Original Paper

Ghrelin Stimulates Hepatocyte Proliferation via Regulating Cell Cycle Through GSK3 β /B-Catenin Signaling Pathway

Qin Wang^a Ming Zheng^a Yue Yin^a Weizhen Zhang^{a,b}^aDepartment of Physiology and Pathophysiology, School of Basic Medical Sciences, Peking University, Beijing, China, ^bDepartment of Surgery, University of Michigan Medical Center, Ann Arbor, USA**Key Words**

Gut hormones • GHS-R1a • X/A like cells • Steatosis • PCNA

Abstract

Background /Aims: Obesity is associated with a reduction in ghrelin, a 28 aa gastric hormone. Whether reduced ghrelin contributes to the impaired proliferation of hepatocytes associated with obesity-related steatosis remains largely unknown. Here we examined the effects of ghrelin on the proliferation of hepatocytes derived from lean and obese mice. **Methods:** AML 12 cells or hepatocytes isolated from mice fed normal chow diet (NCD) or high fat diet (HFD) were used. Effects of ghrelin on hepatocyte proliferation were detected with CCK8 assay and EdU staining. Cell cycle was analyzed by flow cytometry. Levels of proliferation markers was examined by Western blot. **Results:** Growth hormone secretagogue receptor 1a (GHS-R1a) mRNA and protein were present in hepatocytes. Levels of GHS-R1a were increased upon ghrelin treatment. Ghrelin significantly increased hepatocyte proliferation measured by Cell Counting Kit-8(CCK8) assay and EdU staining in a dose- and time-dependent manner. Proportion of cells in S phase was markedly increased upon treatment with ghrelin. Ghrelin significantly increased levels of proliferating cell nuclear antigen (PCNA) and cyclin D1, while reducing p27 in hepatocytes from mice fed NCD or HFD. Deletion of GHS-R1a completely abolished the effects of ghrelin in cultured hepatocytes. Ghrelin stimulated the phosphorylation of glycogen synthase kinase 3 beta (GSK3 β), leading to subsequent increase of nuclear β -catenin in hepatocytes derived from lean and obese mice. This effect was dependent on the GHS-R1a. **Conclusion:** Ghrelin activates GHS-R1a to stimulate hepatocyte proliferation via GSK3/ β -catenin signaling pathway.

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Introduction

Liver has a remarkable capacity of regeneration, especially after surgical resection or chemical injury [1]. The capacity of liver regeneration is significantly decreased in the setting of steatosis which is commonly associated with obesity [2]. Relative to the lean subjects, obese patients with liver steatosis demonstrates a detrimental liver regeneration because of impaired proliferation of hepatocytes [3]. The reduction in self-repair of hepatocytes in the context of fatty liver occurs likely due to the growth arrest and inhibition of DNA damage-inducible protein GADD34 (GADD34, also known as PPP1R15A) [4]. The factors critical for the inhibition of hepatocyte proliferation in the setting of steatosis remain largely unknown, although it is a subject of increasing clinical relevance due to the global epidemic of obesity.

Ghrelin, a 28 aa peptide hormone produced by X/A like cells in the gastric fundus [5], is known as an endogenous ligand of the growth hormone secretagogue receptor 1a (GHS-R1a) [6]. Besides stimulating appetite, ghrelin plays a pivotal role in regulating the proliferation of a variety of cells including hippocampal neural stem cells [7, 8], intestinal epithelial cell [9], H9c2 cardiomyocytes [10], adipocytes [11] and murine T cells [12]. Further studies have suggested that ghrelin exercises its mitotic effect through promoting cell cycle progression [8]. Levels of circulating ghrelin are significantly reduced in obese rodents and human subjects [13]. This observation indicates that reduced circulating ghrelin may contribute to the decrease of self-repair of hepatocytes in the setting of obesity-related liver steatosis. Demonstrating that ghrelin can directly alter the hepatocyte proliferation is a crucial precondition for testing this hypothesis. For this purpose, we examined the mitotic effect of ghrelin on AML12 hepatocytes, a murine cell line, as well as primary hepatocytes isolated from mice fed with normal chow diet (NCD) or high fat diet (HFD). Using a loss-of-function genetic approach, we also determined whether GHS-R1a is required for the effects ghrelin on hepatocyte proliferation and glycogen synthase kinase-3 (GSK-3)/ β -catenin signaling.

Materials and Methods

Materials

The ghrelin peptide was purchased from Phoenix Pharmaceuticals, Inc. (Burlingame, CA). Mouse anti- β -actin, rabbit anti-PCNA, rabbit anti-cyclinD1, rabbit anti-p27, rabbit anti- β -catenin and rabbit anti-pGSK3 β (Ser9) were obtained from Cell Signaling Technology (Beverly, MA). Rabbit anti-GHS-R1a was purchased from Santa Cruze Inc. (Santa Cruze, CA).

Animals

Male wild type and GHS-R1a (-/-) mice littermates were used in the present study. Four-week-old wild type or GHS-R1a (-/-) mice were assigned to receive a normal chow diet or high-fat diet (60% fat, D12492; Research Diets, New Brunswick, NJ, USA) ad libitum for 12 weeks. Mice were housed in standard plastic rodent cages and maintained at a regulated environment (24°C, 12hr light and 12hr dark cycle with lights on at 7:30 AM and off at 7:30 PM). Animals were handled in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996). All experimental protocols were approved by the Animal Care and Use Committee of Peking University.

Cell culture

Isolation and culture of primary hepatocytes: Hepatocytes were isolated from mouse liver as previously described [14]. Briefly, male C57BL/6 mice were anesthetized with 1% Nembutal (7ml/g body weight) and injected intraperitoneally with 1000 IU heparin. The liver was perfused with 20ml pre-warmed 37°C DHANKS buffer, followed by 15-20ml of 3.3% collagenase IV (Sigma Aldrich, CO. St. Louis. MO) at a flow rate of 4ml/min. After perfusion, liver tissues were removed and washed with 15ml high glucose DMEM. Hepatocytes were centrifuged at 50g for 3min and washed thrice with DMEM medium to remove

tissue dissociation enzymes, damaged cells, and non-parenchymal cells. Hepatocytes were cultured in high glucose DMEM medium supplemented with 10% FBS and 1% penicillin/streptomycin at 37°C in a humidified atmosphere with 5% CO₂.

AML12 cells were cultured in humid atmosphere (5% CO₂) with high glucose DMEM medium supplemented with 10% FBS and 1% penicillin/streptomycin at 37°C.

Cell proliferation assay

Cell proliferation was measured by Cell Counting Kit-8 (CCK8, Dojindo, Japan) assay according to the manufacturer's instructions. Experiments for CCK8 were performed in 96-well plates. Cells were seeded at a density of 1×10^4 cells/mL. 20 μ L CCK-8 solution was added into each well (containing 200 μ L medium), and further cultured for 2 hr at 37°C. The absorbance of each group at 450 nm was detected (n=5) using an absorbance microplate reader. This absorbance is directly proportional to the number of living cells.

EdU staining

For EdU staining, cultured hepatocytes were planted in slides with a density of 5×10^3 cells/mL. Cells were exposed to ghrelin for 6 hr before treatment with EdU for 2 hr. Cells were then washed with 3% BSA three times, fixed with 4% paraformaldehyde for 10 min. After washing with 3% BSA three times, cells were permeabilized with 0.4% Triton X-100 for 15 min. Cells were then incubated with EdU staining cocktail kept from lights at room temperature for 30 min. After washing with 3% BSA, samples were then counterstained with 1x Hoechst 33342 for 10 min. Images were acquired by fluorescence microscope.

Flow cytometric analysis of cell cycle

Primary hepatocytes or AML12 cells were seeded in 6-well plates and grown in DMEM supplemented with 10% FBS. Cells were subjected to serum-starvation for 24 hr in order to synchronize the cell cycle. The culture medium was then replaced with fresh medium with saline control or ghrelin. After 6 hr, adherent cells were harvested and fixed at -20°C by 70% ethanol in phosphate-buffered saline (PBS) for 18 hr. Cell aliquots were then treated with 50 μ g/mL RNase A (Sigma; St Louis, MO) for 2 hr and stained with 50 μ g/mL propidium iodide. Percentages of cells in G₀/G₁, S and G₂/M phases were determined by flow cytometric analysis (Beckman Coulter Epics XL-MCL). The cell cycle was analyzed using software BD ModFit™ LT (B.D. Biosciences, San Diego, CA).

Nuclear Fraction Extraction

Primary hepatocytes cultured in a 6-well plate were washed twice with cold PBS and collected in 100 μ L of 1x hypotonic buffer containing 20 mM Tris-HCl of pH 7.4, 10 mM NaCl and 3 mM MgCl₂, followed by incubation on ice for 15 min. Five microliter detergent (10% NP40) was added and vortexed for 10 s, then centrifuged for 10 min at 300 rpm at 4°C. The supernatant contained the cytoplasmic fraction. The remained nuclear pellets were re-suspended in 50 μ L cell extraction buffer containing 100 mM Tris-HCl of pH 7.4, 2 mM Na₃VO₄, 100 mM NaCl, 1% Triton X-100, 1 mM EDTA, 10% glycerol, 1 mM EGTA, 0.1% SDS, 1 mM NaF, 0.5% deoxycholate and 2 mM Na₄P₂O₇, pre-supplemented with 1 mM PMSF and protease inhibitor cocktail. The nuclear pellets were then ultra-sonicated, followed by centrifugation for 30 min at 14,000g at 4°C. Supernatant was collected as the nuclear fraction.

Western Blot

Cells were homogenized with cell lysis buffer to obtain protein lysates. Proteins were separated on SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membrane. Membranes were incubated for 1 hr at room temperature with 5% fat-free milk in Tris-buffered saline containing Tween 20, followed by incubation overnight at 4°C with primary antibodies. The membranes were probed with the following antibodies: GHS-R1a, Cyclin D1, PCNA, P27, p-GSK3, β -catenin and β -actin.

Statistical analysis

Differences between groups were assessed by an unpaired two-sample t-test. Multiple comparisons between more than two groups were conducted by one-way ANOVA. Statistical analysis was performed by using Graph Pad Prism version 5.0. Data represents means $\pm$ standard error of the mean (SEM). P value < 0.05 was considered significant.

Results

Expression of GHS-R1a in hepatocytes

Ghrelin exerts its functions mainly through binding with its receptor: GHS-R1a. To determine whether GHS-R1a is expressed in hepatocytes, we analyzed the protein and mRNA levels of this receptor in primary hepatocytes and AML12 cell line. As shown in Fig. 1, GHS-R1a protein (A) and mRNA (B) were detected in primary hepatocytes (HPC) and AML12 at a level comparable to hypothalamus.

Ghrelin stimulates proliferation of hepatocytes

As shown in Fig. 2, ghrelin time- and dose-dependently promoted proliferation of cultured primary hepatocytes (A) and AML12 cells (B). The optimal concentration and time of ghrelin were 10^{-8} M and 3hr for primary hepatocytes, 5nM and 24hr for AML12 cells. The mitotic effect of ghrelin on primary hepatocytes (Fig. 2C) and AML12 cells (Fig. 2D) was further demonstrated by EdU staining.

To further determine the proliferative effects of ghrelin on primary hepatocytes, we examined levels of GHS-R1a, along with PCNA and P27. As shown in Fig. 3A, ghrelin stimulated the expression of GHS-R1a. This effect peaked at the dose of 10^{-8} M and 6hr of treatment. We then measured the protein levels of PCNA and P27 after treating cells with ghrelin for 6hr. Ghrelin dose-dependently upregulated PCNA, while downregulated P27 levels (Fig. 3B). Consistently, GHS-R1a deficiency significantly decreased the hepatocyte proliferation measured by CCK8 and EdU assays (Fig. 3C). Further, ghrelin demonstrated no effect on the proliferation of hepatocytes with GHS-R1a deficiency (Fig. 3C).

Ghrelin promotes cell cycle progression in hepatocytes

Primary hepatocytes and AML12 cells were used to analyse the effects of ghrelin on cell cycle progression. Cells were serum starved for 24hr to synchronize the cell cycle and subsequently stimulated with 10^{-8} M ghrelin for 6hr in primary hepatocyte or 24hr in AML12 cells. Ghrelin stimulation resulted in an increment in the percentage of cells in the S phase with concurrent decrease in the percentage of cells in G1 phase. The G2 phase remained largely unaltered (Fig. 4). This observation indicates that ghrelin shifts hepatocytes from G1 to S phase, thereby promoting synthesis of nascent DNA for subsequent cell division. This result is consistent with the observation that ghrelin has a pro-proliferative effect.

Fig. 1. Expression of GHS-R1a in HPC and AML 12 cells. Protein and mRNA were extracted from cultured hepatocytes isolated from C57BL/6 mice and AML12 cells. Hypothalamus was used as positive control. Results shown are representative of at least three individual experiments. (A) Expression of GHS-R1a protein in hepatocytes, AML12 cells and hypothalamus. β -actin was used as internal controls. Intensity of GHS-R1a was quantified, normalized to internal control and expressed as mean $\pm$ SEM. (B) Expression of GHS-R1a mRNA in hepatocytes, AML12 cells and hypothalamus. Results were normalized to β -actin and expressed as mean $\pm$ SEM.

Fig. 2. Effects of ghrelin on hepatocyte proliferation. Cell proliferation was assayed using the CCK-8 proliferation assay (A,B) and EdU staining (C,D) in cultured primary hepatocytes (A,C) and AML12 cells (B,D). To determine the time-dependent effect, cells were treated with 10^{-8} M ghrelin for 3, 6, 12, 24hr. To investigate the dose-dependent effect, cells were treated with different concentration of ghrelin for 24hr. The absorbance value of 450nm was measured and expressed as mean $\pm$ SEM. Experiments were repeated for at least three times.*denotes $P < 0.05$ vs. control. Representative EdU staining images of primary hepatocytes treated with 10^{-8} M ghrelin for 6hr (C) or AML12 cells exposed to 10^{-8} M ghrelin for 24hr (D) were shown.

Fig. 3. Effects of ghrelin on the expression of proliferation regulators. (A) Protein levels of GHS-R1a were detected after treating cells with different concentrations of ghrelin for 6hr or 10^{-8} M ghrelin for 3, 6, and 12hr. (B) Protein levels of PCNA and P27 were measured 6hr after treatment of cultured primary hepatocytes with different concentrations of ghrelin. Results were expressed as mean $\pm$ SEM. Experiments were repeated for at least three times. * denotes $P < 0.05$ vs. control. (C) Effects of GHS-R1a deficiency on proliferation. Cell proliferation was assayed using the CCK-8 proliferation assay (Left panel) and EdU staining (Right panel). Primary hepatocytes from WT or GHS-R1a $^{-/-}$ mice were treated with 10^{-8} M ghrelin or vehicle for 6hr. The absorbance value of 450nm was measured and expressed as mean $\pm$ SEM. EdU was added into cultured cells for 2hr. Experiments were repeated for at least three times. *denotes $P < 0.05$ vs. wild type (WT) without ghrelin.

Identical effect of ghrelin-GHS-R1a signaling on PCNA, Cyclin D1 and p27 in hepatocytes from lean and obese mice

To determine whether steatosis affects hepatocyte proliferation in response to ghrelin, we compared the effects of ghrelin on PCNA, cyclin D1 and p27 in cultured primary hepatocytes isolated from lean and obese mice. Ghrelin demonstrated an identical effect on the up-regulation of PCNA and cyclinD1, as well as down-regulation of P27 in hepatocytes isolated from NCD (Fig. 5A) and HFD (Fig. 5B) mice. No significant difference was observed for the effect of ghrelin between these two groups.

Fig. 4. Ghrelin promotes cell cycle progression. Cells were grown in 6-well plates. After serum starvation for 24h to synchronize the cell cycle, cells were subsequently stimulated with 10^{-8} M ghrelin for 6hr in primary hepatocytes (A) or 24h in AML12 cells (B). The cells were harvested and stained with propidium iodide for cell cycle analysis by flow cytometry. Experiments were repeated for three times.

Fig. 5. Effects of ghrelin on PCNA, cyclin D1 and p27 in hepatocytes from lean and obese mice. Male C57B6LJ mice were fed with NCD or 60% HFD for 12 weeks. Hepatocytes were isolated and treated with ghrelin (10^{-8} M) for 6hr. Protein levels of PCNA, cyclin D1 and P27 were detected in hepatocytes isolated from NCD (A) or HFD (B) mice. The upper panel is the representative Western blot, whereas the lower panel shows mean $\pm$ SEM of signal intensity. Nine samples were examined for each condition. * indicates $P < 0.05$ vs. control without ghrelin treatment.

Fig. 6. Effects of GHS-R1a deletion on PCNA, cyclinD1 and p27 in hepatocytes from lean and obese mice. (A). Levels of GHS-R1a in hepatocytes from mice fed NCD or HFD. Proteins were extracted from cultured hepatocytes isolated from C57BL/6 mice fed NCD or 60% HFD for 12 weeks. Shown is representative Western blot of GHS-R1a. β-actin was used as internal controls. Intensity of GHS-R1a was quantified, normalized to internal control and expressed as mean±SEM. * denotes $P < 0.05$. (B) Validation of absence of GHS-R1a in liver by Western blotting. (C) Effects of endogenous ghrelin. Hepatocytes were isolated from male GHS-R1a (-/-) or wild type littermates fed with NCD or 60% HFD for 12 weeks. Protein levels of PCNA, cyclin D1 and P27 were detected with Western blot. The upper panel shows the representative Western blot and the lower pane is quantitative result of signal intensity normalized to internal control β-actin. Nine samples were examined for each condition. Results were expressed as mean±SEM. *, $P < 0.05$ vs. wild type mice fed with NCD or HFD.

Interestingly, hepatocytes isolated from steatotic liver demonstrated a significant increase of GHS-R1a expression levels (Fig. 6A). We thus examined the effect of endogenous ghrelin using GHS-R1a null mice. Absence of GHS-R1a protein was validated by Western blotting in Fig. 6B. Deletion of GHS-R1a resulted in a significant decrease in PCNA, cyclin D1 with concurrent increase in P27. Hepatocytes from NCD and HFD (Fig. 6C) showed an identical response to the absence of GHS-R1a. These results suggest that endogenous ghrelin regulates the proliferation and survival of hepatocytes in a degree identical between lean and obese mice.

Fig. 7. Effects of ghrelin on GSK3β and β-catenin in hepatocytes from lean and obese mice. (A) Effects of exogenous ghrelin. Hepatocytes isolated from C57B6LJ fed NCD or 60% HFD were treated with exogenous ghrelin (10^{-8} M) for 6 hr. (B) Effects of endogenous ghrelin. Hepatocytes were isolated from male GHS-R1a (-/-) and wild type littermates fed with NCD or 60% HFD for 12 weeks. Protein levels of p-GSK3β(Ser9) or nuclear β-catenin were examined by Western blot with the signal intensity quantified and normalized to β-actin or lamin B1. Results were expressed as mean ± SEM. * denotes $P < 0.05$ vs. control or wild type mice fed with NCD. # indicates $P < 0.05$ vs. control or wild type mice fed with HFD. Eight samples were examined for each condition.

Effects of ghrelin on GSK3β/β-catenin

To determine whether ghrelin promotes hepatocyte proliferation by β-catenin signaling pathway, we examined the effects of ghrelin on levels of p-GSK3β(Ser9) and nuclear β-catenin in hepatocytes from both lean and obese mice. As shown in Fig. 7A, exogenous ghrelin markedly stimulated phosphorylation of GSK3β, leading to subsequent increase in nuclear β-catenin. This effect was comparable between hepatocytes from mice fed NCD or HFD. On the other hand, absence of GHS-R1a significantly reduced levels of phosphorylated GSK3β(Ser9) and nuclear β-catenin in hepatocytes derived from both lean and obese mice (Fig. 7B).

Discussion

Ghrelin is a hormone secreted mainly by X/A like cells located in the acid gland of gastric fundus [15]. The molecular structure of ghrelin is unique because its third amino acid serine is octanoylated by a membrane bound enzyme named ghrelin O-acyltransferase. This octanoylation is required for the binding of ghrelin to its receptor, GHS-R1a [16]. Activation of GHS-R1a accounts for the physiological functions of ghrelin ranging from release of endocrine hormones such as growth hormone, adrenal hormone and corticosteroids

[17], initiation of ingestion [18], increase of body weight, regulation of glucose and lipid metabolism, to promotion of arousal [19]. It has been long recognized that ghrelin exercises these physiological functions through a central nervous system mechanism involving the hypothalamus. For example, the orexigenic effect of ghrelin occurs through its activation of GHS-R1a in the hypothalamic neurons [20]. Consistently, high level of GHS-R1a is expressed in hypothalamic nuclei, pituitary gland and the spinal cord [21]. Emerging evidences also indicate the presence of GHS-R1a in the peripheral tissues such as enteric nervous system [22], pancreatic islets [23], adipose tissue [24] and skeletal muscle [25]. The expression of GHS-R1a in peripheral tissues indicates a direct peripheral mechanism for the physiological functions of circulating ghrelin. In pancreatic islets, ghrelin inhibits glucose-stimulated insulin secretion by activation of cAMP/TRPM2 signaling [26]. This effect is absent in the GHS-R1a null mice, suggesting a mechanism dependent on GHS-R1a. In skeletal muscle, both pharmacological or genetic antagonism of GHS-R1a completely abolishes the suppression of GLUT4 expression and membrane translocation induced by ghrelin [25]. For adipose tissue, ghrelin has been demonstrated to alter the adipogenesis or lipolysis of adipocytes through GHS-R1a dependent or independent mechanism [27]. Our study extends the peripheral location of GHS-R1a to hepatocytes. Both GHS-R1a mRNA and protein are present in isolated hepatocytes, whereas GHS-R1a null mice demonstrate no detectable signal. GHS-R1a expression levels increase significantly in hepatocytes isolated from steatotic liver as well as upon exposure to ghrelin, indicating the plasticity nature of GHS-R1a. Consistent with this observation, our previous study demonstrates the cytoplasm membrane binding of rhodamine-labeled ghrelin in hepatocytes stained positively for albumin [28]. Further, incubation of hepatocytes with rhodamine-ghrelin demonstrates rapid receptor internalization typical of G protein coupled receptor [28].

Our study also demonstrates that GHS-R1a in hepatocytes is functionally active. This concept is supported by following observations: (1) Ghrelin directly acts on cultured hepatocytes to stimulate cell proliferation measured by CCK-8 and EdU staining assays. (2) Upon exposure to ghrelin, hepatocytes demonstrate an increase in cell cycle progression with shifting cells from G1 to S phase. This alteration is associated with an up-regulation of cyclin D1, a key inducer of transition from the G1 to S phase of cell cycle. (3) Ghrelin significantly increases levels of PCNA in hepatocytes, while decreasing expression of p27 which functions to trigger cell cycle exit. On the other hand, deletion of GHS-R1a decreases PCNA with concurrent increase of p27 in isolated hepatocytes, suggesting that endogenous ghrelin is critical for the hepatocyte proliferation. (4) Deletion of GHS-R1a blocks the stimulatory effect of ghrelin on hepatocyte proliferation. Consistent with these observations, our previous experiments demonstrate a direct physiological function for ghrelin on the lipogenesis in hepatocytes [28]. In addition, exogenous ghrelin has been reported to attenuate hepatocyte apoptosis induced by lipopolysaccharide (LPS) or palmitic acid (PA) [29]. Taken together, a series of evidence has supported the presence of functionally active GHS-R1a in hepatocytes. However, it is noteworthy that ghrelin has been recognized to stimulate food intake and hepatic lipid accumulation by a mechanism involving the hypothalamus [28]. Further studies should thus aim to identify how the direct peripheral action of ghrelin integrates with the central pathway to modulate self-repair and lipid metabolism in hepatocytes.

The finding that ghrelin stimulates hepatocyte proliferation has physiological relevance to liver regeneration. Liver regeneration initiated by hepatocyte proliferation or stem cells occurs after injury or resection. The process of liver regeneration is precisely controlled by a group of endocrine, paracrine/autocrine growth factors such as hepatocyte growth factor and receptor c-Met, as well as ligands of the EGFR (EGF, TGF, Heparin Binding-EGF and Amphiregulin) [30]. Our previous study has identified circulating ghrelin as a potent stimulator of hepatocyte protection after ischemia/reperfusion injury [31]. Consistently, our present observation further supports the concept that ghrelin may promote liver regeneration by stimulating hepatocyte proliferation. It has been reported that inhibition of GSK-3 β promotes, while β -catenin overexpression increases the proliferation of progenitor cells [32]. In liver, β -catenin signaling cascade is mostly quiescent in hepatocytes except in the

centrizonal region of a hepatic lobule. This small rim of hepatocytes around the central vein shows constitutive β -catenin activation [33]. Our data suggests that ghrelin facilitates GSK-3 β phosphorylation which leads to nuclear translocation of β -catenin. We thus propose that circulating ghrelin may directly activate β -catenin signaling in hepatocytes located in the centrizonal region to promote cell proliferation. Ghrelin thus is an important mitotic signal from gut, whose activity is linked to the liver self-repair. Importantly, we observe no difference in the alteration of PCNA, cyclin D1 and p27 in response to both endogenous and exogenous ghrelin between normal and steatotic hepatocytes. The significance of this finding is that ghrelin provides a potential strategy for the improvement of self-repair following injury or resection of liver in the setting of steatosis.

Conclusion

In conclusion, our study demonstrates that ghrelin could stimulate hepatocyte proliferation via promoting cell cycle progression through the GSK3 β / β -catenin signaling pathway (Fig. 8). Ghrelin may be beneficial for liver regeneration in both lean and obese subjects.

Acknowledgements

This research was supported by grants from the National Natural Science Foundation of China (81700516, 81730020, 81330010) and the National Key R&D Program of China (2017YFC0908900).

Disclosure Statement

The authors declare that no conflict of interests exists.

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Fig. 8. Mechanism by which ghrelin stimulates hepatocyte proliferation.

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