attractive functions for cancer cells and would be an essential factor for malignant tumors. Therefore, various anti-cancer therapies targeting transmembrane mucins have been attempted in various ways (Fig. 1).

CLINICAL TRIALS AND A PRECLINICAL MOUSE MODEL FOR MCC THERAPY

MCC is mainly characterized by the high expression of mucin, which is categorized into over 20 subtypes. However, their functions especially in MCC pathology are not completely understood. Additionally, MCC shows malignant features including highly accumulated DNA damage, resistance to cancer therapy, invasive characteristics, and poor prognosis (3, 4, 12). Nonetheless, the roles of mucins in MCC remain ambiguous, mainly because of the numerous subtypes of mucins, highly complex protein expression, and absence of proper mouse models. Recent studies suggested that MCC exhibits high mucin expression, MSI (3, 40), frequent mutation of *KRAS*, *BRAF*, *MAPK*, and *TOP-1*, hyperactivation of the PIK3CA signaling pathway (13, 14, 18, 40), and increased inflammation. Thus, it is imperative to unveil the molecular mechanism for MCC tumorigenesis and to establish MCC mouse models. In this section, we discuss past/current clinical trials for MCC treatment. Moreover, we introduce mucin-related mouse models and a novel mouse model for MCC research.

Therapeutic targeting of mucin-related oncogenic effects
EGFR receptor inhibitors: As pointed out above, mucins mainly upregulate the EGFR receptor to activate the MAPK pathway (Table 2). Thus, Cetuximab (NIH clinical trial numbers [clinicaltrials.gov]: NCT01198535, NCT00835679, and NCT00100841), Gefitinib (Iressa; NCT00052585), Erlotinib (Tarceva, NCT00060411), and Panitumumab (NCT01285102) were tested to downregulate MCC growth. Cetuximab is an EGFR specific mono-

clonal antibody, that competitively inhibits the binding of epidermal growth factor (EGF) and other ligands that are secreted by tumor cells (94, 95). Gefitinib is the first selective inhibitor of the EGFR tyrosine kinase, which it inhibits by binding to the adenosine triphosphate (ATP)-binding site of the enzyme. Gefitinib is also referred to as Her1 or ErbB-1. Erlotinib inhibits the intracellular phosphorylation of tyrosine kinase associated with the EGFR, but the mechanism of action has not been fully characterized (96). Panitumumab (ABX-EGF) is a recombinant human IgG2 monoclonal antibody, binding to EGFR. Panitumumab competes with the EGF ligand to bind EGFR and shows decreased VEGF production. Although EGRF inhibitors show anti-cancer effects, they exhibit several side effects such as acneform rash, vomiting, diarrhea, skin change, and loss of appetite (97, 98).

Akt inhibitor: Mucins activate PI3K/Akt signaling to survive under various cytotoxicity conditions (55-57, 99). MK2206 (NCT01802320) is an orally available inhibitor of pan Akt (protein kinase B) that inhibits the activity of Akt in a non-ATP competitive manner, resulting in the inhibition of the PI3K/Akt signaling pathway and cell proliferation. Akt contains a Pleckstrin Homology (PH) domain, which binds with high affinity to phosphoinositides including PIP3 and PIP2. Although the mode of action of MK2206 is not clear, it may interfere with binding sites for Akt substrates (100). MK2206 also shows side effects such as skin rash and gastrointestinal upset (101).

Angiogenesis inhibitors: Vascular endothelial growth factor (VEGF) plays a crucial role in angiogenesis, lymphangiogenesis, and tumor growth. Thus, VEGF inhibitors are suggested as a potential therapeutic target for anti-cancer therapies. Bevacizumab (Avastin; NCT00217737, NCT00060411, and NCT00100841) and Aflibercept (Ziv-aflibercept; NCT01652196 and NCT02235324) are employed to test the effect of VEGF inhibitors on MCC progression. Bevacizumab gained FDA approval as the first antiangiogenic agent, which is a humanized monoclonal IgG antibody and neutralizes VEGF-A (102, 103). As a recombinant protein, Aflibercept acts as a decoy receptor for VEGF-A and placental growth factor (PIGF), resulting in suppression of VEGFR-1 and VEGFR-2. However, VEGF inhibitors increase the risk of stroke, and myocardial infarction with various side effects including diarrhea, neutropenia, and thrombocytopenia.

Topoisomerase I inhibitor: MCC shows a frequent amplifying mutation in *TOP-1*, which might increase genetic instability such as MSI. Irinotecan (Onyvyde; NCT00005036, NCT00052585, NCT01285102, NCT01643499, NCT01923337, and NCT04088786) is an antineoplastic enzyme inhibitor, which is derived from camptothecin which inhibits the action of TOP-1 (104). Irinotecan prevents re-ligation of the DNA strand by binding to the TOP-1-DNA complex, which results in lethal double-strand DNA breakage. Irinotecan exhibits toxicity such as nausea, vomiting, abdominal cramping, diarrhea, and infection.

Other anti-cancer drugs: Fluorouracil, an inhibitor of DNA synthesis, is a classical anti-cancer drug. Fluorouracil blocks the formation of thymidylate from uracil, leading to the inhibi-

Table 2. Current and past clinical trials for MCC-related therapy

Drug	Mechanism of action	Phase	Indentifier
MK2206	Akt inhibitor	Phase2	NCT01802320
Alisertib	Aurora A kinase inhibitor	Phase1	NCT01923337
Oxaliplatin	DNA synthesis inhibitor	Phase1	NCT00005036
		Phase2	NCT00060411
		Phase3	NCT00217737
			NCT01643499
			NCT01652196
6,8-Bis(benzylthio)octanoic acid	E1α PDH modulator	Phase1	NCT02232152
Cetuximab	EGFR inhibitor	Early phase1	NCT00100841
		Phase1	NCT00835679
		Phase2	NCT01198535
Dasatinib	EGFR inhibitor	Early phase1	NCT00835679
Erlotinib	EGFR inhibitor	Phase1	NCT00060411
Gefitinib	EGFR inhibitor	Phase2	NCT00052585
Panitumumab	EGFR inhibitor	Phase2	NCT01285102
Gamma-seretase inhibitor RO4929097	Gamma-seretase inhibitor	Phase1	NCT01198535
Recombinant inferferon gamma	Macrophage activation factor	Phase1	NCT00002796
		Phase2	
Fluorouracil	Thymidylate synthase blocking	Phase1	NCT00002796
		Phase2	NCT00005036
		Phase3	NCT00052585
			NCT00060411
			NCT00217737
			NCT01285102
			NCT01643499
			NCT01652196
			NCT02232152
			NCT02235324
Irinotecan	Topoisomerase inhibitor	Phase1	NCT00005036
		Phase2	NCT00052585
		Phase3	NCT01285102
			NCT01643499
			NCT01923337
			NCT04088786
Aflibercept	VEGF inhibitor	Phase2	NCT01652196
			NCT02235324
Bevacizumab	VEGF inhibitor	Phase1	NCT00060411
		Phase2	NCT00100841
		Phase3	NCT00217737

tion of DNA and RNA synthesis. RO4929097 is an orally available gamma-secretase (GS) inhibitor, which directly binds to GS and blocks activation of the Notch receptor. These drugs also lead to severe side effects including trouble sleeping, irritability, temporary hair loss, and abnormal taste.

Preclinical mouse model for MCC research
Despite the poor prognosis and metastatic characteristics of MCC (7), the genetic mechanism of MCC development is unknown. Several genetically engineered mouse models (GEMMs) of *Muc1*, *Muc2*, *Muc5ac*, *Muc5b*, *Muc6*, and *Muc16* have been employed for MCC study (Table 3). However, those GEMMs are highly involved in the maintenance of tissue homeostasis and inflammation without mutations of genes

frequently found in the human MCC (*KRAS*, *BRAF*, *MAPK*, and *TOP-1*) (12-16). It is noteworthy that despite the hyperactivation of the PIK3CA signaling pathway in human MCC, genetic mutations in *KRAS*, *BRAF*, *MAPK*, and *TOP-1* do not lead to MCC development in mouse models (105-107). For example, the $KRAS^{G12D}$ mouse displays tumorigenesis such as non-small cell lung cancer and pancreatic cancer without MCC development (106).

Recently, it was shown that *Cancer-related Regulator of Actin Dynamic* (*CRAD*) is highly mutated in small cell lung cancer, CRC, and melanoma (40, 108). Additionally, the *Crad* KO mouse initiates tumorigenesis in the pancreas and lung with MCC development (40). Thus, it is highly likely that inactivation of the *CRAD* gene might be associated with MCC

Table 3. Mucin-related GEMMs for MCC study

Gene	Allele symbol	Allele attributes	Reported phenotypes	Reference
Muc1	*Muc1<em1Smoc>*	Null/knockout	No abnormal phenotype observed	Shanghai Model Organisms Center
	Muc1<tm1(cre/ERT2)Lcm>	Inducible recombinase	No abnormal phenotype observed	Kopinke and Murtaugh, 2010 BMC Dev Biol
	Muc1<tm1(KOMP)Vlcg>	Null/knockout, reporter	No abnormal phenotype observed	Velocigene MGI Direct Data Submission
	Muc1<tm1.1(cre/ERT2)Lcm>	Inducible recombinase	No abnormal phenotype observed	Kopinke and Murtaugh, 2010 BMC Dev Biol
	Muc1<tm1a(EUCOMM)Wtsi>	Conditional ready, null/knockout, reporter	No abnormal phenotype observed	Skarnes et al., 2011 Nature
	Muc1<tm1e(EUCOMM)Wtsi>	Null/knockout, reporter	No abnormal phenotype observed	Skarnes et al., 2011 Nature
	Muc1<tm1Gend>	Null/knockout	Digestive/alimentary, homeostasis, liver/biliary, neoplasm	Spicer et al., 1995 J Biol Chem
	Muc1<tm1Smoc>	Conditional ready	No abnormal phenotype observed	Shanghai Model Organisms Center
Muc2	*Muc2<eey>*	Chemically induced (ENU)	Cellular, digestive/alimentary, endocrine/exocrine, hematopoietic, immune, mortality/aging	Heazlewood et al., 2008 PLoS Med
	Muc2<keny>	Chemically induced (ENU)	Digestive/alimentary, immune	The Australian Phenomics Facility at The Australian National University
	Muc2<M1Btlr>	Chemically induced (ENU)	Digestive/alimentary, immune	Brandl K et al., MGI Direct Data Submission
	Muc2<m2Btlr>	Chemically induced (ENU)	Digestive/alimentary, immune	Brandl K et al., MGI Direct Data Submission
	Muc2<m3Btlr>	Chemically induced (ENU), no specific	Digestive/alimentary, immune	McAlpine W et al., MGI Direct Data Submission
	Muc2<tm1a(KOMP)Wtsi>	Conditional ready, null/knockout, reporter	No abnormal phenotype observed	Skarnes et al., 2011 Nature
	Muc2<tm1Avel>	Null/knockout		Velcich et al., 2002 Science
	Muc2<tm1e(KOMP)Wtsi>	Null/knockout, reporter	No abnormal phenotype observed	Skarnes et al., 2011 Nature
	Muc2<wnn>	Chemically induced (ENU)	Cardiovascular, cellular, digestive/alimentary, endocrine/exocrine, growth/size/body, hematopoietic, homeostasis, immune, mortality/aging	Robinson et al., 2017 Am J Physiol Gastrointest Liver Physiol
Muc5ac	*Muc5ac<em1Smoc>*	Null/knockout	No abnormal phenotype observed	Shanghai Model Organisms Center
	Muc5ac<tm1.1Evns>	Null/knockout	Digestive/alimentary, homeostasis, immune, vision/eye	Morgan et al., 2021 Nat Commun
	Muc5ac<tm2a(EUCOMM)Hmgu>	Conditional ready, null/knockout, reporter	No abnormal phenotype observed	Helmholtz Zentrum Muenchen GmbH
	Muc5ac<tm2b(EUCOMM)Hmgu>	Null/knockout, reporter	No abnormal phenotype observed	International Knockout Mouse Consortium
	Muc5ac<tm2e(EUCOMM)Hmgu>	Null/knockout, reporter	No abnormal phenotype observed	Helmholtz Zentrum Muenchen GmbH

Table 3. Continued

Gene	Allele symbol	Allele attributes	Reported phenotypes	Reference
Muc5b	*Muc5b<em1(IMPC)Mbp>*	Null/knockout	No abnormal phenotype observed	International Mouse Phenotyping Consortium (IMPC) Database Release
	Muc5b<Gt(EUCE0173a08)Hmgu>	Gene trapped	No abnormal phenotype observed	Mouse Genome Informatics (MGI) and National Center for Biotechnology Information (NCBI)
	Muc5b<tm1(NCOM)Mfgc>	Null/knockout, reporter	No abnormal phenotype observed	Mammalian Functional Genomics Centre
	Muc5b<tm1.2Evns>	Null/knockout	Cellular, growth/size/body, hearing/vestibular/ear, hematopoietic, homeostasis, immune, mortality/aging, respiratory	Roy et al., 2014 Nature
Muc6	*Muc6<tm1(Hbegf)Koo>*	Inserted expressed sequence, reporter	No abnormal phenotype observed	Han et al., 2019 Cell Stem Cell
Muc16	*Muc15<tm1Lex>*	Null/knockout	No abnormal phenotype observed	Tang et al., 2010 Nat Biotechnol
	Muc16<em1Smoc>	Null/knockout	No abnormal phenotype observed	Shanghai Model Organisms Center
	Muc16<m1Mhda>	Null/knockout	No abnormal phenotype observed	Sabrautzki S et al., MGI Direct Data Submission
	Muc16<tm1Bhr>	Null/knockout	Reproductive	Shirai et al., 2014 Invest Ophthalmol Vis Sci
	Muc16<tm1Bhr>	Null/knockout	No abnormal phenotype observed	Tang et al., 2010 Nat Biotechnol

tumorigenesis. The oncogenic mutations and signaling amplification such as *KRAS*, *BRAF*, *MAPK*, and *TOP-1*, might result from concomitant MCC progression in the MCC-specific tumor suppressor-deficient condition. Therefore, the genetic and molecular basis of MCC development and MCC-specific tumor suppressor needs to be resolved. Currently, anti-MCC therapies, including IR and chemotherapy, are not successful, because of the high resistance of MCC. However, the anti-cancer therapy resistance mechanism of MCC is not understood.

A major reason that these issues have not been successfully addressed was the lack of a preclinical MCC animal model. Mucins interact with each other and utilize another signaling pathway to develop MCC, but the GEMM of each mucin does not mimic the environment of MCC patients. However, *Crad* KO displays overexpression of several mucins at the same time, and MCC markers such as MSI and Top-I are upregulated (40). Therefore, *Crad* KO is proposed as a novel preclinical mouse model for MCC study.

DISCUSSION

Metastasis is accompanied by multiple events and requires ideal timing. Further, tumor suppression mechanisms including the immune system, tightly function in the organism to kill cancer cells, thus it is difficult to acquire a wealth of growth factors and nutrients for growth. Hence even malignant tumor cells would often fail to metastasize. The selected malignant tumor cells are more likely to succeed in metastasis. MCC may be the selected cells. Given that MCC maintains enough mucins that could be called a 'stealth cloak', MCC can take a stealth strategy and metastasize while being protected by a cloak (Fig. 1). Mucins interact and support MCC efficiently acquire factors necessary for growth and metastasis. Furthermore, mucin provides sanctuary to escape from the surveillance of the immune system. These demonstrate that MCC thoroughly exploits the superior abilities of mucins. For example, mucins are tightly controlled by a regulatory mechanism such as promoter methylation and transcription factor (10, 11). The expression patterns of MUC2 and MUC5 are similar, but the function appears to be independent, which could be utilized by MCC. Transcription factors such as Sp1, commonly regulate the expression of MUC2 and MUC5, but MUC5-induced β-catenin inhibits the expression of MUC2 (43). In the early stage of MCC tumorigenesis, the expression of MUC2 and MUC5 indicates an incompatible pattern. Reduced MUC2 might lead to the inflammatory response that is necessary for MCC development or promote oncogenic mucins. Subsequently upregulated MUC5 might add sup-

pression force for MUC2 via β-catenin. During MCC progression, MUC5 is downregulated as MSI completion, resulting in MUC2 upregulation to escape from immune surveillance through MUC2's protective function. Additionally, following the loss of cell polarity during MCC tumorigenesis, mucins are expressed all over the cell surface and become available to interplay with several growth factor receptors to modulate their downstream signaling.

CRAD stabilizes the cadherin-catenin-actin filament (CCA) complex (40), which means control of cell adhesion and Wnt/β-catenin signaling by CRAD. We have already discussed the role of mucin in cell adhesion and Wnt/β-catenin signaling. The destabilized CCA complex disrupts epithelial cell polarity, which would trigger an inflammatory response and cell proliferation. It is plausible that abnormal polarity, inflammation, and Wnt/β-catenin signaling might foster an oncogenic environment for MCC via elevated mucins. It still unclear how inactivated CRAD increases mucins, but it is clear that a GEMM in which several mucins are simultaneously overexpressed would helpful for future MCC research and the development of anti-MCC therapeutic strategies.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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