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学　位　論　文　要　旨

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Estrogen accelerates cell proliferation through estrogen receptor α during rat liver regeneration after partial hepatectomy

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[要　旨]

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. 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.

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. 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. Similar sex-based differences are also observed in rodents. The survival rate in male mice after hepatic surgery is significantly improved by treatment with E_2. 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. Interestingly, most sex-based differences in the liver are diminished after menopause suggesting that female sex hormones, especially estrogen might have an important role in the differences.

Estrogen receptor alpha (ERα) and ERβ 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. ERα, but not ERβ, is expressed in hepatocytes, and involved in regulation of glucose and lipid metabolism in the liver. Although estrogen is implicated in the regulation of cell growth and differentiation in various organs, the exact mechanism in liver regeneration is not completely known.

Therefore, we investigated the effect of estrogen in liver regeneration by using 70% partial hepatectomy (PHx) rat model.

Material and methods

Eight-week-old male (240-260 g) and female (190-210 g) Wistar rats were used in the study. To evaluate the effect of estrogen, male rats were given a single intraperitoneal injection of E_2 at a dose of 9 μg/g bodyweight and ICI 182,780 at a dose of 2 μg/g bodyweight. E_2 and E_2+ICI 182,780 were dissolved in 500 μl of corn oil and injected on the day before PHx. 70% PHx was performed in male and female Wistar rats and remained liver was sampled after 6, 12, 24, 36, 48, 72, 120 and 168 h.

The liver tissues were fixed in 4% paraformaldehyde and embedded into paraffin for immunohistochemistry and Southwestern histochemistry (SWH), and some were snap frozen and used for western blot analysis. Cell proliferation activity was examined by proliferation cell nuclear antigen (PCNA) and the expression of ERα was examined by immunohistochemistry and western blotting. The localization of estrogen responsive elements (ERE) binding proteins was examined by SWH. Co-localization of ERα and PCNA was examined by double-staining.

For quantitative analysis, at least 2000 cells were counted at × 400 magnification in random fields, and the percentage of positive cells per total number of counted cells was represented by a labeling index (LI).

Results

PCNA positive cells were found in zones 1 and 2 at 24-36 h after PHx in male rats, but at 12-24 h in female rats. PCNA positive cells were increased in all zones at 48 h after PHx in male rats, but at 36 h in female rats. PCNA-LI indicates that the number of positive cells reached a peak at 48 h in male rats, while the peak in female rats occurred at 36 h after PHx.

In normal liver, ERα was expressed in zones 1, 2 in male rats, but in all zones in female rats. Interestingly, ERα was not detected at 6-12 h, but found at 24-168 h in male rats after PHx. However, ERα expression was found at all sampling time-points in female rats. Activity of ERE binding proteins was detected from 12 h in male rats but was found from 6 h in female after PHx. ERα was co-expressed with PCNA during the liver regeneration, especially at the peak time-points of liver regeneration after PHx.

Male rats were treated with E_2 or E_2+ICI 182,780 on the day before PHx. PCNA positive cells were found in zones 1 and 2 at 12-24 h after PHx and the number of positive cells reached a peak at 36 h in E_2-treated male rats. On the other hand, in

E_2+ICI 182,780 treated male rats, PCNA-positive cells were found in zone 1 at 24-36 h and peaked at 48 h after PHx. The expression of PCNA and ERα was up-regulated by E_2 treatment, but it was inhibited treatment with E_2+ICI 182,780 in male rats after PHx. In addition, The localization of ERE binding proteins was detected from 12 h after PHx in E_2+ICI treated rats, but was found from 6 h in rats treated with E_2.

Discussion and conclusion

The results indicate that the peak number of proliferating hepatocytes in S phase in female and E_2-treated male rats occurred 12 h earlier than in male rats after PHx. Our data suggests that female and E_2-treated male rat livers have a higher regenerative potential than livers from untreated male rats. Estrogen may have an essential role in liver regeneration after PHx.

ERα 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. ERα expression was not detected at 6-12 h after PHx in male rats but was observed at 24-168 h. In female and E_2-treated male rats, ERα expression was found in all zones during the liver regeneration after PHx. Our findings suggest that significantly different expression of ERα after PHx might affect the hepatic regenerative response in male and female rats after PHx. Moreover, ERα expression was up-regulated by treatment with E_2 in male rats and was inhibited in rats treated with E_2+ICI 182,780 after PHx. Therefore, it is suggested that E_2-treatment accelerates liver regeneration after PHx through ERα expression.

The activity of ERE binding proteins was significantly higher at 6 h after PHx in female and E_2-treated male rats compared to male and rats treated with E_2+ICI. Thus, our results suggest that ERα may exert transcriptional activation in regenerating rat liver after PHx. ERα and PCNA were co-expressed during liver regeneration, especially at peak time points of cell proliferation after PHx. PCNA gene contains half-palindromic ERE sequences (TGACC) that can bind to ERs and regulate the transcriptional activity of various genes. 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.

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

備考　論文要旨は、和文にあっては 2，000 字程度、英文にあっては 1，200 語程度とする。