

Expression of Androgen Receptor in Mouse Eye Tissues

Masayoshi Tachibana,¹ Yasubito Kobayashi,² Takashi Kasukabe,¹ Kaname Kawajiri,¹ and Yoshibumi Matsushima¹

PURPOSE. To test the possibility that androgen directly affects the corneal cells, the possible occurrence of androgen receptor (AR) in the cornea and other eye tissues of mice was examined.

METHODS. To examine the occurrence of AR protein in the mouse eye tissues, an immunocytochemical method was used. To examine the occurrence of AR mRNA in the cornea and lens, reverse transcription-polymerase chain reaction (RT-PCR) was used.

RESULTS. Immunocytochemical examination revealed that antigenicity for AR antibody exists in cell nuclei of cornea, lens, iris, and ciliary body of both male and female mice. RT-PCR revealed that mRNA of AR occurs in the cornea and lens of both male and female mice.

CONCLUSIONS. It is concluded that AR occurs in cells of cornea, lens, iris, and ciliary body of the mouse eye. Androgen may affect cells in these tissues directly through interaction with AR. (*Invest Ophthalmol Vis Sci.* 2000;41:64-66)

We are currently establishing a line of mice whose corneas abnormally grow to show keratoconus-like appearance (authors' unpublished data). The phenotype of these mice is overt only in males. However, the phenotype develops in females when they are administered a single dose (2 mg, intramuscularly) of androgen at around 4 weeks of age. We postulated, although some other scenario is equally possible, that androgen affects eye corneal cells directly in this event. As a first step toward proving this hypothesis, we examined whether androgen receptor (AR) occurs in the mouse cornea. Studies by two independent methods revealed that AR occurs in the cornea. During the course of this experiment, we noticed that AR occurs also in the lens and ciliary body.

MATERIALS AND METHODS

BALB/cByJ mice of both sexes were purchased from a local vender (CLEA Japan, Tokyo, Japan) and used at approximately 4 months of age. All animals were treated according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Immunocytochemical Detection of AR

Three female and three male mice approximately 4 months of age were killed by overdose amounts of pentobarbital, and eyeballs were dissected. Tissues were immersed in 10% buffered formalin overnight, dehydrated in graded series of ethanol, and embedded in paraffin. Sections of approximately 4- μ m thickness were cut, mounted on glass slides, deparaffinized

with xylene and graded series of ethanol. To enhance antigenicity, they were immersed in 10 mM citrate buffer (pH 7.4) and treated with 800 W microwave at boiling temperature for 8 minutes. Next, to inhibit endogenous peroxidase activity, sections were treated with 0.3% hydrogen peroxide contained in methanol. Immunostaining was performed by streptavidin-biotin complex method using rabbit polyclonal antibody to AR (Chemicon International, Temecula, CA). This antibody was raised against a synthetic peptide corresponding to the amino terminus of mice,¹ which is highly homologous to AR of other species including the rat and humans but not homologous to that of other steroid hormone receptors.^{2,3} The antibody was used at a concentration of 1:160 together with Histofine SAB-PO(R) kit (Nichirei, Tokyo, Japan) according to the manufacturer's recommendation. After visualization of reaction complex by 0.05% 3,3'-diaminobenzidine tetrahydrochloride and 0.03% hydrogen peroxide contained in 50 mM Tris-HCl buffer (pH 7.6), sections were observed under microscope. In control slides, antibody was replaced by normal rabbit serum.

Reverse Transcription-Polymerase Chain Reaction

Under deep anesthesia with pentobarbital, eyeballs were isolated from male and female mice approximately 4 months of age. Cornea and lens were further dissected under the stereomicroscope. Testes and submandibular glands, which have been shown to be enriched with AR,⁴ were obtained from male mice and used for a positive control. RNAs were extracted from these tissues using Catrimox-14 RNA isolation kit (Takara Biomedicals, Kyoto, Japan). Approximately 500 ng of total RNAs were reverse-transcribed, and resultant cDNA was polymerase chain reaction (PCR)-amplified using RNA PCR kit (Takara). PCR was performed for 30 cycles at 95°C for 1 minute and 65°C for 1 minute, and finally for one cycle at 72°C for 5 minutes in 20 μ l of reaction mixture containing the cDNA template, sense and antisense primers, 1 mM deoxyribonucleotide triphosphates (dATP, dTTP, dGTP and dCTP), and 2.5 U of Taq polymerase (Takara). Primers used for amplification of AR cDNA were designed from the 5' region of the open reading frame of mouse AR cDNA sequence, because this

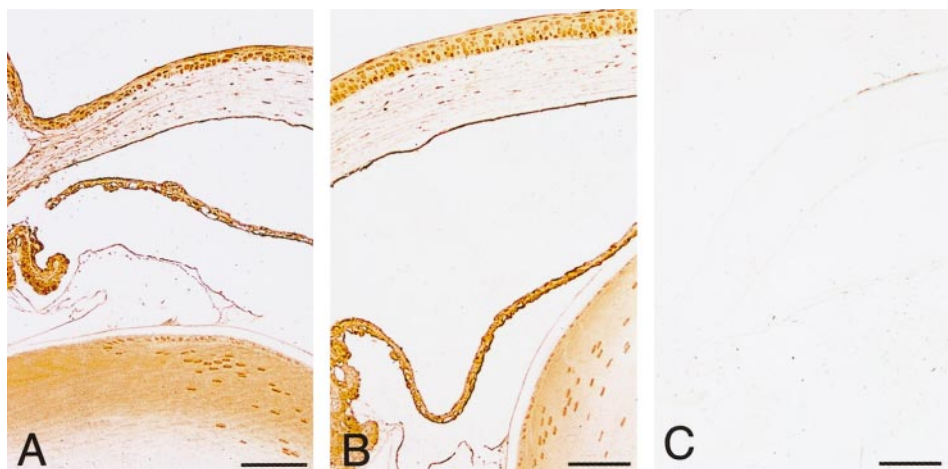
From the ¹Research Institute and the ²Laboratory of Clinical Pathology, Saitama Cancer Center, Saitama 362-0806, Japan.

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Corresponding author: Masayoshi Tachibana, Research Institute, Saitama Cancer Center, 818 Komuro, Ina, Saitama 362-0806, Japan. mtachiba@cancer-c.pref.saitama.jp

FIGURE 1. Immunocytochemical demonstration of AR in eye tissues of female (A) and male (B) mice at approximately 4 months of age. Scale bars, 100 μ m. (A and B) Immunoreactivity for AR was observed in nuclei of corneal cells (i.e., epithelial, stromal and endothelial cells) and lens cells (i.e., subcapsular epithelium and cortical cells). Reactivity was also observed in the nuclei of iris and ciliary body. (C) Replacement control section. No specific staining was observed.



region is not homologous to other steroid hormones.¹ Forty nanograms each of sense primer, CCATCCAAGCCTATC-GAGG (position 75-94), and anti-sense primer, CTCAGAT-CAGGATGACTCA (position 424-443), was used for amplification of AR cDNA. Primers used for glyceraldehyde 3-phosphate dehydrogenase (G3PDH) cDNA amplification were each one nanomole of oligonucleotides ATGGTGAAGGTCGGT and GC-CTTGACTGTGCCGTTGAAT, which were designed from a reported G3PDH cDNA sequence.⁵ PCR products were separated in 2% agarose gel stained with ethidium bromide. For radiolabeling of PCR products, half of the cDNA was amplified in the reaction mixture containing 55.5 KBq of [α -³²P]dCTP. PCR products were separated on 5% polyacrylamide gel, which was dried and applied to x-ray film.

For direct sequencing, PCR products were separated on 1% agarose gel, purified using QIAEX II gel extraction kit (Qiagen, Hilden, Germany), and used for templates of sequencing. The sequencing was carried out using ABI PRISM cycle sequencing kit (Perkin-Elmer, Norwalk, CT) and ABI PRISM 310 gene analyzer.

RESULTS

Immunocytochemistry

Androgen receptor was localized by immunocytochemistry in mouse eyeballs. Immunoreactivity was observed in the nuclei of epithelial, stromal, and endothelial cells of the cornea of both male and female mice (Figs. 1A, 1B). Nuclei of subcapsular epithelial cells and cortical lens cells were also immunostained. In addition to these cells, cells of the iris and ciliary body were stained. Virtually no immunostaining was observed in control sections (Fig. 1C).

Reverse Transcription-Polymerase Chain Reaction

The expression of AR at the transcript level was examined by reverse transcription-polymerase chain reaction (RT-PCR). Amplified transcripts were detected as a single band on an agarose gel in cornea, lens, and submandibular gland (Fig. 2A). The sizes of the amplicons were identical among these tissues and were identical with the expected size (374 bp). DNA sequences of amplicons were identical with that of reported mouse AR cDNA,¹ encoding N-terminus amino acids (position

15-137) of mouse AR. Further examination revealed that AR transcript was expressed in mice cornea and lens of both sexes (Fig. 2B).

DISCUSSION

The possible involvement of androgen in physiology or pathology of eye tissues has attracted interest of researchers, and evidence to support the involvement has accumulated. For example, androgen treatment of female MRL/Mp-*lpr/lpr* (MRL/*lpr*) and NZB/NZW F1 mouse models of Sjögren syndrome has been shown to suppress the inflammation of the lacrimal gland.^{6,7} To support this hormone effect, AR was localized in epithelial cells of lacrimal glands in MRL/*lpr* mice.⁸

In 1995 we found a male mouse with an unusual corneal appearance, which resembled human keratoconus. In an effort to establish mouse models of keratoconus, we inbred this mouse and have currently established an F12 generation, named spontaneous keratoconus (SKC) mice. In this procedure, we noticed that keratoconus-like phenotype appeared solely in male SKC mice. However, female SKC mice develop the phenotype when administered testosterone. Previous studies suggest the occurrence of AR in the cornea. For example, it has been shown that androgen is taken up and bound by bovine corneal epithelial cells.^{9,10} These data led us to postulate that AR occurs in mice cornea, where it interacts with androgen, and that the interaction involves corneal physiology and pathology. In an effort to prove this hypothesis, we examined the occurrence of AR in mice cornea. BALB/c mice (rather than SKC mice) were used, because we wanted to look for the occurrence of AR in normal corneas rather than pathologic corneas.

As we expected, immunocytochemical experiments localized AR in the corneas of male and female mice; AR was localized in epithelial, stromal, and endothelial cells. Localization of AR in lens cells was not surprising, when considering the fact that both cornea and lens derive developmentally from the surface ectoderm.¹¹ Unexpectedly, AR was also localized in cells of the iris and ciliary body, which derive from mesoderm.¹¹

To confirm the expression of AR at the RNA level, we used the RT-PCR method. The transcript for AR was amplified from cornea, lens, submandibular gland, and testes. All RT-PCR

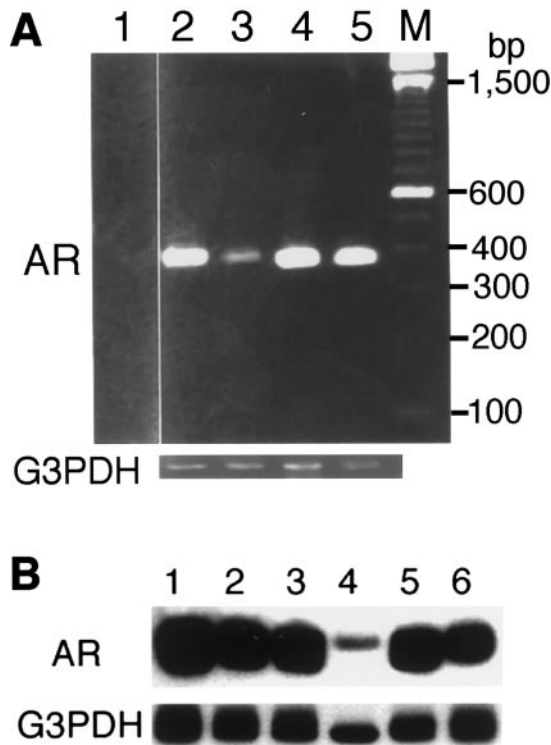


FIGURE 2. Demonstration of AR transcript (*top*) and G3PDH transcript (*bottom*) by RT-PCR in eye and other tissues. **(A)** RNA of tissues from three female and three male mice were mixed and used for RT-PCR. Amplified products were electrophoresed on 2% agarose gel and stained with ethidium bromide. In non-RT controls, RNA of cornea was PCR-amplified without RT. *Lane 1*, Non-RT control; *lane 2*, cornea; *lane 3*, lens; *lane 4*, submandibular gland; and *lane 5*, testes; *lane M*, size marker. **(B)** RNAs of tissues from four female and four male mice were separately used for RT-PCR. Radiolabeled amplicons were electrophoresed on a 5% polyacrylamide gel, which was dried and exposed to x-ray film. *Lane 1*, cornea of male mice; *lane 2*, cornea of female mice; *lane 3*, lens of male mice; *lane 4*, lens of female mice; and *lanes 5* and *6*, testes.

products from these tissues were of the same size, which is consistent with the notion that AR in these tissues does not form distinct isoforms.

Recently, similar results were reported for rat, rabbit, and human corneas, along with other eye tissues.¹² Occurrence of AR in the cornea, lens, and iris may have some developmental, functional, or pathologic significance across mammalian species. We are now examining the possible effects of androgen

on corneal wound healing mitosis of corneal cells in BALB/c and SKC mice. In the present study, we found no evidence of gender difference for the occurrence of AR. However, we did not use the quantitative methods for immunocytochemistry and RT-PCR, so further study is necessary in this regard. Occurrence of AR in SKC mice cornea is currently being examined.

Acknowledgments

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