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Age-Related Changes of the Prostate Gland in the Senescence-Accelerated Mouse

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ABSTRACT: Aging is of utmost importance in the pathogenesis of the prostate gland (i.e., benign prostate hyperplasia or prostatic carcinoma). The object of this study was to examine the morphological and histological changes of the aging prostate of the so-called senescence-accelerated mouse (SAM). Ventral and dorsolateral lobes of prostate glands of SAM were microdissected into two-dimensional ductal arrays. Gross morphology, ductal branching patterns, and histology were examined in these microdissected specimens. Wet weight and numbers of ductal tips in ventral and dorsolateral prostate glands in senescence accelerated-prone (SA-P) mice were significantly smaller than those of senescence accelerated-resistant (SA-R) mice, although the changes of patterns of gross ductal morphology were virtually identical in these groups. High incidence of stromal hyperplasia with fibrosis and inflammation was observed in the dorsal lobe of the aged SA-P mouse. Atypical glandular epithelial cells and cribriform glandular deformity were observed in the dorsal and lateral lobe of aged SA-P mice. Marked heterogeneity in age-related pathological changes was observed between prostatic lobes. These data suggest that the aging process occurs heterogeneously within the prostate gland, and that SA-P mice may be an important model for the study of age-related changes in the prostate gland.

KEY WORDS: **microdissection, prostate ductal pattern, age-related pathology**

INTRODUCTION

It is well known that aging is an important component of the pathogenesis of the prostate gland (i.e., benign prostate hyperplasia [BPH] or prostatic carcinoma). Because only dogs and humans spontaneously develop BPH with increasing age, the canine prostate has been investigated in detail as an animal model for prostate hyperplasia [1,2]. However, a suitable model for the study of age-related changes in the prostate gland has not been established. In our laboratory, we developed a morphological approach for the study of ductal branching patterns in the rodent prostate, using the microdissection technique [3]. With this technique, changes of the prostatic ductal architecture can be rapidly and precisely assessed. Using this technique, Sugimura et al. [3] discovered the postnatal glandular development of mouse prostate from birth to 90 days. Changes in ductal architecture in the prostate gland have not been investigated thoroughly in the rodent prostate during the senile period. Indeed, few studies dealt with male accessory gland in the aged rodent [4–8]. To facilitate our studies of the aging prostate, we have used so-called senescence-accelerated mouse (SAM), established by the Chest Disease Research Institute, Kyoto University, Kyoto, Japan [9]. This unique mouse model demonstrates accelerated senescence [10]. In this study, using this SAM model, age-related morphological changes of the prostate gland are investigated.

MATERIALS AND METHODS

Animals

Five senescence-prone series of mice (SA/P-1, P-2, P-3, P-4, and P-5), and three senescence-resistant series (SA/R-1, R-2, and R-3) were originally obtained

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by continuous sister-brother breeding from five original litters of AKR mice (donated by Jackson Laboratory, Bar Harbor, ME) at the Chest Disease Research Institute, Kyoto University. Two pairs of SA/P-1 (SA-prone series) and SA/R-1(SA-resistant series) from the Chest Disease Research Institute were reared in our laboratory under conventional conditions at 22 ± 2°C, and maintained on a commercial diet (CE-1, Nihon CLEA) and tap water ad libitum. We continued the sister-brother mating, and offspring were weaned usually at 4–6 weeks. To examine prostatic morphology, animals were killed in groups of 15–65 at 3, 6, 9, 12, and 14–15 months of age in SA/P-1 mice, and at 3, 6, 9, 12, 18, and 23–24 months of age in SA/R-1 mice.

Prostatic Dissection

After animals were killed by cervical dislocation, the genital tract of each was exposed by a lower abdominal incision, and removed in block by cutting the ductus deferens, urethra, and ureters. Under a dissecting microscope (Nikon, SMZ-10), ventral and dorsolateral lobes were separated, and the wet weight measured with an electric balance (Shimazu Libror, AEL-40SM). Microdissection of the prostate was performed according to Sugimura et al. [3]. Briefly, individual ductal networks of the prostate gland were microdissected after 10 min of incubation in 0.5% collagenase (Wako, Osaka, Japan). All microdissections were performed using a dissecting microscope, fine forceps, and a von Graefe knife. Microdissected prostatic ducts were photographed and then fixed with 10% formalin. The numbers of ductal tips were determined from careful examination from the photographs. Portions of the fixed ductal networks were embedded in paraffin, and stained with hematoxylin and eosin for histological examination.

Measurement of Serum Testosterone and Estradiol Levels

Serum testosterone was measured by a radioimmunoassay (RIA) kit (testosterone Eiken) after extraction with medium of n-hexan and ethylether (3:2). Serum estradiol was measured by an RIA kit (DPC estradiol kit). All measurements were performed in duplicate, and values were expressed as mean ± standard deviation. Statistical analysis was done by Student's *t* test.

RESULTS

Body Weight

At 6 months postnatal, the body weights of SA-R and SA-P mice were 32.9 ± 1.7 g and 33.0 ± 2.8 g, respectively. However, the body weight of SA-P mice was significantly decreased at the level of 30.4 ± 2.8 g, after 9 months of age, in contrast with that of SA-R mice, which was gradually increased even after 15 months of age.

Wet Weight of the Prostate Gland

Wet weight of the ventral (VP) and dorsolateral prostate (DLP) lobes was gradually increased according to the age until 12 months. Both ventral and dorsolateral lobes of SA-R mice weighed more than those of SA-P mice at all ages (Fig. 1).

Microdissection of the Prostate Gland

There was no difference of the gross size and morphology of the ductal network of the VP of the SA-R and SA-P mice (Fig. 2a,b). The VP consisted of two to three main ducts per side which originated from the urethra and arborized distally into a complex ductal-acinar network of ductal branchings. The pattern of ductal branching did not change with increasing age, but ductal caliber was slightly decreased in aged animals. For both SA-R and SA-P mice, the DLP was composed of 15–20 main ducts with three to four distal branches. No differences in ductal branching were observed in SA-R vs. SA-P mice. However, the prostatic ducts of 62% (25/40) of SAM-P aged 15 months were surrounded with thick connective tissue, which prevented complete microdissection (Fig. 2c,d).

The numbers of the ductal tips in the VP were counted according to the age. Figure 3 demonstrates that the numbers of ductal tips were significantly lower at all ages in the SA-P vs. SA-R mice. The numbers of tips did not vary with increasing age in both strains of SA mice.

Histological Examination

Microdissected prostatic lobes of SA-R mice (at the age of 3 months and 24 months) and SA-P mice (at the age of 3 months and 15 months) were examined for histological changes according to the age.

VP

At age 3 months, high columnar secretory epithelial cells were observed in both strains of SA mice. At age 24 months, the same histological profile was observed in both strains of SA mice. However, in SA-R mice at 24 months of age, 24% (11/46 lobes) of prostatic ducts were distended, and enlarged caliber contained an eosinophilic secretion. SA-P mice did not exhibit such cystic dilated ducts (Fig. 4).

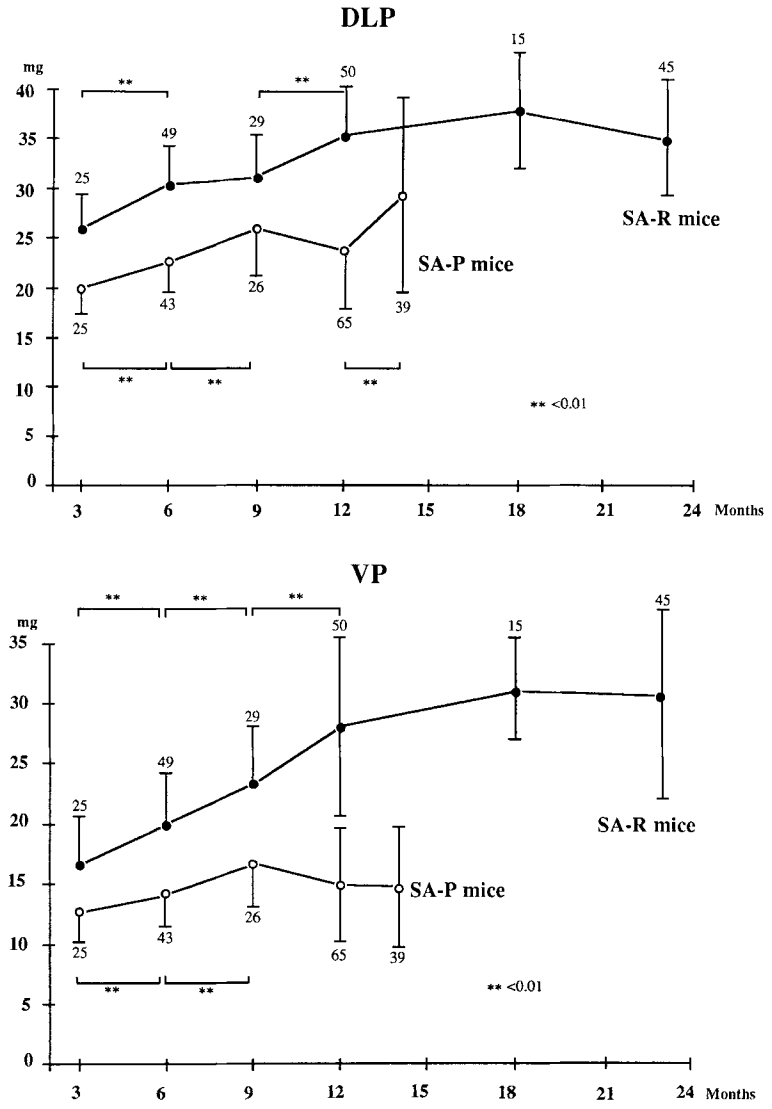

Fig. 1. A plot of wet weights of dorsolateral (DLP) and ventral (VP) prostates of SA-R and SA-P mice with increasing age (mean ± SD). Numbers described at the standard deviation bar indicate the numbers of animals examined in each time point. Wet weights of VP and DLP in SA-R mice are always greater than those of SA-P mice at all time points ($P < 0.01$).

Lateral Lobe

At age 3 months, relatively low columnar epithelial cells were observed in both strains of SA mice. Characteristic eosinophilic crystalloid secretory products were observed in the relatively dilated ducts. Desquamated epithelial cells and eosinophilic secretory products were frequently observed at age 15 and 24 months of SA-R mice. At age 15 months, the distal tips of the ducts of SA-P mice frequently had cribriform patterns of small luminal spaces (Fig. 5).

Dorsal Lobe

At age 3 months, relatively low columnar epithelial cells secreting eosinophilic product were observed in both strains of SAM mice. Heterogeneity of the secretory activity was noted within the DP, as some of those ducts were distended with secretion, while others were empty. In SA-R mice, the histological profile was the same at age 3 and 24 months, with the exception that a slight infiltration of inflammatory cells was observed at age 24 months. In SA-P mice at 15 months, severe stromal fibrosis and inflammatory changes were observed in the DP of 62.5% lobes examined (Figs. 6, 7). Some ducts associated with stromal fibrosis had atypical stratified epithelial cell with pleomorphic nuclei. However, neoplasm was not observed. Stromal fibrotic changes and ductal epithelial atypia were only observed in the dorsolateral lobe in SA-P mice (Table I). The incidence of fibrosis and

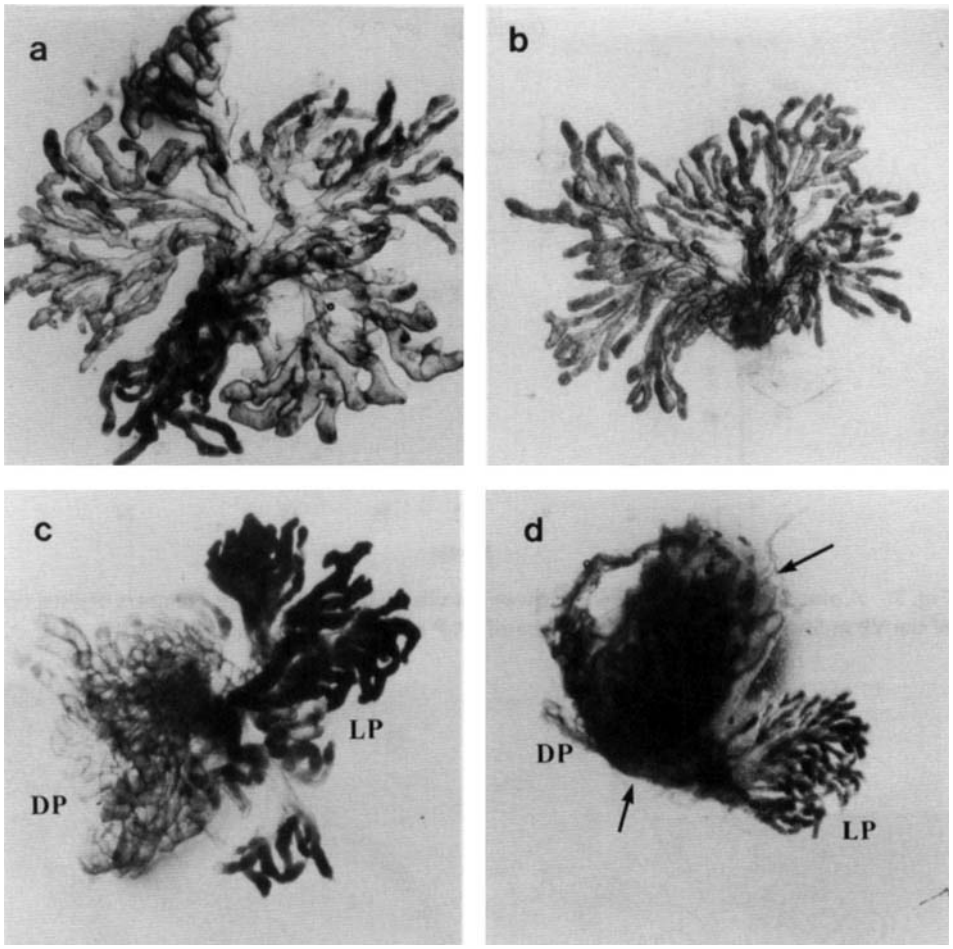

Fig. 2. Gross morphological appearance of microdissected ventral and dorsolateral prostate (right or left side only) (DP, dorsal prostate; LP, lateral prostate) (× 10). **a:** Ventral lobe, SA-R mice at age 24 months. **b:** Ventral lobe, SA-P mice at age 15 months. Note well arborized ductal branching system in both strains. **c:** Dorsolateral lobe, SA-R mice at age 24 months. **d:** Dorsolateral lobe, SA-P mice at age 15 months. Note thick stroma between the ducts in SA-P mice (arrows).

atypical epithelium was increased with increasing age in SA-P mice (Table. 2).

Serum Testosterone and Estradiol Levels

Testosterone levels of SA-R and SA-P mice at age 3 months were 437 ± 340 and 630 ± 640 ng/dl, respectively, although both serum testosterone levels varied between individual animals in both strains. However, significantly decreased levels of serum testosterone were observed at 23 months in both strains (Fig. 8). The serum estradiol levels of 20 animals at age 3 months (both strains), 15 months (SA-P mice), and 24 months (SA-R mice) were all less than 10 pg/ml.

DISCUSSION

Previously we investigated the normal postnatal development of the mouse prostate gland from birth to adulthood through the use of the microdissection technique, demonstrating the precise ductal-acinar patterning [3]. In this study, our interest was focused on the age-related pathological changes in the prostatic ductal system in the SAM.

The SAM was developed through inbreeding as a model of spontaneous senescence in mice. The aging pattern in this model is considered to be due to an accelerated senescence rather than to premature aging or senescence [9]. Characteristics of the aging phenomena in the SA-P mice are an earlier onset and irreversible advancement of senescence manifested by several clinical signs and gross lesions such as the loss of general behavior, various skin lesions, cataracts, and increased lordokyphosis [9]. The average life span of SA mice is reported as 12.4 ± 0.22 months in SA-R mice, and 10.4 ± 0.17 months in SA-P mice. The end points of this study for aged animals were set at age 24 months for SA-R mice and age 15 months

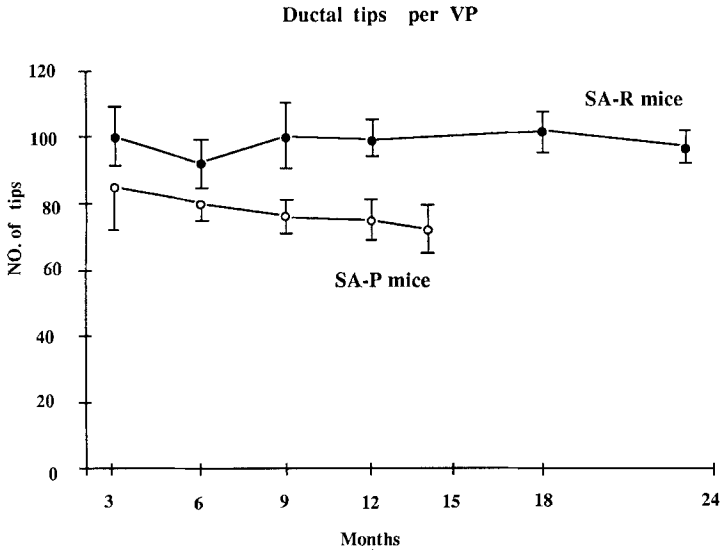

Fig. 3. A plot of ductal tips of the VP (right or left side) with increasing age. Numbers of distal tips of the VP in SA-R mice always exceed those of SA-P mice at all time points ($P < 0.01$).

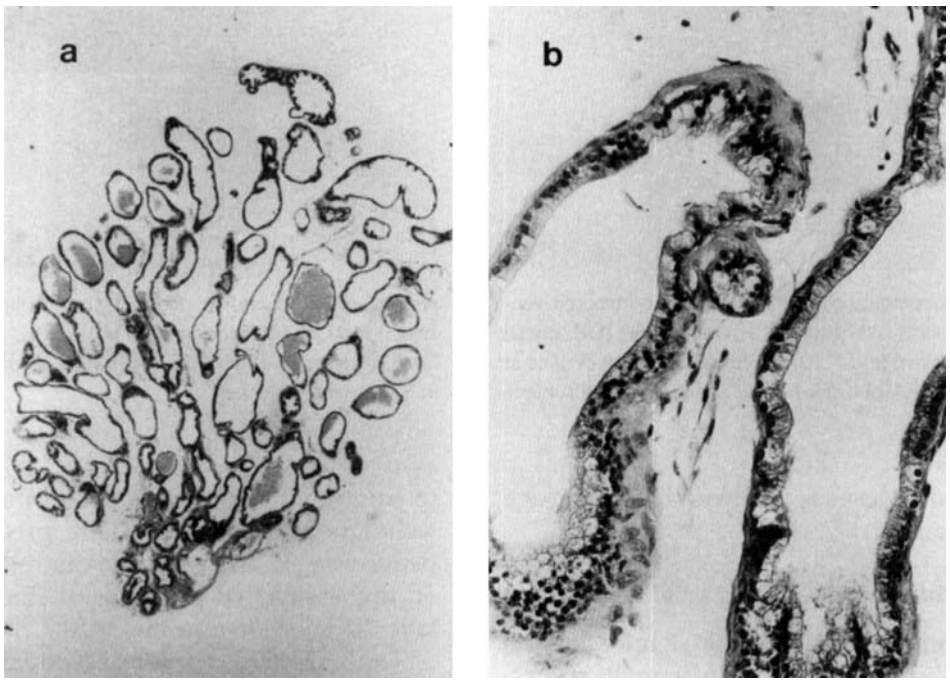

Fig. 4. Histological sections of dissected prostatic ducts from the ventral lobe in SA-P mice at age 15 months (**a:** ×20, **b:** ×200). Note relatively loose connective tissue around the ducts and relatively normal epithelium.

for SA-P mice, which gave equal scores of senescence in the both strains [10].

The histological changes of the prostate gland in the aged rat involve a reduction in epithelial cell height, disappearance of mucosal infoldings, and stromal hyperplasia [4]. Retention of the secretory products within ductal lumen was also observed with aging [5]. The nuclear pleomorphism, mitotic cells, and giant cells have been seen in the mouse VP at 24 months [6]. Ultrastructural studies in the aging rat VP have shown a progressive deposition of electron-dense bodies or pigments in the endoplasmic reticulum in the supranuclear zone of secretory epithelial cells with increasing age [7,8]. Although spontaneous

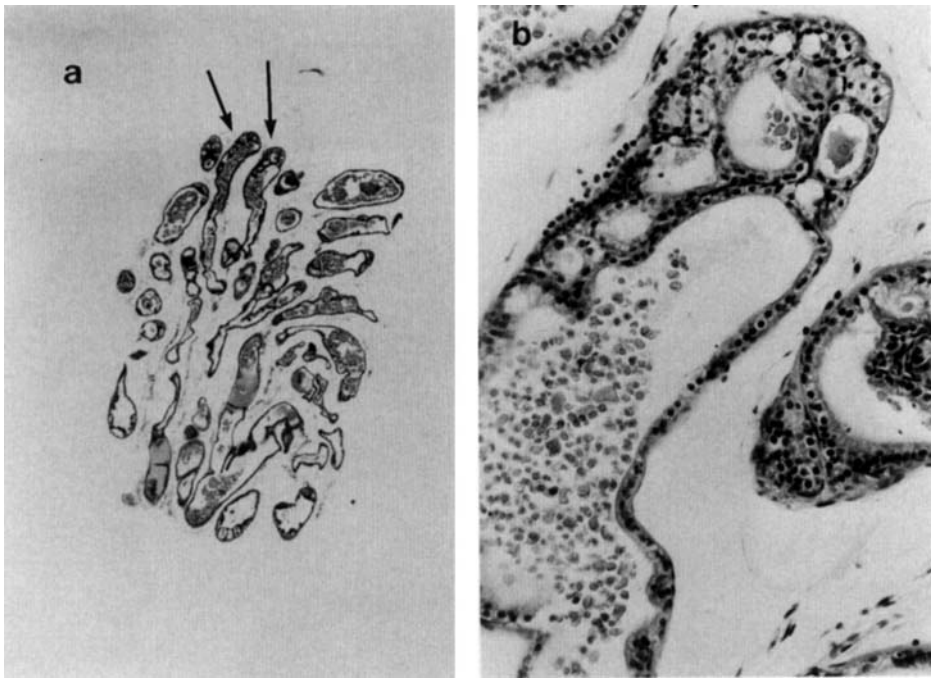

Fig. 5. Histological sections of dissected prostatic ducts from the lateral lobe in SA-P mice at age 15 months (**a:** ×20, **b:** ×200). Note ducts are filled with particulate secretory products. Cribriform changes are observed in the distal ductal tips (arrows).

cancer or stromal hyperplasia is frequently reported in the aged rats [11,12], cancerous lesions are rarely observed in the mouse prostate gland [13]. In this study, atypical epithephial cells were observed in 25% of ducts in the dorsal lobe of SA-P mice, however cancer cells were not observed. The incidence of fibrosis and atypical epithelium was increased with increasing age in SA-P mice; however, spontaneous development of prostate cancer is considered a very rare event.

The histological changes observed in our study of SA-R mice at age 24 months are in keeping with previous reports. In SA-P mice, the most striking histological changes were stromal hyperplasia and inflammation in the dorsal prostate at age 15 months. The pathophysiology of those lobe-specific age-related changes is not understood. We consider that the dorsal prostate of SA-P mice may differ from other lobes in regard to androgen responsiveness. Supporting this hypothesis, many studies emphasize the marked heterogeneity of biological activities within the prostate gland, which has come to light in studies using the microdissection technique [3,14–18]. Hormone sensitivity to androgen is known to differ in the various prostatic lobes [19]. DNA synthesis also varies regionally throughout the gland [14]. The prostatic ductal system in the rat is also morphological and functionally heterogeneous [15,20]. Recent studies demonstrate heterogeneity of expression of mitotic activity, prostate binding protein, cathepsin D, and TRPM-2 gene product along the proximal to distal axes of ducts of the rat VP [16]. Localization of androgen receptor is also different in cell types and lobes of the adult rat prostate [21]. These biological heterogeneities within the prostate gland may ultimately relate to the lobe-specific pathogenesis of the prostate.

Inflammatory reactions were another age-related change observed in this study, and usually coexisted with stromal hyperplasia in the dorsal lobe in SA-P mice. Spontaneous prostatitis has been reported in the lateral prostatic lobe in the rat [22,23]. The age-related androgen depletion is considered to reduce fluid formation, decrease glandular lumen, and reduce epithelial proliferation [22]. Focal inflammatory changes within the prostatic lobes may also relate the heterogeneous sensitivity to androgen and/or estrogens. Although a cause-and-effect relationship among androgen depletion, inflammatory change, and stromal hyperplasia is not understood, these factors may play an important role in the age-related pathological changes of the prostate gland in SA-P mice.

Based on these data, it is clear that age-related changes occur heterogeneously within the mouse prostate gland. Clinically, human BPH also occurs focally in the transition zone around the urethra,

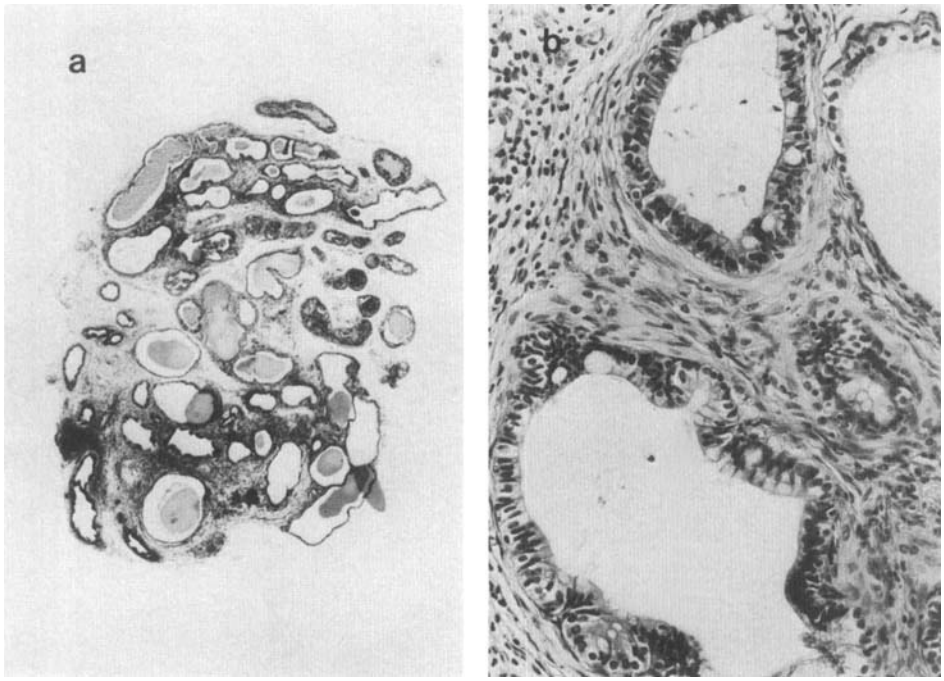

Fig. 6. Histological sections of dissected prostatic ducts from the dorsal lobe in SA-P mice at age 15 months (**a:** ×20, **b:** ×200). Note dilated ducts lined with active secretory epithelial cells in a hyperplastic fibrous stroma.

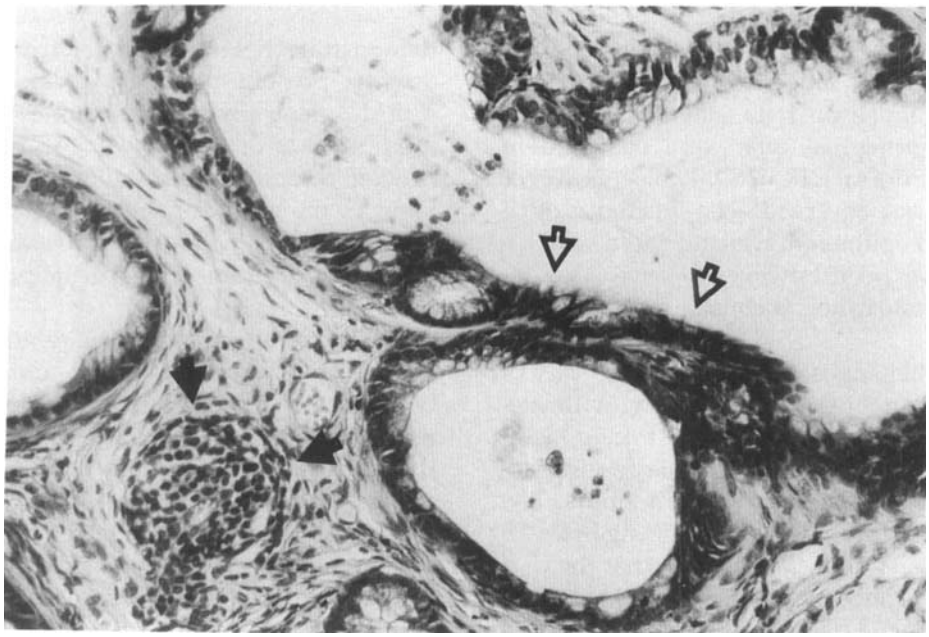

Fig. 7. Histological sections of dissected prostatic ducts from the dorsal lobe in SA-P mice at age 15 months (×200). Note pleomorphic atypical epithelial cells (open arrows) in association with hyperplastic stroma. Inflammatory reaction was focally seen in the fibrous stroma (arrows).

while prostatic cancer usually occurs in the peripheral zone [22]. These pathological differences may relate to the functional heterogeneity in the epithelial and stromal components of the prostate gland.

The importance of the stromal-epithelial interactions in normal prostatic development, and maintenance of adult organ is described elsewhere [24,25]. Embryonic "reawakening" of prostatic stroma has

TABLE I. Incidence of Stromal Fibrosis and Ductal Epithelial Atypia in the Prostatic Lobes of SA Mice

Prostate lobe	Fibrosis	Atypical epithelium
SA-P mice		
Dorsal	25/40 (62.5%)	10/40 (25%)
Lateral	0/40	0/40
Ventral	0/40	0/40
SA-R mice		
Dorsal	0/22	0/22
Lateral	0/22	0/22
Ventral	0/22	0/22

TABLE II. Age-Relatated Incidence of Fibrosis and Atypical Epithelium in the Dorsal Prostate Lobe of SA Mice

	Fibrosis (%)	Atypical epithelium (%)
SA-R mice (24 months)	0	0
SA-P mice (12 months)	14	9
SA-P mice (15 months)	63	24

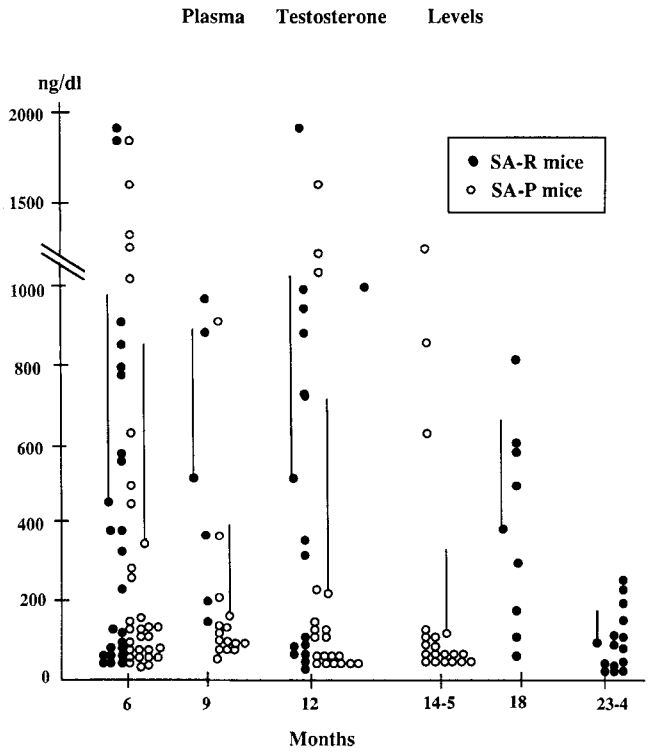

Fig. 8. A plot of serum testosterone levels in SAM-P and SAM-R with increasing age. Bars indicate standard deviation.

been proposed for the pathogenesis of human BPH by McNeal [26]. The hyperplastic secretory epithelial cells associated with the hyperplastic stroma in the dorsal lobe in SA-P mice are consistent with this hypothesis. Recent molecular biological studies indicate the importance of fibroblast growth factor and oncogenes in the pathogenesis of BPH. The age-related changes of the levels of basic fibroblast growth factor (bFGF) [27] or the oncogene, int-2 [28], in the stromal cells of SA-P mice are of great interest. Further characterization of this hyperplastic stroma needs to be determined.

In summary, it is clear that the aging phenomena occur heterogeneously in the prostate gland. Further investigation of these lobe-specific stromal hyperplasia and the cribriform pattern of epithelial changes seen in the dorsal and lateral prostate gland in SA-P mice may shed light on the pathophysiology of age-related changes in the prostate gland.

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