***MUC2* expression in primary mucinous and nonmucinous adenocarcinoma of the prostate: an analysis of 50 cases on radical prostatectomy**

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The expression of mucin (*MUC2*) in prostate cancer has not been well studied previously and may be of prognostic and pathobiologic significance. It is, however, well known that *MUC2* expression in mucinous pancreatic and breast cancer represents an indolent pathway since these tumors have a significantly better outcome than their conventional counterparts. Twenty-five cases each of Gleason pattern 3 and 4 mucinous adenocarcinoma of the prostate defined by greater than 25% mucinous component and nonmucinous adenocarcinoma of the prostate were obtained from the surgical pathology files of the Johns Hopkins Hospital and Emory University Hospital. Immunohistochemical stains were performed for *MUC2* on all 50 cases. Mean patient age was 60 years (range 44–72 years). *MUC2* was expressed in all 25 cases (100%) of mucinous adenocarcinoma of the prostate, irrespective of the Gleason pattern. The nonmucinous component of these cases was negative for *MUC2*. In contrast, *MUC2* expression was significantly lower in nonmucinous adenocarcinoma of the prostate, detected in only 6/25 cases as a focal finding, while 19/25 (76%) of nonmucinous adenocarcinoma of the prostate were completely negative for *MUC2* ($P < 0.01$). In six cases that showed focal positivity, *MUC2* was expressed in areas with Gleason pattern 3 cancer with extensive mucinous fibroplasia (one case) and prominent intraluminal mucin (five cases). Other areas of these tumors were negative for *MUC2*. Mucinous adenocarcinoma of the prostate shows diffuse expression of *MUC2*, a known tumor suppressor, which is not present in either normal prostate or the majority of conventional adenocarcinomas of this organ. This indicates that mucinous adenocarcinoma of the prostate is indeed of the ‘colloid type’ akin to those in other exocrine organs. It is highly conceivable that this *de novo* expression of *MUC2* has a role, not only in the mucinous differentiation of these tumors and their colloid pattern, but also in their relatively indolent behavior that has been recently elucidated.

Modern Pathology (2008) 21, 789–794; doi:10.1038/modpathol.2008.47; published online 16 May 2008

Keywords: *MUC2*; mucinous adenocarcinoma; nonmucinous adenocarcinoma

Prostate cancer is the most common noncutaneous cancer in men worldwide.^{1–3} The vast majority of prostate cancers are acinar ‘nonmucinous’ adenocarcinomas.⁴ Mucinous adenocarcinoma of the prostate, also referred to as colloid adenocarcinoma, is rare and one of the least common morphologic variants of prostate cancer.^{4–9} The incidence of mucinous adenocarcinoma of the prostate, defined

by the presence of more than 25% extravasated mucin, is approximately 0.2%.^{6,7,9,10} In exocrine organs, including breast, skin and pancreas, the expression of *MUC2* (the goblet-type mucin) is limited to mucinous colloid-type carcinomas, not seen in conventional adenocarcinomas of these organs, or their usual type preinvasive precursors.^{11–13} In these organs, colloid carcinoma (pure mucinous carcinoma), characterized by well-circumscribed lakes of mucin that contain scanty, detached malignant cells has a significantly better prognosis than conventional ductal carcinomas.¹¹ We sought to determine the expression of *MUC2* in both mucinous adenocarcinoma of the prostate and nonmucinous adenocarcinoma of the prostate in

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Received 03 January 2008; revised and accepted 24 January 2008; published online 16 May 2008

lieu of the prognostic and pathobiologic significance of *MUC2* expression in other previously described organs.

Materials and methods

Case Selection

Twenty-five cases each of radical prostatectomy specimens with Gleason patterns 3 and 4 mucinous adenocarcinoma of the prostate defined by greater than 25% mucinous component (of the entire tumor in the specimen) and nonmucinous adenocarcinoma of the prostate were obtained from the surgical pathology files of the Johns Hopkins Hospital and Emory University Hospital.

Immunohistochemistry

Immunohistochemical stains were performed for *MUC2* on all 50 cases. Immunohistochemical stains were performed using the avidin–biotin–peroxidase methodology. The primary immunohistochemical stain is a monoclonal antibody: MUC-2 (Glycoprotein clone: Ccp58 Novocastra, Newcastle upon Tyne, UK), used at 1/100 dilution. Antigen retrieval was performed at pH 6 in citrate buffer, in a pressure cooker for 3 min at a pressure of 15 pounds per square inch. The EnVision plus dual link detection kit (Dako Corp, Carpinteria, CA) was used. Positive stain was present in the cytoplasm. Normal large bowel was used as the positive control. Negative controls had specific antibody replaced by buffer.

Results

Mean patients age was 60 years (range 44–72 years). *MUC2* was expressed in all 25 cases (100%) of mucinous adenocarcinoma of the prostate, irrespective of the Gleason pattern, characterized by diffuse intracytoplasmic staining (Figures 1a–2b). The non-mucinous component of these cases was negative for *MUC2* (Figure 3a and b). In contrast, *MUC2* expression was of significantly lower frequency in nonmucinous adenocarcinoma of the prostate, detected in only 6/25 (24%) cases as a focal finding, while 19/25 (76%) cases were completely negative for *MUC2* ($P < 0.01$; Figure 4a and b). In six cases that showed focal positivity for *MUC2*, staining was in areas with Gleason pattern 3 cancer with extensive mucinous fibroplasia (one case) (Figure 5a and b) or with prominent intraluminal mucin (five cases) (Figure 6a and b). Other areas of these tumors were negative for *MUC2*. In all 50 cases (100%), *MUC2* was negative in benign prostate glands.

Figure 1 (a) Mucinous adenocarcinoma of the prostate, cribriform pattern 4. (b) Positive *MUC2* expression, corresponds to focus in panel a.

Discussion

MUC2 was initially isolated from a human jejunum cDNA library and is thought to be one of the major intestinal mucin genes. *MUC2* is a mucin epitope referred to as 'gel forming' or 'secretory' and its expression in normal tissues is largely limited to intestinal goblet cells, though it is also expressed in the trachea.¹⁴ One of its key functions is to form disulfide bonds and unusually large mucin globules. Although *MUC2* is not typically expressed in the breast or pancreas, its expression has been previously documented in mucinous/colloid carcinomas of both organs, but not in their conventional ductal counterparts.^{11,15–17}

It has been shown that *MUC2* expression is highly specific for mucinous/colloid carcinoma, especially in the breast and pancreas. In a study by Adsay *et al*,¹¹ *MUC2* labeling was detected in all 30 and 18 cases of mammary and pancreatic colloid carcinomas, respectively. In their study, conventional ductal carcinomas of these organs very rarely

Figure 2 (a) Mucinous adenocarcinoma of the prostate Gleason pattern 3. (b) Positive *MUC2* expression, corresponds to focus in panel a.

expressed this marker (3 of 44 (7%) and 1 of 136 (1%), respectively). Other papers have also demonstrated this specificity of *MUC2* for colloid carcinoma.^{17,18} Studies have also shown that the mucin in colloid carcinoma is distinctive, showing o-acylated forms of sialomucin, not generally formed in other carcinomas.^{11,19}

The specific association of *MUC2* with colloid is likely to have a key role in the morphogenesis of this selected group of tumors. In normal tissue, the expression of this marker is largely limited to intestinal goblet cells, hence the name 'secretory'-type mucin. *MUC2* is also known to have specific adhesive properties. It functions as a selective physiologic barrier because of its tendency to form large chains of glycoproteins by disulfide linkage, forming a gelatinous film lining the intestinal epithelium, hence its other synonym 'gel-forming' mucin. Therefore, it is no surprise that it may be a key factor in the morphogenesis of 'gelatinous' (colloid) carcinoma. In addition to accounting for the distinctive morphology of these neoplasms,

Figure 3 (a) Mucinous adenocarcinoma of the prostate with cribriform pattern 4 with adjacent nonmucinous Gleason pattern 3 prostate cancer. (b) Positive *MUC2* expression in cribriform pattern 4 mucinous prostate cancer and negative in adjacent Gleason pattern 3 nonmucinous prostate cancer, corresponds to focus in panel a.

MUC2 may also be a biological factor in the relatively slow growth of colloid carcinoma.¹¹ The fact that *MUC2* has been found to have tumor suppressor function is also suggestive of this hypothesis.²⁰ Absence of *MUC2* induces increased cell proliferation, decreased apoptosis and increased migration of intestinal epithelial cells, ultimately leading to a spectrum of neoplastic transformation, ranging from aberrant crypt foci to adenomas to frank carcinomas.^{11,20} The findings of Adsay *et al*¹¹ suggest that, along with its tumor suppressor activity documented in the gastrointestinal tract, the exocrine pancreas and the breast, *MUC2* may have a role in diverting the process of carcinogenesis toward a more favorable prognostic pathway.

Mucinous adenocarcinoma of the prostate of the prostate, similar to usual acinar adenocarcinoma, is associated with elevated serum prostate-specific

Figure 4 (a) Gleason patterns 4 and 3 nonmucinous prostate cancer. (b) Negative *MUC2* expression in both Gleason patterns 4 and 3 nonmucinous prostate cancer, corresponds to focus in panel a.

Figure 5 (a) Gleason patterns 3 and 4 prostate cancer with extensive mucinous fibroplasia. (b) Focal patchy *MUC2* expression in areas with Gleason pattern 3 and 4 prostate cancer with extensive mucinous fibroplasia, corresponds to focus in panel a.

antigen (PSA) values, metastasize to bone and respond to hormonal therapy.^{21,22} However, its prognosis relative to typical acinar carcinoma remains controversial with divergent data based on isolated case reports or small case series.^{5,8,23–26}

We recently showed in a study of 47 cases of mucinous adenocarcinoma of the prostate that this group of patients tends to do better, stage for stage and grade for grade, than patients with conventional nonmucinous adenocarcinoma of the prostate treated by radical prostatectomy.²⁷ The mean follow-up of mucinous adenocarcinoma of the prostate patients without progression was 5.6 years (median 6 years, range: 1–15). One patient (2%) had PSA progression 3 years after his radical prostatectomy, without the evidence of local recurrence or distant metastasis. The 5-year actuarial PSA progression-free rate was 97% for all patients with mucinous adenocarcinoma of the prostate. Using the Kattan nomogram, the predicted mean 5-year actuarial PSA progression-free rate for nonmucinous adenocarcinoma of the prostate with the same PSA and

postoperative findings as those with mucinous adenocarcinoma of the prostate in the study was 85%.²⁷

This study has demonstrated, from the immuno-histochemical standpoint, that *MUC2* is expressed predominantly in mucinous adenocarcinoma of the prostate. Cappello *et al*²⁸ studied cases of conventional prostate cancer with *PCNA*, *p53*, *HSP60*, *HSP10* and *MUC2*. Their study showed that while *PCNA*, *p53*, *HSP60* and *HSP10* were variably expressed depending on the grade and stage of prostate cancer carcinogenesis, *MUC2* was negative in both tumoral and nontumoral prostatic tissue.

Cozzi *et al*²⁹ examined the expressions *MUC1*, *MUC2*, *MUC4*, *MUC5AC* and *MUC6* in prostate cancer tissues using tissue microarrays (TMAs). There was overexpression of *MUC1* in 58% of primary prostate cancer and 90% of lymph node metastases, but no expression in normal prostate or benign tissues. The expression of *MUC2*, *MUC4*, *MUC5AC* and *MUC6* was found to be negative in both normal and neoplastic tissues. They, however,

Figure 6 (a) Gleason pattern 3 prostate cancer with prominent intraluminal mucin. (b) Focal patchy *MUC2* expression in areas with Gleason pattern 3 prostate cancer with prominent intraluminal mucin, corresponds to focus in panel a.

did not specify whether they had any cases of mucinous adenocarcinoma of the prostate.

In summary, to the best of our knowledge, our study is the largest to date, comparing the immunophenotypic profile of primary mucinous adenocarcinoma of the prostate and nonmucinous adenocarcinoma of the prostate in radical prostatectomy specimens. It is highly conceivable that this *de novo* expression of *MUC2* has a role, not only in the mucinous differentiation of prostate cancers and their colloid pattern, but also in their relatively indolent behavior. Additional studies are warranted from the molecular biologic standpoint to further our understanding of this unique variant of prostate cancer.

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