—**Photogravure**—

Immunohistochemistry for ESR2 with a Mouse Monoclonal Antibody (PPZ0506)

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(J Nippon Med Sch 2023; 90: 138–140)
Key words: mouse, rat, estrogen receptor, immunohistochemistry

Lack of reliable antibodies against estrogen receptor β (ESR2), a nuclear estrogen receptor, has stalled ESR2 research. Thus, the recent discovery of a well-validated antibody against human ESR2 (PPZ0506)[1] provided momenta to develop new ESR2 detection systems. To utilize PPZ0506 for immunohistochemical detection of rodent ESR2 proteins, our previous studies confirmed its specific cross-reactivity against rat and mouse ESR2 proteins and optimized protocols for immunohistochemical staining of rat and mouse sections with PPZ0506[2–4]. Here, we present the immunohistochemical procedures optimized in our previous study with some modifications (Supplementary Text; https://doi.org/10.1272/jnms.JNMS.2023_90-209) and show representative photomicrographs obtained following the procedures in **Figure 1, 2**. Specific immunoreactive signals were observed in granulosa cells of rat and mouse ovaries (**Fig. 1A**), epithelial cells of rat dorsal and ventral prostate (**Fig. 1B**), and neurons of rat and mouse brain subregions containing the principal nucleus of the bed nucleus of the stria terminalis, paraventricular nucleus, and posterodorsal part of the medial amygdala (**Fig. 2**).

Acknowledgments: This work was supported by KAKENHI Grants-in-Aid [Grant Nos. 21K20659 (M.M.), 20K17544 (Y.H.), 21K06775 (S.H.), 18K06879 (H.I.), 22 and 18K06860 (H.O.)]. We thank Edanz (https://jp.edanz.com/ac) for editing a draft of this manuscript.

Grants: KAKENHI Grants-in-Aid [Grant Nos. 21K20659 (M.M.), 20K17544 (Y.H.), 21K06775 (S.H.), 18K06879 (H.I.), and 18K06860 (H.O.)]

Approval of Institutional Review Board: All animal experimental procedures in this study were approved by the Nippon Medical School Animal Care and Use Committee and conducted in compliance with the institutional guidelines (approval number, 2020-032).

Conflict of Interest: The authors declare no competing financial interests.

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https://doi.org/10.1272/jnms.JNMS.2023_90-209
Journal Website (https://www.nms.ac.jp/sh/jnms/)

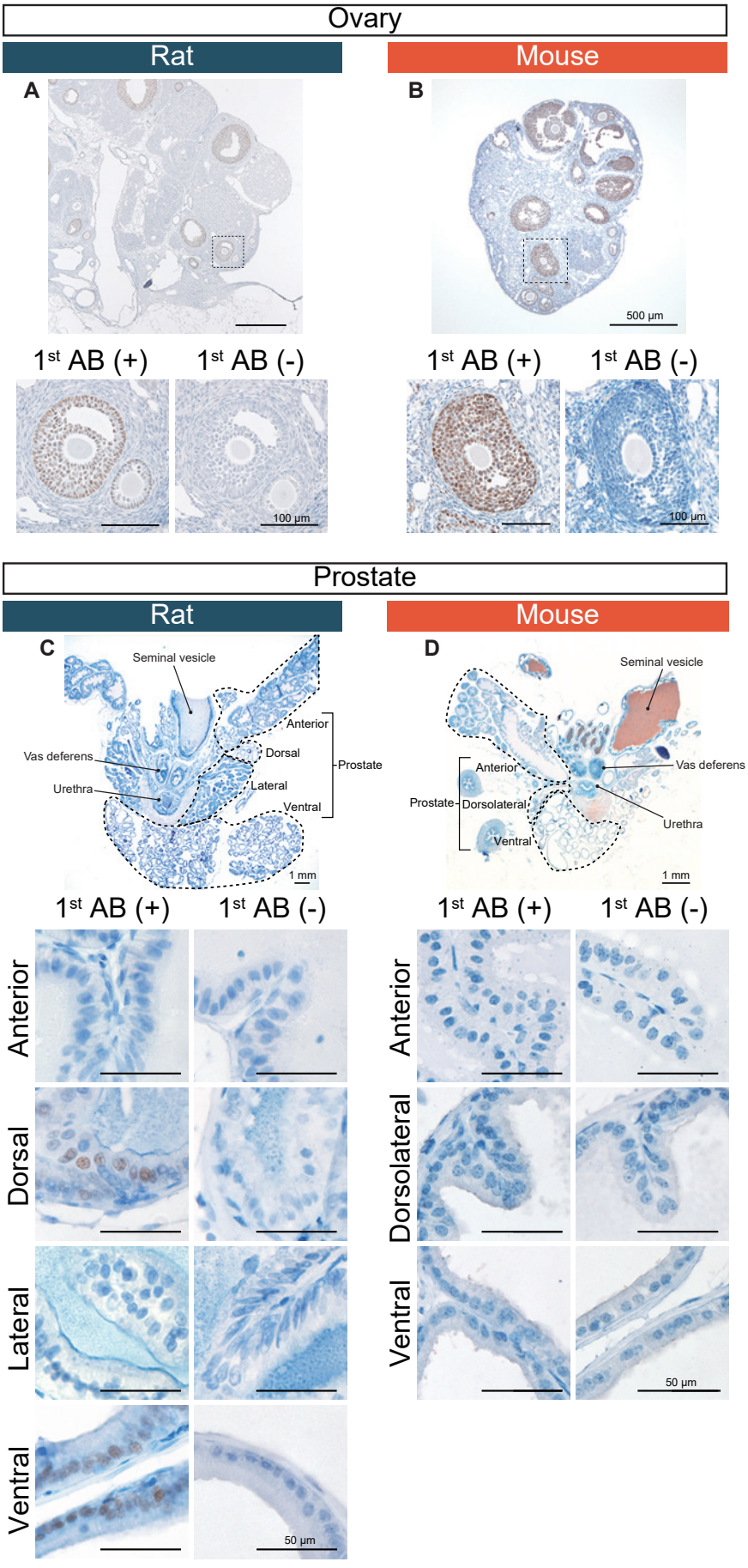
Ovary
Rat
Mouse
A
B
500 μm
1st AB (+)
1st AB (-)
1st AB (+)
1st AB (-)
100 μm
100 μm
Prostate
Rat
Mouse
C
Seminal vesicle
Anterior
Dorsal
Lateral
Ventral
Prostate
Vas deferens
Urethra
1 mm
D
Seminal vesicle
Anterior
Vas deferens
Prostate
Dorsolateral
Urethra
Ventral
1 mm
1st AB (+)
1st AB (-)
1st AB (+)
1st AB (-)
Anterior
Dorsal
Lateral
Ventral
Anterior
Dorsolateral
Ventral
50 μm
50 μm

Fig. 1

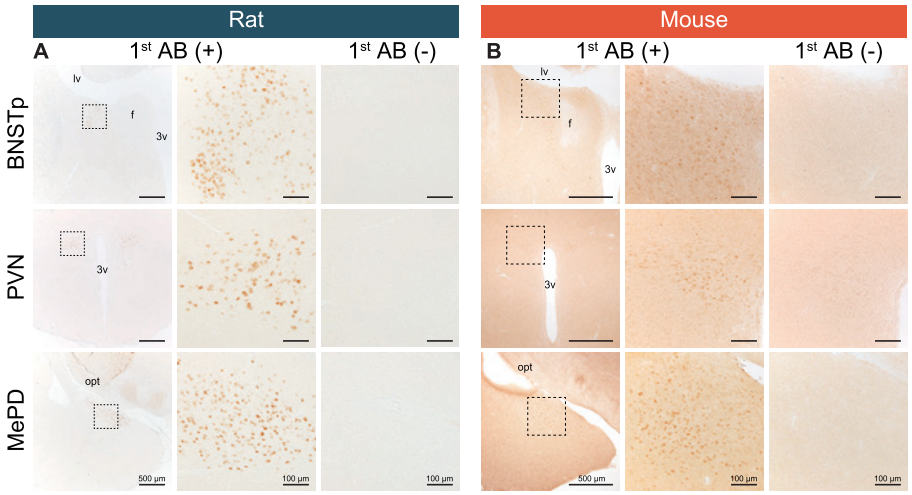

Fig. 2

Fig. 1 Distribution of ESR2-immunopositive cells in the genital organs

Immunostained paraffin-embedded sections prepared from ovaries (A, B) and male urethra-surrounding organs (C, D) of rats (A, C) and mice (B, D). Sections were reacted with and without PPZ0506 [1st AB (+) and (-)], and then counterstained with hematoxylin. The dashed squares in upper panels A and B indicate the location of corresponding magnified images in lower left panels. The boundaries of each lobe of the prostate are indicated by dashed lines in upper panels of C and D. Magnified images of the respective lobes of the prostate are represented in lower panels of C and D. Although staining was observed in mouse seminal vesicles, these signals were not attributed to immunoreaction of PPZ0506, as similar signals were observed in the corresponding antibody-omitted controls (Supplementary Fig. 1; https://doi.org/10.1272/jnms.JNMS.2023_90-209). Scale bars, 500 μm in upper images of A and B, 100 μm in lower images of A and B, 1 mm in upper images of C and D, and 50 μm in other images of C and D.

Fig. 2 Distribution of ESR2-immunopositive cells in the brain

Immunostained frozen brain sections containing the principal nucleus of the bed nucleus of the stria terminalis (BNSTp), paraventricular nucleus (PVN), and posterodorsal part of the medial amygdala (MePD) from rats (A) and mice (B). Sections were reacted with or without PPZ0506 [1st AB (+) and (-)]. The dashed squares in low-magnification images (left panels) indicate the location of high-magnification images (middle panels). Scale bars, 500 μm in low magnification images and 100 μm in high magnification images. 3v, third ventricle; f, fornix; lv, lateral ventricle; opt, optic tract.

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(Received, October 19, 2022)
(Accepted, December 2, 2022)
(J-STAGE Advance Publication, February 21, 2023)