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MUC1-C ACTIVATES POLYCOMB REPRESSIVE COMPLEXES AND DOWNREGULATES TUMOR SUPPRESSOR GENES IN HUMAN CANCER CELLS

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Summary

The PRC2 and PRC1 complexes are aberrantly expressed in human cancers that have been linked with decreases in patient survival. MUC1-C is an oncoprotein that is overexpressed in diverse human cancers and is associated with a poor prognosis. Recent studies have supported a previously unreported function for MUC1-C in activating PRC2 and PRC1 in cancer cells. In the regulation of PRC2, MUC1-C (i) drives transcription of the *EZH2* gene, (ii) binds directly to EZH2, and (iii) enhances occupancy of EZH2 on target gene promoters with an increase in H3K27 trimethylation. Regarding PRC1, which is recruited to PRC2 sites in the hierarchical model, MUC1-C induces *BMI1* transcription, forms a complex with BMI1, and promotes H2A ubiquitylation. MUC1-C thereby contributes to the integration of PRC2- and PRC1-mediated repression of tumor suppressor genes, such as *CDH1*, *CDKN2A, PTEN* and *BRCA1.* Like PRC2 and PRC1, MUC1-C is associated with the epithelial-mesenchymal transition (EMT) program, the cancer stem cell (CSC) state, and the acquisition of anticancer drug resistance. In concert with these observations, targeting MUC1-C downregulates EZH2 and BMI1, inhibits EMT and the CSC state, and reverses drug resistance. These findings emphasize the significance of MUC1-C as a therapeutic target for inhibiting aberrant PRC function and reprogramming the epigenome in human cancers.

Keywords

MUC1-C; PRC2; PRC1; epigenome; EMT; CSC; DNA repair

Polycomb complexes and repression of gene expression

The Polycomb Group (PcG) proteins constitute Polycomb Repressive Complexes (PRCs; PRC1, PRC2) that function as epigenetic suppressors of gene expression in cell fate, development and cancer [1–3]. The PRCs are recruited throughout the genome by transcription factors and non-coding RNAs, and at sites with CpG islands [4, 5]. Components of PRC2 are

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EZH2, SUZ12 and EED, among others [6]. EZH2 is a histone methyltransferase, which in association with SUZ12 and EED, catalyzes the mono-, di- and tri-methylation of histone H3 on K27 (H3K27me1, H3K27me2 and H3K27me3) and thereby the repression of target genes [7]. In the canonical hierarchical model, H3K27me3 marks function as sites for the recruitment of PRC1, such that PRC2 and PRC1 co-localize with the resulting maintenance of chromatin in a transcriptionally suppressed state [4]. BMI1 is a component of PRC1, which in concert with RING1 binds to the catalytic RING 2 subunit to form a ubiquitin E3 ligase [4, 8]. In this way, PRC1 catalyzes the ubiquitylation of histone H2A and confers silencing of *homeobox (HOX)* genes and the *CDNK2A* locus, which encodes the $p16^{INK4a}$ and $p14^{ARF}$ tumor suppressors [8–10]. In addition to the recruitment of PRC1 to sites of H3K27 trimethylation, PRC2 interacts with DNA methyltransferases (DNMTs) and directly controls DNA methylation with the downregulation of tumor suppressor genes (TSGs), including *CDH1* [11–13]. PRC2 thus integrates H3K27 trimethylation with recruitment of PRC1 and DNMTs in a hierarchical program of epigenetic gene silencing. Of note, the canonical model is likely an oversimplification given the complexities of PRC interactions that are cell context dependent [3, 4].

Aberrant expression of PcG proteins in cancer

EZH2 is overexpressed in human tumors and promotes the proliferation of transformed human cells [14]. Increases in EZH2 have been associated with aggressive breast cancers with a poor prognosis [15–19]. Overexpression of EZH2 has also been linked to poor outcomes for patients with non-small cell lung cancer (NSCLC), prostate cancer and other types of carcinomas [20–26]. Like EZH2, BMI1 is overexpressed in breast, lung and other carcinomas, and is associated with poor survival outcomes [8, 27–29]. In this context, BMI1 induces a gene signature that is associated with highly invasive tumors which are resistant to treatment [30]. Based on these findings, EZH2 and BMI1 have emerged as attractive targets for cancer treatment. Tazemetostat, CPI-1205 and GSK2816126 are SAM-competitive inhibitors of EZH2, which are presently in early stages of evaluation for the treatment of different cancer types [26, 31]. PTC-209 is an inhibitor of BMI1 expression that is active preclinically against colorectal and lung adenocarcinomas [32, 33], but has yet to undergo clinical evaluation. Another potential approach for inhibiting EZH2, BMI1 or other essential PRC components is to target upstream effectors that contribute to their aberrant expression in cancer. Along these lines, E2F and MYC have been identified as activators of *EZH2* and *BMI1* transcription, respectively [14, 34]. Moreover, recent studies have shown that the oncogenic MUC1-C protein drives *EZH2* [35] and *BMI1* [36] expression and thereby contributes to their regulation of the epigenome in human cancer cells.

MUC1-C transduces stress signals from the cell membrane to the nucleus

Mucin 1 (*MUC1*) emerged in mammals to protect the integrity of polarized epithelia from stress at the interface with the external environment [37, 38]. Implicit to our understanding of *MUC1* is that it encodes two subunits in epithelial cells; an extracellular N-terminal subunit that contributes to a physical mucous barrier at apical borders, and a C-terminal transmembrane subunit (MUC1-C) that activates stress responses for repair, proliferation and survival of the critical epithelial cell layer [37, 39]. *MUC1* is overexpressed in diverse

human carcinomas [37, 39]. Moreover, the initial finding that the MUC1-C subunit induces transformation [40] provided the basis for defining how MUC1-C functions as an oncoprotein [39, 41]. MUC1-C interacts with receptor tyrosine kinases (RTKs), such as EGFR [42, 43], FGFR3 [44], PDGFR [45], MET [46] and HER2 [47] and, in certain settings, activates the downstream AKT and ERK signaling pathways [39, 41]. As one example, MUC1-C→AKT signaling increases glucose uptake, lactate production and pyruvate kinase M2 activity [48], findings in concert with the stimulation of glycolysis. In addition, MUC1-C→AKT signaling upregulates the TIGAR protein, providing further evidence that MUC1-C controls the glycolytic and pentose phosphate pathways [49–51]. MUC1-C also interacts with the cell membrane xCT light chain of the cysteine/glutamate transporter and thereby contributes to the dependency of cancer cells on glutamine metabolism [52, 53].

In addition to its impact on cell membrane signaling, MUC1-C is imported to the nucleus [54], where it associates with multiple transacting factors, including β-catenin/TCF4 [55, 56], p53 [57], NF-κB p65 [58], STAT1/3 [59, 60] and HIF-1α [61, 62]. In this respect, MUC1-C activates gene signatures associated with tumorigenesis, which are significantly predictive of clinical outcomes [63, 64]. MUC1-C induces gene signatures linked to metabolic reprogramming [53, 61, 65]. The interaction between MUC1-C and NF-κB is of interest in that MUC1-C activates the proinflammatory TAK1→IKK→NF-κB p65 pathway, binds directly to these effectors and promotes the induction of NF-κB target genes [58, 66, 67]. For example, MUC1-C associates with NF-κB p65 on the *ZEB1* promoter with the induction of *ZEB1* transcription [68]. MUC1-C also binds directly to ZEB1 and contributes to the function of ZEB1 as an EMT-inducing transcription factor by suppressing *miR-200c* expression [68]. These direct interactions with transcription factors and the initial observations that MUC1-C recruits the p300 histone acetyltransferase to target gene promoters [56, 69] supported the notion that this oncoprotein plays a role in reprogramming the epigenomes of cancer cells.

MUC1-C activates EZH2 expression and PRC2 function

Aberrant EZH2 expression has been linked to breast and other types of carcinomas [7]. Downregulating MUC1-C with genetic and pharmacologic approaches in triple-negative breast cancer (TNBC) cells results in the suppression of EZH2 expression and, interestingly, that of SUZ12 and EED, demonstrating that MUC1-C induces multiple components of the PRC2 complex (Fig. 1A) [35]. MUC1-C is also necessary for EZH2 expression in NSCLC and prostate cancer cells, indicating that this MUC1-C function is broadly applicable to different types of carcinomas. MUC1-C has been associated with the activation of CDK4 and phosphorylation of pRB [69]. In this way, MUC1-C induces *EZH2* transcription by a pRB→E2F-mediated mechanism (Fig. 1B) [35]. MUC1-C also enhances *EZH2* transcription by associating with NF-κB p65 on NF-κB consensus sites in the *EZH2* intron 1 enhancer region (Fig. 1B). Interestingly, MUC1-C-induced *EZH2* expression is mediated by both E2F and NF-κB p65 [35]. The *EZH2* promoter and enhancer regions include CpG islands with CTCF binding motifs that may contribute to a promoter-enhancer loop structure in an insulated neighborhood (Fig. 1B). MUC1-C also activates (i) *SUZ12* by a mechanism involving E2F and NF-κB, and (ii) *EED* by E2F, but not NF-κB (Fig. 1C). Of further interest, the MUC1-C cytoplasmic domain interacts directly with EZH2 through binding to the EZH2 CXC region adjacent to the catalytic SET domain (Figs. 2A and 2B). The

functional significance of MUC1-C binding to EZH2 is supported by the demonstration that MUC1-C increases *CDH1* promoter H3K27 trimethylation in concert with repression of E-cadherin expression (Fig. 2C), a hallmark for passage through the EMT program. Breast cancer cells that overexpress EZH2 tend to have associated EMT gene signatures and phenotypic characteristics of cell invasion and metastases [17, 70]. Aberrant EZH2 expression has also been linked to BRCA1 downregulation and DNA repair defects [71, 72]. Importantly, the MUC1-C→EZH2 pathway is upstream to repression of the *BRCA1* and *RAD51* genes, which encode essential components of the homologous recombination (HR) DNA repair pathway [35]. These findings and the suppression of other DNA damage response pathways, including non-homologous end-joining (NHEJ), have provided new insights into potential a potential role for MUC1-C in promoting genomic instability of cancer cells [35].

MUC1-C integrates PRC2 with PRC1 and DNA methylation

In the hierarchical model described above, a CBX-containing protein in PRC1 binds to the PRC2-mediated H3K27me3 mark with recruitment of PRC1 at PRC2-specified target sites [3, 7]. As found for PRC2, MUC1-C also induces expression of the PRC1 components, BMI1, RING1 and RING2 [36]. MUC1-C interacts with the β-catenin/TCF4 pathway [55, 56, 73] and drives the WNT target gene, *MYC* [69, 74]. In turn, MYC activates *BMI1* and *RING2* (Figs. 3A and 3B)[36, 75]. In contrast, MUC1-C→NF-κB p65 signaling induces *RING1* expression (Figs. 3A and 3B) [36, 75]. Interestingly, and as reported for EZH2, the MUC1-C cytoplasmic domain interacts directly with BMI1 [36]. In addition, MUC1-C promotes H2A ubiquitylation, downregulation of *HOX* genes, and BMI1 occupancy on the *CDKN2A* promoter, supporting a direct role in repressing BMI1 target genes (Fig. 3C). Consistent with this paradigm, targeting MUC1-C induces the $p16^{INK4a}$ tumor suppressor [36]. These findings collectively support the premise that MUC1-C represses TSGs by activating and integrating PRC2 and PRC1 functions.

PcG-mediated repression of gene expression has been linked to the DNA methylation process [11, 76]. Specifically, EZH2 interacts with DNMTs and is necessary for methylation of EZH2-target gene promoters [11]. The H3K27me3 mark recruits DNMTs to CpG islands, leading to DNA methylation [12]. Of potential relevance here is that MUC1-C→NF-κB p65 signaling activates the *DNMT1* and *DNMT3b* genes in human cancer cells [77, 78]. Targeting MUC1-C also induces changes in DNA methylation patterns in concert with depression of the *CDH1, PTEN* and *BRCA1* TSGs [77, 78]. These findings, when taken with the demonstration that MUC1-C activates EZH2, hold potentially important implications for MUC1-C involvement in integrating the PRC and DNA methylation systems in repression of TSG expression (Fig. 4).

MUC1-C activates PRCs in concert with induction of the EMT program

A considerable body of evidence has supported the premise that epigenetic regulatory mechanisms control epithelial-mesenchymal plasticity in cancer [79]. For instance, aberrant EZH2 expression promotes EMT, invasion and metastasis in diverse carcinomas [31]. As a result, targeting EZH2 with an inhibitor downregulates EMT signaling [80]. MUC1-C drives EZH2 expression, and increases EZH2 occupancy and H3K27 trimethylation on the *CDH1*

promoter [35]. These findings and the role of MUC1-C in suppressing E-cadherin expression have given traction for the concept that MUC1-C could integrate PRC2 function with induction of the EMT program [35]. Along these lines, MUC1-C induces EMT by activating the inflammatory TAK1→IKK→NF-κB pathway, which in turn drives *ZEB1* and repression of the ZEB1-target gene *miR-200c* [68]. The MUC1-C/ZEB1 interaction has also been associated with repression of the *CDH1* gene, and downregulation of genes encoding cell polarity factors, such as CRB3, necessary for apical-basal polarity [68, 81]. Moreover and in concert with induction of EZH2 and EMT, MUC1-C has been widely linked to cancers with more aggressive, invasive and metastatic phenotypes [37, 39].

Of additional interest, EZH2 and the EMT program are closely associated with the cancer stem cell (CSC) state by presently unclear mechanisms [82–84]. Indeed, the role of MUC1-C in activating PRC2 and PRC1 may provide new insights into this association. EZH2 is linked to EMT, invasion and metastases [31]. Additionally, aberrant BMI1 expression promotes self-renewal and tumorigenic potential of CSCs [8, 32, 85, 86]. BMI1 has also been linked to EMT induction [8, 87, 88], and EZH2 to the CSC state [84], further strengthening the associations among PRCs, EMT and CSCs. Along these lines, MUC1-C signaling could contribute to the integration of PRCs, EMT and the CSC state. As such, in a simplified model, MUC1-C-induced PRC2 activation contributes to EMT induction of EMT and recruitment of PRC1 to PRC2 target sites. In turn, MUC1-C-induced activation of PRC1 promotes acquisition of the CSC state. In support of such a model, targeting MUC1-C in cancer cells results in downregulation of PRCs, reversal of the EMT phenotype and decreases in self-renewal capacity [68, 81, 89, 90].

MUC1-C is a target for inhibiting immune evasion in cancer

The EMT program has been linked to the induction of programmed death ligand 1 (PD-L1) expression and adverse clinical outcomes in patients with breast, lung and other types of cancers [91–93], suggesting that immune evasion promotes an invasive and metastatic phenotype. Immune evasion of tumors has also been associated with EZH2-mediated suppression of chemokines and effector T-cell recruitment [94, 95]. Accordingly, the involvement of MUC1-C in inducing EMT and *EZH2* invoked a possible role in immune evasion. Indeed, recent work has shown that MUC1-C→NF-κB signaling, which drives EZH2 [35], EMT [68], and the CSC state of self-renewal [81, 89, 90], also induces the *CD274* gene and PD-L1 expression [96]. In addition, the MUC1-C→NF-κB→ZEB1 pathway represses effectors of the immune response, including IFNγ and GM-CSF [96]. Inhibiting MUC1-C function with GO-203 thus results in downregulation of PD-L1, induction of IFNγ, and activation of anti-tumor immunity in an immune competent MUC1 transgenic mouse model [97]. MUC1-C also protects cancer cells from killing by TRAIL, FAS ligand and perforin/granzyme B-mediated lysis [98, 99]. These findings hold important implications for MUC1-C in the accumulating evidence that EMT and CSCs are associated with the induction of PD-L1 and other events linked to immune evasion [92, 100, 101].

MUC1-C confers anticancer drug resistance

The association between EMT and the CSC state has also been linked to development of drug resistance by mechanisms that have been largely unexplored [83, 102]. MUC1-C contributes to the development of resistance to cytotoxic [62, 103] and targeted [47, 90, 104] agents. How MUC1-C has the capacity to promote such pleiotropic mechanisms for anticancer drug resistance is in part related to activation of multidrug resistance (MDR) genes, including *ABCB1*, which encodes the P-glycoprotein [105]. MUC1-C-induced drug resistance could also be related to epigenetic regulation of genes that confer the resistant phenotype, and/or a previously unrecognized effect on genomic instability that selects for cell populations refractory to drug treatment. Of potential importance for this paradigm is the finding that the MUC1-C→EZH2 pathway suppresses expression of BRCA1, which functions in cell cycle checkpoint activation and DNA repair, and RAD51, which directs homologous strand exchange [35, 106]. DSBs are repaired by HR or by the more error-prone NHEJ pathway. With a defective HR pathway, for example in the setting of MUC1-C→EZH2-mediated BRCA1 and RAD51 suppression, insufficient repair of DSBs could result in genomic instability and thereby the selection of cells with anticancer drug resistance.

The repair of DSBs is facilitated by BMI1-mediated ubiquitylation of H2A and γH2AX [107]. BMI1-induced suppression of DSB-induced CHK1 and CHK2 activation has further implicated PRC1 in promoting genomic instability and transformation [107]. Thus, the role of MUC1-C in activating *BMI1* and suppressing *CDKN2/p16*^{INK4a} expression could thereby enhance MUC1-C-mediated genomic instability [36]. Additionally, RNA-seq studies have demonstrated that MUC1-C suppresses multiple effectors of DNA damage response (DDR) pathways, including HR, NHEJ, mismatch repair and transcription-coupled repair, among others [35]. How MUC1-C regulates these additional genes and whether there is involvement of the MUC1-C→EZH2 or MUC1-C→BMI1 pathways is not presently known. Nonetheless, these observations highlight the potential importance of MUC1-C function in integrating histone modifications, genomic instability and the propensity for acquiring anticancer drug resistance.

MUC1-C is a highly promising target for cancer treatment

Emerging evidence has thus demonstrated that MUC1-C promotes hallmarks of the cancer cell, including epigenetic regulation, EMT, the CSC state, immune evasion and anticancer drug resistance. Accordingly, MUC1-C has emerged a promising target for cancer therapy. However, to date, there are no approved agents that target MUC1-C function. This situation derives in part from the undruggable nature of this target. The MUC1-C cytoplasmic domain is devoid of a kinase function and is an intrinsically disordered protein [108], a characteristic that provides for interactions with multiple signaling pathways [109], but represents a challenge for drug development. Despite this hurdle, a CQC motif in the MUC1-C cytoplasmic domain is necessary for MUC1-C homodimerization, nuclear localization and oncogenic function, and is the Achilles' heel of this oncoprotein [37, 39]. Thus, MUC1-C with a CQC→AQA mutation functions as a dominant-negative for transformation [110]. In addition, blocking the MUC1-C CQC motif with the cell-penetrating GO-203 peptide

inhibits MUC1-C function [39]. Thus, in concert with targeting MUC1-C by genetic approaches, treatment with GO-203 inhibits MUC1-C-induced EZH2 and BMI1 expression [35, 36], EMT signaling [68, 81] and self-renewal capacity [81, 89, 90]. Significantly, GO-203 treatment of cancer cells is also synergistic with cytotoxic drugs [111] and reverses acquired resistance to targeted agents [47, 90, 104], consistent with the premise that MUC1-C confers pleotropic drug-resistant phenotypes.

The findings that MUC1-C function is blocked by GO-203 provided the experimental basis for the clinical evaluation of this agent. A Phase I trial of GO-203 in patients with advanced carcinomas demonstrated an acceptable safety profile and early evidence of anti-tumor activity. Pharmacokinetic studies further demonstrated a circulating GO-203 half-life of 5–7 h that necessitated daily delivery to maintain drug levels in a therapeutic range. Accordingly, GO-203 has been developed in a nanoparticle formulation for less frequent and more sustained delivery in the clinic [112]. In parallel and based on the findings that (i) MUC1 is expressed by AML stem-like cells [113], and (ii) GO-203 is highly synergistic with the DNA methylation inhibitor decitabine [78], a Phase II trial of GO-203 in combination with decitabine is now underway for the treatment of patients with relapsed/refractory AML (ClinicalTrials.gov Identifier: NCT02204085).

MUC1 has also emerged as an attractive target for the immunotherapy of cancer. In this regard, vaccines targeting MUC1 for the treatment of malignancies, including NSCLC and breast cancer, have been advanced to late-stage clinical trials, but have had limited success to date [114, 115]. Indeed, a challenge for an effective anti-cancer MUC1 vaccine is overcoming tolerance to MUC1, which is widely expressed by normal epithelial cells. In the course of addressing this challenge, a dendritic cell (DC)-based vaccine was found to be effective in reversing tolerance to MUC1 in a MUC1 transgenic mouse background, and eradicating MUC1-positive tumors in the absence of autoimmunity against normal tissues [116]. This DC-based vaccine is also effective in reversing tolerance to MUC1 in patients with solid tumors and the hematologic malignancies, multiple myeloma and AML [117, 118]. In addition, the findings that induction of anti-MUC1 immunity is associated with anti-tumor activity [117, 118] provided the basis for advancing this vaccine to national multi-center Phase II trials (ClinicalTrials.gov Identifiers: NCT02728102 and NCT03059485). Moreover, the finding that targeting MUC1-C with GO-203 in cancer cells inhibits immune evasion also provides the experimental rationale for combining GO-203 treatment with the above DC-based or other anti-cancer vaccines.

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Abbreviations

MUC1	mucin 1
MUC1-C	MUC1 C-terminal subunit

PcG	Polycomb Group
PRC	Polycomb Repressive Complex
EZH2	enhancer of zeste homolog 2
SUZ12	the suppressor of zeste 12 homolog
EED	embryonic ectoderm development
BMI1	B-cell-specific Moloney murine leukemia virus integration site 1 protein
HOX	homeobox
DNMT	DNA methyltransferase
TSG	tumor suppressor gene
RTK	receptor tyrosine kinase
EMT	epithelial-mesenchymal transition
CSC	cancer stem cell
CTCF	CCCTC-binding factor
DDR	DNA damage response
DSB	DNA double strand break
HR	homologous recombination
NHEJ	non-homologous end-joining
IDR	intrinsically disordered region

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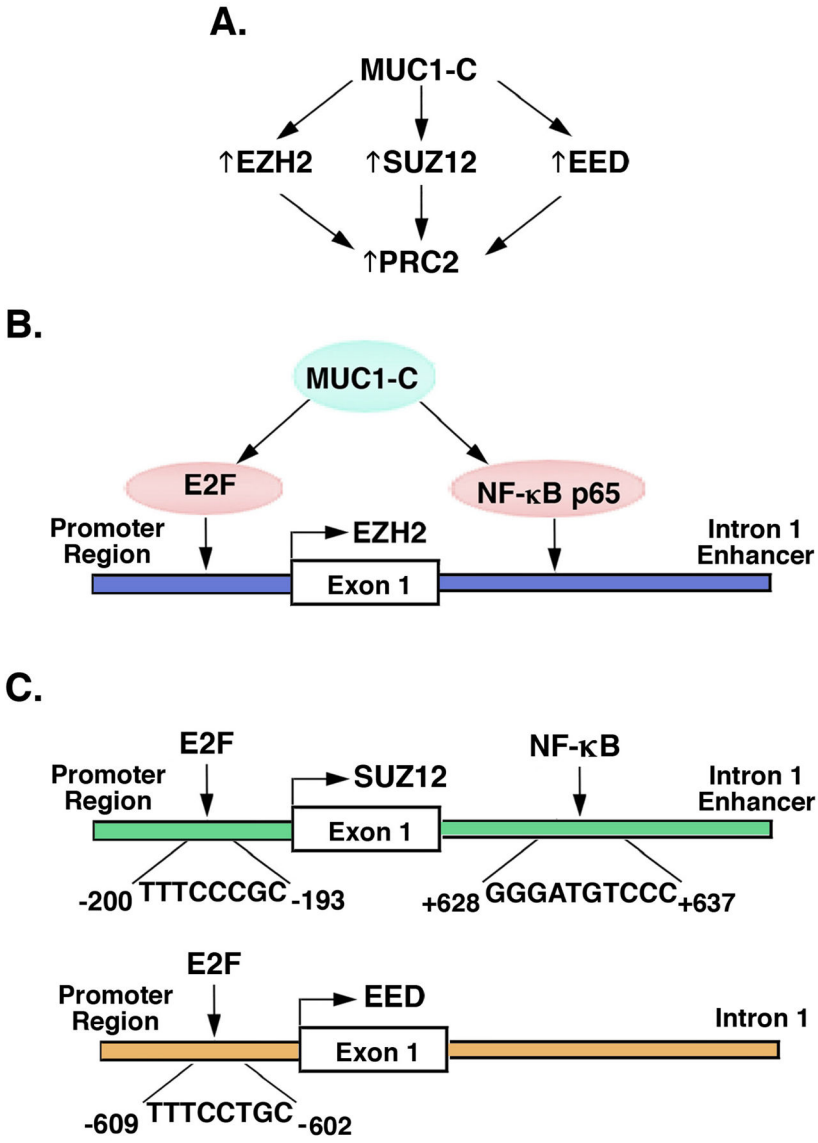

Figure 1. MUC1-C induces expression of PRC2 components, EZH2, SUZ12 and EED

A. Targeting MUC1-C genetically or pharmacologically with the GO-203 inhibitor downregulates EZH2, SUZ12 and EED expression in TNBC, NSCLC and prostate cancer cells, demonstrating that MUC1-C induces multiple PRC2 components. B. MUC1-C drives *EZH2* transcription by two mechanisms; (i) E2F-mediated activation of the *EZH2* promoter, and (ii) binding of NF-κB p65 complexes to consensus sites in the *EZH2* intron 1 enhancer region [35]. The *EZH2* promoter and enhancer regions include CpG islands (−1046 to −56; +161 to +914) [119] and CTCF binding sites (−603 to −598, −468 to −463; +292 to +297, +702 to +707) for forming a potential loop structure by the CTCF-cohesin complex. C. MUC1-C also activates *SUZ12* and *EED* transcription by E2F, in concert with previous work [14, 120], and by NF-κB p65 (unpublished data). MUC1-C thereby integrates EZH2, SUZ12 and EED expression by E2F signaling and by the inflammatory NF-κB pathway.

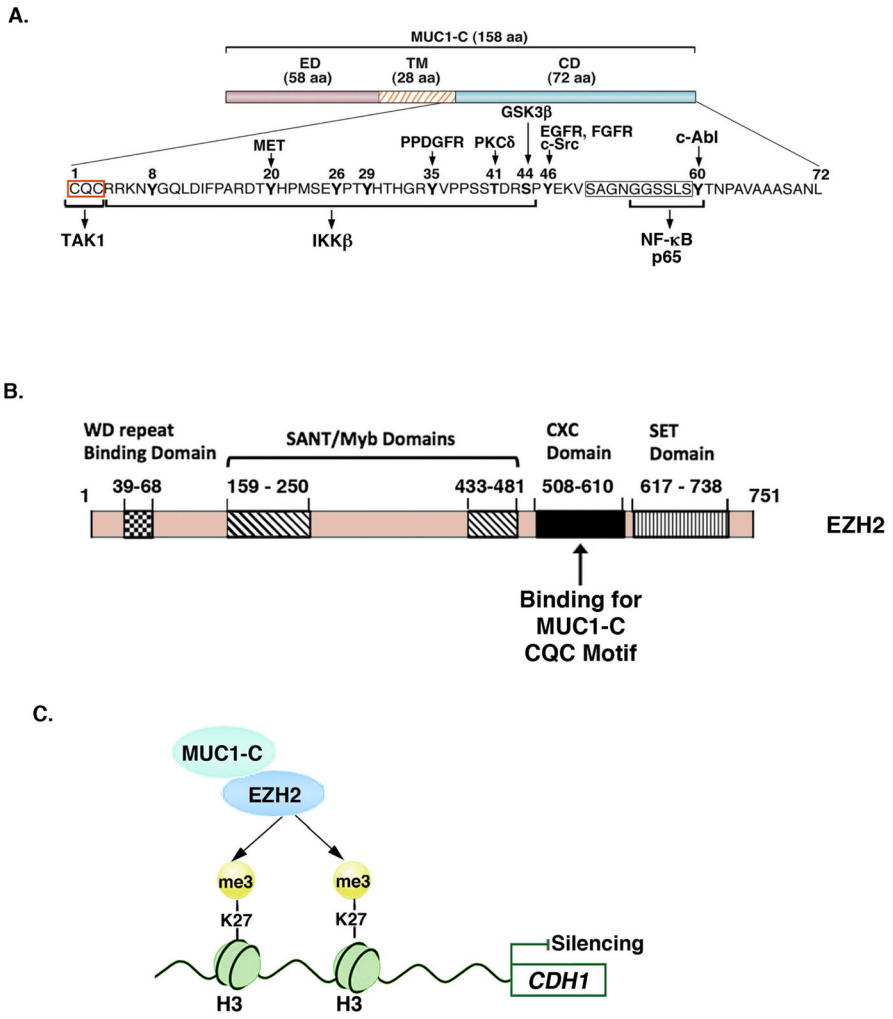

Figure 2. MUC1-C binds directly to EZH2 and promotes EZH2-induced H3K27 trimethylation

A. Structure of the MUC1-C subunit, which includes a 58-aa extracellular domain (ED) and a 28-aa transmembrane domain (TM). The MUC1-C 72-aa cytoplasmic domain (CD) includes a CQC motif located immediately following the TM region that is necessary for MUC1-C homodimerization in the response to oxidative stress and for nuclear import [39, 54, 108]. The CQC motif is the target for the GO-203 inhibitor. The remainder of the MUC1-C cytoplasmic domain is an intrinsically disordered region (IDR). IDRs have been identified in other oncoproteins, such as p53, that function as nodes for the integration of signaling cascades and are also prevalent in transcription factors and the transcriptional coactivators, p300 and CBP [109, 121]. The MUC1-C cytoplasmic domain IDR is modified by diverse kinases and, as highlighted, interacts directly with multiple effectors of the inflammatory NF-κB p65 pathway [39, 108]. The MUC1-C cytoplasmic domain CQC and the SAGNGGSSLS (boxed) motifs also bind directly to TCF4 and β-catenin, respectively, with activation of the WNT pathway. B. Schema of the EZH2 protein and the indicated domains. The MUC1-C CQC motif binds directly to the EZH2 CXC domain [35]. C. MUC1-C forms a complex with EZH2, enhances EZH2 occupancy on the *CDH1* promoter, and thereby increases H3K27 trimethylation with repression of E-cadherin expression [35].

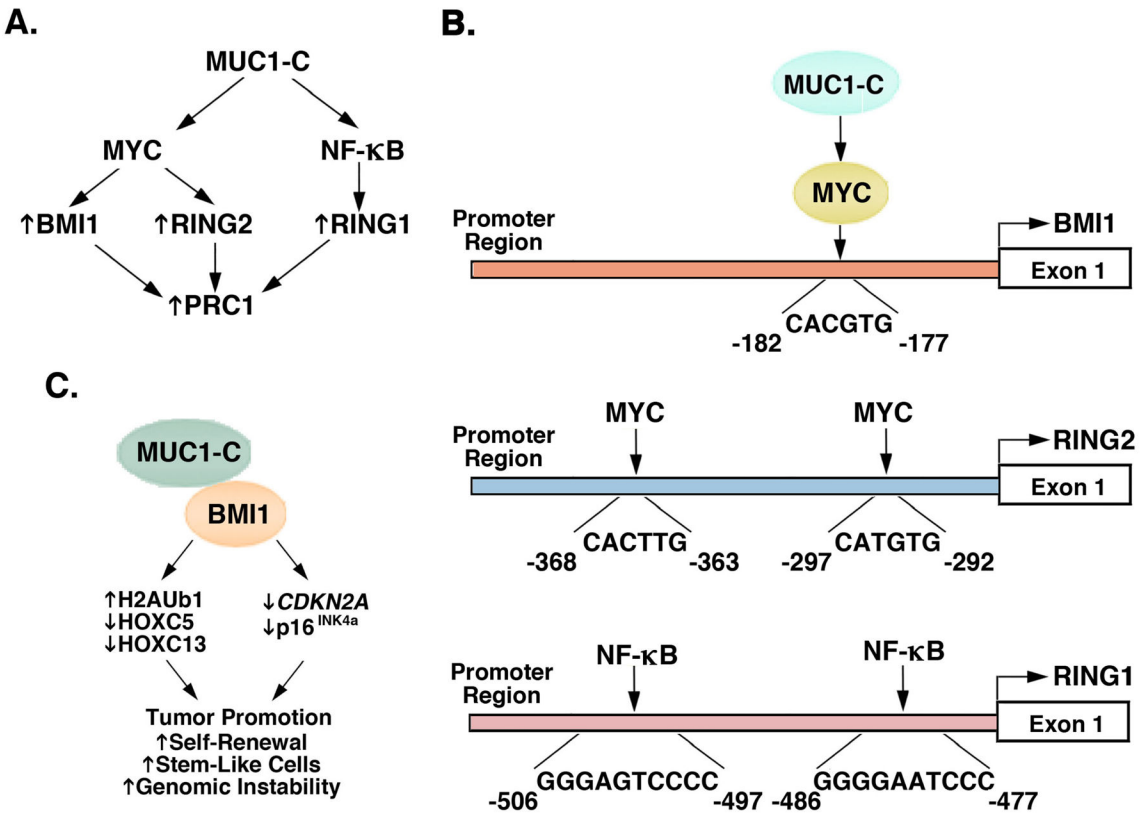

Figure 3. MUC1-C drives expression of PRC1 components, BMI1, RING1 and RING2

A. MUC1-C activates (i) *BMI1* and *RING2* by MYC-mediated mechanisms, and (ii) *RING1* through the NF-κB p65 pathway [36]. Thus, targeting MUC1-C is associated with downregulation of BMI1, RING2 and RING1 expression in TNBC and NSCLC cells [36]. B. Schemas of the *BMI1, RING2 and RING1* promoters with highlighting of the MYC and NF-κB binding sites. C. MUC1-C binds directly to BMI1 by an interaction dependent of the MUC1-C CQC motif [36]. Complexes of MUC1-C and BMI1 have been detected on the *CDKN2A* promoter [36]. In support of the depiction, targeting MUC1-C genetically or with the GO-203 inhibitor (i) decreases H2A ubiquitylation, (ii) increases HOXC5 and HOXC13 expression, and (iii) activates *CDKN2A* and expression of $p16^{INK4a}$ [36]. The findings thus support a role for MUC1-C in contributing to BMI1-driven tumor promotion, self-renewal capacity, the CSC state, and genomic instability.

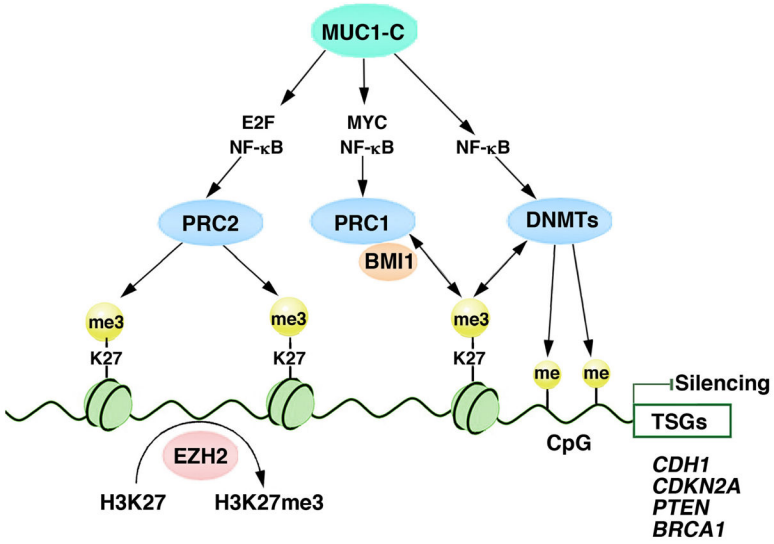

Figure 4. Schema of the proposed model in which MUC1-C integrates functions of PRC2, PRC1 and DNA methylation in the repression of tumor suppressor genes

In this model, MUC1-C induces expression of the PRC2 components, EZH2, SUZ12 and EED, and thereby drives H3K27 trimethylation on target gene promoters [35]. In addition, MUC1-C induces expression of the PRC1 components, BMI1, RING2 and RING1, and in turn the potential recruitment of PRC1 to H3K27me3 sites [36]. In addition, MUC1-C activates DNMT1 and DNMT3b expression and changes in DNA methylation patterns [77]. EZH2 and the H3K27me3 mark recruit DNMTs, leading to DNA methylation [11, 12]. In concert with this model of gene repression, targeting MUC1-C with the downregulation of EZH2 [35], BMI1 [36, 75], and DNMT1/3b [77] is associated with induction of the target *CDH1, CDKN2A, PTEN* and *BRCA1* TSGs.