LOCALIZATION OF ESTROGEN RECEPTOR- β IN RAT TISSUE BY IMMUNOHISTOCHEMISTRY

Hisako Yuasa¹⁾, Yoshitatsu Fukabori¹⁾, Katsuya Nakano¹⁾,

Yoshihiro Ono¹⁾, Nao Tomita¹⁾, Keiji Suzuki²⁾

and Hidetoshi Yamanaka¹⁾

1) Department of Urology, Gunma University School of Medicine

2) Department of Laboratory Sciences, Gunma University School of Health Sciences

Abstract : Steroid hormones regulate cell functions via specific receptors. We have raised a polyclonal antiserum using a peptide specific for estrogen receptor- β in order to determine the cellular sites of expression of the receptor. In the 6 week old rat, estrogen receptor- β was localized to the cell nuclei in both male and female reproductive organs, such as the ventral, lateral and dorsal prostate, seminal vesicle, coagulating and ampullary gland, spermatic duct, testis, uterus and ovary. In the ventral and lateral prostate, estrogen receptor- β was present in glandular epithelial cells and strong staining was observed. In the dorsal prostate, seminal vesicle, coagulating gland, ampullary gland and uterus, epithelial cells were immunopositive and moderate staining was observed. In the spermatic duct, estrogen receptor- β positive nuclei were observed in the epithelial cells. In the stromal cells of many tissue, estrogen receptor- β positive nuclei were also observed. In the testis, nuclei of Sertoli cells were immunopositive. In the ovary, estrogen receptor- β was present in the follicles and corpus luteum and strong staining was observed. Thus immunohistochemistry was shown to be an important investigative tool for visualising specific sites of expression of estrogen receptor- β in rat male and female reproductive organs.

Key words : Estrogen, Estrogen receptor β , Immunohistochemistry, Rat
(Kitakanto Med.J. 49 (6) : 409~412, 1999)

INTRODUCTION

Steroid action is mediated by specific intracellular receptors. Recently, a novel nuclear receptor with ligand specificity for estrogen was discovered and named estrogen receptor- β (ER β)^{1~3)}. We previously demonstrated immunohistochemically that the classical estrogen receptor- α (ER α) was expressed in the seminal vesicle of estrogen-treated immature castrated rats, but not in the ventral prostate^{4~7)}. In the present study, we immunohistochemically examined localization of ER β in rat male and female reproductive organs.

MATERIALS AND METHODS

Animal

Male and female Wistar rats were obtained from

Imai Animal Co.(Kodama, Saitama) and housed under conditions of controlled temperature ($22 \pm 3^\circ\text{C}$) and lighting (lights on from 7:00 am to 7:00 pm). The rats were sacrificed under ether anesthesia at 6 weeks of age.

Antibody

A peptide (MTFYSPAVMNYSVP) corresponding to amino acids 1-14 (A/B region) of rat ER β ¹⁾ was synthesized and used to immunize rabbits following a standard procedure. Peptide-specific antibody was purified on protein A chromatography from serum collected after 5 or more months of immunization, and coupling of the antibody to protein A after differential precipitation with ammonium sulfate. The purified antibody was concentrated and dialyzed against 0.01M phosphate buffer-saline (PBS) pH 7.4 containing 0.1M NaN_3 using an Amicon stirred cell concentrator.

Purified antibody was analyzed by SDS-PAGE (Fig. 1).

Fig. 1. SDS-PAGE analysis of ER β antibody. Lane 1, 2, 3 and 4 indicate MW standard, crude antiserum, purified antibody and purified antibody+DTT.

Immunohistochemistry

Rat male and female reproductive organs were removed and immediately fixed using 20% formalin in 0.1M phosphate buffer at pH 7.4 for 6 hours, before processing into paraffin wax. The 3 μ m thick sections were dewaxed, rehydrated and immersed in 0.01M citrated buffer pH 6, autoclaved under sterile conditions of 121°C, 2 atmospheric pressures at 5 minutes for antigen retrieval. Sections were immersed in 0.3% hydrogen peroxide/methanol for 15 minutes to block endogenous peroxidase. Next, sections were blocked with protein blocking solution (DAKO Co., Carpinteria, CA, USA) for 20 minutes at room temperature, and then incubated for 48 hours at 4°C with the estrogen receptor- β polyclonal antibody, diluted 1:800 (1.7 μ g IgG/ml) in 0.01M PBS pH 7.4. After washing in PBS, sections were incubated with EnVision plus™ (DAKO Co., Carpinteria, CA, USA), goat anti-rabbit immunoglobulin, conjugated to peroxidase labelled-dextran polymer for 60 minutes at room temperature. The sections were treated with a chromogen, 3,3'-diaminobenzidine tetrahydrochloride (DAB, Sigma Chemical Co., St. Louis, MO, USA) / 0.05M Tris-HCl pH 7.6 for 5 minutes. Sections were then lightly counterstained with hematoxylin for 3 seconds. Specificity of immunostaining was confirmed using preimmune rabbit IgG-fraction of serum or diluted antiserum centrifuged after incubation with immunopeptide for 16 hours at 4°C.

RESULTS

Localization of estrogen receptor- β (ER β)

Sections of different tissue from the rats were stained. ER β was immunolocalized to the cell nuclei in the various tissue. In both the ventral prostate and lateral prostate, nuclear ER β positive staining was observed in the majority of glandular epithelial cells and the staining intensity in both tissue was strong (Fig. 2-a, b). In the dorsal prostate, seminal vesicle, coagulating gland and ampullary gland, nuclear ER β positive staining was observed, as was the case for the ventral prostate, but the observed staining intensity here was moderate (Fig. 2-c, d, e, f). Moderate positive staining was also observed in the uterus (Fig. 2-k). In the spermatic duct, ER β positive staining was observed in the nuclei of epithelial cells and the staining intensity was weak (Fig. 2-g). For reference, ER β weak positive staining was also observed in the urinary bladder (Fig. 2-h). In the testis, Sertoli cells were immunopositive, but the expression did not appear to be stage-dependent (Fig. 2-i). In the ovary, nuclear ER β positive staining was observed in the small, medium and large follicles and corpus luteum and the staining intensity was strong (Fig. 2-j). In addition, the thecal cells and interstitial cells were also immunopositive, but the observed staining intensity here was moderate. In the stromal cells of many tissue, estrogen receptor- β positive nuclei were also observed.

Positively stained stromal cells in the seminal vesicle and uterus were demonstrated (Fig. 2-l, m). Non specific cytoplasmic staining was observed in the epithelial cells of many tissue. Specific nuclear staining was almost completely abolished by incubation with the peptide used for immunization and almost no staining was observed by incubation with the preimmune serum aside from low background staining. The ventral prostate, seminal vesicle and testis were stained with the antibody after preabsorption with the antigen (Fig. 2-n, p, q), and the ventral prostate was shown to be stained with the preimmune serum (Fig. 2-o).

DISCUSSION

The occurrence of estrogenic effects in female estrogen-target organs has been thought to be mediated by the intracellular estrogen receptor^{8,9)}. The precise physiological role of the estrogen receptor in male reproductive organs has not yet been clarified. The discovery of a second estrogen receptor (ER β) has redefined the estrogen signaling pathway and may have broad implications for estrogen-responsive tissue^{1,2)}. In our previous study, an immunohistochemical analysis demonstrated that estrogen treatment increased

Localization of ER β

Fig. 2. Immunohistochemical findings of ER β in rat tissue. In the ventral prostate (VP) and lateral prostate, ER β was immunolocalized to almost nuclei of the glandular epithelial cells and the staining intensity was strong (a, b). In the dorsal prostate, seminal vesicle (SV), coagulating gland, ampullary gland and uterus, the nuclei of epithelial cells were immunopositive and the staining intensity was moderate (c, d, e, f, k, arrowhead). In the spermatic duct and urinary bladder, ER β positive nuclei were observed in the epithelial cells (g, h, arrowhead). In the testis (T), Sertoli cells were immunopositive (i, arrow). In the ovary, nuclear ER β positive staining was observed in all sizes of follicles (openstar) and corpus luteum (solidstar), and the staining intensity was strong (j). Thecal and interstitial cells were also immunopositive. The specificity of the ER β antibody was confirmed by absorption with antigen (n, p, q : VP, SV, T) and checked using preimmune serum (o : VP). ER β positive nuclei were observed in the stromal cells of SV and uterus (l, m, arrowhead). magnification $\times 360$, bar $20\mu\text{m}$.

estrogen receptor- α (ER α) levels in the seminal vesicle, but did not increase them in the ventral prostate of immature castrated rats^{6,7)}. In the present study, we immunohistochemically examined localization of ER β in the rat reproductive organs. The present results agreed with other immunohistochemical studies done on ER β in rat tissue^{10,11)}.

Immunohistochemistry has proved to be an important investigative tool for visualising specific sites of expression of ER β in the rat male and female reproductive organs. In our recent study, both the ventral prostate and seminal vesicle showed a distinct pattern of localization of ER α and ER β in the estrogen-treated immature castrated rats. In future studies, we will focus on the expression and interplay of ER α and ER β in the male reproductive organ, primarily in the ventral prostate and seminal vesicle.

ACKNOWLEDGMENTS

This study was supported in part by a 1997-1999 Grant-in-Aid for Scientific Research (No. 09470339, 09671602, 10671458, 10877248) from the Ministry of Education, Science, Sports and Culture of Japan.

REFERENCES

- 1) Kuiper GGLM, Enmark E, Peltö-Huikko M, et al.: Cloning of a novel estrogen receptor expressed in rat prostate and ovary. *Proc Natl Acad Sci USA* 1996; 93: 5925-5930.
- 2) Kuiper GGLM, Carlsson B, Grandien K, et al.: Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β . *Endocrinol* 1997; 138: 863-870.
- 3) Mosselman S, Polman J and Dijkema R: ER β : identification and characterization of a novel human estrogen receptor. *FEBS Letters* 1996; 392: 49-53.
- 4) Yuasa H, Fukabori Y, Mashimo T, et al.: The characteristics of hormone responsiveness of glandular epithelium and stroma in male accessory sex organs. *Acta Urol Jap* 1993; 39: 631-637.
- 5) Yuasa H, Ono Y, Ohma T, et al.: The estrogen-induced changes of estrogen receptor in seminal vesicle of immature castrated rat. *Acta Urol Jap* 1996; 42: 33-37.
- 6) Yuasa H, Ono Y, Fukabori Y, et al.: Effect of sex hormones on the tissue localization of nuclear estrogen receptor positively stained cells in the seminal vesicle of immature castrated rats. *Tohoku J Exp Med* 1997; 181: 297-309.
- 7) Yuasa H, Ono Y, Fukabori Y, et al.: Usefulness of the estrogen releasing silastic tubing -estrogenic effect on seminal vesicle in immature castrated rats -. *Kitakanto Med J* 1998; 48: 15-18.
- 8) King RJB and Mainwaring WIP: Oestrogen. In: King RJB, Mainwaring WIP (eds). *Steroid-cell interactions*. Butterworths London 1974; pp.190-255.
- 9) Gorski J and Gannon F: Current models of steroid hormone action- A critique. *Annu Rev Physiol* 1976; 38: 425-450.
- 10) Saunders PTK, Maguire SM, Gaughan J and Millar MR: Expression of oestrogen receptor beta (ER β) in multiple rat tissues visualised by immunohistochemistry. *J Endocrinol* 1997; 154: R13-R16.
- 11) Sar M and Welsch F: Differential expression of estrogen receptor- β and estrogen receptor- α in the rat ovary. *Endocrinol* 1999; 140: 963-971.