Pathol. Oncol. Res. (2010) 16:371–375
DOI 10.1007/s12253-009-9231-4

MUC1 Expression in Incidental Prostate Cancer Predicts Staging and Grading on the Subsequent Radical Prostatectomy

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Received: 5 October 2009 / Accepted: 16 November 2009 / Published online: 27 November 2009

Abstract The behavior of Incidental prostate cancer (IPC) cannot be reliably predicted by means of conventional histomorphology. MUC1 (episialin) expression has been linked to poor outcome in peripheral prostate cancer (PC). We aimed to determine the so far neglected prognostic role of MUC1 expression in IPC which most commonly represents transition zone cancer. Using Tissue microarray (TMA), we assessed the association between MUC1 expression recorded in transurethral resection specimens of the prostate (TURP chips) and histopathologic outcome parameters (Gleason scores and histologic staging) performed on the subsequent radical prostatectomies (RPs) in a study cohort of 54 patients. Due to tissue loss during arraying and sectioning, a total of 44 (81.5%) tumor samples remained available for immunostaining which was dichotomized by two independent clinical pathologists as being absent or present. MUC1 expression was present in 7 (15.9%) of the 44 IPC immunohistochemically investigated with a striking overrepresentation in high stage tumors, and was significantly correlated with histopathologic staging (ρ=0.4; p=0.02) and Gleason scores (ρ=0.3; p=0.03) performed on the corresponding RPs. These data were confirmed by means of the McNemar test (staging: p=0.01; grading: p=0.04). Our findings suggest that MUC1 might become a valuable adjunct to enable individual prognostic ramification prior to radical surgery in prostate cancer histologically detected in TURP chips. This interesting observation clearly awaits validation by larger studies surveying clinical follow-up data.

Keywords Incidental prostate cancer · MUC1 (episialin) · Immunohistochemistry · Histopathologic outcome parameters · Radical prostatectomy

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Introduction

Incidental prostate cancer (IPC) is defined as a clinically unapparent tumor discovered after histologic examination of the specimen obtained by open prostatectomy or transurethral resection (TURP chips) of the prostate [1]. Its overall incidence in TURPs performed for clinically benign prostatic hypertrophy varies between 13% and 22% [2]. The currently established subdivision of the disease into two histopathologic stages (T1a and T1b) according to the latest TNM classification system has gained wide acceptance [3].

However, owing to its substantially variable natural history, clinical management ("wait and watch" versus radical surgery) remains challenging since the clinical course of individual IPC cannot be predicted by means of conventional histopathology. Especially, there is often disagreement between the Gleason score at TURP and prostatectomy [4]. Therefore, advanced predictive factors are clearly needed in order to better define the individual course of the disease.

Mucins are increasingly used as diagnostic markers as well as possible therapeutic targets due to their aberrant expression pattern during cancer progression. Seven genes

for the protein component of epithelial mucin have been cloned [5, 6]. Amongst these, the MUC1 (episialin) gene codes for a non-secretory membrane-associated mucinous glycoprotein with complex prognostic implications in various human neoplasms [7]. Recently, MUC1 has been suggested as a target in breast cancer immunotherapy [8]. In the prostate, MUC1 expression was increasingly observed from benign to prostatic intraepithelial neoplasia (PIN) and malignant glands [9]. It has been found to play a pivotal role in prostate cancer (PC) progression, and its over-expression has been reported to occur in PC with poor outcome after radical prostatectomy (RP), and also to correlate with high Gleason score [10–12]. However, its prognostic role in IPC, which most commonly represents transition zone cancer, has not been assessed yet.

Since RP is only very rarely performed after the histologic detection of IPC in TURP chips, we conducted a multi-center study which enabled us to present the first immunohistochemical study aiming to evaluate the so far neglected prognostic role of MUC1 expression in IPC.

Material and Methods

Patient Selection

Retrospective computerized database analysis was performed in order to identify all patients with newly diagnosed clinically unapparent (neither palpable nor visible by imaging using transrectal 7-MHz ultrasonography) IPC based on histologic examination of TURP specimens resected due to symptomatic prostatic hypertrophy. In all cases, the resected tissue was entirely subjected to histopathologic examination. Histologic tumor staging was straightforward in all cases based upon the histologic estimation of tumor spread because equivocal cases (still T1a versus just T1b) did not occur. All patients subsequently underwent RP in the period of time between 1999 and 2008 at the Departments of Urology affiliated to two hospitals in Berlin (Charité-University Medicine and Vivantes Klinikum am Urban) and five hospitals in Brandenburg (HELIOS Klinikum Bad Saarow, Klinikum Hoyerswerda, Röhn Klinikum Frankfurt/Oder, Klinikum Bautzen, and Ernst-von-Bergmann Klinikum Potsdam). This analysis yielded a total of 54 patients (mean age: 65.9 years; range: 49–80 years). None of the patients received radiotherapy, androgen deprivation, five alpha-reductase inhibitor (i.e., Finasteride) therapy, repeat TURP, or needle biopsy prior to radical surgery. The following data were recorded: serum PSA level at the time of TURP (unavailable in three patients), histopathologic staging (performed on TURP chips and on RPs) and Gleason scores (performed on RPs) as well as surgical margin and nodal status. In RPs harboring multifocal cancer, the composition of the dominant tumor nodule was representative of the entire tumor.

Since Gleason scores have initially been assigned to 17 of the TURP chips evaluated, the remaining cases were subsequently graded on their corresponding Tissue microarray (TMA) spots.

Since the majority of archivated wax-embedded tissue blocks from the corresponding RPs have been discarded, a total of 12 (22.2%) wax-embedded archivated RP specimens could be retrieved for comparative MUC1 immunostaining. Pertinent clinicopathologic metrics of the study cohort are summarized in Table 1.

Pathology

Hematoxylin and eosin (HE)-stained TURP chips were reviewed by two independent clinical pathologists (S.G. and A.E.) in order to select one representative section from

Table 1 Pertinent clinicopathologic metrics (serum PSA levels unavailable in three patients)

Intervals of serum PSA levels at the time of TURP	Absolute (relative) number of cases
Serum PSA≤4.0 ng/m/4.1–10 ng/ml/>10 ng/ml	24(47%)/13(25.5%)/14(27.5%)
Histopathologic staging (n=54) and Gleason grading (n=17) performed on TURP chips	
T1a/1b	27(50%)/27(50%)
Gleason sum 4–6	17(100%)
Gleason sum 7	0
Gleason sum 8–10	0
Histopathologic staging (n=54) and Gleason grading (n=54) performed on RP specimens	
Gleason sum 4–6	45(83.3%)
Gleason sum 7	5(9.3%)
Gleason sum 8–10	4(7.4%)
pT2	48(88.9%)
pT3a/3b	2(3.7%)/4(7.5%)
pN0/pN1	54(100%)/0(0%)
R0/R1	54(100%)/0(0%)

each patient to be used for subsequent Tissue microarray (TMA) construction. During this review, special attention was paid to the presence and distribution of electrocautery artifacts which might affect immunohistochemical results. Notably, cautery artifacts were negligible in the tumor bearing areas of all TURP chips investigated (Fig. 1). All tumors investigated were found to be acinar adenocarcinomas. Ductal adenocarcinomas or other variants of PC were not investigated in our cohort.

Then, the corresponding paraffin wax-embedded tissue blocks were retrieved from the files involved. Concerning TURP chips, tumor bearing areas were marked on HE-stained sections, punched out of the paraffin block (1.5 mm punch diameter), and inserted into a recipient block as previously described [13]. Due to tissue loss during arraying and sectioning, a total of 44 (81.5%) tumor samples remained available for immunostaining.

Immunohistochemistry

Freshly cut 4 μm thick TMA sections were used for immunohistochemistry. From each of the 12 archivated wax-embedded RP specimens retrieved, one representative tumor bearing section from the transition zone of the gland with neigbouring areas of its peripheral zone was also subjected to immunohistochemistry. Following deparaffinization with graded alcohols and xylene, antigen demasking was achieved by heat retrieval (100°C) in 0.01 M citrate buffer for 30 min, automatically performed by bond™ system (Visionbiosystems, Australia). Then, the primary antibody directed against MUC1 (MUC-1 Core Glycoprotein clone Ma552; monoclonal; 1:100 dilution; Novocastra, UK) was employed and incubated at 25°C for 1 h. Subsequently, sections were washed with PBS and incubated with rabbit anti-mouse IgG 1:50 and, following that, mouse peroxidase–antiperoxidase conjugate 1:200. The enzymatic reaction was developed in a freshly prepared solution of diaminobenzidine (0,5 mg/ml; Sigma, Deisenhofen, Germany) and 0.01% hydrogen peroxide in water.

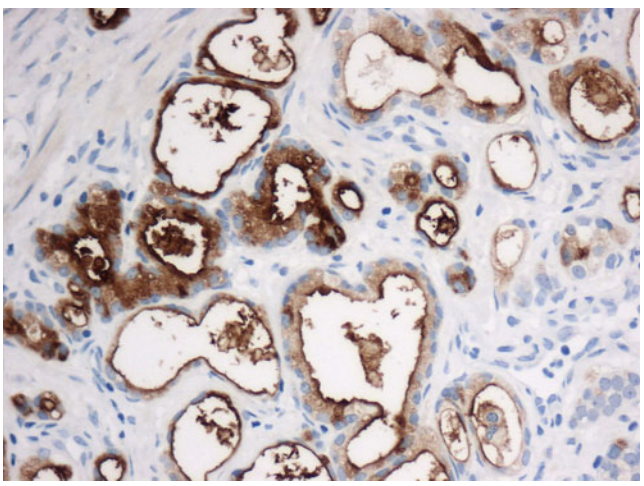

Fig. 1 Incidental prostate cancer showing membrane-associated and variable cytoplasmic MUC1 immunostaining. Anti-MUC1, ×200 magnification

Negative controls were prepared by omitting the primary antibody. Sections from a RP specimen not belonging to the study cohort that contained acinar adenocarcinoma as well as benign prostatic glands served as positive controls.

The immunostained sections were examined by two independent clinical pathologists (S.G. and A.E.) blinded with respect to outcome parameters as well as to the staining evaluation previously signed out by the other clinical pathologist. Assessment of marker expression (dichotomized as being present or absent) was accomplished at ×200 magnification. Briefly, positive staining was defined as to having at least 10% of tumor cells showing membrane-associated and variable cytoplasmic immunostaining (Fig. 1).

Statistical Analysis

Contingency tables and the McNemar test have been employed in order to evaluate the association of the dichotomized MUC1 expression with other parameters.

The Spearman correlation was used to determine the magnitude and direction of the association between parameters. The non-parametric Wilcoxon test was used in order to evaluate differences between the Gleason scores performed on TURP and RP specimens.

Cohen weighted Kappa (K)-statistics were employed to assess inter-observer agreement concerning staining evaluation. Briefly, K-statistics are a measure of overall agreement without requiring assumptions concerning the "correct" grade of marker expression. The value of K ranges from −1.0 to +1.0. A value of 0 indicates chance agreement only, while a value of +1.0 indicates perfect agreement. A negative value implies systematic disagreement between observers. It is generally accepted that a value of 0.001–0.20 indicates slight agreement, 0.201–0.40 fair agreement, 0.401–0.60 moderate agreement, 0.601–0.80 substantial agreement, and 0.801–0.99 excellent agreement, respectively.

The level of significance was set at 0.05. All calculations were performed using the statistical software package SPSS 13.0.

Results

Correlation Between Conventional Clinicopathologic Parameters

Histologic staging performed on TURP chips (T1a and T1b) failed to correlate with Gleason scores (ρ=0.08; p=0.6) and

Table 2 Dichotomized MUC1 expression recorded in the TURP chips in relation to the histopathological tumor stage (n=44)

MUC1 staining	T1a	T1b	pT2a	pT2b	pT2c	pT3a	pT3b
Absent	17	20	20	4	11	1	1
Present	4	3	2	0	2	0	3

with histologic staging (ρ=0.03; p=0.8) performed on their corresponding RPs.

Gleason scores assigned either on TURP chips or TMA spots failed to correlate with histologic staging performed on the TURP chips (ρ=0.072; p=0.61) and on their corresponding RPs (ρ=0.197; p=0.154) as well as with the patients' age (ρ=0.19; p=0.168) and with serum PSA levels at the time of TURP (ρ=0.165; p=0.247).

Gleason scores performed on TURP chips or TMA spots (mean: 5.26; range: 3–7) differed significantly from the Gleason scores assigned on their corresponding RP specimens (mean: 5.48; range: 3–9; p=0.016).

In comparison with the Gleason scores assigned on TURP chips or TMA spots, a total of 16 cases (29.6%) required downgrading on their corresponding RP specimens while the remaining 38 cases (70.4%) were found to have identical Gleason patterns on their subsequent RPs.

The patients` age at the time of prostatectomy failed to correlate with Gleason scores (ρ=0.25; p=0.07) and with histologic staging (ρ=0.03; p=0.08) performed on the corresponding RPs. However, there was a trend toward increased patient age in advanced cancer on RP in our study cohort (mean age in organ-confined disease 64 years versus 67.4 years in organ-exceeding cancer).

Serum PSA levels at the time of TURP showed a significant correlation with Gleason scores (ρ=0.63, p=0.01) and with histologic staging performed on the TURP chips (ρ=0.34, p=0.02) but failed to correlate with Gleason scores (ρ=0.19, p=0.17) and with histologic staging (ρ=0.23, p=0.12) performed on their corresponding RPs, respectively.

MUC1 Expression in the TURP Chips

Staining results are summarized in the Tables 2 and 3. Briefly, MUC1 expression showed diffuse membrane-associated and variable cytoplasmic staining throughout the majority of tumor cells in a total of 7 (15.9%) of the 44 TURP chips immunohistochemically investigated (Fig. 1). Histomorphologically, MUC1 expression did not show any affection to certain conventional criteria such as tumor gland size or arrangement, sheets, or cribriform areas, respectively. The MUC1 expressing cases did not show any association to the different institutions from which the tissues were retrieved. None of the 12 wax-embedded RP specimens comparatively assessed showed any MUC1 expression as did the corresponding TMA spots obtained from their preceding TURP chips.

MUC1 expression recorded in the TURP chips showed a significant positive correlation with histologic staging (ρ=0.4; p=0.02) and with Gleason scores (ρ=0.3; p=0.03) performed on the corresponding RPs. These data were confirmed by means of the McNemar test (staging: p=0.01; grading: p=0.04).

The event of histopathological downgrading, which occurred in a total of 16 (29.6%) RP specimens, failed to show any significant association with dichotomized MUC1 expression.

Concerning dichotomized staining evaluation based upon a threshold of 10% of tumor cells showing MUC1 expression, disagreement between both independent clinical pathologists occured in three (6.8%) of the 44 cases immunohistochemically evaluated. These three discrepant cases were critically discussed in order to establish consensus decisions to be used for subsequent statistical calculations.

Table 3 Dichotomized MUC1 expression recorded in the TURP chips in relation to the Gleason sum assigned on subsequent prostatectomy (n=44; the accumulation of MUC1 expressing IPC in the group of low grade tumors is attributable to their relative over-representation in our study cohort compared with their high grade counterparts)

MUC1 staining	Gleason sum 4–6	Gleason sum 7	Gleason sum 8–10
Absent	31	4	2
Present	4	1	2

Discussion

Paul et al. reported TURP not to represent an adverse prognostic factor compared with needle biopsy, and suggested radical surgery to be performed in selected patients [14]. However, clinical management of individual patients diagnosed with Incidental prostate cancer (IPC) remains challenging due to unpredictability of its speed of progression. Especially, the frequently encountered disagreement between the Gleason score at TURP and prostatectomy strongly limits conventional histopathology to be employed for prognostic evaluation in ICP [4]. In keeping with this phenomenon, a total of 16 cases (29.6%) required subsequent downgrading on the corresponding RP specimens in our study cohort.

We focused on the identification of so far neglected prognostic parameters in IPC. According to our data, expression of Prostatic acid phosphatase (PSAP) in ICP is

predictive of histopathologic tumor stage but not of Gleason scores performed on the subsequent radical prostatectomies [15]. Therefore, in the present study, we aimed to broaden the limited panel of predictive markers by evaluating the so far neglected role of MUC1 expression in transition zone cancer. Tissue microarrays (TMAs) which recently have been reported to represent a useful means for immunohistochemically investigating mucin expression patterns were employed in our study in order to standardize immunostaining procedures [16].

Notably, the determination of cut-off points might impair reproducibility of immunohistochemistry studies. Aiming to minimize this problem, we have used a 10% cut-off threshold to reproducibly delineate MUC1 negative from positive cases. Indeed, the substantial interobserver agreement in terms of its dichotomized staining evaluation (present vs. absent) among independent examinors backs our observation up. However, our data clearly need to be validated by a larger independent study cohort since our investigation was restricted to 44 patients. Also, our observation is felt to merit further validation in terms of possible associations between MUC1 expression in IPC and clinical follow-up (e.g., postsurgical PSA failure).

Looking at our study cohort, approximately one third of clinically unapparent tumors were found to be at least stage pT2c on the subsequent RPs. This figure might be explained by the frequent multicentricity of ICP. Moreover, a subset of IPC was assigned Gleason sum≥7 on the corresponding RPs. Although definite allocation of the zonal origin of cancer is felt to be inappropriate in our cohort, these tumors might have arisen in the peripheral aspect of the gland with subsequent intraglandular spread toward its transition zone.

At first glance, a possible limitation of our study might be that only a minority (15.9%) of the IPC immunohistochemically investigated showed MUC1 expression. This finding is likely to be attributable to the fact that roughly half of the MUC1 expressing cases were found to be stage pT3b on subsequent RPs which represented a striking minority (7.4%) in our study cohort, and therefore, these data are strongly supportive of the suggested prognostic value of MUC1 expression in terms of predicting histologic staging performed on the corresponding prostatectomy.

Notably, the complete concordance between TURP chips and their corresponding RPs in terms of lacking MUC1 expression strongly supports the concept that MUC1 negativity encountered in the TURP chips represents “true” negativity rather than sampling error.

In conclusion, conventional histopathology fails to predict clinical outcome in Incidental Prostate Cancer. MUC1 expression recorded in the TURP chips might become a valuable prognostic adjunct in terms of predicting histologic grading and staging on the subsequent radical prostatectomy, and therefore, might enable individual prognostic ramification prior to radical surgery. However, these observations clearly await validation by larger studies surveying clinical follow-up data.

Acknowledgements We gratefully thank Frank Dietrich,MD, Conrad Flössel,MD, Jan Jander,MD, Helmut Knispel,MD, Hartmut Lobeck,MD, Volker Loy,MD, Roland Pauli,MD, Volkmar Rosenthal,MD, and Wilko Weichert, MD, for their contribution to this work.

We also thank the HELIOS research center for supporting the present study.

References

1. Van Andel G, Vleeming R, Kurth KH, de Reijke TM (1995) Incidental carcinoma of the prostate. Semin Surg Oncol 11:36–45
2. Sheldon CA, Williams RD, Fraley EE (1980) Incidental carcinoma of the prostate: a review of the literature and critical reappraisal of classification. J Urol 124:626–631
3. Sobin LH, Wittekind CH (eds) (2002) TNM classification of malignant tumors. 6th edition. New York: Wiley-Liss, pp 172–175
4. Melchior S, Hadaschik B, Thüroff S, Thomas C, Gillitzer R, Thüroff J (2008) Outcome of radical prostatectomy for incidental carcinoma of the prostate. BJU Int Dec 5 [Epub ahead of print]
5. Gum JR (1992) Mucin genes and the proteins they encode: structure, diversity, and regulation. Am J Respir Cell Mol Biol 7:557–564
6. Jass JR, Roberton AM (1994) Colorectal mucin histochemistry in health and disease: a critical review. Pathol Int 44:487–504
7. Baldus SE, Palmen C, Thiele J (2007) MUC1 (EMA) expressing plasma cells in bone marrow infiltrated by plasma cell myeloma. Histol Histopathol 22:889–893
8. Yang E, Hu XF, Xing PX (2007) Advances of MUC1 as a target for breast cancer immunotherapy. Histol Histopathol 22:905–922
9. Garbar C, Mascaux C, Wespes E (2008) Expression of MUC1 and sialyl-Tn in benign prostatic glands, high-grade prostate intraepithelial neoplasia and malignant prostatic glands: a preliminary study. Anal Quant Cytol Histol 30:71–77
10. Burke PA, Gregg JP, Bakhtiar B, Beckett LA, Denardo GL, Albrecht H, De Vere White RW, De Nardo SJ (2006) Characterization of MUC1 glycoprotein on prostate cancer for selection of targeting molecules. Int J Oncol 29:49–55
11. Li Y, Cozzi PJ (2007) MUC1 is a promising therapeutic target for prostate cancer therapy. Curr Cancer Drug Targets 7:259–271
12. Strawbridge RJ, Nister M, Brismar K, Li C, Lindström S (2008) Influence of MUC1 genetic variation on prostate cancer risk and survival. Eur J Hum Genet 16:1521–1525
13. Kononen J, Bubendorf L, Kallioniemi A, Bärlund M, Schraml P, Leighton S, Torhorst J, Mihatsch MJ, Sauter G, Kallioniemi OP (1998) Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 4:844–847
14. Paul R, Knebel C, van Radenborgh H, Kübler H, Alschibaja M, Günther M, Hartung R (2005) Incidental carcinoma of the prostate: can we and should we recommend radical prostatectomy? Urologe A 44:1054–1058
15. Gunia S, Koch S, May M, Dietel M, Erbersdobler A (2009) Expression of prostatic acid phosphatase (PSAP) in transurethral resection specimens of the prostate is predictive of histopathologic tumor stage in subsequent radical prostetctomies. Virchows Arch 454:573–579
16. Morrison C, Merati K, Marsh WL Jr, De Lott L, Cohn DE, Young G, Frankel WL (2007) The mucin expression profile of endometrial carcinoma and correlation with clinical-pathologic parameters. Appl Immunohistochem Mol Morphol 15:426–431