

Estrogen Accelerates Cell Proliferation through Estrogen Receptor α during Rat Liver Regeneration after Partial Hepatectomy

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Although estrogen is implicated in the regulation of cell growth and differentiation in many organs, the exact mechanism for liver regeneration is not completely understood. We investigated the effect of estrogen on liver regeneration in male and female Wistar rats after 70% partial hepatectomy (PHx) and performed immunohistochemistry, western blotting and Southwestern histochemistry. 17β -estradiol (E_2) and ICI 182,780 were injected into male rats on the day before PHx. The proliferating cell nuclear antigen (PCNA) labeling index reached a maximum at 48 hr after PHx in males, and at 36 hr in females and E_2 -treated male rats. Estrogen receptor α (ER α) was expressed in zones 1 and 2 in male rats, but was found in all zones in female rats. Interestingly, ER α was not detected at 6–12 hr after PHx but was found at 24–168 hr in male rats. However, ER α expression was found at all sampling time-points in female and E_2 -treated male rats. The activity of estrogen responsive element binding proteins was detected from 12 hr after PHx in male rats but was found from 6 hr in female and E_2 -treated male rats. ER α was co-expressed with PCNA during liver regeneration. These results indicate that estrogen may play an important role in liver regeneration through ER α .

Key words: estrogen, estrogen receptor α , liver regeneration, partial hepatectomy, cell proliferation

I. Introduction

Although hepatocytes in adult livers rarely divide under normal conditions, the liver possesses a remarkable ability to restore its original mass and size following surgical removal or after various chemical injuries [7, 29]. This regenerative capacity allows the removal of tumor masses from the liver without impairment of its function. However, the potential for liver regeneration is limited in chronic liver diseases such as cirrhosis, hepatocellular carcinoma (HCC), non-alcoholic steatohepatitis, and excessive resection leads to liver failure [8, 28]. Recently there has been

much interest in rational therapies to reduce the factors which inhibit liver regeneration or to stimulate remnant liver regeneration [10].

In a clinical setting, there are substantial sex-based differences such as enzyme activity, gene expressions and steroid hormone responsiveness which can modulate the liver's capacity to metabolize certain drugs and hormones [27, 33, 37]. Chronic liver diseases are more severe and occur more frequently in males compared to females, and women have a significantly lower incidence of HCC than men [23, 30]. Similar sex-based differences are also observed in rodents. It was reported that the survival rate following partial hepatectomy or portal branch ligation was significantly higher in female compared to male rodents [14, 18, 43]. The liver is protected from injury due to reduced-size ischemia and reperfusion by 17β -estradiol

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(E₂). The survival rate in male mice after hepatic surgery is significantly improved by treatment with E₂ [14]. Although several factors are suggested to influence gender-based differences, sex steroid hormones such as estrogen and androgen may be closely associated with sex-based differences in the liver [42]. It was reported that estrogen prevents and androgen enhances DNA damage and oxidative stress during hepatocarcinogenesis [23, 26]. Interestingly, most sex-based differences in the liver are diminished after menopause [3] suggesting that female sex hormones, especially estrogen might have an important role in the differences.

Estrogen receptor alpha (ER α) and ER β bind to the estrogen response element (ERE), which is present in the promoter region of estrogen-target genes and regulates the transcriptional activity of various genes [4, 5, 21]. ER α , but not ER β , is expressed in hepatocytes, and involved in regulation of glucose and lipid metabolism in the liver [9, 11, 40]. Although estrogen has been implicated in cell proliferation in the intestine and skin [6, 38], its effect on liver regeneration is not completely understood.

In this study, we investigated the effect of estrogen on liver regeneration using a 70% partial hepatectomy (PHx) model in rats. Cell proliferation activity was determined using proliferating cell nuclear antigen (PCNA) and ER α expression in immunohistochemistry and western blot analysis. The activity of estrogen responsive elements (ERE) was analyzed using Southwestern histochemistry (SWH). Finally, to confirm the effect of estrogen on liver regeneration, we treated male rats with E₂ or estrogen receptor antagonist ICI 182,780 and analyzed cell proliferation activity and ER α expression after PHx.

II. Materials and Methods

Chemicals and biochemicals

Paraformaldehyde (PFA) was purchased from Merck (Darmstadt, Germany). ICI 182,780, 17 β -estradiol (E₂), bovine serum albumin (BSA), 2-mercaptoethanol, 3-aminopropyl-triethoxysilane, Brij L23 were from Sigma Chemical Co. (St Louis, MO). Diethyl ether, sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) reagents were from Wako Pure Chemical Industries, Ltd. Protein marker was from Bio Dynamics Laboratory Inc, Ltd. Polyvinylidene fluoride membrane (PVDF) was from Millipore (Bedford, MA). 3,3'-Diaminobenzidine-4 HCl (DAB) was from Dojindo Chemicals (Kumamoto, Japan) and 4-Cl-1-naphthol was from Tokyo Kasei Kogyo (Tokyo, Japan). All other chemicals used in this study were from Wako Pure Chemicals (Osaka, Japan).

Animals and tissue preparation

Eight-week-old male (240–260 g) and female (190–210 g) Wistar rats were used in the study. Rats were kept under constant 12 hr dark/light conditions and fed normal chow and drinking water *ad libitum*. The experimental protocol was approved by the Animal Ethics Review

Committee of the University of Miyazaki (2012-502-5). After sacrifice, liver tissue was cut into several small pieces and some were snap frozen and kept at -80°C until use in western blot analysis. The remaining pieces of liver tissue were fixed in 4% PFA in phosphate-buffered saline (PBS) at room temperature (RT) overnight and paraffin-embedded using standard methods. Each rat was anesthetized by inhalation of isoflurane and 70% PHx was performed using the technique described by Higgins and Anderson [15]. After PHx, rats were sacrificed at 0, 6, 12, 24, 36, 48, 72, 120 and 168 hr and tissues were sampled as described above. Male, female, E₂ and E₂+ICI 182,780 treated groups consisting of 3–5 rats for each time-point were used in these experiments.

Estrogen and estrogen receptor antagonist administration

Male rats were given a single intraperitoneal injection of E₂ at a dose of 9 $\mu\text{g/g}$ bodyweight [14] and ICI 182,780 at a dose of 2 $\mu\text{g/g}$ bodyweight [19, 39]. E₂ and E₂+ICI 182,780 were dissolved in 500 μl of corn oil and injected on the day before PHx.

Antibodies

Mouse monoclonal antibody against PCNA (PC-10; 2 $\mu\text{g/ml}$) and normal mouse IgG were purchased from Dako (Glostrup, Denmark). Mouse monoclonal antibody against ER α (6F11; dilution 1:50) and horseradish peroxidase (HRP)-goat anti-mouse IgG (dilution 1:100) were from Thermo scientific (Rockford, IL), and mouse monoclonal antibody against β -actin (AC-15; dilution 1:12,000) and normal goat IgG were from Sigma.

Immunohistochemistry

Immunohistochemistry was performed as reported previously [2, 4, 31, 36]. Paraffin-embedded tissues were cut into 5 μm sections and placed onto silane-coated glass slides. The sections were deparaffinized with toluene, and rehydrated through a graded ethanol series, and then heated to 120°C for 15 min in 10 mM citrate buffer (pH 6.0). After inhibition of endogenous peroxidase activity with 0.3% H₂O₂ in methanol for 15 min, the sections were pre-incubated with 500 $\mu\text{g/ml}$ normal goat IgG and 1% BSA in PBS for 1 hr to block non-specific antibody binding. Unless otherwise specified, all reactions were conducted at RT. The sections were then reacted with the primary antibodies for overnight. After washing with 0.075% Brij L23 in PBS, slides were reacted with HRP-goat anti-mouse IgG for 1 hr. After washing in 0.075% Brij L23 in PBS, the HRP site was visualized with DAB and DAB, Ni, Co, and H₂O₂ according to the method of Adams [1]. Normal mouse IgG was used at the same concentration instead of the primary antibodies for each experiment as a negative control.

Double-staining for ER α and PCNA

For simultaneous detection of ER α and PCNA, double-staining was performed as described previously [4].

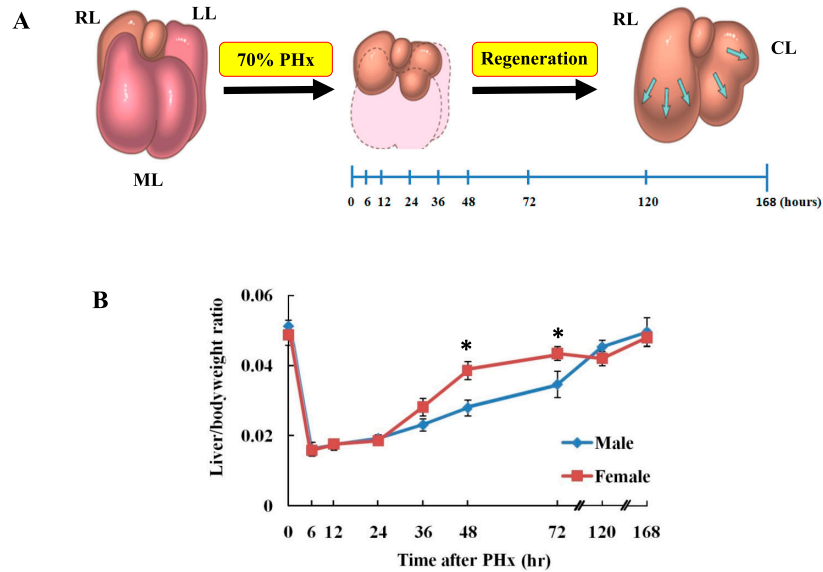


Fig. 1. Model of liver regeneration and the liver weight/bodyweight ratio of male and female rats after PHx. **A:** Schema of 70% PHx in Wistar rats [13]. RL: right lobe, LL: left lobe, ML: median lobe, CL: caudate lobe. **B:** Liver weight/bodyweight ratio of male and female rats after PHx. Asterisks indicate statistically significant differences ($*p < 0.05$). Data represent the mean \pm SE of 3–5 rats.

Briefly, after antigen retrieval, the sections were stained with anti-ER α overnight and HRP sites were visualized as brown deposits of DAB and H₂O₂. The slides were then immersed and stirred in 0.1 M glycine-HCl buffer (pH 2.2) to remove immuno-complexes. After washing with double distilled water (DDW) once and PBS three times, the sections were reacted with anti-PCNA overnight. HRP sites were visualized by the purple-blue product of 4-Cl-1-naphthol and H₂O₂ solution.

Western blot analysis

Lysate containing 20 μ g of protein was mixed with loading solution [200 mM Tris-HCl (pH 8.0), 0.5 M sucrose, 5 mM EDTA, 0.01% bromophenol blue, 10% 2-mercaptoethanol, and 2.5% SDS], boiled for 5 min, separated by SDS-PAGE with 8% polyacrylamide gel and electrophoretically transferred onto PVDF membranes. The membranes were blocked with 5% nonfat milk in Tris-buffered saline (TBS; 20 mM Tris buffer, pH 7.6, and 150 mM NaCl) for 1 hr at RT and then incubated overnight with mouse monoclonal antibody anti-ER α diluted 1:500 in TBS buffer. As a secondary antibody, HRP-goat anti-mouse IgG was diluted with TBS buffer for 1 hr and membranes were washed six times for 15 min each with TBS/0.05% Triton X-100 buffer. The bands were visualized with DAB, Ni, Co, and H₂O₂.

Southwestern histochemistry (SWH) for localization of ERE binding proteins

To confirm the localization of ERE binding proteins, SWH was performed as described previously [16]. Briefly, paraffin sections from male, female, E₂ and E₂+ICI 182,780 treated rats were deparaffinized and heated to 120°C for 15

min in 0.01 M citrate buffer (pH 6.0). The sections were then reacted with a digoxigenin labeled double-stranded DNA probe which contained a complete palindromic estrogen responsive element (vERE: 5'-GATCCAGGTCACAG TGACCTGGATC-3') of the chicken vitellogenin gene, and a mutated estrogen responsive element (mERE: 5'-GATC CAGATCACAGTGATCTGGATC-3') with 2 base mutations and a digoxigenin label at the 3'-end. For the detection of hybridized oligo-DNA probes, the sections were immunohistochemically stained with HRP-conjugated sheep anti-digoxigenin antibody using a chromogen solution with DAB, Ni, Co, and H₂O₂, as described previously [1].

Quantitative analysis

At least 2000 cells were counted at $\times 400$ magnification in random fields, and the percentage of positive cells per total number of counted cells was represented by a labeling index (LI).

Statistical analysis

All data were expressed as mean \pm SE. Differences between experimental groups were assessed by Student's *t*-test. $P < 0.05$ was considered statistically significant. All analyses were performed with The Statistical Package for Social Sciences (version 11.5; Chicago, IL, USA).

III. Results

Liver weight/bodyweight (LW/BW) ratio in male and female rats after PHx

A model of liver regeneration [13] and the liver weight/bodyweight ratio of male and female Wistar rats after PHx was shown in Fig. 1. Liver weight/bodyweight

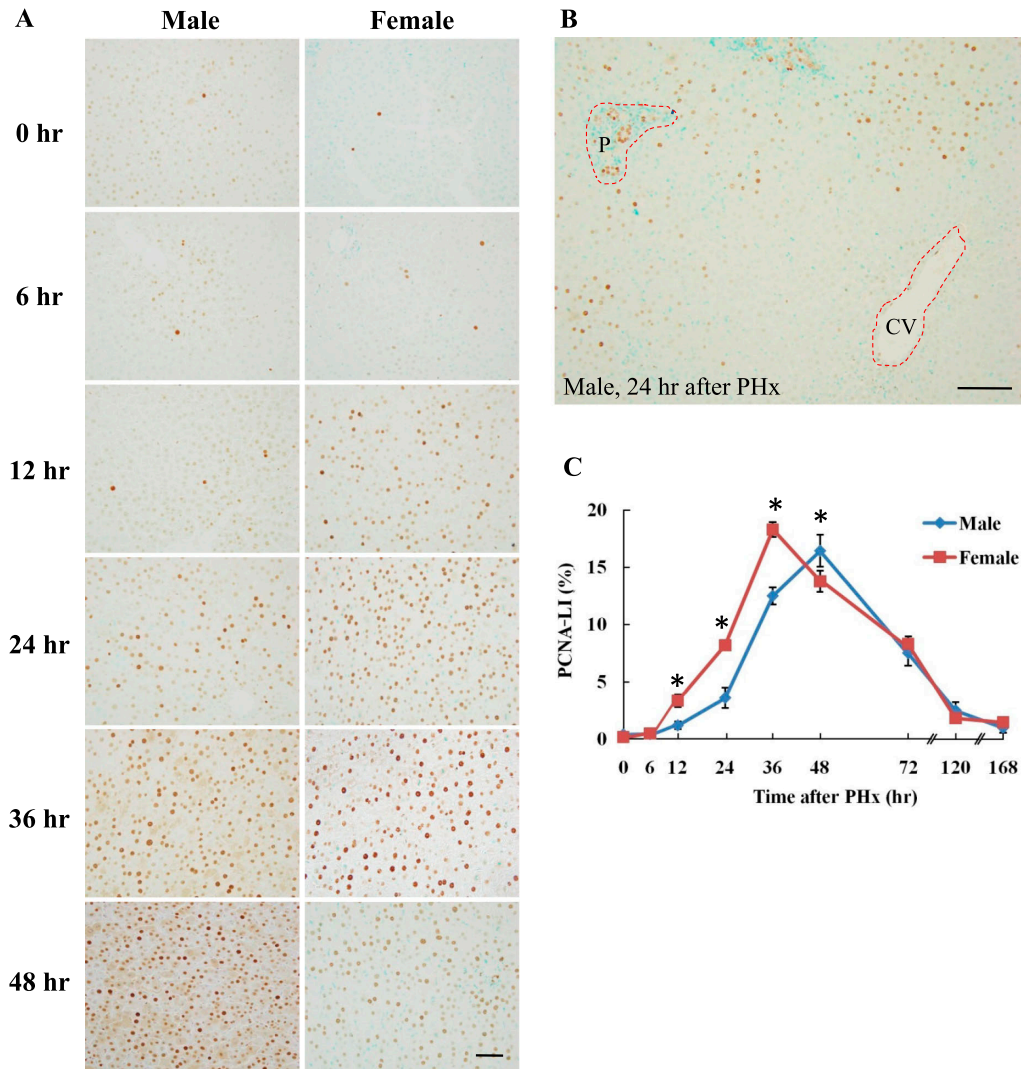


Fig. 2. Immunohistochemical detection of PCNA in male and female rat liver after PHx. **A:** Liver tissue was collected from male and female rats at 0, 6, 12, 24, 36 and 48 hr after PHx. Paraffin-embedded rat liver sections were analyzed by immunohistochemistry. Magnification $\times 400$. Bar = 50 μm . **B:** Zonal distribution of PCNA-positive cells at 24 hr in male rat liver after PHx. P: portal area, CV: central vein. Magnification $\times 200$. Bar = 100 μm . **C:** PCNA-LI in male and female rats after PHx. The number of PCNA positive hepatocytes was counted at each time-point after PHx. Blue and red lines represent male and female, respectively. Asterisks indicate statistically significant differences ($*p < 0.05$). Data represent the mean \pm SE of three independent experiments.

ratio was evaluated following 70% PHx. The results indicated that liver weight in females was increased significantly at 48 and 72 hr after PHx compared to the males. The liver weight was fully restored at 168 hr after PHx in both male and female rats (Fig. 1B).

Immunohistochemical detection of PCNA in male and female rat liver after PHx

PCNA expression was a marker of late G1 to S phase in regenerating liver and was investigated using immunohistochemistry. PCNA positive cells were found in zones 1 and 2 at 24–36 hr after PHx in male rats, but at 12–24 hr in female rats (Fig. 2A, 2B). PCNA positive cells were increased in all zones at 48 hr after PHx in male rats, but at 36 hr in female rats (Fig. 2A). PCNA-LI indicates that the

number of positive cells reached a peak at 48 hr in male rats, while the peak in female rats occurred at 36 hr after PHx. PCNA-LI was significantly higher at 12–36 hr after PHx in female rats compared to male rats (Fig. 2C). These results indicated that after PHx, cell proliferation starts 12 hr earlier in female rats compared to male rats. PCNA expression was significantly reduced at 72–168 hr after PHx and significant differences were not found between male and female rats (data not shown).

Immunohistochemical detection of ER α in male and female rat liver after PHx

The expression of ER α was examined in the regenerating liver using immunohistochemistry and western blot analysis. In normal liver, ER α was only expressed in zones

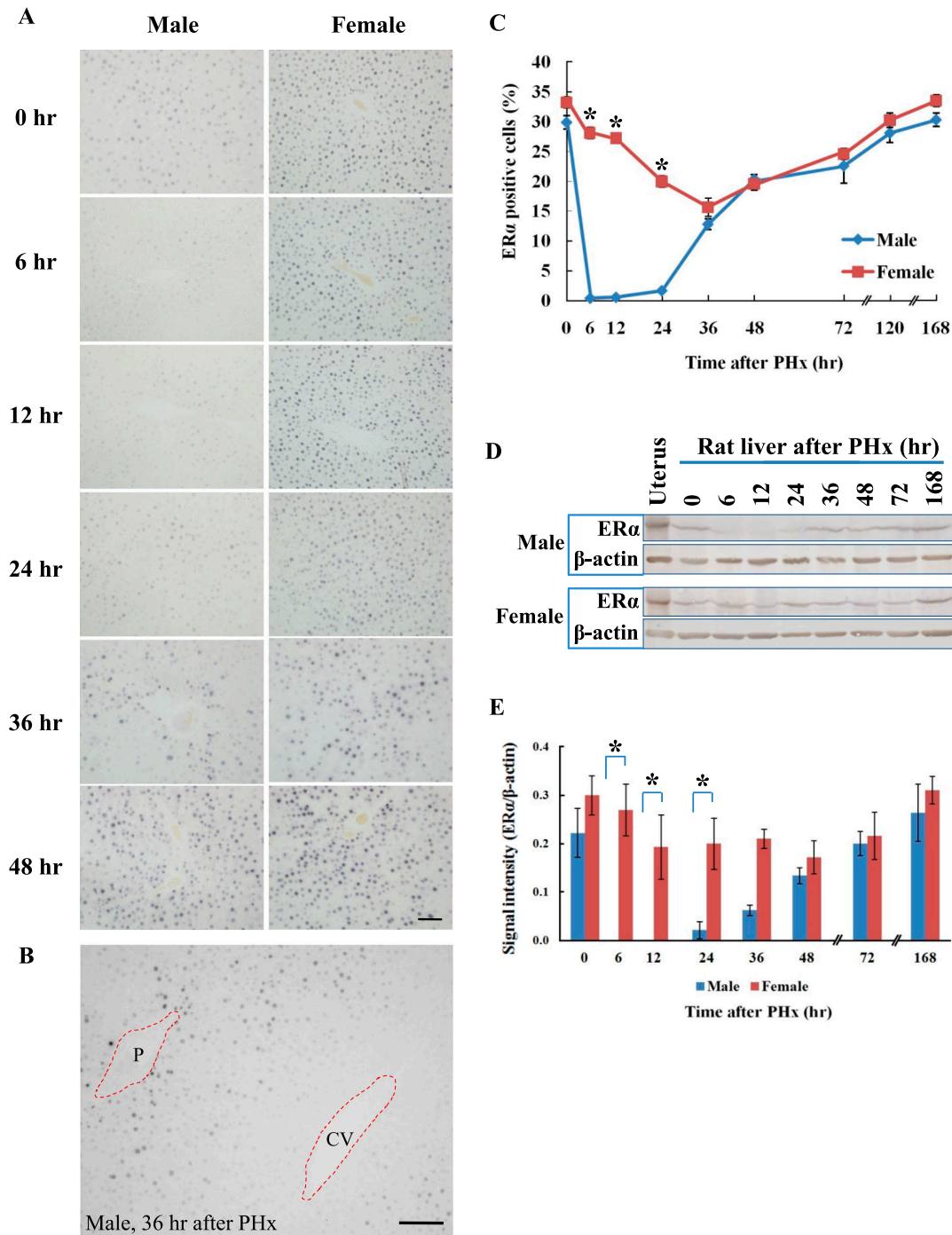


Fig. 3. ERα expression in rat liver after PHx. **A:** Immunohistochemical detection of ERα in male and female rat liver at various time-points after PHx. Paraffin-embedded liver sections were analyzed by immunohistochemistry. Magnification ×400. Bar = 50 μm. **B:** Zone dependent ERα expression at 36 hr in male rat liver after PHx. P: portal area, CV: central vein. Magnification ×200. Bar = 100 μm. **C:** The number of ERα positive cells in the liver sections of male and female rats at various time-points after PHx. Blue and red lines represent male and female, respectively. **D:** Western blot analysis of ERα in male and female rats. ERα (66 kDa) and β-actin (42 kDa). **E:** Densitometry analysis of western blot. Asterisks indicate statistically significant differences (**p* < 0.05). Data represent the mean ± SE of three independent experiments.

1 and 2 in male rats, but was found in all zones in female rats (Fig. 3A). In male rats, ERα was not detected from 6–12 hr after PHx but was weakly found at 24 hr in zone 1. ERα expression was increased in zones 1 and 2 at 36 hr after PHx (Fig. 3B), and was found in all zones from 48 to

168 hr (Fig. 3A). On the other hand, ERα expression was found in zones 1, 2 and 3 at all time-points after PHx in female rats (Fig. 3A). The number of ERα positive cells was significantly higher at 6–24 hr after PHx in female rats compared to male rats (Fig. 3C). ERα expression was

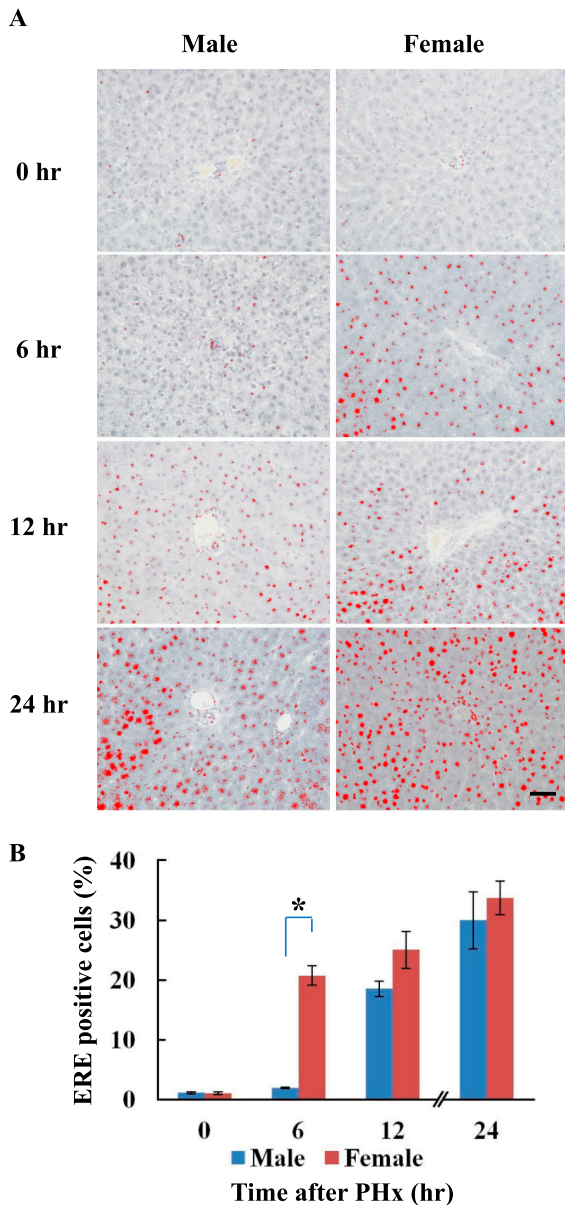


Fig. 4. Localization of ERE binding proteins in male and female rats after PHx. Paraffin-embedded liver sections were used for detection of ERE binding proteins by Southwestern histochemistry at various time-points after PHx. **A:** The localization of ERE binding proteins was detected from 12 hr after PHx in male rats, but was found from 6 hr in female rats. Positive staining was processed using a DAB-image analyzer. **B:** The number of ERE-positive cells in the liver sections of male and female rats at various time-points after PHx. *Blue* and *red* columns represent male and female, respectively. Asterisk indicates statistically significant difference ($*p < 0.05$). Magnification $\times 400$. Bar = 50 μm .

increased at 72–168 hr in male and female rats but significant differences were not found (data not shown). The amount of ER α protein in the regenerating liver was analyzed by western blot at various time-points (Fig. 3D) using mouse uterus as a positive control. In male rats, ER α protein was detectable at 0 hr, but not at 6–12 hr after PHx and gradually increased from 24–168 hr. In female rats, ER α

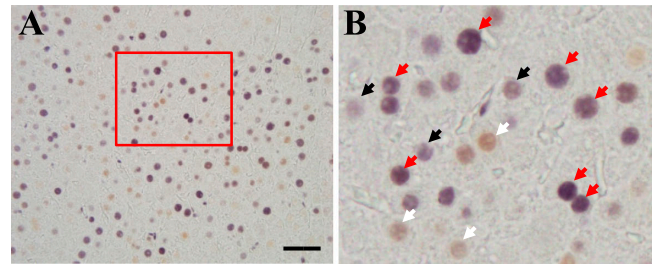


Fig. 5. Double staining for PCNA and ER α in male and female rat liver after PHx. The paraffin-embedded sections were analyzed by immunohistochemistry. **A:** ER α -positive cells were stained brown (DAB), whereas PCNA positive cells were stained purple-blue (4-Cl-1-Naphtol). Boxed area is enlarged in **B**. Arrows indicate white (ER α), black (PCNA) and red (double staining), respectively. Magnification $\times 400$. Scale Bar = 50 μm .

was continuously expressed at all time-points after PHx (Fig. 3D). Densitometry analysis revealed that the amount of the ER α protein was significantly higher at 6–24 hr after PHx in female rats compared to male rats (Fig. 3E).

Localization of ERE binding proteins in male and female rat liver after PHx

ERE binding proteins were detected from 12 hr after PHx in male rats, but were found from 6 hr in female rats. Positive staining was processed using a DAB-image analyzer (Fig. 4A). The activity of ERE binding proteins was significantly higher at 6 hr after PHx in female rats compared to male rats (Fig. 4B). Interestingly, the staining pattern and localization of ERE binding proteins by SWH were highly similar to the ER α expression in regenerating rat liver after PHx.

Proliferating cells express ERα in regenerating liver

To determine whether ER α affects liver cell proliferation, double staining was performed for ER α and PCNA using immunohistochemistry. The results revealed that ER α was co-expressed with PCNA during the liver regeneration especially at the peak time-points of liver regeneration after PHx (Fig. 5A). Arrows indicated black (PCNA), white (ER α) and red (double staining), respectively (Fig. 5B).

Estrogen induces and ICI 182,780 inhibits liver regeneration in rats after PHx

Male rats were treated with E₂ or E₂+ICI 182,780 on the day before PHx. PCNA positive cells were found in zones 1 and 2 at 12–24 hr after PHx and the number of positive cells reached a peak at 36 hr in E₂-treated male rats. On the other hand, in E₂+ICI 182,780 treated male rats, PCNA-positive cells were found in zone 1 at 24–36 hr and peaked at 48 hr after PHx (Fig. 6A, B). PCNA-LI indicated that the positive cell number was significantly higher at 36 hr in E₂-treated male rats compared to rats treated with E₂+ICI 182,780. These results indicated that the peak number of proliferating hepatocytes at S phase in E₂-treated male rats after PHx occurred 12 hr earlier than in male rats

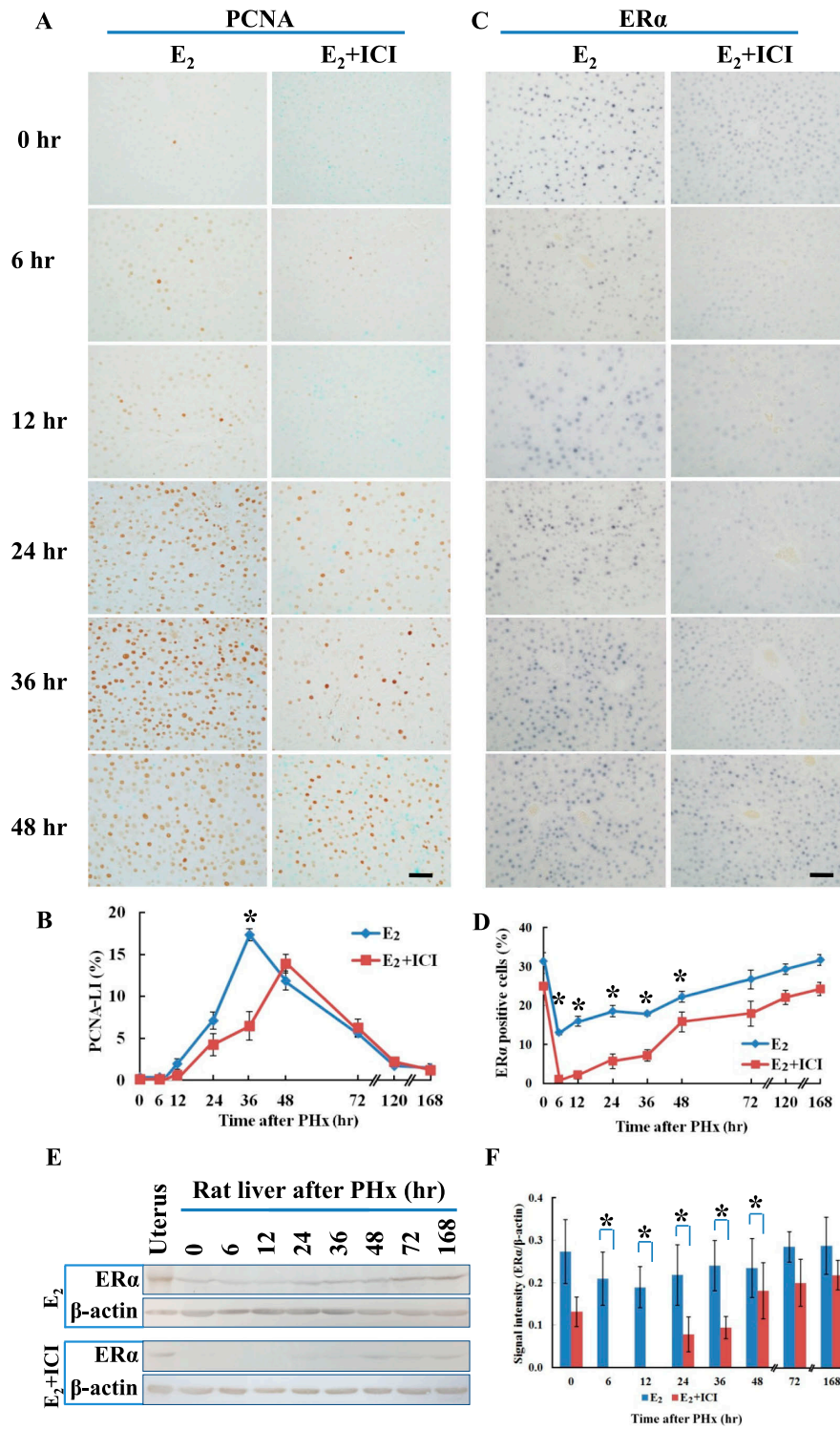


Fig. 6. PCNA and ERα expression in E₂ and E₂+ICI treated male rat liver after PHx. **A:** Immunohistochemical detection of PCNA in E₂ and E₂+ICI treated male rat liver after PHx. **B:** PCNA-LI in E₂ and E₂+ICI treated rats after PHx. The number of PCNA-positive hepatocytes was counted at each time-point after PHx. Blue and red lines represent E₂ or E₂+ICI treated male rats, respectively. Data represent the mean ± SE of three independent experiments. **C:** Immunohistochemical detection of ERα in E₂ and E₂+ICI treated rats at various time-points after PHx. Magnification ×400. Bar = 50 μm. **D:** The number of ERα-positive cells in the liver sections of E₂ and E₂+ICI treated rats at various time-points after PHx. Blue and red lines represent E₂ or E₂+ICI treated male rats, respectively. **E:** Western blot analysis of ERα in E₂ or E₂+ICI treated male rats after PHx. ERα (66 kDa) and β-actin (42 kDa). **F:** Densitometry analysis of western blot. Asterisks indicate statistically significant differences (**p* < 0.05). Data represent the mean ± SE of three independent experiments.

treated with E_2 +ICI 182,780 (Fig. 6B). ER α expression was found in zones 1 and 2 at 6–12 hr after PHx and the number of positive cells was increased in zone 3 from 24–168 hr in E_2 treated male rats. However, in E_2 +ICI 182,780 treated male rats, ER α expression was not detected at 6–12 hr, but was found at 24–168 hr after PHx (Fig. 6C). In E_2 -treated male rats, ER α -positive cells were significantly higher at 6–48 hr after PHx compared to rats treated with E_2 +ICI 182,780 (Fig. 6D). The results of immunohistochemistry were confirmed by western blot analysis. ER α protein was continuously expressed in E_2 -treated male rats at all time points after PHx (Fig. 6E). Densitometry analysis revealed that the amount of the ER α protein was significantly higher at 6–48 hr after PHx in E_2 -treated rats compared to E_2 +ICI 182,780 treated male rats (Fig. 6F).

Localization of ERE binding proteins in rats treated with E_2 or E_2 +ICI 182,780 after PHx

The localization of ERE binding proteins was detected from 12 hr after PHx in E_2 +ICI treated rats, but was found from 6 hr in rats treated with E_2 . Positive staining was processed by a DAB-image analyzer (Fig. 7A). The activity of ERE binding proteins was significantly higher at 6 hr after PHx in E_2 -treated male rats compared to rats treated with E_2 +ICI 182,780 (Fig. 7B). The staining pattern and localization of ERE binding proteins by SWH were highly similar to the ER α expression in regenerating rat liver after PHx.

IV. Discussion

In this study, we found that estrogen accelerates liver regeneration in rats after PHx. Our results indicate that the peak number of proliferating hepatocytes in S phase in female and E_2 -treated male rats occurred 12 hr earlier than in male rats after PHx. Although aging affects liver regeneration, the peak number of PCNA, Ki-67 and 5-bromo-2'-deoxyuridine positive cells was previously shown to occur at 48 hr in young (7–8 weeks) male rats and mice after 70% PHx [2, 25, 35]. Our data suggests that female and E_2 -treated male rat livers have a higher regenerative potential than livers from untreated male rats and that estrogen may have an essential role in liver regeneration after PHx.

In this study, ER α was the predominant ER type in rat liver and was found in zones 1 and 2 in male rats, and in all zones in female rats. Surprisingly, ER α expression was not detected at 6–12 hr after PHx in male rats but was observed at 24–168 hr. In female and E_2 -treated male rats, ER α expression was found in all zones during the liver regeneration after PHx. It has been reported that the plasma estradiol level was 2.5-fold higher in female compared to male mice [32]. Moreover, in male rats, estrogen was mainly produced in testicular Sertoli cells and was also converted from testosterone by aromatase. However, the serum testosterone level was reduced to 25–80%, but the estradiol level increased and the peak occurred in male rats at 3 days after

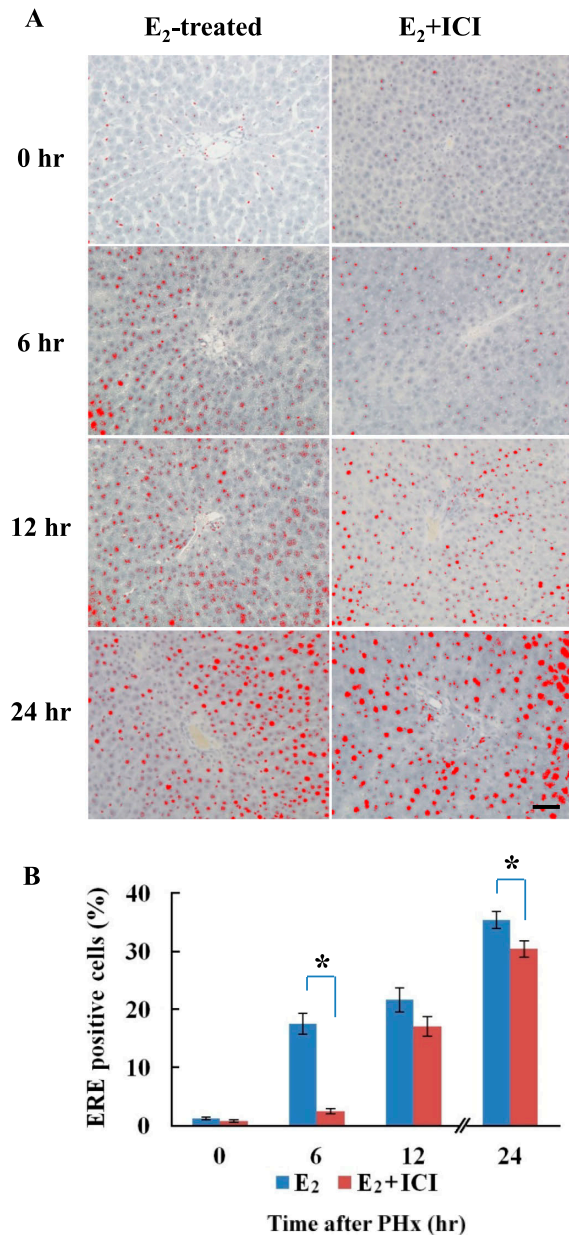


Fig. 7. Localization of ERE binding proteins in E_2 or E_2 +ICI treated male rats after PHx. **A:** The localization of ERE binding proteins was detected from 12 hr after PHx in E_2 +ICI treated rats, but was found from 6 hr in rats treated with E_2 . Positive staining was processed using a DAB-image analyzer. **B:** The number of ERE positive cells in the liver sections of E_2 and E_2 +ICI treated male rats at various time-points after PHx. Blue and red columns represent E_2 or E_2 +ICI treated male rats, respectively. Asterisks indicate statistically significant differences ($*p < 0.05$). Magnification $\times 400$. Bar = 50 μ m.

PHx. Similar changes in plasma estradiol and testosterone levels were observed in male patients after PHx [12, 22, 24]. Alteration of ER α expression in male rats after PHx indicates that conversion from testosterone to estradiol might be important in the initiation of the liver regenerative response in male rats after PHx. Our findings suggest that significantly different expression of ER α might affect

the hepatic regenerative response in male and female rats after PHx. Moreover, ER α expression was up-regulated by treatment with E₂ in male rats and was inhibited in rats treated with E₂+ICI 182,780 after PHx. Our findings suggest that E₂-treatment accelerates liver regeneration after PHx through ER α expression.

ER-ligand complexes bind to specific consensus sequences known as EREs which are located in various target gene promoters and stimulate gene transcription [16]. Our results demonstrated that the activity of ERE binding proteins was significantly higher at 6 hr after PHx in female and E₂-treated male rats compared to male and rats treated with E₂+ICI. Emmerson *et al.* [6] reported that ER α induces ERE-mediated signaling in skin regeneration in female mice. Thus, our results suggest that ER α may exert transcriptional activation in regenerating rat liver after PHx.

ER α and PCNA were co-expressed during liver regeneration, especially at peak time points of cell proliferation after PHx. It has been reported that the PCNA gene contains half-palindromic ERE sequences (TGACC) that can bind to ERs and regulate the transcriptional activity of various genes [21, 41]. Moreover, Schultz-Norton *et al.* [34] reported that PCNA interacts with ER α and enhances receptor-DNA interaction in a breast cancer cell line. Taken together, these findings suggest that estrogen might be involved in the initiation of DNA synthesis through the transcriptional activation of the PCNA gene, which harbors ERE in the promoter region.

Orphan nuclear receptors such as estrogen-related receptor alpha (ERR α), ERR β and ERR γ are also involved in estrogen signaling. Increased liver damage through impaired mitochondrial energy production has been associated with hepatocytes lacking ERR α [17]. Moreover, ERR γ is upregulated in HCC and its inhibition suppressed cancer cell proliferation [20]. Although the similar expression pattern of ERE binding proteins paralleled to ER α in regenerating rat liver, indicating that ER α may be the main receptor for estrogen pathway, it is also possible that orphan nuclear receptors may have the potential effects during liver regeneration.

In conclusion, we found that estrogen may play an important role in liver regeneration through ER α expression and that cell proliferation in male and female rats after PHx is differently affected. Taken together, these results suggest that estrogen treatment can induce liver regeneration after PHx.

V. Acknowledgments

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