Tenascin expression and postnatal development of the human prostate

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ABSTRACT   We investigated the distribution of tenascin during postnatal development of the human prostate. Monoclonal antibody specific to human tenascin was applied to paraffin sections by the avidin-biotin-complex method to examine its localization. Infantile prostates showed two distinct zones. The inner zone had sparse acini with fibromuscular bundles. The peripheral zone had similar acini and lighter stroma as compared with inner zone. Tenascin was found diffusely but weakly. The periglandular area occasionally showed immunoreactivity. The prostates from 9- to 13-year-old subjects had a morphology similar to that of the infantile gland. The difference was increased density of the acini. Immunoreactivity was low. In the prostates from 14- to 21-year-old subjects, the acini distribution was more crowded, and the epithelial lining had become taller in both zones. Tenascin distributed preferentially in the peripheral zone during this period. Simultaneously, the percent of glandular area in the peripheral zone rose abruptly. The dynamics of tenascin expression are closely associated with the development and maturation of the gland. The distribution of tenascin during the post-puberal period may suggest its participation in the preferential occurrence of prostatic carcinoma in the peripheral zone.

KEY WORDS: *tenascin, postnatal development, normal prostate, infantile prostate, puberal prostate*

Introduction

Tenascin is a significant extracellular matrix (ECM) glycoprotein. It was isolated and characterized by several independent research groups from diverse sources, including chicken embryo fibroblasts, developing central nervous system of the chicken and mouse (Erickson and Bourdon, 1989). Its expression was also noted in reactive, hyperplastic and neoplastic tissues, while tenascin was said to decrease markedly or to disappear from normal adult tissue (Koukoulis et al., 1991; Sakakura et al., 1991). As the sites in which tenascin was found grew in number and diversity, it became evident that its function(s) might be similarly diverse and/or more fundamental. Although it is produced in response to epithelial-mesenchymal interactions that initiate organogenesis, its expression in tissues during postnatal development has not been examined so much as in the embryonic period. Most of the aforementioned reports limited observation to the early postnatal period with conjunction of fetal stage. Generally, tenascin expression is simply considered to decrease or disappear postnatally.

There has been only one report of immunohistochemical analysis of tenascin expression during development of the mouse urogenital sinus. Its distribution in human prostate has been reported in only one case (Ibrahim et al., 1993). However, the main focus of that report concerns prostatic lesions, including hyperplasia,

intraepithelial neoplasia and carcinoma. The authors examined only two fetal prostates obtained during autopsy, and found diffuse stromal staining by immunohistochemistry. Chronological or developmental analysis including the postnatal period was not done.

The purpose of this study was to investigate tenascin localization in the human prostate by immunohistochemistry, and correlate these findings with postnatal development of the gland.
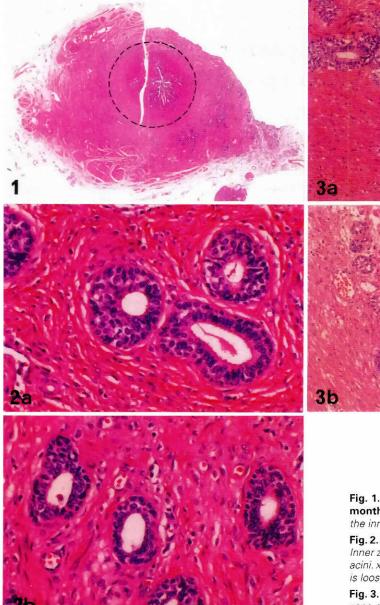
Results

Morphology

Three infantile prostates showed two distinguishable zones. The inner zone occupied the inner half of the gland, containing longitudinal and circular fibromuscular stroma with scattered small round acini (Fig. 1). The distribution of the acini was sparse. They were lined by cuboidal pseudostratified epithelium, and had a small or no lumen. In the outer zone, the arrangement of the fibromuscular bundles was somewhat irregular and loose. Most acini were small and round in shape and some were elongated. They were lined by an epithelium similar to the kind seen in the inner zone (Fig. 2a,b).
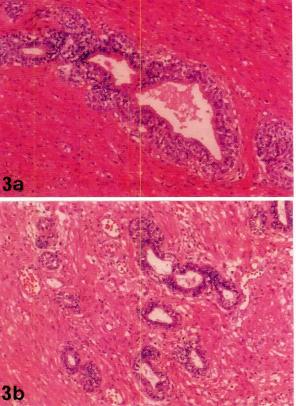

Fig. 1. Transverse section of the prostate gland from a two-month-old infant. *The infantile prostate is divided into two zones, the inner zone (I) and the peripheral (P) one. x4.*

Fig. 2. Human infantile prostate from a 2-month-old infant. (a) *Inner zone has dense fibromuscular bundles with scattered small acini. x200.* **(b)** *Peripheral zone has similar findings, but the stroma is looser. x200.*
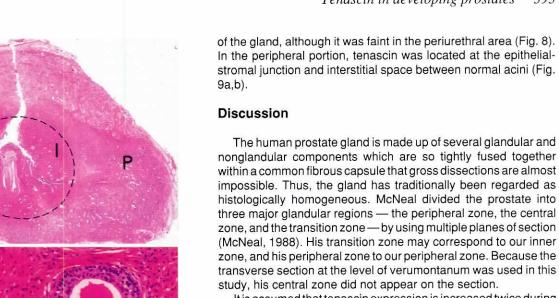
Fig. 3. Prepuberal prostate gland from a 12-year-old. (a) *Inner zone shows elongated acini with columnar epithelium. x100.* **(b)** *Peripheral zone has more crowded acini. x100.*

The prostates obtained from 9- to 13-year-old boys showed basically the same picture as those from infants. However, the distribution of acini was more crowded, and they had low columnar epithelium with stratification. In the peripheral zone, acini were larger and their distribution was less crowded. Stromal bundles were sparse as compared with the inner zone (Fig. 3a,b).

In the prostates from 14- to 21-year-old subjects, the gland could be divided into the two zones. However, the boundary was not clear as in infant specimens (Fig. 4). Inner zone acini were small and round in shape and lined by tall columnar epithelium. Stroma in the inner zone was composed of compact interlacing bundles. Compared to the inner half, the peripheral zone had grown and increased in volume. The acini became larger and revealed papillary projections into the lumen. The peripheral zone epithelium was simple columnar and composed of pale cells. Its stroma was loosely woven with randomly arranged fibromuscular bundles (Fig. 5a,b).

Percent of glandular area

The ratio of epithelium was always higher in the peripheral zone than in the inner one (Fig. 6). The ratio in both zones tended to increase as the age of subjects progressed. The age-related increase in the peripheral zone was more obvious. It rose abruptly during the puberal period, i.e., between the ages of 13 and 14.

Fig. 4. Transverse section of the prostate gland from a 14-year-old boy, the inner zone (I) and the peripheral (P) one. *x3.*

Fig. 5. Human prostate from a 14-year-old. **(a)** *Inner zone shows round shaped acini with tall columnar epithelium. x100.* **(b)** *Peripheral zone has large acini with papillary projections into the lumen. x100.*

Localization of tenascin

Tenascin was diffusely but weakly distributed within the prostates from three infants. The epithelial-stromal junction occasionally showed immunoreactivity, especially at budding sites (Fig. 7). The prostates from 9- to 13-year-old boys seldom expressed tenascin. The prostates from subjects between 14 and 21 years of age showed immunoreactivity for tenascin in the peripheral portion of the gland, although it was faint in the periurethral area (Fig. 8). In the peripheral portion, tenascin was located at the epithelial-stromal junction and interstitial space between normal acini (Fig. 9a,b).

Discussion

The human prostate gland is made up of several glandular and nonglandular components which are so tightly fused together within a common fibrous capsule that gross dissections are almost impossible. Thus, the gland has traditionally been regarded as histologically homogeneous. McNeal divided the prostate into three major glandular regions — the peripheral zone, the central zone, and the transition zone — by using multiple planes of section (McNeal, 1988). His transition zone may correspond to our inner zone, and his peripheral zone to our peripheral zone. Because the transverse section at the level of verumontanum was used in this study, his central zone did not appear on the section.

It is assumed that tenascin expression is increased twice during the normal development and maturation of the prostate gland. The first increase occurs during the fetal period, followed by a decrease at an early postnatal stage. A quiescent period follows up to the puberal age (approximately 13-14 years old). Then, tenascin expression is raised with maturation of the gland, lasting from several years to more than ten, and gradually decreases as maturation is completed. Its expression during this period corresponds with the marked rise in percent of glandular area.

A similar biphasic increase in tenascin expression was observed in the mammary gland. Inaguma *et al.* (1988) reported on it in the mouse mammary gland. Tenascin was located in the mesenchyme surrounding epithelium in 14- to 17-day embryos and in 3-week-old glands. During the former period, the mammary bud proliferates, rapidly elongating and forming mammary sprouts. During the latter period, many endbuds have formed at the tip of the ducts. This staining pattern was also observed in the infantile prostate.

There have been a few reports investigating the postnatal development of the human prostate (Xia *et al.,* 1990; Aumuller,
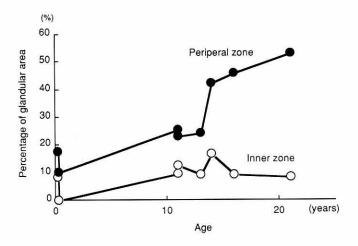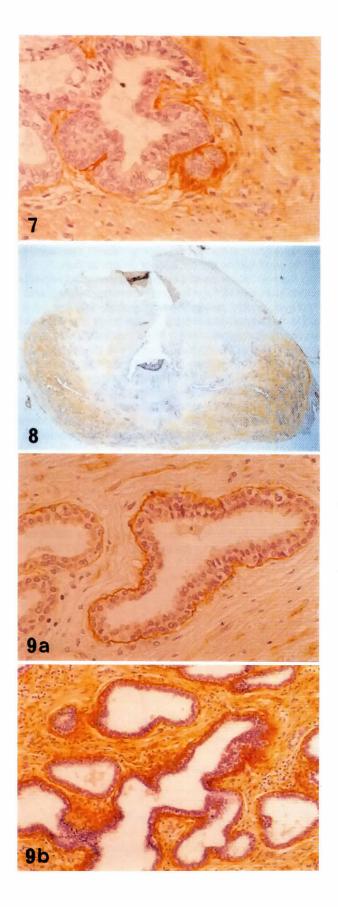

Fig. 6. Chronological changes of percent of glandular area in the inner and peripheral zones.

1991). Aumuller (1991) divided it into the three periods, including a regression, quiescent and maturation periods. His model is quite consistent with our results. However, he reported that differentiation of the epithelium is completed by about 17-18 years, while our data suggest that it lasts at least until 21 years of age. The ratio of glandular area in the inner zone remains at a low level in this study. That portion is known to grow at middle age or later. At the same time the proportion of glandular area is expected to increase, and benign hyperplasia will appear within the inner zone (McNeal 1988).

Clinically, the prostate gland has been divided into two parts, the periurethral or inner zone and the peripheral zone according to biological difference. The former is the main site of origin of prostate hyperplasia, while most cancer arises in the latter area (McNeal et al., 1988). The distribution of tenascin during the post-puberal period may correlate with the preferential distribution of prostate carcinoma in the peripheral zone. Prostatic carcinoma may initiate at this time, since it is known to be slow growing and to have a long natural history, whereas the expression of tenascin is heterogeneous or focal in prostatic hyperplasia (Ibrahim, et al., 1993). This suggests that tenascin could be responsible for stromal alterations associated with malignant prostatic epithelial growth processes rather than with benign ones.

Ibrahim et al. (1993) were the first to report on tenascin expression in prostatic hyperplasia and carcinoma. They also examined two fetal and four normal adult prostates, although chronological analysis was not done because of the limited case number. They found diffuse stromal staining throughout fetal prostates with an increased immunoreactivity adjacent to developing ducts and acini. Normal adult prostates demonstrated only weak and focal staining in the stroma. Unfortunately, the age of the subjects was not specified, but just mentioned as <40 years of age.

Materials and Methods

Twelve prostates obtained at autopsy were examined. The subjects included three infants (2 to 3 months old), and nine boys (9 to 21 years old).

Examination was limited to those who had had no prostatic surgery or diagnosis of prostatic cancer. Examination procedures of the prostates were essentially the same as those reported previously (Yatani et al., 1982). Briefly, the prostates were fixed in 10% formalin and step-sectioned at 3 mm intervals vertically to the lower urethra. Each slice was systematically numbered. The slice located most caudal to the gland was regarded as the first one. Each slice was embedded in paraffin. The blocks were cut 5-7 μm in thickness and stained with hematoxylin and eosin.

Immunohistochemical method

Sections were deparaffinized in xylene and rehydrated by gradual changes of ethanol. They were incubated with 0.3% hydrogen peroxide in methanol for 30 min to block endogenous peroxidase activity. After three

Fig. 7. Immunolocalization of tenascin in the prostate from a 3-month-old infant. *Focal immunostaining in the stroma surrounding the gland. x200.*

Fig. 8. Immunolocalization of tenascin in transverse section of the prostate gland from a 14-year-old boy. *Preferential localization of tenascin was noted in the peripheral zone. x3.*

Fig. 9. Immunolocalization of tenascin in the prostate from a 16-year-old subject. (a) *Inner zone shows weak, heterogeneous immunostaining. x200.* **(b)** *Peripheral zone reveals diffuse immunostaining in the stroma with intense periglandular area. x100.*

washes with phosphate buffered saline (PBS), the sections were treated with 1% normal rabbit serum for 30 min to block nonspecific binding of immunoglobulin. They were incubated with monoclonal rat antibody against human tenascin (Oike *et al.,* 1990). After overnight incubation at 4°C, the sections were washed three times with PBS, incubated in biotinylated anti-rat IgG antibody (Vector Laboratories, CA, USA) for 30 minutes. They were washed with PBS, and incubated with avidin-biotinylated horseradish peroxidase complex (ABC; Vector Laboratories) for 30 min. The color reaction was developed with a freshly prepared 0.1 mg/ml diaminobenzidine tetrahydro-chloride (Wako Pure Chemical, Osaka, Japan) in PBS containing 0.1% hydrogen peroxide. The sections were washed in PBS, lightly counterstained with Mayer's hematoxylin and mounted in Marinol (Muto Chemicals, Tokyo, Japan).

Morphometry

The epithelial-stromal ratio was estimated by the point counting method with the aid of a transparent grid attached to the eye-piece of the microscope. The section bearing verumontanum was selected from each case for the estimation, and the count was done in the anterior periurethral and subcapsular regions.

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