Characterization of MUC1 glycoprotein on prostate cancer for selection of targeting molecules

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Abstract. MUC1 glycoprotein that is overexpressed in aberrant forms in epithelial cancers has been used for diagnosis, staging and therapy. As normal prostate and prostate cancer tissues express MUC1, it represents a potential target, but MUC1 epitopes specific to prostate cancer have not been well characterized. In order to assess MUC1 epitopes in prostate cancer, and their correlation with Gleason grades, binding of 7 well-characterized anti-MUC1 monoclonal antibodies (MAbs) (BrE-3, SM3, BC2, EMA, B27.29, HMFG-1 and NCL MUC1 core), were studied on a prostate tissue microarray. This microarray contained 197 prostate tissue cores representing: i) normal/benign prostate; ii) prostatic intraepithelial neoplasia and Gleason grades 1 and 2; and iii) Gleason grades 3-5. These MAbs bind the MUC1 extracellular domain, but have variable sensitivity to MUC1 glycosylation. To further characterize the effect of glycosylation on their binding, MAb reactivities with unglycosylated MUC1 core peptide and breast and prostate cancer cell lysates were compared. These studies demonstrated strong binding of BrE-3, BC2 and EMA to the peptide core and recognition by BrE-3, SM3, BC2 and EMA of hypoglycosylated MUC1. The results for the microarray indicated that higher Gleason grades were associated with markedly increased cellular staining by MAbs that preferentially recognize less glycosylated MUC1 (BrE-3, $p<0.001$; SM3, $p<0.004$; EMA, $p=0.009$; and BC2, $p<0.001$). Staining by MAbs that bind preferentially to hyperglycosylated MUC1 (B27.29, $p=0.33$; HMFG-1, $p=0.89$; and NCL MUC1 core, $p=0.96$) did not correlate with Gleason grade. These results demonstrated that hypoglycosylated MUC1 expression increased with Gleason grade, thus supporting the targeting of hypoglycosylated MUC1 epitopes in prostate cancer for more specific imaging and therapy applications.

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Introduction

Prostate cancer is the most common cancer in men with 232,000 new cases and 30,350 deaths estimated for 2005 in the USA (1). The 5-year survival rate is 34% for patients with metastatic prostate cancer. For this reason, new modalities are needed to selectively target and treat prostate cancer. Specific targeted treatment for this disease requires sensitive methods for early detection and therapy. Identification of epitopes solely available on the cancer cells is needed. The mucin, MUC1, a high molecular weight glycoprotein, is a good candidate for providing such epitopes. MUC1 is a heterodimer composed of a N-terminal extracellular region with a variable number (20-120) of 20 amino acid tandem repeats (VNTR) that are substituted with sugars (2). The extracellular protein subunit is bound to a membrane associated subunit with transmembrane and intracellular domains (3). MUC1 is distributed apically on normal glandular epithelial tissue (4,5) and its glycosylation patterns have been observed to be tissue specific (3). MUC1 expression has been correlated with a worse patient outcome in studies of several cancers (6-14) and is thought to play a role in progression and metastasis (3,15). Higher expression of aberrant forms of the MUC1 glycoprotein have been observed in a number of cancers (15-20). Expression of MUC1 epitopes with reduced branching of O-glycans has been observed in breast cancer tissue (18,21) and was reflected by increased staining with MAbs recognizing hypoglycosylated MUC1 (17,20,22-27). However, not all cancers express high levels of hypoglycosylated MUC1 epitopes and cancer progression is not always associated with this MUC1 form (11,28,29).

Use of MUC1 targeting requires analysis of the MUC1 epitopes present in the cancer of interest. No in depth characterization of MUC1 expression on prostate cancer has been performed to correlate MUC1 epitope variation with tumor

grade. In a study utilizing MAb B27.29, increased diffuse cytoplasmic MUC1 correlated with increased prostate cancer grade (30). Zhang *et al* (31) also showed increased staining of metastatic compared to primary prostate cancer and normal prostate glandular epithelial tissue using MAb HMFG-2 (32). Other studies, utilizing a single antibody against MUC1 extracellular epitopes, have shown an association between disease progression and MUC1 expression (33-36). In a study utilizing an antibody to the MUC1 cytoplasmic tail, no association with disease progression was found (37). MUC1 overexpression is also associated with tumor angiogenesis in prostate cancer (38), and may be modulated by androgens and estrogens (39,40).

In the present study, mapping of MUC1 epitopes in prostate cancer was performed by analysis of the binding of 7 well-characterized anti-MUC1 MAbs with prostate cancer tissue. The results provide a broader picture of the differences in MUC1 epitopes detected on prostate cancer vs. normal prostate tissues in respect to glycosylation and levels of MUC1 representation and allow more informed selection of targets on prostate cancer cells for molecular imaging and treatment.

Materials and methods

Tissue specimens. Tissue fixed in formalin and embedded in paraffin were obtained from the University of California at Davis Human Biological Specimen Repository. Specimens, categorized in terms of pathology and evaluated for Gleason grade (41), were chosen to include samples from benign/normal prostate tissues and a range of prostate cancers from PIN to Gleason grade 5. Benign/normal tissues included normal prostate tissue, benign prostatic hyperplasia (BPH), benign prostate tissue with atrophy and benign prostate tissue with inflammatory response.

After identification of areas of interest by a pathologist, 197 cores with a diameter of 0.6 mm were punched from tissue blocks and assembled in a recipient paraffin block using a Tissue Microarrayer (Beecher Instruments, Silver Spring, MD); 32 tissue blocks from 24 patients were utilized to construct the tissue microarray that consisted of 77 cores with benign/normal (39%), 31 cores with PIN and Gleason grades 1 and 2 (16%) and 89 cores with Gleason grades 3-5 (45%).

Anti-MUC1 MAbs. Seven mouse MAbs were chosen to evaluate MUC1 epitopes: B27.29, EMA, BC2, NCL-MUC1 Core, HMFG-1, BrE-3 and SM3 (Table I). With the exception of BrE-3, all of these MAbs were characterized extensively in the 1998 Workshop on MUC1 MAbs (42); *in vitro* and *in vivo* binding of BrE-3 has been described elsewhere (19,22,23,27, 43-45). MAb characterization included comparison for core peptide epitopes (46), VNTR affinity (47), effect of glycosylation on binding to VNTR (48), ability to bind to highly glycosylated (49) and hypoglycosylated cell lines (50) and binding to normal MUC1-expressing tissues (breast, small intestine, colon) (51). In addition to their MUC1 epitope diversity, these MAbs were also selected based on published information reflecting their usefulness for imaging, therapy and immunopathology correlations with cancer stage and prognosis. Sources of MAbs were: BrE-3 (gift from R. Ceriani); SM3 (Cancer Research UK); B27.29 (Rebiodiagnostics, Edmonton, Canada); BC2 (Accurate Chemical, Westbury, NY); EMA (Dako, Carpinteria, CA); HMFG-1 (Novocastra, Newcastle upon Tyne, UK); and NCL MUC1 Core (Novocastra).

ELISA. A MUC1 100-mer synthetic peptide with 5 VNTR, $(GVTSAPDTRPAPGSTAPPAH)_5$ (O. Finn, Pittsburgh, PA), was conjugated to BSA using EDC reagent (Pierce Chemicals, Rockford, IL). Triplicate wells (Falcon flexible 96-well plates, Becton-Dickinson, Franklin Lakes, NJ) were coated overnight at 4˚C with 0.13 μg of BSA-MUC1 conjugate per well in coating buffer (15 mM $NaHCO_3$, pH 9.6). Non-specific binding was blocked by incubation with 3% non-fat milk in phosphate-buffered saline containing 0.05% Tween-20 (PBST) for 1 h at 37˚C. Anti-MUC1 MAbs and the control IgG_1 (R&D Systems, Minneapolis, MN) were incubated at 5 μg/ml in PBST containing 0.03% non-fat milk or for HMFG-1 and NCL MUC1 core at 1:10 dilutions in PBS/0.03% non-fat milk of tissue culture supernatants for 1 h at 37˚C. The detection MAb, an anti-mouse conjugated to horseradish peroxidase (HRP) (Promega, Madison, WI), was used diluted at 1:500 in PBST/0.03% non-fat milk for 1 h at 37˚C. After each incubation, the wells were washed 5 times with PBST. Following the last wash, the 2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) (Sigma, St. Louis, MO) substrate containing 0.03% H_2O_2 was added. After development of the colorimetric reaction, plates were read at A_{405nm} in a MRX microplate reader (Dynatech Laboratories, Inc., Chantilly, VA).

Cell lines. All cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA). DU145 prostate cancer cells were grown in RPMI medium (Gibco, Invitrogen Corp., Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS) (Gibco). MCF-7 breast cancer cells were grown in DMEM medium (Gibco) supplemented with 5% FBS.

Cell and tissue lysates. Cell pellets rinsed with PBS were incubated for 30 min on ice in lysis buffer (150 mM NaCl, 20 mM Tris-HCl, pH 7.6, 2 mM EDTA, 1% Nonidet P-40, 0.1% SDS, 0.1% sodium deoxycholate, 1 mM PMSF, 10 μg/ml leupeptin, 10 μg/ml pepstatin and 10 μg/ml aprotinin), followed by centrifugation for 5 min at 3000 rpm (Eppendorf microfuge) to remove debris. Protein concentrations of the cell lysates were measured by BCA protein reagent (Pierce Chemicals). For comparison, a frozen human prostate biopsy sample, obtained from the UC Davis tissue repository, was ground using a mortar and pestle in lysis buffer and treated similarly to cell lysates. Cell lysates were used immediately or stored at -20˚C.

Immunoblotting. Proteins were separated on 4-12% non-reducing PAGE gels, followed by transfer onto PVDF membranes (Millipore, Bedford, MA). After blocking with 10% non-fat milk in PBST (0.5%), the membrane was cut into strips for incubation overnight at 4˚C with anti-MUC1 MAbs in PBST containing 0.3% non-fat milk. The following MAb concentrations were used: 5 μg/ml for BrE-3; 3.3 μg/ml for SM3; 0.4 μg/ml for B27.29; 0.17 μg/ml for BC2; 0.8 μg/ml for EMA. For HMFG1 and NCL MUC1 core, tissue culture supernatants were diluted at 1:75. Following rinses in PBST,

Table I. Characteristics of the anti-MUC1 MAbs used to assess MUC1 on prostate cancer.

MAb	MUC1 epitopes to which antibody binds[b]	Isotype	Core epitope[a]
BrE-3	Moderately and hypoglycosylated (19)	IgG1	TRP
SM3	Hypoglycosylated (17,18,26)	IgG1	APDTRP
B27.29	Hyperglycosylated/normal (59)	IgG1	DTRPAP
BC2	Moderately and hyperglycosylated (47,49,50)	IgG1	APDTR
EMA	Hypo and hyperglycosylated (47,50)	IgG2a	PDTRP
HMFG-1	Hyperglycosylated (32,60)	IgG1	PDTR
NCL-MUC1 core	Hyperglycosylated/normal (47,50,61)	IgG1	PDTRPAP(G)

[a]All of these MAbs target epitopes in the VNTR region (42,44,46). [b]Epitope recognized by MAb as described in References.

the anti-mouse-HRP MAb was added diluted at 1:1000. ECL reagent and hyperfilm™ ECL (Amersham Biosciences Inc., Piscataway, NJ) were used for detection of HRP activity.

Tissue microarray immunohistochemistry (IHC). Slides at room temperature were deparaffinized through xylenes and alcohols, and rinsed in PBS. Endogenous peroxidases were quenched by 15 min incubation in 0.3% H_2O_2 in methanol followed by rinsing in PBS. Antigen retrieval was performed by microwaving the slides 3 times for 5 min at 600 W in 10 mM sodium citrate buffer, pH 6.0.

Slides were blocked for 30 min with 10% goat serum in PBS prior to incubation overnight at 4˚C with anti-MUC1 MAbs at the concentrations listed; 5 μg/ml BrE-3, 10 μg/ml SM3, 0.5 μg/ml B27.29, 2 μg/ml BC2, and 6.4 μg/ml EMA. Antibodies HMFG1 and NCL MUC1 core (tissue culture supernatants) were diluted 1:10 and 1:20, respectively in PBS. After rinsing in PBS, the slides were incubated for 40 min with biotinylated anti-mouse antibody (1:250), followed by rinsing in PBS. ABC reagent was added according to the manufacturer's instructions, followed by rinsing in PBS, with DAB used for detection. The slides were dipped in Mayer's modified hematoxylin followed by dehydration and mounting. After IHC, images were captured using a Kontronic camera model 8102 with an Olympus BH2 microscope. Images were then stored electronically for review and scoring by the pathologist.

Microarray data analysis. Core images from arrays were assigned scores based upon percent of tumor cells stained, apical vs. cytoplasmic staining, and intensity of stain. Cores without tumor cells (normal/benign) were scored to reflect the percentage of epithelial glandular cells stained. The percentages of apically and cytoplasmically positive cells were noted along with the intensity of the stain on a scale from 0-3 where no stain was scored as 0, light stain as 1, moderate stain as 2 and dark stain as 3. The scores were then grouped by tissue status (normal/benign; PIN, Gleason grades 1 and 2; and Gleason grades 3-5) and assessed by hematoxylin and eosin staining of a separate matching slide. Cores were considered positive if ≥ 50% of tumor cells (stained either apically or cytoplasmically) were positive with a minimum staining intensity of 1. The data were analyzed using SAS software

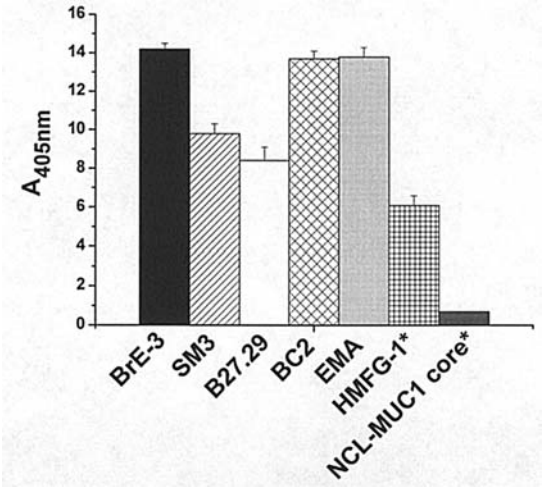

Figure 1. ELISA with anti-MUC1 MAbs and 100 mer unglycosylated synthetic MUC1 peptide. MAbs were added to microtiter plate wells coated with the 100 mer peptide conjugated to BSA (0.13 μg/well), at 5 μg/ml or for HMFG-1 and NCL MUC1 core (*) 1/10 dilutions of tissue culture supernatant. MAb binding was measured by HRP activity on ABTS substrate with an HRP-anti-mouse MAb conjugate, normalized to binding by control IgG1 MAb. Results from duplicate experiments performed in triplicate are shown as mean ± SE.

(SAS version 8.0, SAS Institute, Inc., Cary, NC) for trend of increased staining and Gleason grade using the highest raw percent score obtained apically or cytoplasmically. Analysis was performed using a linear regression model with Gleason grade as a predictor. The means were also compared using analysis of variance (ANOVA) with Tukey's protected multiple comparisons procedure and α=0.05.

Results

Binding of anti-MUC1 MAbs to unglycosylated MUC1 core peptide. Binding of the panel of selected anti-MUC1 MAbs to a 100 mer unglycosylated MUC1 peptide [(GVTSAPDTRPAP GSTAPPAH)$_5$] was assessed by ELISA (Fig. 1). MAbs BrE-3, BC2 and EMA bound readily to the synthetic MUC1 VNTR core peptide while moderate binding was observed for MAbs SM3, B27.29 and HMFG-1 and no binding for NCL-MUC1 core.

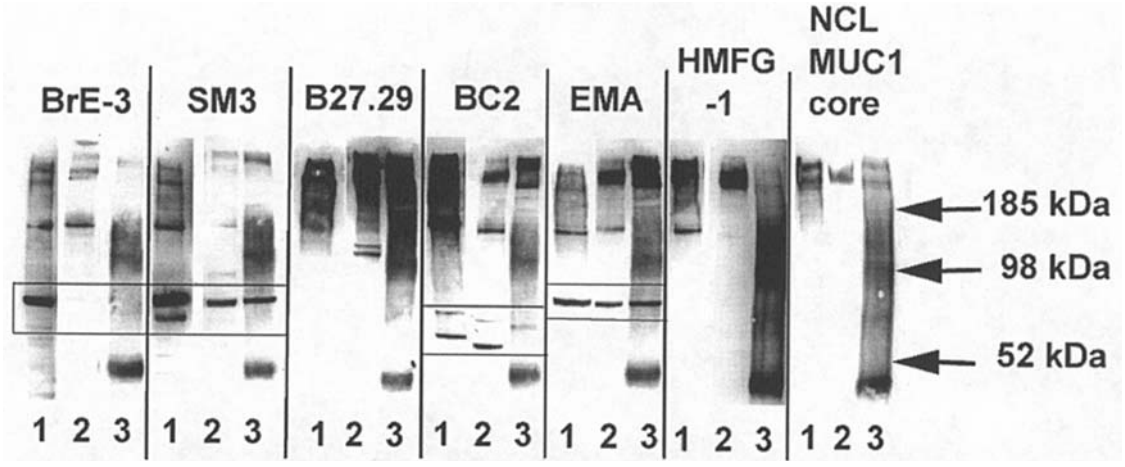

Figure 2. Western blot immunodetection of MCF-7 and DU145 cell and prostate tumor tissue lysates. Cell (5 μg) or tissue lysate proteins (50 μg) were separated by SDS-PAGE and transferred to a PVDF membrane for immunodetection with the anti-MUC1 MAbs. For MAbs HMFG-1 and NCL MUC1 core, 25 μg/lane DU145 cell lysate and 70 μg/lane prostate tumor lysate were used. For each MAb, lane 1 corresponds to MCF-7 cell lysate, lane 2 to DU145 cell lysate and lane 3 to prostate tumor tissue lysate. MW standards are indicated on the right side. Proteins running above the 185-kDa standard were considered hyperglycosylated whereas proteins below the 98-kDa standard were considered hypoglycosylated. The boxes indicate the selective reactivity of MAbs BrE-3, SM3, BC2 and EMA with hypoglycosylated MUC1 present in the MCF-7 and DU145 cell lysates.

Binding of anti-MUC1 MAbs to MUC1 in lysates of breast and prostate cancer cells and tissue. Proteins present in lysates from breast cancer MCF-7 cells, prostate cancer DU145 cells and prostate tissue were separated by SDS-PAGE, transferred to a PVDF membrane and reacted with each of the selected 7 anti-MUC1 MAbs (Fig. 2). Since mucins do not run on SDS-PAGE strictly according to molecular weight (MW), but are influenced by additional negative charges from sialylation (52), no apparent MW was assigned to each of the MUC1 proteins detected on the immunoblots (Fig. 2). Instead, MUC1 glycosylation states were assigned to 3 mobility zones: MUC1 forms with less mobility than the 185-kDa MW standard were assumed to be hyperglycosylated, MUC1 species with a mobility between that of the 98- and 185-kDa MW standards were considered as moderately glycosylated and MUC1 forms with a mobility higher than the 98-kDa MW standard were considered to be hypoglycosylated. Proteins with mobilities of approximately 98 and 50 kDa, exclusively detected in the prostate tissue lysate by all 7 anti-MUC1 MAbs, were most likely not MUC1 related.

Hyperglycosylated MUC1, present in all the lysates, reacted strongly with MAbs B27.29, BC2, EMA and HMFG-1, whereas weaker reactions were observed for MAbs BrE-3, SM3 and NCL-MUC1 core. Hypoglycosylated MUC1 in the MCF-7 and DU145 cell lysates was only recognized by MAbs BrE-3, SM3, BC2 and EMA (Fig. 2, boxed portions). It is notable that MAbs recognizing an apparent low molecular weight protein, consistent with poorly glycosylated MUC1, also bound well to unglycosylated MUC1 core peptide (Fig. 1).

IHC on prostate cancer tissue. Two stages of prostate cancer and benign/normal tissue were represented among the 197 tissue cores and their distribution was as follows: benign/normal, 77 cores; PIN and Gleason grades 1 and 2, 31 cores; Gleason grades 3-5, 89 cores. The results of IHC with the 7 anti-MUC1 MAbs are illustrated in Fig. 3 and summarized in Table II. Tissue cores were scored as staining positively when >50% of the cells were stained. A correlation between increase in hypoglycosylated MUC1 and Gleason grade was supported by the staining patterns of MAbs BrE-3 and SM3, which preferentially recognized hypoglycosylated MUC1 (Fig. 3 and Table II). Regression analysis confirmed the association between increased staining with BrE-3 and SM3 and higher Gleason grade ($p<0.001$ for both MAbs). Two, BC2 and EMA, of the 3 MAbs recognizing hyper and moderately glycosylated forms of MUC1 showed increased staining with higher Gleason grade ($p<0.001$ and $p=0.009$, respectively), whereas B27.29, characterized as recognizing normal and hyperglycosylated MUC1, did not ($p=0.33$). NCL MUC1 core and HMFG-1, MAbs preferentially recognizing hyperglycosylated MUC1, also showed no increased staining with increased Gleason grade ($p=0.89$ and $p=0.96$, respectively). These observations were more apparent when the ratios of percent positive cells in

Table II. Percent[a] of positive cells in prostate tissue biopsies stained by anti-MUC1 MAbs.

MAb	B/N	PIN 1, 2	3-5
BrE-3[b]	16.8±30.7	26.0±33.6 (2)	60.9±31.9 (4)
SM3[b]	37.5±43.2	70.5±40.9 (2)	70.4±38.8 (2)
B27.29	33.0±38.2	43.8±36.1 (1)	39.4±39.0 (1)
BC2[b]	21.4±35.6	39.1±34.5 (2)	55.3±35.6 (3)
EMA[b]	32.7±38.4	42.3±40.1 (1)	51.1±34.3 (2)
HMFG-1	13.1±26.2	9.8±17.5 (1)	13.6±25.0 (1)
NCL MUC1 core	13.8±29.6	9.8±17.2 (1)	13.5±25.0 (1)

[a]Mean percent (± SD) obtained by averaging percents of stained (apical or cytoplasmic) cells. [b]MAbs demonstrating a significant correlation of staining with Gleason grade. Numbers in parentheses indicate the ratio of percent positive cancer cells to percent positive benign/normal cells.

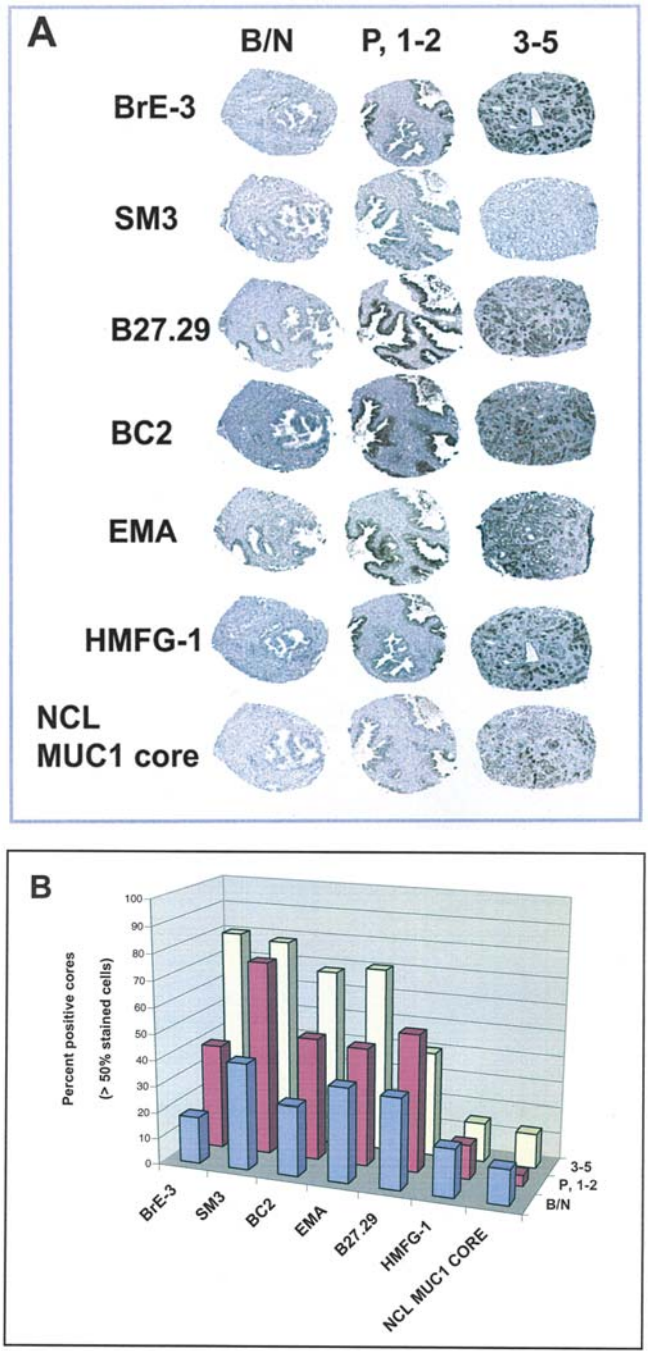

Figure 3. IHC evaluation of MUC1 present on prostate tissue. (A) Illustration of stained tissue cores. The same core, for each tissue grade, stained by each anti-MUC1 MAb is depicted. (B) Percent of cores with ≥ 50% of the cancer cells or, for benign/normal tissue, glandular epithelial cells stained (see Materials and methods and Table II for scoring details). Higher Gleason grades were associated with markedly increased staining by the MAbs (BrE-3, SM3, BC2 and EMA) that preferentially recognize less glycosylated MUC1. B/N: benign/normal; P, 1-2: PIN and Gleason grades 1-2; and 3-5: Gleason grades 3-5.

prostate cancer to percent positive cells in normal/benign tissue were compared (Table II). BrE-3, SM3 and BC2 had the highest ratios, indicating more specific targeting of prostate cancer compared to normal tissue. These MAbs bound the unglycosylated MUC1 peptide core and appeared to bind a low MW protein in MCF-7 and DU145 cell lysates. Taken together, these data are consistent with less hyperglycosylated MUC1 and more hypoglycosylated MUC1 on prostate cancer tissue that correlated with higher Gleason grade.

Discussion

Epitopes of MUC1 on breast cancers have provided molecular targets for imaging and therapy (45,53,54). Despite growing interest, no clear picture of MUC1-specific epitopes in prostate cancer has yet emerged. Previous studies have used a single anti-MUC1 MAb to characterize MUC1 expression and disease progression, though extensive studies of anti-MUC1 MAbs, selected with a focus on breast cancer (19,42), concluded that binding was due not only to recognition of the MUC1 peptide core, but is also affected by glycosylation. Because targeted therapy requires careful selection of the target to be used, we evaluated MUC1 epitopes as a function of prostate cancer grade using a panel of 7 anti-MUC1 MAbs (SM3, BrE-3, BC2, EMA, HMFG-1, B27.29 and NCL MUC-1 core), previously characterized on MUC1 present in breast tissue (19,42). To standardize our study of prostate tissue, the MUC1 binding of these MAbs was reassessed by ELISA on unglycosylated MUC1 core peptide as well as immunoblotting of cancer cell lysates. The MAbs were classified according to their preferential recognition of various MUC1 epitopes. BrE-3 and SM3 recognize epitopes on hypoglycosylated forms of MUC1; BC2, and EMA recognize epitopes available on hyperglycosylated and some hypoglycosylated forms of MUC1; and B27.29, HMFG-1 and NCL MUC1 core recognize epitopes on hyperglycosylated and normal forms of MUC1. This panel of anti-MUC1 MAbs was then utilized to characterize and compare MUC1 epitopes on a prostate tissue array (197 cores) composed of normal and prostate cancer tissues that included various malignant grades (PIN, Gleason grades 1, 2, and 3-5). On the prostate tissue array, the highest percent of prostate cancer tissue was stained by MAbs binding to moderately and hypoglycosylated MUC1: BrE-3, SM3, EMA and BC2. The reactivity observed for these MAbs is in agreement with previously published studies (42,44,46). Preferential binding of BrE-3 and SM3 to hypoglycosylated MUC1 has been exploited in the detection of metastatic breast cancer by imaging (45,53) including metastasis to lymph nodes (55,56). Preferential binding of EMA to prostate cancer in comparison to normal tissue has also been reported (4). Each of these MAbs, BrE-3, SM3, EMA and BC2, recognizing hypoglycosylated MUC1 showed increased staining of prostate cancer tissue with increased Gleason grade, which was in contrast to the MAbs recognizing only hyperglycosylated MUC1, strongly suggesting a higher representation of hypoglycosylated MUC1 on prostate cancer. MAbs B27.29, HMFG-1 and NCL MUC1 core, preferentially binding to epitopes on hyperglycosylated forms of MUC1, did not show increased staining with increased Gleason grade.

Our results with B27.29, showing no correlation of expression with Gleason grade, are in disagreement with those reported by Kirschenbaum *et al* (30). We found no correlation when considering either cytoplasmic staining or overall staining. Furthermore, Western blot analysis with B27.29 showed that it preferentially recognized hyperglycosylated MUC1 on prostate cancer cells and tissue. Similarly, no difference was observed for cytoplasmic staining between

tumors of different nuclear grade in a study of breast cancer stained by B27.29; interestingly, breast cancer with low B27.B29 staining had a higher nuclear grade (57). In our study, the overall percent of positive cores in Gleason grades 3-5 was lower for B27.29 than for BrE-3, SM3, BC2 and EMA, consistent with lower expression of hyperglycosylated MUC1 epitopes on prostate cancer tissues. With HMFG-1, we observed more staining on normal prostate cores in comparison to cores of Gleason grades 3-5 whereas Zotter *et al* (4) found staining in 9/10 prostate cancers and only weak focally positive staining on normal prostate tissue. Sampling size may account for these discrepancies as our sample size was larger than those reported by others. Our prostate tissue microarray IHC data support an overall increase of MUC1 epitopes in prostate cancer compared to normal prostate tissue, as well as a higher representation of hypoglycosylated MUC1 epitopes on higher grade prostate cancer. Based on the reactivity of HMFG-2, a MAb characterized as binding to less hypoglycosylated MUC1 on breast cancer cells (32), Zhang *et al* (31) reached a similar conclusion for normal, primary and metastatic prostate cancer tissues.

Lapointe *et al* (34) showed by gene expression profiling that primary prostate cancer falls into 3 subtypes that are associated with distinct clinicopathological features, and that MUC1 is expressed in the most clinically aggressive subtypes (II and III). In a comparison between gene expression in primary and metastatic prostate cancer tissues, the MUC1 gene was not found to be differentially expressed (58). Using a MAb against a cytoplasmic epitope of MUC1, O'Connor *et al* (37) concluded that MUC1 expression was heterogeneous in both normal and malignant prostate epithelia. On the other hand, Arai *et al* (36) found a correlation between the levels of sialylated MUC1 and the histological grade and clinical stage of the prostate cancer using MAb MYE12, which detects sialylated MUC1. Finally, a correlation of MUC1 expression with perineural invasion using the EMA MAb was reported by Tsuzuki *et al* (33), with the conclusion that MUC1 could be useful for diagnosis of perineural invasion.

The data presented in our study are consistent with an increase of MUC1 epitopes in prostate cancer tissue compared to normal prostate tissue; a majority of these epitopes are present as hypoglycosylated forms of MUC1.

In conclusion, this study, using a panel of anti-MUC1 MAbs with specificities for various MUC1 glycoforms, supports the following conclusions: i) extracellular MUC1 is present at higher levels on prostate cancer than on normal or benign prostate tissue, and ii) increased presence of hypoglycosylated MUC1 in prostate cancer tissue is associated with higher Gleason grade. Since hypoglycosylated MUC1 epitopes are greatly increased in higher grade prostate cancers, targeting these epitopes should allow MUC1-directed imaging and therapy in aggressive prostate cancer. Though our results support the targeting of hypoglycosylated forms of MUC1, there is not yet a consensus regarding MUC1 gene expression and protein representation in prostate cancer.

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