Comparative Medicine
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Vol 66, No 4
August 2016
Pages 286–289

Case Report

Incidence of Mucinous Metaplasia in the Prostate of FVB/N Mice (*Mus musculus*)

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Prostate epithelium in mice is considered to be relatively resistant to aged-related changes, as compared with human prostate epithelium, which is prone to spontaneous hyperplasia and cancer, for example. In addition, the incidence of metaplasia in mouse prostate typically is considered to be low. Here we report the incidence of mucinous metaplasia in the prostates of wild-type FVB/N mice. Our histologic study shows that mucinous metaplasia involving goblet cells occurs much more frequently (incidence as high as 50%) in the prostates of aged mice (17-24 mo) than has been reported previously. Mucinous metaplasia in the prostates of laboratory mice may be considerably more frequent than previously appreciated.

Abbreviation: PAS, periodic acid–Schiff

Spontaneous pathologic changes occur at low incidence in the prostates of wild-type mice.[12] These changes include neoplastic proliferations, such as hyperplasia and mouse prostatic intraepithelial neoplasia, as well as nonneoplastic alterations. Even in geriatric mice, pathologic changes of the prostate are rare. In a large survey of 612 wild-type B6C3F1 mice involved in a 2-y toxicology and carcinogenicity study, not a single spontaneous carcinoma of prostate was observed.[13] Importantly, mucinous metaplasia was detected in only a single dorsolateral prostate, thus representing less than 0.2% of the mice studied.[13] To our knowledge, no further data for genetically wild-type mice have been published, and mice of different genetic backgrounds have been considered the same in this regard.[12]

The most prominent characteristic of mucinous metaplasia (also called intestinal metaplasia) in the prostate is the appearance of goblet cells, which typically do not occur in the prostatic epithelium. Goblet cells are specialized secretory cells that are a normal component of mucosal epithelium, producing lubrication and acting as a barrier against outside pathogens and debris.[9] Goblet cells occur most frequently in the intestinal and pulmonary epithelia and are prominent in the conjunctival epithelium of the eye.

In the human prostate, well-recognized forms of metaplasia include transitional urothelial metaplasia, mucinous metaplasia, and squamous metaplasia. Mucinous metaplasia in human prostate is usually a focal process, involves all ages, and mostly is located in the inferior periurethral area.[1,6] Benign lesions with mucin-secreting cells are found in both normal and hyperplastic human prostates.[5] In addition, mucinous adenocarcinoma, a rare form of prostate cancer, has been described in human samples.[1]

Received: 16 Nov 2015. Revision requested: 17 Dec 2015. Accepted: 21 Jan 2016.
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Mucinous metaplasia in the prostate reportedly occurs after genetic manipulation in several mouse prostate cancer models. Transgenic mice overexpressing RAS in the prostate exhibit low-grade prostatic intraepithelial neoplasia and intestinal metaplasia with goblet cells.[11] In mice null for *Pten* and overexpressing human MYC showed adenocarcinoma of the prostate with focal intestinal metaplasia in a background of high-grade prostatic intraepithelial neoplasia,[2] despite the fact that metaplasia has not been reported for either of the parental transgenic lines. A similar phenotype of prostate adenocarcinoma with focal intestinal metaplasia in a background of high-grade prostatic intraepithelial neoplasia affected mice with conditional *Pten* loss, lacking an allele on *Nkx3.1* and expressing an activated K-ras mutant ($Nkx3.1Cre^{ERT2/+};Pten^{flox/flox};Kras^{LSL/+}$).[7] Deletion of androgen receptor from the prostate epithelial cells of *Pten*-null mice, reported to develop adenocarcinoma in dorsolateral lobes,[10] induced focal intestinal metaplasia.[8] Mucinous metaplasia was noted in the prostates of old mice transgenic for *FGF8b*, and the rate was markedly increased in the prostates of FGF8b-Tg-$BERKO_{FVB}$ mice also deficient for estrogen receptor β (82% compared with 38%).[3,4]

To ensure the correct interpretation of results obtained from genetically modified mouse models, it is critical that age-related alterations in genetically wild-type mice are thoroughly recorded. Even though previous authors[13] screened numerous mice, all of the animals represented a single genetic background. Here we report that the incidence rate of mucinous metaplasia in the prostate is considerably higher in mice of the FVB/N genetic background than reported previously for wild-type B6C3F1 mice.[13]

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We histologically examined the ventral, dorsal and lateral prostates of 48 male FVB/N mice (age, 11 to 24 mo; Table 1). Ethical approval for animal experimentation has been admitted

Table 1. Rate of mucinous metaplasia in FVB/N mice

	Age of mice (mo)		
	11–12	17–18	22–24
No. of mice	18	14	16
% of mice with PAS-positive areas	0	43	50
Average no. of PAS-positive areas per mouse	—	1.2	1.5
% of lesions in each lobe			
Lateral	—	100	67
Dorsal	—	0	17
Ventral	—	0	17

by the Regional State Administrative Agency for Southern Finland (ESAVI/6271/04.10.03/2011). The mice were predominantly of the FVB/NHanHsd substrain (Envigo, Horst, Netherlands) that were 1 to 4 generations descendant to mice purchased from the supplier; the remaining 21% (n = 10) of the mice had one parent of the substrain FVB/NCrl (Charles River, Sulzfeld, Germany). The mice were housed in 2 different semibarrier facilities routinely sampled for microbiologic status according to FELASA guidelines.[5] Aspen bedding (Tapvei, Paekna, Estonia) was used, and the mice were fed a commercial diet (RM 3[E] soya free, Special Diets Services, Essex, UK).

Tissues were fixed in either formalin or PAXgene molecular fixative (PreAnalytiX, Hombrechtikon, Switzerland) according to manufacturer's recommendations and embedded in paraffin. The tissue blocks were sectioned (thickness, 5 µm), and sections at 50-µm intervals were stained with hematoxylin and eosin to study the overall histology throughout the prostate. Adjacent sections of any suspect areas of metaplasia detected in sections stained with hematoxylin and eosin were stained with periodic acid–Schiff (PAS) and counterstained with hematoxylin to detect mucin secreted by goblet cells (Figure 1).

We found 19 lesions of mucinous metaplasia in 14 mice that ranged in age between 17 to 24 mo (47% of the mice had lesions; 1.4 lesions on average; Table 1). Of the 14 mice affected, 4 had 2 or more separate metaplastic areas. The incidence of metaplasia was 50% in 22- to 24-mo-old mice compared with 43% in 17- to 18-mo-old mice. The lateral prostate was the most common lobe for mucinous metaplasia: all of the lesions in the 17- to 18-mo age group affected the lateral prostate. In the 22- to 24-mo age group, the majority (67%) of the metaplastic lesions were found in the lateral prostate, with 17% each in the dorsal and ventral lobes (Table 1). Mucinous metaplasia was not observed in the prostates of 11- to 12-mo-old wild-type FVB/N mice (n = 18; Table 1).

Mucinous metaplasia often involved scattered areas of epithelium in several glands (Figure 1 A). In some regions, the metaplastic epithelium was increased in height, becoming columnar or pseudostratified, with apparent goblet cells (Figure 1 B and E). Staining with PAS confirmed the metaplastic changes to be mucinous metaplasia (Figure 1 B, D, and F), as PAS-staining indicates the mucins produced by the goblet cells both within cells and within the lumen of the prostatic acini. In addition, the goblet cells exhibited typical densely stained nuclei with a compressed or even triangular appearance compared with the round or elliptically shaped nuclei of normal cuboidal to columnar prostatic epithelium (Figure 1 C through F).

Discussion

Here we report an increased incidence rate of mucinous metaplasia in the mouse prostate compared with rates previously reported in the literature.[7,13] We studied mice of the FVB/N strain and found that 43% to 50% of 17- to 24-mo-old mice had at least one area of mucinous metaplasia in the prostatic epithelium. These lesions included apparent goblet cells in the epithelium and prominent mucinous secretions in the lumen of the acini. We found no evidence of goblet cells in 11- to 12-mo-old mice. In addition to the mucin-filled goblet cell vesicles, the mucinous metaplasia we observed often involved heightened, even pseudostratified, epithelium and exhibited typical, compressed, or triangular-shaped goblet-cell nuclei.

Previous reports of mucinous metaplasia with goblet cells in the mouse prostate have mostly involved genetically modified mouse models of prostate cancer. In some cases, the original reports did not mention metaplasia, but rescreening of the material has revealed the lesion.[7] Perhaps the incidence of metaplasia in the previous tumor models was low or that mucinous metaplasia as such may not have been considered a noteworthy alteration in a tumor model, thus explaining the lack of reports of this lesion. It is noteworthy that most mouse prostate tumor models that develop mucinous metaplasia lack Pten or ERβ, and it was recently shown that the prostate tumorigenesis due to *Pten* deletion involves ERβ repression.[8] Therefore, it is tempting to speculate that the Pten–ERβ pathway governs the differentiation state of the epithelial cells or the homeostasis of the prostate epithelial tissue and acts as a barrier against mucinous metaplasia in mouse prostate epithelium.

In a previous report involving the B6C3F1 strain, mucinous metaplasia was detected in less than 0.2% of the mice studied.[13] According to our current data, FVB/N mice are considerably more prone to mucinous metaplasia of the prostate than has been reported for B6C3F1 animals, thus suggesting strain-dependent differences in the incidence of this abnormality. In addition, the number of slides evaluated per mouse may account for in the divergent results between the current and earlier reports. Here, the entire prostate (all 3 lobes) was sectioned and examined every 50 µm throughout the entire organ. This density of inspection ensured that all acini of the prostate were screened for histologic alterations in these mice.

In addition, various environmental factors may influence the rate of metaplasia in the murine prostate, for example, differences in inflammatory burden or diet between mice housed in different facilities. In the current study, the mice evaluated were housed in 2 different semibarrier facilities and were fed the same diet. The facilities were monitored for infectious agents according to FELASA guidelines.[5] In one facility, no major infections were found during the period when these mice were housed, only infrequent positive results for *Staphylococcus aureus*. In the second facility, infrequent positive findings during the housing time of these mice were obtained for several bacteria (*Staphylococcus aureus*, *Pasteurella pneumotropica*, *Helicobacter ganmani*, *Helicobacter hepaticus*, *Helicobacter typhlonius*, and *Klebsiella oxytoca*), 2 parasites (*Trichuris muris* and *Entamoeba muris*), and mouse norovirus. Yet, the incidence of mucinous metaplasia did not differ between the mice housed in the 2 facilities (data not shown).

We report here that mucinous metaplasia can be far more common in the mouse prostate than earlier reported. Whether

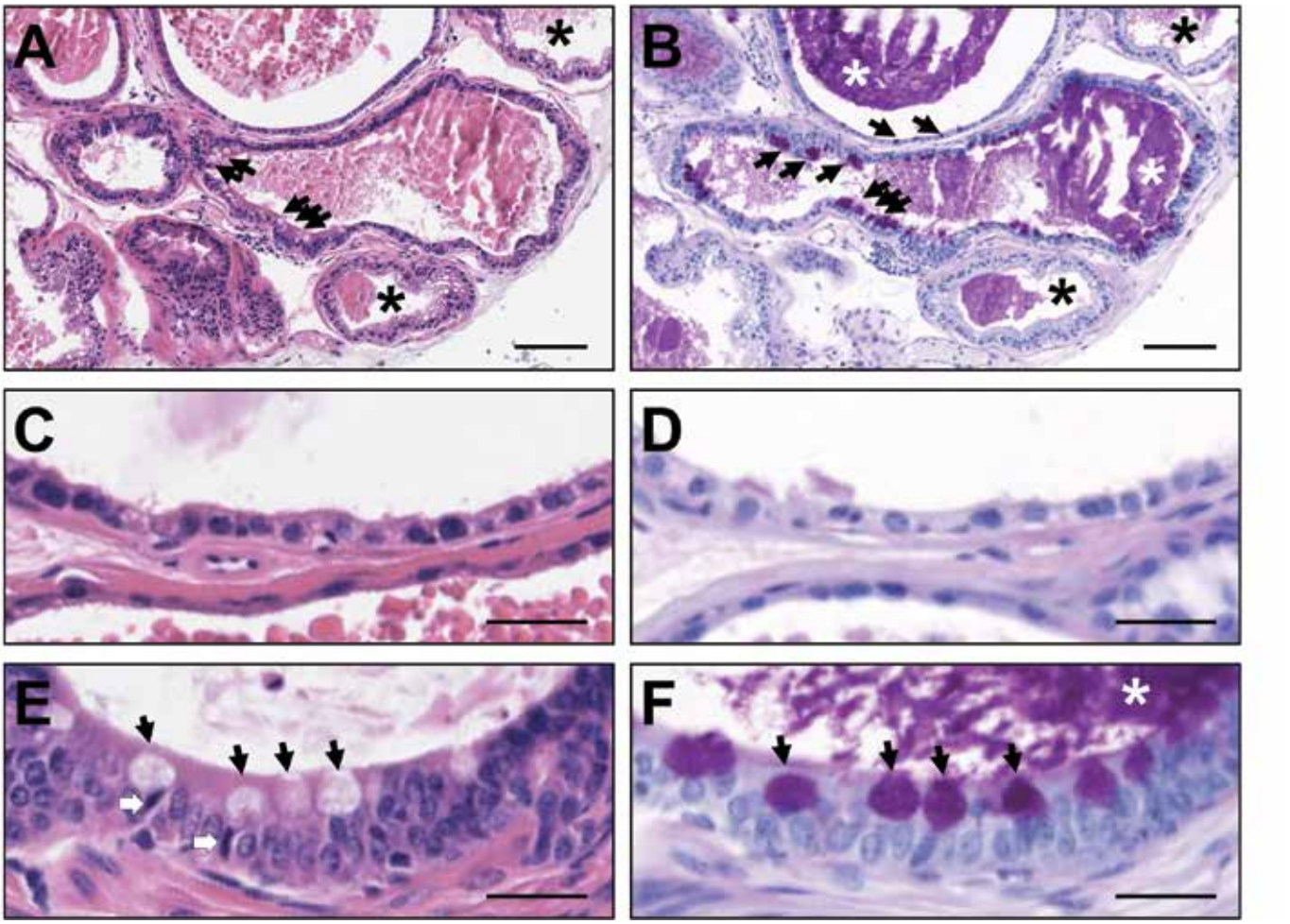

Figure 1. Histology of mucinous metaplasia in mouse prostate. (A) A section of mouse ventral prostate showing normal glands (asterisks) as well as a gland with goblet cell metaplasia (black arrows). (B) Section adjacent to that in panel A showing mucin-containing goblet cells (black arrows) and secretions (purple areas; white asterisks). (C and D) Normal and (E and F) metaplastic lateral prostatic epithelium. Normal cuboidal epithelium is replaced with heightened, columnar to pseudostratified epithelium including goblet cells with apparent mucinous secretion (black arrows) and densely stained nuclei that have a 'compressed' appearance (white arrows). Hematoxylin and eosin stain: A, C, E; periodic acid–Schiff stain with hematoxylin counterstain: B, D, F; scale bars, 100 µm (A and B) and 25 µm (C through F).

the differences between our current and previous studies[12] are dependent on genetic or environmental differences between the mice evaluated remains to be investigated. Future studies are also required to address the incidence of mucinous metaplasia in the prostates of other commonly used laboratory mouse strains.

Acknowledgments

We thank Mrs Katja Liljeström, Mr Osku Alanen, and Ms Sonja Koivukoski for skillful technical assistance. Funding support was obtained from the Academy of Finland (grant number 279270), the Cancer Society of Finland, the Sigrid Juselius Foundation, and the Medical Research Fund of Tampere University Hospital.

References

1. **Bohman KD, Osunkoya AO.** 2012. Mucin-producing tumors and tumor-like lesions involving the prostate: a comprehensive review. Adv Anat Pathol **19:**374–387.
2. **Clegg NJ, Couto SS, Wongvipat J, Hieronymus H, Carver BS, Taylor BS, Ellwood-Yen K, Gerald WL, Sander C, Sawyers CL.** 2011. MYC cooperates with AKT in prostate tumorigenesis and alters sensitivity to mTOR inhibitors. PLoS One **6:**e17449.
3. **Elo TD, Valve EM, Seppänen JA, Vuorikoski HJ, Mäkelä SI, Poutanen M, Kujala PM, Härkönen PL.** 2010. Stromal activation associated with development of prostate cancer in prostate-targeted fibroblast growth factor 8b transgenic mice. Neoplasia **12:**915–927, IN17–IN19
4. **Elo T, Yu L, Valve E, Mäkelä S, Härkönen P.** 2014. Deficiency of ERβ and prostate tumorigenesis in FGF8b transgenic mice. Endocr Relat Cancer **21:**677–690.
5. **FELASA working group on revision of guidelines for health monitoring of rodents and rabbits, Mähler Convenor M, Berard M, Feinstein R, Gallagher A, Illgen-Wilcke B, Pritchett-Corning K, Raspa M.** 2014. FELASA recommendations for the health monitoring of mouse, rat, hamster, guinea pig, and rabbit colonies in breeding and experimental units. Lab Anim **48:**178–192.
6. **Gal R, Koren R, Nofech-Mozes S, Mukamel E, His Y, Zajdel L.** 1996. Evaluation of mucinous metaplasia of the prostate gland by mucin histochemistry. Br J Urol **77:**113–117.
7. **Ittmann M, Huang J, Radaelli E, Martin P, Signoretti S, Sullivan R, Simons BW, Ward JM, Robinson BD, Chu GC, Loda M, Thomas G, Borowsky A, Cardiff RD.** 2013. Animal models of human prostate cancer: the consensus report of the New York meeting of the Mouse

Models of Human Cancers Consortium Prostate Pathology Committee. Cancer Res **73:**2718–2736.

8. **Mak P**, **Li J**, **Samanta S**, **Chang C**, **Jerry DJ**, **Davis RJ**, **Leav I**, **Mercurio AM.** 2015. Prostate tumorigenesis induced by PTEN deletion involves estrogen receptor β repression. Cell Rep **10:** 1982–1991.
9. **McCauley HA**, **Guasch G.** 2015. Three cheers for the goblet cell: maintaining homeostasis in mucosal epithelia. Trends Mol Med **21:**492–503.
10. **Mulholland DJ**, **Tran LM**, **Li Y**, **Cai H**, **Morim A**, **Wang S**, **Plaisier S**, **Garraway IP**, **Huang J**, **Graeber TG**, **Wu H.** 2011. Cell autonomous role of PTEN in regulating castration-resistant prostate cancer growth. Cancer Cell **19:**792–804.
11. **Scherl A**, **Li JF**, **Cardiff RD**, **Schreiber-Agus N.** 2004. Prostatic intraepithelial neoplasia and intestinal metaplasia in prostates of probasin–RAS transgenic mice. Prostate **59:**448–459.
12. **Shappell SB**, **Thomas GV**, **Roberts RL**, **Herbert R**, **Ittmann MM**, **Rubin MA**, **Humphrey PA**, **Sundberg JP**, **Rozengurt N**, **Barrios R**, **Ward JM**, **Cardiff RD.** 2004. Prostate pathology of genetically engineered mice: definitions and classification. The consensus report from the Bar Harbor meeting of the Mouse Models of Human Cancer Consortium Prostate Pathology Committee. Cancer Res **64:**2270–2305.
13. **Suwa T**, **Nyska A**, **Haseman JK**, **Mahler JF**, **Maronpot RR.** 2002. Spontaneous lesions in control B6C3F1 mice and recommended sectioning of male accessory sex organs. Toxicol Pathol **30:**228–234.