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Review

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Sex disparity in the liver regeneration focusing on sex hormones

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Abstract

The liver is known as a sexually dimorphic organ because it has both androgen and estrogen receptors and responds to sex hormones. Specifically, the unique ability of the liver to regenerate is under the control of sex hormones. In human patients, liver recovery after resection occurs more quickly in women than in men. Accumulating evidence shows that change in the amount of sex hormones occurs quickly after partial hepatectomy (PHx) and impacts the expression of genes associated with liver regeneration. Increased estrogen promotes liver regeneration by regulating liver cell proliferation and energy metabolism, whereas estrogen depletion delays liver restoration. Implantation of estrogen in male mice with PHx improves liver regeneration. In addition, a few studies report that androgen is involved in enhancing liver regeneration, but its role in this process is not fully elucidated. This review briefly describes the change of estrogen and androgen during liver regeneration after PHx and discusses their feasible relevance to liver regeneration based on the results reported so far. Therefore, this review helps to improve our understanding of the sex-related physiological difference in liver restoration and develop a sex-specific therapeutic approach for liver regeneration.

Keywords: Sex difference, liver regeneration, estrogen, androgen, hepatocyte

INTRODUCTION

Sex disparity is a fundamental factor contributing to the physical, behavioral, and physiological differences between men and women[1]. Many physiological aspects of sex differences are derived from not only genetic difference, but also from the action of distinct sex hormones in men and women[2]. Sex-based gene expression is regulated by sex hormones in various tissues, including bone, adipose tissue, heart, muscle, and liver[3-6]. In addition, sex hormones are associated with the regulation of oncogenesis in several cancers, such as esophageal, gastric, pancreatic, colorectal, and liver cancer[7-9]. In particular, the liver is a highly dimorphic organ that accounts for more than 72% of sexually differentiated genes[10,11]. Sex hormones regulate many physiological processes such as metabolism, immune response, and cell proliferation in the liver[6,12]. Thus, gender differences affect liver homeostasis as well as the progression of liver diseases such as non-alcoholic fatty liver disease (NAFLD), liver fibrosis, and hepatocellular carcinoma (HCC)[13-15]. Epidemiological studies have shown that men are more susceptible to chronic liver disease compared with women of reproductive age[16,17]. For example, NAFLD prevalence is higher in men than in women (41% *vs.* 18%)[18]. HCC also predominantly affects men, with an incidence two to four times higher in men than women[19]. Liver transplantation affords the chance of a life-saving treatment for patients with chronic liver disease or liver cancer, based on the regenerative ability of the liver[20,21]. Sex-specific responses also occur in both the live liver donors and the transplant recipients during liver regeneration[22]. Following partial hepatectomy, women have faster liver regeneration and a higher survival rate than men[23-25]. In addition, the amount of estrogen in the serum increases, but androgen decreases after hepatectomy[26]. Given that estrogen promotes hepatocyte proliferation and impacts the survival rate in male mice, sex hormones could have a critical effect on liver regeneration[12,27]. In addition, a few studies have reported that androgen influences liver restoration after hepatic surgery[28,29]. Based on these findings, this review summarizes the hormonal changes and discusses the roles of sex hormones in physiological differences observed in the liver regeneration process.

Alteration of estrogen in serum level during liver regeneration

During liver regeneration, the levels of sex hormones change dramatically. It has been shown that estradiol level is elevated, whereas testosterone level is alleviated in serum of both humans and rodents after partial hepatectomy (PHx)[30]. Francavilla *et al.* reported that the serum level of estrogen in men who underwent 40%-60% PHx increased significantly 24 and 48 hours after PHx, while the serum level of testosterone decreased 96 hours after PHx, compared with men without PHx[12]. In male mice with PHx, serum estrogen level was enhanced rapidly 3 hours and peaked 24 hours after PHx[31]. Elevated estrogen was reported to regulate the cell cycle regulatory protein cyclin D1, whose expression was upregulated during liver regeneration[32]. Mullany *et al.* presented that cyclin D1 upregulated enzymes involved in the conversion of androgens to androstenedione and downregulated the enzymes involved in the conversion of estradiol to estrone, resulting in E2 accumulation in the liver[32]. With increased concentration of hepatic estrogen, translocation of estrogen receptors (ERs) from the cytoplasm to the nucleus in the hepatocytes impacts the expression of genes involved in initiating the regenerative response. These results indicate that elevation of hepatic estrogen is associated with enhanced liver regeneration.

Estrogen improves hepatic regeneration

Accumulating evidence shows that estrogen orchestrates liver regeneration [Figure 1]. Umeda *et al.* reported that ovariectomized female mice had dramatically reduced level of estrogen, less hepatocyte proliferation, and lower recovery of liver mass than sha m-operated female mice did after PHx[33]. It was reported that estrogen elevated hepatic expression of the miR-17-92 cluster targeting p21 and pTEN, cell cycle inhibitors, and suppression of the miR-17-92 cluster hindered liver regeneration in female mice after PHx[34]. In PHx-given male rodents, estrogen treatment is shown to promote liver regeneration. Estradiol administration increased proliferating cell nuclear antigen (PCNA)-positive proliferating cells in

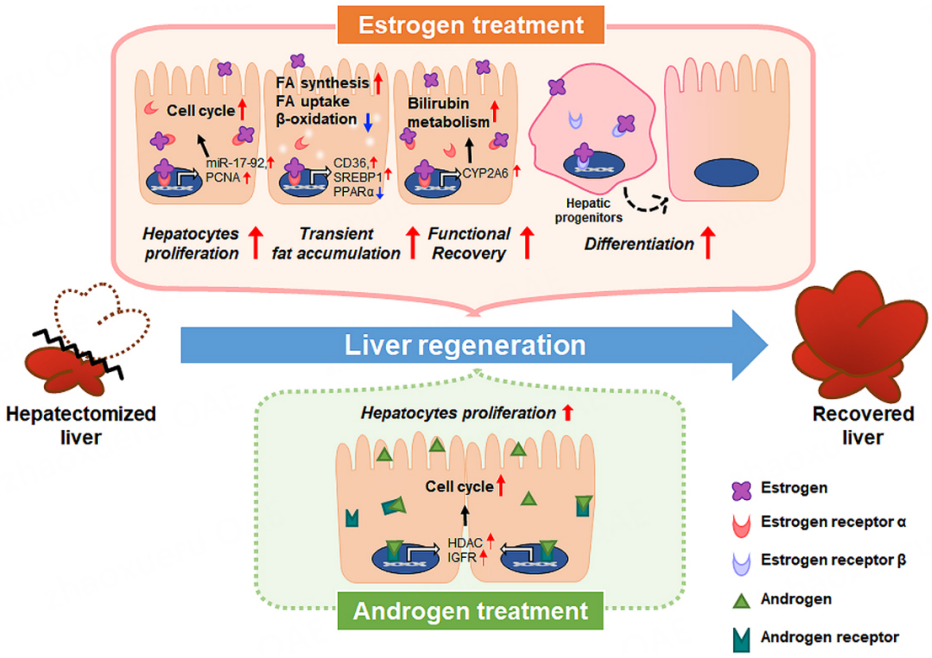

Figure 1. A schematic depicting of potential effect of estrogen and androgen on liver regeneration after partial hepatectomy.

hepatectomized male rats by upregulating ERα expression, a predominant subtype of ERs in hepatocytes[35]. Exogenous estradiol interacted with ERα and enhanced liver weight recovery and total DNA amounts in the liver, while ERα antagonist ICI182,700 blocked the regenerative effect of estrogen in PHx-receiving male mice[31]. Increased level of serum bilirubin caused by hepatocyte loss after PHx impeded liver regeneration[36]. However, estrogen bound to ERα directly induced the expression of bilirubin oxidase cytochrome P450 2A6, which reduced bilirubin levels by stimulating bilirubin oxidation in the liver[37]. The lowered amount of bilirubin attenuated toxicity to hepatocytes, contributing to their functional recovery. In addition, it was shown that increased estrogen after PHx upregulated ERα expression in $CD11c^+$ liver dendritic cells, and recruited them into the liver. And these cells induced local immunosuppression by upregulating anti-inflammatory IL-10 and downregulating pro-inflammatory IFN-γ, contributing to the enhanced proliferation of hepatocytes[38].

Estrogen also promotes energy metabolism supporting the massive energy supply needed to compensate for liver loss in hepatectomized rodent models. Estrogen supplementation increased the activities of glycolytic enzymes and improved the recovery of liver mass in ovariectomized female mice[39]. Srisowanna *et al.* reported that transient steatosis appeared more rapidly in female rats than in male rats after PHx[40]. They also revealed that estrogen treatment enhanced lipid accumulation in the liver of ovariectomized female rats by upregulating CD36 and sterol regulatory element-binding transcription factor 1 (SREBP1), which are involved in fatty acids (FAs) synthesis and FAs import into the liver, and downregulating PPARα, a key regulator for FAs oxidation, and induced the liver regeneration. However, many other studies have reported that estrogen alleviates de novo lipogenesis and lipid uptake and elevates β-oxidation, thereby preventing the progression of fatty liver disease[41,42]. Estrogen seems to have different effects on lipid metabolism in the liver depending on pathophysiological conditions. Hence, further detailed studies are needed to unveil the role of estrogen in hepatic lipid metabolism during liver regeneration.

ERβ is recently shown to be involved in modulating liver restoration, although its level is lower in hepatocytes compared with ERα[31,43]. Kao *et al.* described that delayed liver regeneration was common in both Erα-knockout (KO) and Erβ-KO mice, but the effect of the knockout on liver regeneration is mediated by distinct events during regenerative processes[44]. Bioinformatic analyses reported that the interaction of ERα with chromodomain helicase DNA-binding protein-1 facilitates cell growth and proliferation by upregulating cell cycle regulators such as cystatin 11 and crystallin gamma C, whereas ERβ stimulates hepatic differentiation by interaction with ubiquitin-protein ligase E3A, which is known to enhance differentiation of hepatic progenitor cells into hepatocytes. However, ERβ is not a predominant isotype expressed by hepatocytes, and in fact, many studies have demonstrated that ERβ is not expressed in hepatocytes. Therefore, before elucidating the role of ERβ in liver regeneration, it is necessary to first accurately identify its expression in hepatocytes.

Estrogen and androgen, representative sex hormones, have been reported to influence liver regeneration. Estrogen promotes hepatocyte proliferation by upregulating miR-17-92 clusters and PCNA through binding with estrogen receptor (ER) α. Estrogen-binding to ERα enhances accumulation of transient fat to provide the energy required for liver regeneration. Estrogen elevates fatty acids (FAs) genesis and FA uptake by increasing expression of differentiation cluster 36 (CD36) and sterol regulatory element-binding transcription factor 1 (SREBP1), whereas alleviating FA oxidation by decreasing expression of peroxisome growth factor-activated receptor α (PPARα). In addition, estrogen upregulates cytochrome P450 2A6 (CYP2A6), which lowers bilirubin levels, improving liver function that helps in liver restoration. Furthermore, estrogen stimulates the differentiation of hepatic progenitor cells into hepatocytes in interaction with another receptor, ERβ, and contributes to hepatocyte repopulation. Androgen seems to influence liver regeneration. Androgen upregulates histone deacetylase (HDAC) and insulin-like growth factor I receptor (IGF1R) and improves hepatocyte proliferation.

Androgen is potentially involved in liver restoration

Although many studies have reported that estrogen promotes liver regeneration, a few reports have shown that liver recovery is faster in male than in female mice, suggesting a role of androgen in liver regeneration [Figure 1]. It was found that male mice with PHx had higher levels of hepatic HDAC1, which inhibited B-myc, a suppressor of cell proliferation, and increased hepatocyte proliferation and liver regeneration compared with female mice receiving PHx[28]. Desbois-Mouthon *et al.* demonstrated that androgens regulated the expression of several genes in the liver, such as histone deacetylase (HDAC) and insulin-like growth factor I receptor (IGF1R)[29]. After PHx, male mice contained more Ki67-positive hepatocytes than female mice did, and liver-specific deletion of IGF1R impaired liver regeneration in male mice by inactivating IRS-1/ERK signaling, indicating that IGF1R promoted hepatocyte proliferation in male mice, but not in female mice. These two studies suggest that androgen stimulates liver regeneration. However, there is not much research on this topic, and the role of androgens in liver regeneration is still unclear. Therefore, more in-depth investigation is required to prove its function in liver regeneration.

CONCLUSION

A growing body of evidence has emphasized sex-specific pathophysiology in the liver[11]. During liver regeneration, rapid changes in sex hormones, in this case, estrogen and androgen, are accompanied by alterations in the expression of various genes relating to liver regeneration[31,32]. Female mice have faster liver restoration than male mice, and ovariectomy interrupts hepatocyte proliferation and liver recovery post PHx[33]. Administration of exogenous estrogen facilitates liver regeneration in male mice after PHx[31,35]. These findings support that estrogen has therapeutic potential to promote liver regeneration by impacting hepatocyte proliferation after hepatic surgery. However, supplementation of 17α-ethynyl estradiol (EE), a

synthetic estrogen widely used as an oral contraceptive, inhibited DNA synthesis in the livers of hepatectomized rats and delayed liver regeneration[45]. Long-term treatment for 60 days with EE blocked S phase entry of hepatocytes by downregulating cell cycle promoters, such as PCNA, cyclin A, E, and cdk2, and upregulating cell cycle inhibitors p53 and p21[46]. Furthermore, short-term treatment of EE for 5 days impaired bile acid biosynthesis and secretion via interacting with ERα, and disrupted the process of liver regeneration[47]. In addition, G protein-coupled estrogen receptor (GPER), which is a variant of ER and mediates non-genomic estrogen-related signaling, has been shown to increase hepatocyte proliferation and size of the liver in zebrafishes[48]. However, it also promoted the formation and progression of HCC in zebrafishes treated with 9,10-dimethyl-1,2-benzanthracene. Thus, estrogen is a double-edged sword in liver regeneration, although the positive aspects of estrogen in liver regeneration have been more highlighted. It is necessary to obtain sufficient evidence to define the potential role of estrogen in liver regeneration to use estrogen as a therapeutic agent for liver regeneration. The data on the effect of androgen on liver regeneration are limited. Furthermore, while in the past many studies have been conducted on sex-specific differences in liver regeneration, this topic has not been actively explored recently. Therefore, further in-depth studies on the sex-specific physiological differences in liver regeneration are needed and the findings obtained from these studies will help to develop and apply sex-specific clinical therapy.

DECLARATIONS

Authors' contributions

Literature review and drafting the manuscript: Lee C
Conception, review, drafting, and editing of the manuscript, and supervision: Jung Y

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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