MUCIN 1 in Prostate Cancer

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Abstract: Despite extensive research efforts in prostate cancer for the last several decades, the disease remains a leading cause of cancer death in men in the developed world. A typical feature of prostate cancer initiation and progression is the landscape of genetic alterations, which changes the expression patterns of numerous molecules in prostate epithelial cells, where the disease originates. These aberrantly expressed proteins are tumor-associated antigens. Their uniqueness in tumors offers an avenue not only in advancing our understanding of prostate cancer but also in the search for better diagnostic and therapeutic tools. Mucin 1 is one of the most well-characterized tumor-associated antigens. The protein is overexpressed and aberrantly glycosylated following prostate cancer development, and influences certain disease factors including disease initiation, metastasis, and resistance to therapy. Mucin 1 possesses value as a biomarker in predicting prostate cancer prognosis and has been studied as a therapeutic target. This chapter provides an overview of the impact of Mucin 1 on prostate cancer and its clinical values.

Keywords: biomarkers; mucin 1; prostate cancer; prostate cancer vaccine; therapy resistance

INTRODUCTION

Prostate cancer remains the most prevalent malignancy and the second leading cause of cancer-related death in men in the developed world (1). The disease originates from the prostate epithelial cells as prostatic intra-epithelial neoplasia and progresses to invasive carcinoma and metastatic prostate cancer (2, 3). Metastases occur frequently in the bone (4). Primary prostate cancers are commonly managed by active surveillance, and curative treatments including radical prostatectomy and radiation. Approximately 30% of patients following radical prostatectomy will develop recurrent tumors or biochemical recurrence with rise in serum prostate-specific antigen (PSA) (5). Recurrent tumors are typically resistant to therapy, and relapsed prostate cancers or prostate cancers with resurged PSA are associated with higher risk of metastasis (6). Metastatic prostate cancers are treated with androgen deprivation therapy (ADT), which commonly leads to resistance in the form of castration-resistant prostate cancer (CRPC) (7, 8). There are multiple therapeutic options available for CRPCs, including taxane-based chemotherapy and those targeting androgen receptor signaling such as abiraterone or enzalutamide (8–10), and immunotherapy (11, 12). Despite this variety of treatment options, CRPC remains lethal (8, 13).

Cancer initiation, progression, and development of therapy resistance are regulated by complex processes, owing to the genetic and epigenetic changes that occur during the course of oncogenesis. These alterations result in a large number of unique tumor-associated antigens (TAAs) (14, 15). PSA as a classic prostate cancer TAA has been shown to generate PSA-specific T cells (16, 17). The nature of cancer-specific alterations (overexpression and modification) makes TAAs attractive targets for diagnostic and therapeutic purposes. Mucin 1 (MUC1) is one of the most well-characterized TAAs. MUC1 promotes tumorigenesis by activating PI3K-AKT, MEK-ERK, and other molecular pathways (18). Overexpression, hypoglycosylation, and aberrant glycosylation of MUC1 occur during prostate cancer initiation and progression. These changes are also associated with relapse and CRPC development. Thus, changes in MUC1 can be used as a prognostic biomarker. As a TAA, MUC1 has been explored as a target candidate for prostate cancer vaccine. This chapter provides an overview of the role of MUC1 in prostate cancer. The biology of MUC1, its alterations during prostate cancer development and progression, and its potential as a therapeutic target along with its limitations and future research are discussed.

THE BIOLOGY OF MUC1

The *MUC1* gene at 1q22 encodes mucin 1, a protein belonging to the 21-member mucin family in humans. Mucins are large proteins with extensive O-glycosylation and constitute the mucus barrier on epithelium to protect epithelial cells from external environment (19). MUC1 was first detected in human milk fat globule and a set of breast cancer cell lines using anti-human milk fat globule serum (anti-HMFG) (20); its membrane expression was subsequently observed at the apical surface of many glandular epithelial cells including those of the mammary gland, salivary gland, pancreas, prostate, uterus, as well as gastrointestinal and

respiratory tracts (21, 22). MUC1 plays a critical role in forming the protective mucus barrier on epithelial surfaces, evident by the significant reduction of mucus obstruction in cystic fibrosis mice with MUC1 deficiency (23).

Cell surface MUC1 is a heterodimer consisting of a large N-terminal extracellular subunit (MUC1-N or α-subunit) and a small C-terminal subunit (MUC1-C or β-subunit) containing a small extracellular domain, a transmembrane motif, and a C-terminal intracellular region; dimers are formed via non-covalent association in extracellular regions adjacent to cell membrane (Figure 1) (24). The two subunits are produced from a single polypeptide chain by autocleavage following the GSVVV sequence, which is located within the SEA (Sea urchin sperm protein enterokinase and agrin) domain, during translation (25). The N-terminal fragment contains variable number of tandem repeats (VNTR, n = 40–80) of 20 amino acid residues (26, 27); MUC-N is enriched with proline, threonine, and serine (PTS) motifs and is extensively O-glycosylated such that the peptide core is mostly covered (Figure 1) (28). The heavy glycosylation contributes to MUC1's physiological functions in normal cells (28).

In cancer cells, MUC1 is not only significantly upregulated but also undergoes aberrant glycosylation and hypoglycosylation in most cancers (29). Hypoglycosylation leads to exposure of VNTR peptides, which along with aberrant glycosylation change the biochemical properties and cell distributions of MUC1 (28). These abnormalities underline MUC1's properties as a biomarker and therapeutic target as well as its functionality in promoting cancer progression.

UPREGULATION OF *MUC1* IN PROSTATE CANCER

In a study of 2760 prostate cancer cases and 1722 controls, *MUC1* gene variations in terms of single nucleotide polymorphisms and haplotype were not associated
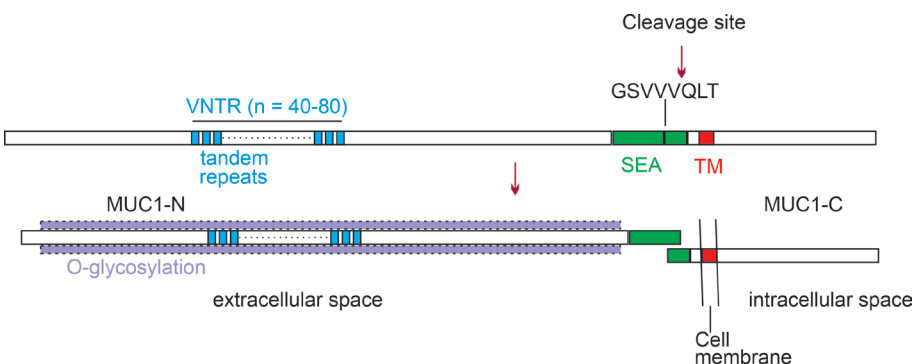

Figure 1. **MUC1 heterodimer structure.** MUC1 is cleaved at the indicated site, i.e., after GSVVV, during translation to generate the MUC1-N and MUC1-C subunits. Both subunits form a heterodimer in the extracellular space adjacent to cell membrane. MUC1-N is extensively O-glycosylated as indicated. SEA (Sea urchin sperm protein enterokinase and agrin) and TM (transmembrane) domains are indicated. VNTR, variable number of tandem repeats.

with prostate cancer risk and disease progression (30). In an investigation of primary prostate cancers (n = 333), metastatic prostate cancers (n = 150), and CRPCs (n = 77), an increase in *MUC1* gene copy number was observed in 35% of CRPCs compared to 6% and 1.8% in mPCs and primary PCs, respectively (31), indicating that *MUC1* gene amplification contributes to MUC1 upregulation in CRPCs.

In a NanoString-based gene expression analysis using 7 pairs of primary prostate cancers and matched non-tumor tissues, MUC1 mRNA was increased in four prostate cancer samples compared to their matched non-tumor controls; 5 of the PC tissues showed elevations of ERG expression (demonstrative of TMPRSS2-ERG fusion) and downregulation of PTEN, both common molecular alterations in prostate cancer oncogenesis (31). However, in an analysis of multiple cohorts consisting of 221 prostate cancers and 92 normal prostate tissues, MUC1 mRNA expression was shown to be reduced (31). Nonetheless, high level of MUC1 mRNA expression likely correlates with TMPRSS2-ERG fusion based on data from the Sueltman dataset (Figure 2A) (32). TMPRSS2-ERG fusion occurs commonly in prostate cancer and plays important roles in its initiation and progression (33, 34). Additionally, microarray-based gene expression profiling of 62 primary prostate cancers and 41 normal prostate tissues revealed increases in MUC1 mRNA expression in high-grade and advanced prostate cancers (35). Collectively, while current evidence does not conclusively support upregulation of MUC1 gene expression during prostate cancer initiation, elevations in MUC1 mRNA largely correlate with prostate cancer progression.

The above concept is supported by increases in MUC1 mRNA expression in metastatic prostate cancers. In two independent cohorts containing 54 metastatic prostate cancers compared to 82 normal prostate tissues, higher levels of MUC1 mRNA were observed in metastatic cases (31). Elevation of MUC1 mRNA in metastatic prostate cancer could also be demonstrated using the well-established Sawyers dataset (36) organized by the R2: Genomics Analysis and Visualization Platform (http://r2.amc.nl http://r2platform.com) (Figure 2B).
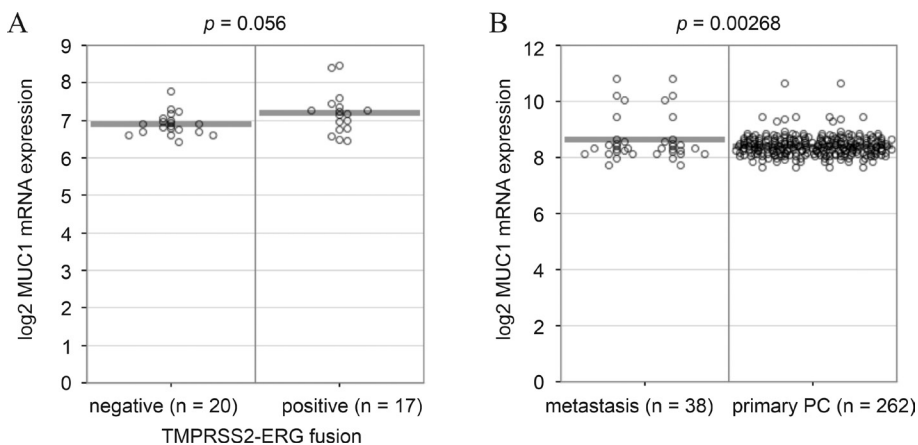

Figure 2. MUC1 expression is associated with adverse features of PC. A. Analyses were performed using the Sueltman dataset (45) in R2: Genomics Analysis and Visualization Platform. **B.** Analyses were performed using the Sawyers dataset (49) in R2: Genomics. Statistical analyses were performed by the R2 Platform using one-way ANOVA.

MUC1 expression was observed in prostate epithelial cells and prostate adeno-carcinoma more than two decades ago using two anti-MUC1 monoclonal anti-bodies (mAb) DF3 and 139H2 (22). Immunohistochemistry staining with B27.29, which recognizes the peptide core (37), showed enhanced MUC1 protein expression in prostate cancer compared to normal prostate glandular epithelial cells (38). Hypoglycosylation of prostate cancer-associated MUC1 was demon-strated by its preferential recognition of prostate cancer cells compared to non-tumor prostate epithelial cells using antibodies BrE-3, BC2, and EMA; these mAbs bind to the peptide core. The upregulation of hypoglycosylated MUC1 positively correlates with Gleason scores (39) and cancer progression (40, 41) (Table 1).

Reduction in O-glycosylation in tumor-associated MUC1 is also caused by premature termination of chain elongation, which is in part attributed to the addi-tion of sialic acid, leading to MUC1 being highly sialylated in tumors (28). In line with this concept, mAb MY.1E12 which reacts with sialylated MUC1 (42, 43), detects MUC1 upregulation and is correlated with prostate cancer grade (44). Elevation of 2 O-linked glycan syalyl Lewis X (sLe^x) MUC1 occurs in prostate cancer (Table 1), which might be in part attributable to the upregulation of GCNT1 glycosyltransferase in prostate cancer (45).

While evidence collectively supports overexpression of aberrantly glycosylated MUC1 in prostate cancer, it remains unclear whether the "upregulation" detected by antibodies recognizing the altered forms truly reflects MUC1 upregulation, as aberrantly modified MUC1 is present in prostate epithelial cells. This limitation is reflected in immunohistochemistry analysis using mAb MBC-2, which revealed MUC1 positivity in 28% of primary prostate cancers (9/32), and 22% of non-tumor prostate tissues (15/68) (46). Similarly, MUC1 protein was detected in 17%

TABLE 1	MUC1 upregulation in prostate cancer[a]			
Population (n)[b]	**MAb**	**% of positive**	**Association[c]**	**Reference**
10	DF3	100%	NA	(22)
	139H2	100%		
5	B27.29	NA	NA	(38)
24	BrE-3, BC2, EMA	NA	Upregulation in PC and higher Gleason grade PC	(39)
120 primary PC	C595	58%	Upregulation in PC and higher Gleason grade PC	(40)
10 LN mPC		90%		
9 mPC	HMFG-2	55.5%[d]	Upregulation in mPC	(41)
57	MY.1E12	NA	Upregulation in PC and higher Gleason grade PC	(44)
10	CHO131	NA	NA	(45)

[a]In comparison to normal prostate and/or BPH (benign prostate hyperplasia) tissues; [b]Primary PCs unless otherwise indicated; [c]Association with PC severity; [d] Positivity was defined by MUC1-positive cells > 50% of total tumor cells. LN mPC, lymph node metastasis; mPC, distant metastasis; NA, not available.

(30/175) of prostate cancer and 41% (42/103) of non-tumor tissues using the VNTR-specific, but glycosylation-insensitive, anti-MUC1 antibody 214D4 (47). It is thus important to further examine MUC1 upregulation using gene expression and genetic approaches.

While the mechanisms responsible for MUC1 upregulation in prostate cancer at either the protein or mRNA level are still largely unknown, prostate cancer stem cells (PCSC) may play a role in this process. Sphere cells derived from DU145 cells possess PCSC properties (48) and display significant upregulation of MUC1 at both the protein and mRNA level compared to their non-stem cancer counterparts (31). Higher levels of MUC1 were also detected in xenografts generated from DU145 PCSC-like sphere cells compared to tumors produced by non-stem cancer DU145 cells (31). Evidence indicates that mechanisms regulating PCSCs might be important in MUC1 upregulation in prostate cancer. This notion is in accordance with the expression of MUC1*, a MUC-1C fragment missing the N-terminal 13 residues from its 58 residues of the extracellular domain in human embryonic stem cells (hESCs) (49). PCSCs are a major driver of prostate cancer progression and development of therapy resistance, including CRPC (50).

MUC1 AND PROSTATE CANCER PROGRESSION

Resistance to ADT or the generation of CRPC remains the inevitable, lethal progression of prostate cancer, to which PCSC is a major contributor (50). Of note, upregulation of MUC1 has been demonstrated in human CRPCs, LNCaP cell-derived CRPC xenografts, and CRPC produced in castrated prostate-specific $PTEN^{-/-}$ mice (31, 51). MUC1 promotes CRPC in part via enhancement of PCSC. MUC1-C induces the expression of the pluripotent genes *OCT4*, *SOX2*, *LKF4*, and *MYC* in prostate cancer cells, facilitates PCSCs, and promotes CRPC development (52). Intriguingly, MUC1* maintains the self-renewal of hESCs via binding to NM23-H1, a metastasis-associated protein (49). MUC1-C enhances prostate cancer plasticity partly through suppression of AR signaling (52). MUC1-C reduces AR signaling via association with ARs and activating miR-135 that downregulates ARs (53). ARs downregulate MUC1 expression in LNCaP cells via binding to the *MUC1* promoter, and also through induction of miR-125b that inhibits MUC1 expression (54). The AR-derived suppression of MUC1 expression might be a contributor for LNCaP cells being MUC1-negative (55). While these observations support mutual inhibition between ARs and MUC1 expression in prostate cancer, their relationship is complex; ectopic expression of ARs in AR-negative PC3 cells upregulated MUC1 following stimulation with 5α-dihydrotestosterone (DHT) (56). Similar observations were also obtained in AR-negative DU145 cells with ectopic AR expression (57).

Induction of MUC1 by androgens in DU145-AR and PC3-AR cells decreased cell adhesion (56, 57). Upregulation of MUC1 in PC3 cells by arctiin also reduced cell adhesion (58), supporting the idea that MUC1 plays an important role in decreasing cell adhesion, which may facilitate metastasis. This possibility is reinforced by the production of sialyl Lewis a (sLe[a]) modification on MUC1 upon its ectopic expression in low MUC1 expression LNCaP and PC3 cells (59).

MUC1 with the sLe^a and sLe^x antigen are selectin ligands (60–62); the interaction between cancer cells and selectin plays a critical role in the extravasation of cancer cells from blood vessel to tissues during metastasis (63). MUC1 may enhance metastasis via multiple mechanisms. For example, MUC1-C can induce the epithelial-mesenchymal transition (EMT) (53), an essential process of metastasis. MUC1 also enhances prostate cancer progression through other mechanisms. The inhibition of AMPKα activity by MUC1 *in vivo* promotes CRPC development; conversely, AMPKα suppresses CRPC in part by inhibition of MUC1 expression (64). While the detailed mechanisms are still unclear, MUC1 expression in prostate cancer is associated with angiogenesis (65) and evasion of natural killer cell-derived immunity (66). Downregulation of MUC1 expression by miR-326 inhibited cell proliferation *in vitro* and xenograft formation *in vivo*; the inhibitions were neutralized upon MUC1 re-expression (67). Collectively, a large body of evidence reveals that MUC1 plays a role in promoting prostate cancer progression through modulating multiple oncogenic processes, including angiogenesis, metastasis, and CRPC development. These properties might be attributed to MUC1-C's action in promoting growth factor receptor signaling, PI3K-AKT-mTOR, MEK-ERK, and cancer metabolism (18).

MUC1-MEDIATED PREDICTION OF PROSTATE CANCER PROGNOSIS

The upregulation of aberrant glycosylation along with its functional contributions to prostate cancer underlines MUC1's potential as a prognostic biomarker. MUC1 expression can be used for risk stratification (44), predicting tumor volume, stage, metastasis (68), recurrence-free survival (35, 69) and mortality risk (70). MUC1-mediated prediction of prostate cancer recurrence and fatality can be improved with multiple gene panels consisting of MUC1+AZGP1 (35) and MUC1+AZGP1+p53 (70), respectively. Furthermore, MUC1-associated genes or its network predicted prostate cancer relapse with high level of certainty (51, 71). Collectively, accumulative evidence supports an association of high MUC1 expression with poor prognosis of PC (Table 2).

Nonetheless, the prognostic role of MUC1 in prostate cancer might be much more complex. In a tissue microarray analysis of early-stage prostate cancer (T1a-b, Nx, M0; n = 195) under watchful waiting for 20 years, tumors with either high- or low-MUC1 expression were associated with a higher risk of fatality compared to those with moderate MUC1 expression comparable to normal prostate epithelium (72). MUC1's prognostic potential was independent of Gleason score and tumor stage (72). The observed higher risk of death for early-stage prostate cancers with reduced MUC1 expression needs further investigation. Nonetheless, this study indicates a complex relationship between MUC1 expression and prostate cancer progression, a concept that is in line with the observations that overexpression of MUC1 in LNCaP C4–2B4 cells was neither stimulative nor inhibitive of xenograft formation (73). Collectively, more work is needed to translate the knowledge generated in laboratory into clinical applications.

TABLE 2	MUC1-associated prognostic biomarker value			
Population (n)	Progression	HR (95% CI)[a]	p value	Reference
57	PFS	5.23 (1.83-14.97	0.002**	(44)
225	RFS	2.35 (1.30-4.24)	0.0005***	(35)
119[b]	DSS	3.2 (1.5-7.0)[c]	0.0382*	(68)
1326	RFS	1.24 (1.02-1.49)	0.02*	(69)
315[d]	OS	2.51 (1.14-5.54)	0.02*	(70)
485[e]	DFS	2.38 (1.55-3.58)	3.45E-05***	(51)

[a]Univariate Cox analysis unless otherwise specified; [b]Patients with LN metastasis; [c]Multivariate Cox analysis including Gleason scores; [d]Mortality cases n = 83; [e] A nine-gene panel derived from MUC1-associated genes. DFS, disease free survival; DSS, disease-specific survival; OS, overall survival; PFS, progression free survival; RFS, recurrence free survival; *p<0.05, **p<0.01, and ***: p < 0.001.

MUC1 AS A THERAPEUTIC TARGET FOR PROSTATE CANCER

As a TAA, MUC1 has been examined as a target for immunotherapy for prostate cancer. In an *in vitro* model, chimeric antigen receptor (CAR)-MUC1 T cells were produced and shown to be effective in killing PC3 and DU145 cells; they also increased the cytotoxicity of AR-positive LNCaP cells together with flutamide, an anti-androgen (74). Tecemotide or L-BLP25 is a cancer vaccine targeting the tandem repeats of MUC1 and has been under clinical trials for a variety of cancers, including a phase III trial for non-small cell lung carcinoma (NSCLC) (75, 76). A phase II clinical trial has been conducted on 16 patients who had biochemical recurrence following radical prostatectomy. Of these, six patients showed prolonged PSA doubling time (PSADT) (77). In a phase I/II clinical trial (NCT00852007) on 17 patients with non-metastatic CRPC, autologous dendritic cells were stimulated with a Tn-MUC1 peptide *in vitro*, and upon reintroduction to patients, it significantly improved PSADT in 11 patients and induced Tn-MUC1 specific CD4^{+} and CD8^{+} T cell response in five of the seven patients analyzed (78). In a randomized phase IIa clinical trial on 21 chemonaïve CRPC patients with dendritic cells loaded with NY-ESO-1, MAGE-C2, and MUC1 peptides, specific T cell responses were detected and in patients with IFN-γ^{+} T cells, extension of median radiological progression-free survival was observed (79).

MUC1 has also been targeted using a virus-based vaccine. TG4010 is a recombinant vaccinia virus Ankara expressing MUC1 and IL2. In a phase II clinical trial on 40 prostate cancer patients with PSA progression treated with TG4010, 13 patients had at least a 2-fold improvement in PSADT, and 10 patients had stabilized PSA for more than 8 months (80). Although the primary objective of a 50% PSA reduction from base line was not achieved, inclusion of

MUC1 in the vaccine provided some therapeutic benefits. Collectively, the above observations support MUC1 being a useful TAA for developing prostate cancer vaccine.

CONCLUSION

Since its discovery as a component of human milk fat globule in 1977 (20), MUC1 has been extensively studied in cancer, particularly in epithelium-originated malignancy; it is commonly overexpressed with aberrant glycosylation in numerous cancer types (19), including prostate cancer. Despite some inconsistencies (46), cumulative evidence clearly reveals MUC1 upregulation in prostate cancer, and its possible role in initiation, progression, and metastasis of prostate cancer. While MUC1 expression does show prognostic value, this prediction is not robust and should be strengthened by multigene panels for potential clinical application. In this regard, the multigene panels derived from MUC1's network (51, 71) should be explored for clinical applications. While MUC1 as a TAA has clinical benefits as a vaccine, its therapeutic potential seems limited based on several clinical trials in which MUC1 tandem repeat peptide core and aberrant glycosylation have been used. Approaches to inhibition of MUC1-C warrant more attention. Of note, GO-201, a synthetic peptide that inhibits MUC1-C oligomerization displays anti-prostate cancer activity in preclinical studies (81). Additionally, upon linkage to ZZ-PE38, the Fc-binding ZZ domain of protein A fused to *Pseudomonas* exotoxin (82), a humanized mAb DMB5F3 potently killed MUC1$^+$ cancer cells (83). DMB5F3 recognizes the SEA domain shared between MUC1-N and MUC1-C (83). The therapeutic utility of GO-201 and DMB5F3-ZZ-PE38 in treating prostate cancer should be investigated either alone or together with the current MUC1 vaccines. Further, the role of MUC1 on *MUC1$^{-/-}$* mice and MUC1 transgenic animals should be investigated. Both mouse lines are available (84, 85). Transgenic expression of human MUC1 in mice did not cause tumor formation (85). *MUC1$^{-/-}$* mice were normal (84) but showed delay in mammary tumor formation induced by polyoma middle T antigen (84). It will be interesting to see the impact of these mice on research into prostate cancer formation and progression induced by prostate-specific PTEN deficiency.

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