REVIEW

Pathobiological role of MUC16 mucin (CA125) in ovarian cancer: Much more than a tumor biomarker

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Abstract

MUC16 (CA125) has remained the mainstay for ovarian cancer assessment and management since the early 1980's. With the exception of HE4, it is the only reliable serum biomarker for ovarian cancer. MUC16 belongs to a family of high-molecular weight glycoproteins known as mucins. The mucin family is comprised of large secreted transmembrane proteins that includes MUC1, MUC4 and MUC16. These mucins are often overexpressed in a variety of malignancies. MUC1 and MUC4 have been shown to contribute to breast and pancreatic tumorigenesis. Recent studies have uncovered unique biological functions for MUC16 that go beyond its role as a biomarker for ovarian cancer. Here, we provide an overview of the literature to highlight the importance of MUC16 in ovarian cancer tumorigenesis. We focus on the growing literature describing the role of MUC16 in proliferation, migration, metastasis, tumorigenesis and drug resistance. Accumulating experimental evidence suggest that the C-terminal domain of MUC16 is critical to mediate theses effects. The importance of MUC16 in the pathogenesis of ovarian cancer emphasizes the need to fully understand the signaling capabilities of MUC16 C-terminal domain to develop more efficient strategies for the successful treatment of ovarian cancer.

Key words: MUC16; CA125; Mucin; Ovarian cancer; Tumorigenesis; Biomarker

Core tip: MUC16/CA125 has been a mainstay biomarker for ovarian cancer but its pathobiological role has remained mostly unknown. Recent literature has shown that MUC16 is much more than a biomarker. MUC16 has oncogenic properties and plays an important role in tumorigenesis. Here, we will review the current knowledge regarding the oncogenic role of MUC16.

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INTRODUCTION

Cancer antigen 125 (CA125) is the most widely used and the best characterized biomarker for the management of epithelial ovarian cancer (EOC)[1-16]. CA125 was initially identified in 1981 as an antigen present in the serum of patients with EOC[17,18]. However, it was not until 2001 that the gene was cloned and identified as MUC16[19-22]. It was later realized that the CA125 antibody recognizes a repeating epitope located in the extracellular domain of MUC16[5,19,20]. For the purpose of this review, the term CA125 will be used to designate the cleaved extracellular domain that can be found in serum of EOC and the term MUC16 will designate the full length mucin glycoprotein. For CA125 to be detected in the serum of EOC patients, the MUC16 glycoprotein undergoes a proteolytic cleavage resulting in the release of most of the extracellular domain from the cell surface. Serum levels of CA125 are routinely used in clinic to monitor patients with EOC[2,7,9,10,12,13,15,18]. There is indeed a strong correlation between rising and falling levels of serum CA125 with the progression and regression of the disease. Although the MUC16 mucin may be expressed in other cancers, its use in clinic has been mostly limited to patients with EOC.

Despite the fact that serum CA125 has been the mainstay of EOC assessment and management since the early 1980's, there are considerable gaps in our knowledge regarding the biological functions of MUC16 in normal physiology and particularly in oncogenesis. Not surprisingly, most of the early studies (before 2001) have focused on the clinical applicability of CA125 as a biomarker. However, the cloning of *MUC16* gene and the characterization of its structure, which revealed that MUC16 belong to membrane-bound mucins, has encouraged studies aiming to elucidate its physiological normal function as well as its role in malignant conditions. Because other membrane-bound mucins, such as MUC1 and MUC4, have oncogenic properties, it has been hypothesized that MUC16 may possess oncogenic capabilities as well. However, the very large size of the molecule (MUC16 is the biggest mucin identified to date) has hampered the characterization of MUC16 functional domains leading to an incomplete understanding of the physiological and pathological roles of MUC16. Nonetheless, in the last few years, a number of studies have begun to unravel the biological functions of MUC16. In particular, these studies have demonstrated that MUC16 possesses oncogenic properties. Here, we will review the current knowledge regarding the oncologic role of MUC16 in ovarian cancer. Our discussion will focus on cancer cells signaling, invasion and metastasis, and on regulation of drug-induced apoptosis. This review will also highlight gaps in our knowledge to provide a framework for future research studies.

CA125 AS A BIOMARKER

CA125 is the most widely studied serum biomarker for EOC. CA125 has been recognized as a tumor-associated antigen based on the characterization of the OC125 monoclonal antibody raised against the human ovarian cancer cell line OVCA433 in 1981[17,18]. This antibody detected a molecule that was named CA125 in the serum from patients with EOC[17]. It was later realized that CA125 is an epitope located on a repeated extracellular domain of MUC16 mucin[5,19,20]. Measurement of serum CA125 is an important part of the clinical management for EOC patients[2,10]. Its clinical utility has been evaluated as a screening test for the early detection of EOC, to distinguish benign diseases from malignant conditions, and to monitor EOC progression and regression following treatment.

The early detection of EOC remains a clinical challenge and minimal progress has been achieved to detect early diseases, which are often clinically asymptomatic, at a more curable stage. The use of serum CA125, as a single biomarker, for the early detection of EOC is tempered by several factors. First, MUC16 glycoprotein may be expressed in various malignant and non-malignant conditions. MUC16 is expressed at low levels in the normal airway epithelium and levels can increase in some chronic conditions such as cystic fibrosis[23-25] or vary with dexamethasone treatment[26]. MUC16 is expressed at the apical surface of the ocular and conjonctival epithelium where it is part of the glycocalyx protecting corneal cells from bacterial infections and dryness[27-30]. Immunohistochemistry of human tissues using the OC125 antibody detected MUC16 expression in fetal coelomic epithelia and its derivatives such as Müllerian duct, fallopian tube, endometrium, endocervix[8,14]. MUC16 is expressed by mesothelial cells of the peritoneum, the pleura and the pericardium[8,14]. Furthermore, CA125 serum levels can be elevated in various benign diseases including menstruation, first trimester pregnancy, endometriosis, adenomyosis, salpingitis, uterine fibroids, chronic renal failure or in inflammation of the pleura, peritoneum or pericardium[31-34]. However, MUC16 expression is not found in normal adult colon, rectum, cervix, small intestine, liver, pancreatic ducts, spleen, kidney, skin and ovaries[35]. Secondly, the expression of MUC16 in EOC tissues varies according to the histotype. MUC16 is expressed by 56% to 85% of serous, 65% of endometrioid, 40% of clear cell and 36% of undifferentiated adenocarcinomas of the ovary, but by only 12% of mucinous ovarian cancers[6,7,35]. Therefore, elevated levels of CA125 are most strongly associated with serous EOC subtypes and its expression is more limited in other subtypes. Consequently, the detection of serum CA125 is notably less sensitive for subtypes other than serous EOC. MUC16 is also detected in normal airways as well as in a small percentage of invasive breast carcinomas, lung carcinomas and pancreatic carcinomas[23,36-39]. Therefore, measurements of serum CA125 levels, as a single modality, have a limited utility for screening of EOC because of its lack of sensitivity and specificity.

The clinical utility of monitoring of serum CA125, as a screening test, has been evaluated by the ovarian component of The Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial[40]. Results from this study showed that annual screening with CA125 and transvaginal ultrasound had no mortality benefit. Furthermore, the majority of the screen detected cancers presented in advanced stages. Another large ovarian cancer screening trial is currently ongoing, the UK Collaborative Trial of Ovarian Cancer Screening. The multimodal arm of this study utilizes the Risk of Ovarian Cancer Algorithm (ROCA) to predict the probability of EOC as a first-line screen[41]. The ROCA is based on the slope of serial serum MUC16 measurements. According to ROCA, patients are classified as low, intermediate and high risk of developing EOC. When the risk is intermediate or high, patients undergo a transvaginal ultrasound. Results of this trial are expected soon and it thus remains to be seen whether CA125 serum levels will be useful for screening of ovarian cancer in the general population.

Another potential application of CA125 serum monitoring, is its ability to distinguish benign gyneco-logical conditions from EOC. However, as mentioned previously, the expression of MUC16 in a number of benign gynecological conditions limits the utilization of serum CA125 as a single biomarker. To overcome this limitation, a number of risk prediction scores have been developed to identify women that have high risk of EOC. Examples of risk prediction models include: (1) the Risk of Malignancy Index combines serum CA125 levels with ultrasound and menopausal status to identify women at high risk of having EOC[42]; (2) the Risk of Ovarian Malignancy Algorithm (ROMA), which uses menopausal status with CA125 and HE4 serum values[43-45]; and (3) OVA1, which uses CA125, β2-microglobulin, apolipo-protein A1, transthyretin and transferrin[46]. The use of ROMA added more accuracy for differentiating the benign and malignant conditions compared to CA125 alone. Other algorithms are being investigated and there is an intense effort to search for new potential biomarkers for EOC. Recently, the combination of serum CA125 with IL-6 ascites levels was evaluated for its ability to discriminate between benign conditions and EOC[47]. Despite encouraging data, further evaluation is needed to determine the clinical utility of these panels of markers.

The clinical utility of serum CA125 has been best validated for monitoring of therapy as well as detection of recurrence. Indeed, CA125 is the only biomarker currently recommended for the monitoring of therapy and for the detection of recurrent diseases. Elevated levels of serum CA125 are common in patients with advanced disease of serous histiotype (approximately 90%). It was shown by several groups that rising and falling levels of serum CA125 correlate with progression and regression of the disease and this formed the basis for monitoring CA125 serum levels for patient follow-up[14,15,18]. However, up to 20% of patients with advanced EOC have normal serum level of CA125. CA125 nadir serum values were associated with a significantly longer progression-free survival (PFS) and overall survival[9,13]. Pretreatment CA125 serum level is an independent predictor of PFS in patients with advanced EOC who received a standard chemotherapy regimen[16].

CLONING OF *MUC16* GENE

The cloning of the *MUC16* gene in 2001 revealed that MUC16 is a tethered mucin[19-22]. The deduced amino acid sequence of MUC16 demonstrated that it shares common features with other membrane-bounded mucins with high serine, threonine and proline contents. MUC16 therefore belongs to the family of membrane-bound mucins. Other membrane-associated mucins include MUC1, MUC3A, MUC3B, MUC4, MUC12, MUC13, MUC15, MUC16, MUC17 and MUC20[48]. With a molecular weight of > 2 MDa, MUC16 is the largest membrane-bounded mucin known to date[21,22]. This glycoprotein is composed of a heavily glycosylated N-terminal domain, a large multiple repeat domain (up to 60 tandem repeats of 156 amino acids each), an ectodomain proximal to the putative cleavage site, a transmembrane domain (TM) and a small cytoplasmic domain of 31 amino acids[21] (Figure 1). MUC16 extracellular domain possesses much longer repeats (156 amino acids) as compared to MUC4 (16 amino acids) and MUC1 (20 amino acids). The N-terminal domain (12068 amino acids) and the repeat domain are heavily glycosylated with both O- and N-linked oligosaccharides[22]. In contrast to other membrane-bound mucins such as MUC1, which harbor a single sea urchin sperm protein, enterokinase and agrin (SEA) domain, MUC16 contains approximately 56 SEA domains (Figure 1)[49,50]. SEA domains consist of about 120 amino acids. Sequence analysis of MUC16 SEA modules showed that they display some sequence variability[50,51]. The second MUC16 SEA domain, proximal to the TM, however is relatively conserved and most closely resembles the SEA domain found in other mucins. Thus, MUC16 was speculated to contain a putative proteolytic cleavage site located in a SEA domain proximal to the membrane which could be involved in releasing the ectodomain[21]. Direct experimental validation has been lacking. However, a recent study has challenged this model. The investigators suggest that MUC16 cleavage occurs outside the proximal SEA domains and within the 12 extracellular amino acids proximal to the TM domain[52,53]. According to these authors, MUC16 cleavage takes place in the acidic pH of Golgi/post-Golgi compartments[54]. Notably, these authors did not found any evidence of intramembrane proteolysis with their constructs. The ectodomain of MUC16 has been reported to be released by metalloproteases [matrix metalloproteinase 7 (MMP-7), MMP-9, ZmpC] and neutrophil elastase[28,55]. ZmpC, a zinc metalloproteinase secreted by *S. pneumoniae*, selectively cleaves the ectodomain of
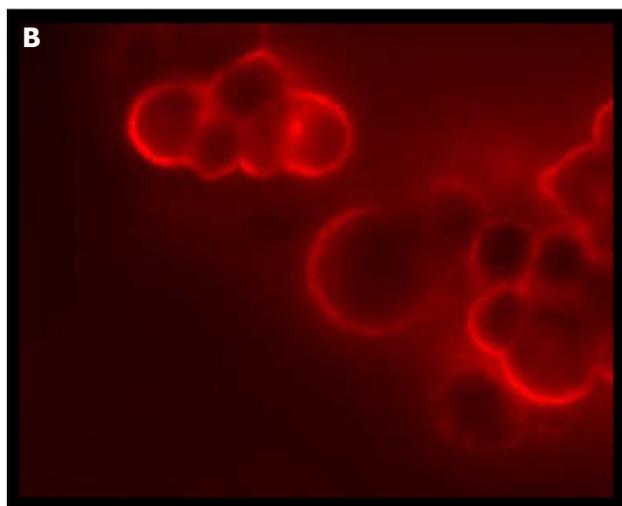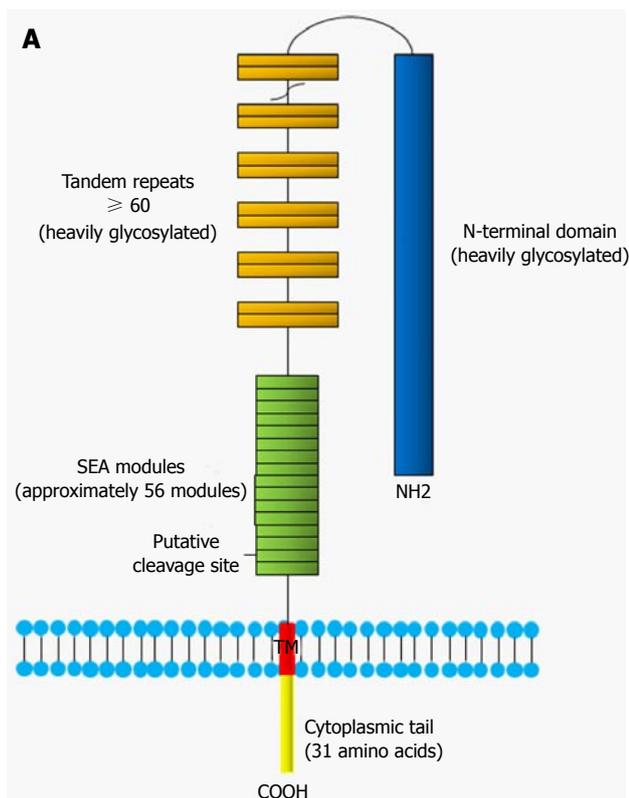
Figure 1 Schematic structure of MUC16 mucin. A: The major domains of MUC16 include the N-terminal domain, the tandem repeat domain and the C-terminal domain. The SEA modules contain a putative proteolytic cleavage site which divides MUC16 in two subunits. The extracellular larger subunit consists of the N-terminal (> 12000 a.a.) and tandem repeat domains (156 a.a. each), and are heavily glycosylated. The smaller subunit contains SEA domains, a transmembrane domain (TM) and the cytoplasmic tail (31 a.a.); B: MUC16 is usually expressed at the apical surface of normal epithelial cells. In EOC cells, this pattern of expression is lost and MUC16 is expressed through the entire surface of the tumor cells. The micrograph represents OVCAR3 cells probed with M11 antibody. SEA: Sea-urchin sperm protein, enterokinase and agrin; a.a: Amino acids.

MUC16 mucin from conjunctival and corneal epithelial cells[55]. The SEA domain is presumably the cleavage site for these proteases[28]. These findings contrast with that of Das *et al*[54] which showed that MUC16 cleavage was independent of neutrophil elastase and MMP-7.

Post-translation modifications such as glycosylation can potentially regulate the release of MUC16 ectodomain and could explain these different findings. Intriguingly, mutation of Ser106Ala or Thr84/85Ala in the cytoplasmic tail did not affect the cleavage of MUC16[54]. According to these results, it would mean that there is no requirement for cytoplasmic cues for MUC16 extracellular cleavage. This is unexpected because previous studies showed that the phosphorylation of MUC16 cytoplasmic tail (Ser/Thr phosphorylation) has been associated with its secretion[56]. The secretion of MUC16 was also reported to be stimulated by epidermal growth factor (EGF) or tyrosine phosphatases[57]. Its shedding is decreased by glucocorticoids[26]. One limitation of the study by Das *et al*[54] is that, because of the lack of specific antibodies to MUC16 CT, they were not able to demonstrate cleavage of endogenous MUC16 in ovarian cancer cells to validate their observations, particularly in the context where most of the data were generated in non-ovarian cancer cell lines. Therefore, although MUC16 cleavage may transit in the Golgi before it reaches the cell surface, it is possible that its cleavage may also be triggered once the full length MUC16 is anchored in the cell membrane. The findings of Das *et al*[54], if confirmed, have important implications from a clinical standpoint. Since the cleavage of MUC16 appears to occur before it reaches the plasma membrane, antibodies raised against CA125 (extracellular portion of MUC16) may not be the most effective means to assess expression of MUC16 and could have been one of the reasons for the limited success in clinical studies of CA125 monoclonal antibodies[58-62].

Unlike MUC1 and MUC4, MUC16 lacks an EGF-like domain. Through their EGF-like motif located at C-terminal domain (extracellular portion), MUC1 and MUC4 bind to growth factor receptor tyrosine kinases (RTKs) such as erbB family and fibroblast growth factor receptor 3[63-66]. The formation of heterodimer with RTKs causes cross-phosphorylation of their respective cytoplasmic domain leading to the activation of various signaling pathways[67]. Because MUC16 lacks an RTK binding motif in its C-terminal domain, it is not clear whether MUC16-induced signaling is affected by RTKs although MUC16 extracellular domain shedding from the cell surface is stimulated by EGF. Consistent with the lack of an RTK binding motif, the intracellular interaction between MUC16 and β-catenin was not affected by EGF[68].

MUC16 31 amino acids long cytoplasmic tail (CT) contains serine/threonine/tyrosine residues that serve as potential phosphorylation sites. MUC16 CT also contains a polybasic sequence of amino acids (RRRKK), which is predicted to bind to the ezrin/radixin/moesin (ERM) family of proteins (Figure 2). This motif is not found in MUC1 and MUC4. The ERM proteins can interact with numerous membrane-associated proteins and the actin cytoskeleton. In line with this, MUC16 was recently shown to interact with E-cadherin and β-catenin,

MUC16

ERM binding domain

VTT**RRRK**KEGE**Y**NVQQQCPGY**Y**SHLDLEDLQ

Potential nuclear
localization signal

MUC1

CQC**RR**KNYGQLDIFPARDTYHPMSEYPTYHTHGR**Y**VPSSTDRSPYEKVSAGNGGSSLS**Y**TN
PAVAATSANL

MUC4

LRFWGCSGARFS**Y**FLNSAEALP
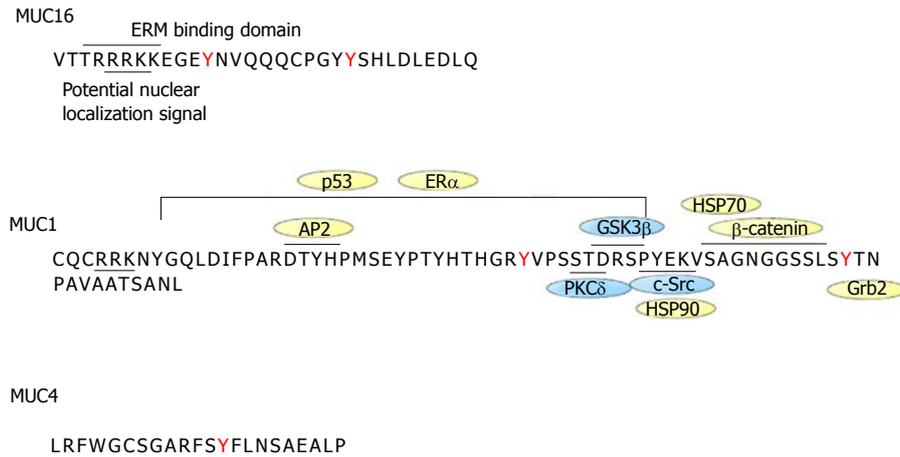
Figure 2 Sequence of MUC1, MUC4 and MUC16 cytoplasmic tails. The intracellular sequence of the different mucins is shown along with protein interaction sites. MUC1 is the best characterized mucin. MUC1 cytoplasmic tail interacts with c-Src, GSK3β, PCKδ, β-catenin, p53, ERα, HSP70/90, Grb2, AP-2. Proteins with kinase activity are in blue whereas those without kinase activity are in yellow. HSP70 binds to MUC1 cytoplasmic tail in the same region as β-catenin. HSP90 binding to MUC1 depends on c-Src-induced Y-46 phosphorylation. MUC16 cytoplasmic tail has an ERM motif for potential interaction with the cytoskeleton. Both MUC1 and MUC16 contain a potential nuclear localization signal motif. MUC4 has no known interaction binding partners. EMR: Ezrin/radixin/moesin.

causing alterations in the actin cytoskeleton[68]. It remains unclear however whether MUC16/β-catenin and MUC16/E-cadherin interactions are mediated through the ERM motif of the MUC16 CT. Janus kinases (JAKs), which are non-receptor tyrosine kinases, contain an ERM domain. Previous studies showed that MUC16 co-immunoprecipitates with JAK2 in breast cancer cells resulting in the activation of STAT3, which may be involved in breast cancer development[69].

MUC1 cytoplasmic tail has been shown to bind to β-catenin and a serine-rich SXXXXXSSL motif in MUC1 CT is responsible for this interaction *in vitro*[70-72]. This motif is notably absent in MUC16. Interestingly however, the binding of MUC1 to β-catenin in cells was independent of the serine-rich motif[72]. These observations suggest that MUC16 interaction with β-catenin is mediated by an indirect mechanism, probably through another protein.

The positively charged R-K rich region of MUC16 CT also constitutes a putative nuclear localization motif[67]. Up until recently, evidence were lacking to support nuclear localization of MUC16 CT. However, a recent study demonstrated that MUC16 CT is cleaved from the cell surface and co-localized with JAK2 in the nucleus[52]. MUC16 CT-mediated increased nuclear JAK2 leads to up regulation of LMO2 and NANOG, two proteins shown to induce stem cell-like properties emphasizing the signaling capabilities of MUC16's CT domain[52].

MUC16 AND TUMORIGENICITY OF CANCER CELLS

MUC16 is overexpressed by most serous EOC, by fallopian tube cancers and by cancers of the uterus[6,11,32,38]. Consistent with the role of MUC16 in tumorigenicity, overexpression of *MUC16 mRNA* and amplification of genomic regions encoding *MUC16* DNA have been observed in The Cancer Genome Atlas (TCGA) ovarian

cancer project that included 316 cases of serous EOC[73]. However, MUC16 expression was not correlated with overall survival or resistance. MUC16 is also aberrantly expressed by several other malignancies, including cancer of the lung, pancreas and breast. Along with MUC16, overexpression of other membrane-bound mucins, such as MUC1 and MUC4, is common in breast and pancreatic cancers[74]. Interestingly, both MUC1 and MUC4 mucins have been shown to possess oncogenic properties and promoted proliferation, invasion and metastasis in pancreatic cancer models[75-77]. These observations suggested that MUC16 could play a role in ovarian cancer progression as well as in other cancers.

Indeed, there are now several studies demonstrating that MUC16 C-terminal domain enhanced proliferation, migration, invasion and tumorigenicity of ovarian, breast and pancreatic cancer cell lines. The first study demonstrating the tumorigenic-enhancing properties of MUC16 was published by Thériault *et al*[78] in 2011. In this study, the knockdown of MUC16 in MUC16 overexpressing EOC cell line OVCAR3 decreased tumorigenicity as shown by the decreased number of colonies in soft agar and the reduction of *in vivo* tumor growth. In the same study, the enforced expression of MUC16 C-terminal domain (last 284 C-terminal residues) enhanced soft agar colony formation and tumor growth in nude mice. Consistently, intraperitoneal injection of MUC16 C-terminal domain-expressing tumor cells significantly reduced the survival of SCID mice compared to those injected with vector-expressing controls[78]. Subsequently, Lakshmanan *et al*[69] showed that MUC16 knockdown in overexpressing breast cancer cell lines decreased cell proliferation and *in vivo* tumor growth. Similarly, Reinartz *et al*[79] demonstrated that MUC16 knockdown in ovarian and breast cancer cells was associated with decreased migration and invasion. In line with these observations, more recent studies showed that ectopic expression of MUC16 C-terminal

domain (last 114 C-terminal residues) enhanced proliferation, migration and *in vivo* tumor growth in pancreatic cancer cells whereas MUC16 knockdown reduced migration and invasion in these cancer cells[52,80]. Collectively, these studies clearly support a critical role for MUC16 in tumor growth and metastasis. Most importantly, expression of the C-terminal domain appears to be sufficient to mediate these effects.

The mechanism by which MUC16 enhances tumorigenicity of cancer cells remains poorly defined however. Although MUC16, as for MUC1 and MUC4, enhances tumorigenicity in cancer cells, the cytoplasmic tails of these three mucins are poorly conserved[50]. Size and amino acid sequence considerably vary suggesting a variety of functions in cell signaling. The ectopic expression of MUC16 C-terminal domain induces an epithelial to mesenchymal transition which has been associated with tumorigenesis and tumor progression[78]. Constitutive MUC16 C-terminal expression was associated with reduced E-cadherin expression which may enhance metastasis[81]. In addition, both Akt and ERK pathways may be activated by constitutive expression of MUC16 C-terminal domain[69,82]. The precise role these pathways was not however further investigated and therefore it is not clear whether their activation is essential to mediate MUC16 effects on tumor progression. However, Akt and ERK activation is frequently observed in serous OC and have been previously shown to be important for tumor progression[73,83,84].

Ectopic expression of MUC16 C-terminal domain in cancer cells has been associated with altered gene expression profile with increased expression of genes coding for proteins involved in invasion such as MMP2, MMP9, CXCL12 and CDH1[85]. MMPs are major proteolytic enzymes that are involved in cancer cell migration, invasion, and metastasis[86]. The CXCL12/CXCR7 axis plays an important role in ovarian cancer as it enhances cancer cell invasion by up-regulating MMP9 expression through the MAPK pathway[87]. MUC16 knockdown in breast cancer cells was associated with decreased expression of cyclin B1 and D1, both involved in regulation of cell cycle control[69]. The cytoplasmic domain of transmembrane mucins is usually embedded in the apical membrane of normal epithelial cells. However, upon transformation, the cytoplasmic tail of mucins such as MUC1 accumulates in the cytoplasm[88-90]. As mentioned above, there is evidence that MUC16 cytoplasmic tail gets cleaved and translocates in the nucleus[52]. However, whether this cleavage is required for MUC16 to enhance tumorigenicity remains to be determined.

MUC16 AND TRANSFORMATION

Membrane-bound mucins such as MUC1 and MUC4 possess transforming properties[91,92]. For example, when the C-terminal portion of MUC1 was stably transfected into 3Y1 fibroblast cells, soft agar colonies and subcutaneous tumors in nude mice were readily obtained[91]. Interestingly, MUC16 is not expressed by normal ovarian surface epithelial (OSE) cell but its expression is induced in transformed OSE cells[93]. These observations suggest that MUC16 may play an important role at early stages in the development of EOC. Two recent studies indeed confirmed the transforming properties of MUC16. Giannakouros et al[82] stably expressed MUC16 C-terminal domain (last 284 C-terminal residues) and a similar construct lacking most of the cytoplasmic domain in immortalized NIH3T3 mouse fibroblast cells using lentiviral vectors. It was observed that MUC16 C-terminal domain-expressing cells displayed enhanced anchorage-dependent and -independent growth and enhanced the formation of sub-cutaneous tumor nodules in athymic nude mice[82]. Notably, these effects were abolished by the deletion of the cytoplasmic tail. In line with these observations, Rao et al[85], using constructs containing either the last 114 C-terminal amino acids or last 344 C-terminal amino acids, showed that both constructs significantly enhanced the number of soft agar colonies compared to vector only in NIH3T3 cells. In addition, both constructs formed larger sub-cutaneous tumors relative to control NIH3T3 cells with no significant differences suggesting that the proximal extracellular part of the molecule has little effect on the oncogenic effects of MUC16. These studies establish that MUC16 is an oncogenic glycoprotein much like MUC1 and MUC4.

There are, however, conflicting data regarding the minimal functional domain of the C-terminal domain required for transformation. Although, the SEA domain appears to be dispensable for tumorigenicity, the respective role of the ectodomain and the cytoplasmic tail are more controversial. Rao et al[85] showed that ectopic expression in NIH3T3 cells of a construct containing the ectodomain (58 amino acids), the TM (22 amino acids) and the first 6 amino acids of the CT had transforming effects mostly similar to a construct carrying the full length CT. In contrast, Thériault et al[78] and Giannakouros et al[82] showed that deletion of the CT in a construct consisting of the last 284 amino acids completely abolished the tumorigenic and transforming effects of MUC16 C-terminal domain. The reasons for this discrepancy are unclear but may reflect differences in integration of extracellular and intracellular signals with the different constructs that were used in these experiments.

MUC16 AND DRUG RESISTANCE

Silencing MUC16 using single-chain antibodies increased the sensitivity of MUC16 overexpressing OVCAR3 cells to genotoxic agents such as cisplatin and doxorubicin but not to microtubule assembly inhibitors such paclitaxel and vinorelbin[94]. Conversely, expression of MUC16 C-terminal domain conferred increased resistance to cisplatin[94]. Consistent with the role of MUC16 in drug resistance, MUC1 has been shown to modulate the

sensitivity of cancer cells to genotoxic agents. Indeed, expression of MUC1 C-terminal domain conferred resistance to cisplatin and etoposide in colon cancer cells[89,95]. The downregulation of MUC16 in OVCAR3 cells activates the PI3K/Akt pathway[68]. The authors also reported that MUC16 knockdown in these cells decreased FOXO3a nuclear localization. FOXO3a function is controlled in part by activation of the Akt pathway. Akt phosphorylates FOXO3a, resulting in binding of FOXO3a to 14-3-3 proteins and retention of FOXO3a in the cytoplasm. In contrast, dephosphorylation of FOXO3a induces its nuclear localization where it transactivates gene expression[96]. FOXO3a modulates the expression of several genes that regulate the cellular response to stress at the G2-M checkpoint. The growth arrest and DNA damage response gene Gadd54a is a target of FOXO3a that mediates part of FOXO3a's effects on DNA repair[97]. Thus, preventing the nuclear localization of FOXO3a contributes to the apoptotic response to genotoxic drugs. It is therefore possible MUC16 knockdown sensitizes tumor cells to genotoxic drugs by activating Akt which in turn prevents FOXO3a nuclear localization. Although MUC16 expression may be associated with cisplatin resistance *in vitro*, no correlation was observed between MUC16 expression and resistance to chemotherapy in the TCGA ovarian cancer project[73]. Interestingly, however, a recent study suggested that the combination of serum CA125 and ascites leptin levels have a high discriminating potential to distinguish clinically resistant patients (intrinsic resistance) to first-line therapy from patients that responded to first-line chemotherapy[47].

Death receptor ligands such as tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) trigger rapid apoptosis in various tumor cell types[98-102]. TRAIL binds to death receptors, TRAIL-R1 (DR4) and -R2 (DR5), whose cytoplasmic death domain (DD) signals downstream caspase activation to mediate TRAIL-induced apoptosis[103]. Upon receptor activation, FADD and pro-caspase-8 are recruited to form a death-inducing signaling complex (DISC)[104]. When recruited to the DISC, pro-caspase-8 becomes activated and subsequently activates downstream effectors caspases-3, -6 and -7, leading to apoptosis. The cellular FLICE inhibitory protein (cFLIP) regulates both recruitment and processing of pro-caspase-8 within the DISC[105]. MUC16 ectopic expression, like MUC1, was shown to attenuate TRAIL-induced caspase-8 and mitochondria activation, resulting in decreased apoptosis[106]. Notably, MUC16 C-terminal expression was sufficient to attenuate TRAIL-induced apoptosis and signaling. MUC16-induced resistance to TRAIL was related to decreased TRAIL-R2 expression and recruitment at the DISC, and by increased cFLIP expression. MUC1-blockade of TRAIL-induced apoptosis has been attributed to localization of MUC1 C-terminal domain to the mitochondria or to the ability of MUC1 to directly bind caspase-8 and FADD, thereby inhibiting recruitment of caspase-8 at the DISC[107]. Thus, although both MUC1 and MUC16

can inhibit TRAIL-induced apoptosis, the mechanisms by which these mucins inhibit TRAIL signaling differ. This could be related to the amino acids composition of their cytoplasmic tail.

CONCLUSIONS AND FUTURE DIRECTIONS

Since its discovery in the late 1970s, MUC16 glycoprotein has been recognized as a useful clinical biomarker in advance diseases. However, evidence now suggests that MUC16 is much more than a biomarker for disease progression. Recent studies have established that MUC16 can act as an oncogene. MUC16 expression leads to the transformation normal fibroblastic cells and it contributes to the pathogenesis and progression of EOC. These phenotypic effects are shared by other membrane-bounded mucins such as MUC1 and MUC4. However, the mechanisms by which these mucins exert their effects differ. The complex biochemical structure of MUC16 is a constant challenge to define functional domains. Although progress has been made toward understanding the role of MUC16 in tumorigenesis, many question remains. Further studies are required to gain a better understanding of the oncogenic role of MUC16. An area of critical importance is deciphering the mechanism by which MUC16 C-terminal domain enhances tumorigenesis and the downstream signaling associated with this effect. A better understanding of the contribution of MUC16 C-terminal domain to tumorigenesis may yield specific intracellular or extracellular signaling targets. Exploiting these targets could provide novel treatment for ovarian cancer which are urgently needed.

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