Nuclear Import of the MUC1-C Oncoprotein Is Mediated by Nucleoporin Nup62*

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The MUC1 heterodimeric transmembrane protein is aberrantly overexpressed by most human carcinomas. The MUC1 C-terminal subunit (MUC1-C) is devoid of a classical nuclear localization signal and is targeted to the nucleus by an unknown mechanism. The present results demonstrate that MUC1-C associates with importin β and not importin α. The results also show that, like importin β, MUC1-C binds to Nup62 (nucleoporin p62). MUC1-C binds directly to the Nup62 central domain and indirectly to the Nup62 C-terminal α-helical coiled-coil domain. We demonstrate that MUC1-C forms oligomers and that oligomerization is necessary for binding to Nup62. The MUC1-C cytoplasmic domain contains a CQC motif that when mutated to AQA abrogates oligomerization and binding to Nup62. Stable expression of MUC1 with the CQC → AQA mutations was associated with targeting to the cell membrane and cytosol and attenuation of nuclear localization. The results further show that expression of MUC1(CQC-AQA) attenuates MUC1-induced (i) transcriptional coactivation, (ii) anchorage-independent growth, and (iii) tumorigenicity. These findings indicate that the MUC1-C oncoprotein is imported to the nucleus by a pathway involving Nup62.

The human MUC1 heterodimeric glycoprotein is expressed on the apical borders of normal secretory epithelial cells (1). The MUC1 protein is translated as a single polypeptide and is cleaved into N- and C-terminal subunits in the endoplasmic reticulum (2–4). The MUC1 N-terminal subunit (MUC1-N) contains a signal sequence for cell membrane localization and includes variable numbers of conserved 20-amino acid tandem repeats (5, 6). The tandem repeats are extensively modified by O-linked glycans that contribute to a structure that extends beyond the glyocalyx of the cell. MUC1-N is tethered to the cell membrane as a heterodimer with the MUC1 C-terminal subunit (MUC1-C), which includes a 58-amino acid extracellular domain, a 28-amino acid transmembrane domain, and a 72-amino acid cytoplasmic tail (7). Aberrant overexpression of MUC1, as found in most human carcinomas (1), confers anchorage-independent growth and tumorigenicity (8–11). Other studies have demonstrated that overexpression of MUC1 confers resistance to apoptosis induced by oxidative stress and genotoxic anti-cancer agents (12–17).

The family of tethered and secreted mucins functions in providing a protective barrier of the epithelial cell surface. With damage to the epithelial layer, the tight junctions between neighboring cells are disrupted, and polarity is lost as the cells initiate a heregulin-induced repair program (18). MUC1-N is shed from the cell surface (19), leaving MUC1-C to function as a transducer of environmental stress signals to the interior of the cell. In this regard, MUC1-C forms cell surface complexes with members of the ErbB receptor family, and MUC1-C is targeted to the nucleus in the response to heregulin stimulation (20, 21). MUC1-C also functions in integrating the ErbB receptor and Wnt signaling pathways through direct interactions between the MUC1 cytoplasmic domain (CD) and members of the catenin family (11, 21–25). Other studies have demonstrated that MUC1-CD is phosphorylated by glycogen synthase kinase 3β, c-Src, protein kinase Cδ, and c-Abl (16, 23, 24, 26).

The mechanisms responsible for nuclear targeting of MUC1-C are unclear. Proteins containing a classical nuclear localization signal (NLS) are imported into the nucleus by first binding to importin α and then, in turn, importin β (27, 28). The cargo-importin α/β complex docks to the nuclear pore by binding to nucleoporins and is transported through the pore by a mechanism dependent on the Ran GTPase. Classical NLSs are monopartite with a single cluster of 4–5 basic amino acids or bipartite with two clusters of basic amino acids separated by a linker of 10–12 amino acids. MUC1-CD contains a RRK motif that does not conform to a prototypical monopartite NLS (29). However, certain proteins containing nonclassical NLSs are transported through the nuclear pore by binding directly to importin β (30). Importin β associates with several nucleoporins (31), including Nup62, which is located on both the cytoplasmic and nucleoplasmic faces of nuclear pore complexes (32). Other studies have indicated that β-catenin is imported into the nucleus by an importin- and nucleoporin-independent mechanism (33).

The present results demonstrate that MUC1 is imported into the nucleus by a mechanism involving binding to Nup62. We also demonstrate that MUC1 forms oligomers through a CQC motif in the MUC1 cytoplasmic domain and that MUC1 oligomerization is necessary for nuclear import.
MATERIALS AND METHODS

Cell Culture—Human ZR-75-1/vector and ZR-75-1/MUC1 small interfering RNA breast cancer cells (13) were grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum, 100 μg/ml streptomycin, 100 units/ml penicillin, and 2 mM L-glutamine. Human HCT116 colon carcinoma and 293 kidney cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated fetal bovine serum, 100 μg/ml streptomycin and 100 units/ml penicillin.

Immunoprecipitation and Immunoblot Analysis—Cell lysates were prepared as described (26). Soluble proteins were subjected to immunoprecipitation as described (26) with anti-importin α (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), anti-importin β (Santa Cruz Biotechnology), anti-Myc (Santa Cruz Biotechnology), or anti-Myc (Santa Cruz Biotechnology). Immunoprecipitates and soluble proteins were analyzed by immunoblotting with anti-MUC1-C (Ab1; NeoMarkers), anti-c-Abl (Santa Cruz Biotechnology), anti-importin α, anti-importin β, anti-Nup62, anti-green fluorescence protein (GFP; Clontech), anti-His (Invitrogen), anti-IkBa (Santa Cruz Biotechnology), anti-proliferating cell nuclear antigen (PCNA) (Upstate Biotechnology), anti-MUC1-N (monoclonal antibody DF3) (1), or anti-β-actin (Sigma). Reactivity was detected with horseradish peroxidase-conjugated secondary antibodies and chemiluminescence (PerkinElmer Life Sciences).

Vector Generation and Cell Transfection—Myc-tagged MUC1-CD (20) and Myc-tagged Nup62 were expressed by cloning into the pCMV-Myc vector (Clontech). Mutants were generated by site-directed mutagenesis and confirmed by sequencing. GFP-tagged MUC1-CD and its mutants were expressed by cloning into the pEGFP-C1 vector (Clontech). 293 cells were transfected with the expression vectors in the presence of Lipofectamine. Glutathione S-transferase (GST) fusion proteins were prepared by expression of pGEX4T1-based vectors in Escherichia coli BL21 (DE3). His-tagged MUC1-CD proteins were prepared as described (26). The EYFP-Nuc vector
was obtained from Clontech. The pIRESpuro2-MUC1(CQC-AQA) mutant was generated as described (20) and transfected into HCT116 cells with Lipofectamine. Stable clones were selected in puromycin (Calbiochem).

Binding Assays—Cell lysates were incubated with 5 μg of GST or GST fusion proteins bound to glutathione beads. After incubation for 2 h at 4 °C, the adsorbates were washed and then subjected to immunoblot analysis. An aliquot of total lysate (2%, v/v) was included as a control. For direct binding assays in vitro, purified GST proteins were incubated with purified His-tagged proteins in binding buffer (250 mM NaCl, 20 mM HEPES, pH 7.0, 5 mM EDTA, 0.5 mM dithiothreitol, and 0.1% Tween 20) for 1 h at room temperature. The adsorbates were analyzed by immunoblotting.

Cellular Fractionation—Nuclear and cytosolic fractions were prepared as described (25).

Luciferase Assays—Cells were transfected with pcyCD1(161)-luc (34) and β-galactosidase in the presence of Lipofectamine. Luciferase assays (Luciferase Assay System; Promega, Madison, WI) were performed at 48 h after transfection. Transfection efficiency was normalized by β-galactosidase expression.

Anchorage-independent Growth—Cells (1 × 10⁵) were suspended in 0.33% Noble agar (Difco) in Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated fetal bovine serum and antibiotics. The cell suspension was layered over 0.5% agar in medium in 100-mm dishes. Colonies of >10 cells were counted at 2 weeks.

Tumorigenicity Assays—Cells (1 × 10⁶) were injected subcutaneously into the flanks of 4–6-week old nude (nu/nu) mice. Tumor volumes were calculated from bidimensional measurements.

RESULTS

MUC1-C Associates with Nup62—To investigate mechanisms responsible for nuclear import of MUC1-C, we first asked if MUC1-C associates with importins in MUC1-positive and, as a control, MUC1-negative ZR-75-1 cells. Immunoblot analysis of anti-importin α immunoprecipitates with an antibody against the C terminus of MUC1 showed no detectable reactivity (Fig. 1A, left). As a control, the c-Abl protein, which contains an NLS, was detectable in complexes with importin α (Fig. 1A, left). When a similar analysis was performed on anti-
importin β immunoprecipitates, reactivity with the anti-MUC1-C antibody was detectable with 15–25-kDa proteins (Fig. 1A, right). The anti-MUC1-C signals detected in the anti-importin β immunoprecipitates corresponded to those obtained with lysates not subjected to immunoprecipitation, indicating that MUC1-C associates with importin β and not importin α. Importin β binds to the C-terminal domain of the nucleoporin p62 (Nup62) (32). Consequently, we asked if MUC1-C associates with Nup62. The results demonstrate that MUC1-C is detectable in anti-Nup62 immunoprecipitates (Fig. 1B). Using 293 cells that express MUC1 and Myc-tagged Nup62, the immunoblot analysis of anti-Nup62 immunoprecipitates with anti-MUC1-C confirmed the association of MUC1-C and Nup62 (Fig. 1C). In other studies, lysates from ZR-75-1 cells were incubated with GST or GST-Nup62. Analysis of the adsorbates with anti-MUC1-C demonstrated binding of MUC1-C to GST-Nup62 and not to GST (Fig. 1D). These findings indicate that MUC1-C associates with Nup62.

**MUC1-CD Associates with the Nup62 T-rich and C-terminal Coiled-coil Regions**—To localize the region of MUC1-C that associates with Nup62, 293 cells expressing a Myc-tagged MUC1-CD were incubated with GST or GST-Nup62. The results demonstrate that Nup62 binds to MUC1-CD (Fig. 2A, left). In the reciprocal experiment, lysates from 293 cells expressing Myc-Nup62 were incubated with GST or GST-MUC1-CD. Immunoblot analysis of the adsorbates with anti-Myc confirmed binding of Nup62 to MUC1-CD (Fig. 2A, right). To define the region of Nup62 involved in the association with MUC1-CD (Fig. 2B), 293 cells were transfected with vectors expressing the Nup62 N-terminal FXFG repeats, the T-rich linker region, and the C-terminal α-helical coiled-coil domain (Fig. 2B). Probing the lysates with GST-MUC1-CD demonstrated that MUC1-CD associates with Nup62-(178–327) and Nup62-(328–522) but not with Nup62-(1–177) (Fig. 2C). To confirm these observations, 293 cells expressing MUC1 and Myc-tagged full-length Nup62 or the deletion mutants were immunoprecipitated with anti-Myc or IgG. Immunoblot analysis of the precipitates with anti-MUC1-C confirmed binding of MUC1 to Nup62-(178–327) and Nup62-(328–522) and not to Nup62-(1–177) (Fig. 2D). These findings demonstrate that MUC1-CD associates with the Nup62 T-rich linker and C-terminal coiled-coil regions.

**MUC1-CD Forms Oligomers**—To assess binding of MUC1-CD to Nup62 in vitro, we purified a His-tagged MUC1-CD. Immunoblot analysis under reducing conditions showed that the ~10-kDa His-MUC1-CD is also detectable as ~20-kDa and higher molecular mass forms (Fig. 3A). Moreover, most of the His-MUC1-CD was found at ~20 kDa or higher under nonreducing conditions, indicating that MUC1-CD forms oligomers in vitro (Fig. 3A). A CQC motif is present at amino acids 1–3 of MUC1-CD (Fig. 2B, scheme). To determine if the cysteines are involved in the formation of MUC1-CD oligomers, we generated MUC1-CD with C-A mutations. Incubation of His-MUC1-CD with GST-MUC1-CD or GST-MUC1-CD(CQC-AQA) and immunoblot analysis of the adsorbates with anti-His demonstrated binding of His-MUC1-CD to GST-MUC1-CD and not GST-MUC1-CD(CQC-AQA) (Fig. 3B). To define the kinetics of oligomer formation, the parameters of His-MUC1-CD binding were determined using His-MUC1-CD immobilized to the sensor chip in a BIAcore. GST-MUC1-CD bound to His-MUC1-CD with a dissociation constant ($K_d$) of 33 nM (Fig. 3C). Substitution of His-MUC1-CD with His-MUC1-CD(CQC-AQA) showed little if any binding (data not shown). To investigate whether MUC1-CD forms oligomers in cells, we coexpressed Myc-MUC1-CD and GFP-MUC1-CD. Immunoblot analysis of anti-Myc immunoprecipitates with anti-GFP showed that Myc-MUC1-CD associates with GFP-MUC1-CD (Fig. 3D).
contrast, there was no detectable association when Myc-MUC1-CD was coexpressed with a GFP-MUC1-CD-(7–72) mutant deleted at the CQCRRK motif (Fig. 3D). There was also no detectable binding of Myc-MUC1-CD with GFP-MUC1-CD(CQC-AQA), indicating that the cysteines are essential for the interaction (Fig. 3D). These findings demonstrate that MUC1-CD forms oligomers in vitro and in cells by a mechanism dependent on the cysteine residues.

**MUC1-CD Binds Directly to Nup62**—Incubation of purified GST-Nup62 and purified His-MUC1-CD demonstrated direct binding of MUC1-CD to Nup62 (Fig. 4A, left). Notably, oligomeric MUC1-CD was detectable in the GST-Nup62 adsorbates (Fig. 4A, left). Moreover, there was no detectable binding of Myc-MUC1-CD with GFP-MUC1-CD(CQC-AQA), indicating that the cysteines are essential for the interaction (Fig. 3D). These findings demonstrate that MUC1-CD forms oligomers in vitro and in cells by a mechanism dependent on the cysteine residues.

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**Nuclear Import of MUC1 Involves Binding to Nup62**—To determine if the interaction with Nup62 contributes to nuclear import of MUC1, immunoblot analysis was performed on nuclear lysates. The results demonstrate the presence of GFP-MUC1-CD and little if any GFP-MUC1-CD-(CQC-AQA) (Fig. 5A). Equal loading and purity of the nuclear fractions were documented by immunoblotting for levels of nuclear PCNA and cytoplasmic IκBα (Fig. 5A). To determine if MUC1 is imported into the nucleus by binding to Nup62, Myc-Nup62-(178–328) was expressed as a decoy...
to interfere with the interaction between GFP-MUC1-CD and endogenous Nup62. Immunoblot analysis of nuclear lysates demonstrated that Nup62-(178–328) blocks nuclear localization of MUC1-CD (Fig. 5B). By contrast, nuclear localization of EYFP-Nuc, which contains an NLS, was unaffected by Nup62-(178–328) expression (Fig. 5C). These findings indicate that MUC1-CD is imported into the nucleus by interacting with Nup62.

Stable Expression of Full-length MUC1(CQC-AQA) Attenuates Binding to Nup62 and Nuclear Localization—To further assess involvement of the MUC1 CQC motif in subcellular localization, MUC1-negative HCT116 carcinoma cells were stably transfected to express the empty vector, wild-type MUC1, or the MUC1(CQC-AQA) mutant. As shown previously (9, 13), expression of MUC1-C was detectable in HCT116/MUC1, but not HCT116/vector, cells (Fig. 6A, left). MUC1-C expression was also detectable in two independently selected clones (A and B) of HCT116/MUC1(CQC-AQA) cells (Fig. 6A, left). Analysis of the transfectants by flow cytometry with anti-MUC1-N further demonstrated that, as found with wild-type MUC1 (13), MUC1(CQC-AQA) is detectable on the surface of HCT116 cells (Fig. 6A, right). To determine if MUC1(CQC-AQA) interacts with Nup62, anti-Nup62 immunoprecipitates were analyzed by immunoblotting with anti-MUC1-C. The results show that binding of MUC1 to Nup62 is abrogated in the HCT116 cells expressing MUC1(CQC-AQA) (Fig. 6B). To extend this analysis, nuclear and cytosolic lysates were subjected to immunoblot analysis with anti-MUC1-C. The results show that MUC1-C is detectable to a greater extent in nuclear lysates from HCT116/MUC1, as compared with HCT116/MUC1(CQC-AQA), cells (Fig. 6C). By contrast, cytosolic MUC1-C levels were similar in the HCT116/MUC1 and HCT116/MUC1(CQC-AQA) cells (Fig. 6C). MUC1 functions as a coactivator of Tcf4-mediated transcription of the cyclin D1 promoter (9, 11). To analyze the effects of MUC1(CQC-AQA), we used the pycD1(−161)−Luc reporter, which contains three Tcf-binding sites (34). As shown previously (9) and as compared with HCT116/vector cells, activation of pycD1(−161)−Luc was increased by MUC1 expression (Fig. 6D). By contrast, MUC1-induced coactivation of pycD1(−161)−Luc was attenuated in the HCT116/MUC1(CQC-AQA) cells (Fig. 6D). These findings indicate that, like MUC1-CD, mutation of the CQC motif in full-length MUC1 attenuates binding to Nup62 and nuclear localization.

Nuclear Import of MUC1 Contributes to Transformation—To determine if attenuation of nuclear localization affects the MUC1 transforming function, the HCT116 cell transfectants were assessed for anchorage-dependent and -independent growth. Expression of MUC1 or MUC1(CQC-AQA) had little if any effect on anchorage-dependent growth in tissue culture flasks (data not shown). As shown previously for growth in soft agar (9, 26), the MUC1 transfectants formed colonies that were substantially larger than those obtained with HCT116/vector cells (Fig. 7A). By contrast, expression of MUC1(CQC-AQA) resulted in the formation of colonies that were similar to those found with HCT116/vector cells (Fig. 7A). Quantification of the number of colonies demonstrated plating efficiencies of ~6% for the HCT116/MUC1 cells and less than 0.1% for the HCT116/
vector and HCT116/MUC1(CQC-AQA) cells (Fig. 7B). To determine if expression of MUC1(CQC-AQA) affects tumorigenicity, 1 x 10^6 HCT116/vector, HCT116/MUC1, or HCT116/MUC1(CQC-AQA) cells were injected subcutaneously into nude mice. As shown previously (9, 13), HCT116/MUC1 cells formed tumors that were somewhat larger than those obtained with HCT116/vector cells (Fig. 7C). Significantly, however, tumors were substantially smaller in mice injected with the HCT116/MUC1(CQC-AQA) cells as compared with those formed with both HCT116/vector and HCT116/MUC1 cells (Fig. 7C). These findings indicate that the function of wild-type MUC1 in supporting anchorage-independent growth and tumorigenicity is attenuated by the MUC1(CQC-AQA) mutant.

**DISCUSSION**

**Nuclear Import of MUC1-C**—The MUC1 protein is expressed as a transmembrane heterodimer that consists of N-terminal and C-terminal subunits. Previous work has shown that the MUC1-C, and not MUC1-N, localizes to the nucleus in diverse cell types (8, 11, 21, 35–39). The mechanisms responsible for nuclear import of MUC1-C, which is devoid of a classical NLS, have been unclear. Mutation of the RRK motif in the MUC1 cytoplasmic tail attenuated nuclear targeting of MUC1-C in the response to heregulin stimulation (21), indicating that this region of MUC1 is of importance to its nuclear import. In concert with the lack of a classical NLS, the present
results show that there is no detectable binding of MUC1-C to importin α. Moreover, the association of MUC1-C with importin β suggested that MUC1-C may be imported by a nonclassical pathway. Indeed, certain proteins, including parathyroid hormone-related protein (40), Smad-3 (41), cAMP-response element-binding protein, Jun, and Fos (42), contain nonclassical NLSs that are transported through the NPC by direct binding to importin β. In this regard, importin α forms a complex with Nup62 (32). Other proteins, such as β-catenin, enter the nucleus by an importin α/β- and nucleoporin-independent mechanism (33). The present studies demonstrate that MUC1-C associates with importin β and with Nup62. These results indicated that MUC1-C could enter the nucleus by forming a complex with Nup62 (Fig. 7D). Little, however, is known about binding of nuclear proteins to the nucleoporins.

FIGURE 7. MUC1(CQC-AQA) attenuates the MUC1 transforming function. A, photomicrographs of the indicated cells (1 × 10^5) after suspension in soft agar and incubation for 2 weeks. B, colonies of >10 cells were counted from three plates of the indicated cells. The results are expressed as the number of colonies (mean ± S.E.) per plate. C, the indicated cells (1 × 10^5) were injected subcutaneously into the flanks of nude mice. Tumor volumes were calculated by bidimensional measurements on day 25. The results are expressed as the tumor volume (mean ± S.D.) obtained from 6 mice/group. D, scheme depicting the proposed interactions of MUC1 oligomers with importin β and Nup62. TRL, T-rich linker region. CTD, C-terminal domain.

MUC1 Oligomers Interact with Nup62—Our studies on binding of purified MUC1-CD and Nup62 indicated that MUC1 forms oligomers. Incubation of purified MUC1-CD in vitro and immunoblot analysis showed the presence of dimers and higher order structures. Pull-down and coimmunoprecipitation studies further demonstrated that Nup62 associates predominantly, if not exclusively, with MUC1 oligomers. These results suggested that the interaction between MUC1 and Nup62 is dependent on the formation of MUC1 oligomers. Previous work had shown that MUC1/Y forms heterodimeric complexes with a 15–20-kDa protein by a mechanism involving the CQC motif (46). However, to our knowledge, there is no previous evidence indicating that MUC1 forms homodimers. To investigate the role of MUC1 oligomers, we mutated the two cysteines in the MUC1-CD CQC motif. MUC1-CD(CQC-AQA) failed to form oli-
gromers in vitro and in cells. MUC1-CD(CQC-AQA) also failed to bind to Nup62. In addition, we found that, in contrast to MUC1-CD, there was no detectable binding of MUC1-CD(CQC-AQA) to importin β (data not shown). These results indicate that the CQC motif and oligomerization are necessary for the formation of MUC1 complexes with importin β and Nup62. In concert with binding of the MUC1 oligomers, and not MUC1-CD(CQC-AQA), to Nup62, nuclear targeting of full-length MUC1 was disrupted by the CQC → AQA mutant. These findings provide the first evidence that MUC1 forms oligomers and that oligomerization is involved in targeting MUC1 to the nucleus.

**MUC1 Is Imported into the Nucleus by Interacting with Nup62**—The finding that MUC1 oligomers bind directly to the T-rich linker region of Nup62 suggested that this interaction may be responsible for transport of MUC1 through the nuclear pore. To address this possibility, the Nup62-(178–328) T-rich linker region was expressed to block binding of MUC1 to endogenous Nup62. The results showed that Nup62-(178–328) blocks nuclear localization of MUC1, indicating that binding of MUC1 to the T-rich linker region of endogenous Nup62 is necessary for nuclear import. To our knowledge, there are no previous reports that provide evidence for nuclear import of a cargo by direct binding to Nup62. Nuclear import of the Ran exchange factor, RCC1, does not require the importins or other soluble factors (47). The E7 transforming protein of the type 16 human papillomavirus also enters the nucleus in the absence of soluble factors (48). However, it is not known if RCC1 or E7 is imported into the nucleus by direct binding to a nucleoporin. Nuclear import of the HIV-1 genome is mediated by the small virally encoded Vpr protein, which binds directly to the nucleoporin CG1 (49). Otherwise, little is known about nuclear import that is directly mediated by nucleoporins.

**Nuclear Localization of MUC1 May Contribute to Transformation**—MUC1-C forms cytosolic and nuclear complexes with β-catenin, γ-catenin, and p120catenin, and p104 (8, 11, 21, 25, 35, 36, 50). The available evidence indicates that MUC1 coactivates β-catenin/Tcf-mediated transcription of the cyclin D1 promoter-reporter (9, 11). Expression of MUC1 with a Tyr→Phe mutation in the cytoplasmic domain attenuates coactivation of the cyclin D1 promoter, fails to confer anchorage-independent growth, and blocks the formation of HCT116 tumors (9). Previous work demonstrated that mutation of the MUC1 CQC motif to AQA blocks localization of MUC1 to the surface of MDCK cells (51). However, the present studies clearly show that the MUC1(CQC-AQA) mutant is expressed on the surface of HCT116 cells. The basis for this discrepancy in findings may be related to cell context. Expression of Tac-MUC1 fusion proteins, as used in other work (52), has shown that the CQC motif is necessary for palmitoylation and cell surface recycling, indicating that the MUC1(CQC-AQA) mutant could accumulate in endosomes. The present studies further demonstrate that binding of full-length MUC1 to Nup62 and nuclear import is attenuated by the CQC → AQA mutations. In concert with these results, expression of the MUC1(CQC-AQA) mutant attenuated MUC1-mediated coactivation of the cyclin D1 promoter. Moreover, like MUC1(Y46F), expression of MUC1(CQC-AQA) was ineffective in supporting anchor-independent growth and attenuated the formation of HCT116 tumors. These findings collectively indicate that MUC1 may contribute to transformation through nuclear localization. Therefore, strategies designed to disrupt the formation of MUC1 oligomers could attenuate localization of MUC1 to the nucleus and thereby its transforming function.

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