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Upregulation of hepatic melanocortin 4 receptor during rat liver regeneration

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ABSTRACT

Background: Melanocortin 4 receptor (MC4R) is predominantly recognized to mediate energy metabolism and anti-inflammation through the central nervous system. However, the expression of MC4R has recently been identified in rat liver and was shown to be upregulated during acute phase response. This study aims to investigate potential roles of MC4R in liver regeneration.

Materials and methods: Rat partial hepatectomy (PH) was performed, and MC4R expression was analyzed at different time points after resection. Sham-operated animals (SH) served as controls. *In vitro* primary hepatocytes (HCs) were isolated from normal rat liver and stimulated with α-melanocyte–stimulating hormone (MC4R agonist). Real-time polymerase chain reaction, Western blot, and immunofluorescence staining were applied to detect gene expression.

Results: Up to 8 h after PH, hepatic messenger RNA of proinflammatory cytokines interleukin 6 and tumor necrosis factor α reached peak values. Between 8 and 72 h after PH, rat liver regeneration was extremely active as assessed by the regeneration indices labeled by Ki-67. Immunofluorescence staining indicated that MC4R was mostly expressed in hepatocyte nuclear factor 4^+ cells (HCs) and upregulated during rat liver regeneration. Concurrently, the expression of hepatic MC4R protein was significantly higher in PH than in SH animals, and phosphorylated extracellular signal–regulated kinase 1/2 was remarkably increased in PH compared with SH animals ($P < 0.05$, respectively). *In vitro* experiments showed that the expression of proliferating cell nuclear antigen was significantly higher in HCs treated with α-melanocyte–stimulating hormone than in control HCs, which was correlated to the increase of phosphorylated extracellular signal–regulated kinase 1/2 and reduction of phosphorylated signal transducer and activator of transcription 3 ($P < 0.05$, respectively).

Conclusions: MC4R is predominantly expressed in HCs and upregulated during rat liver regeneration. *In vitro* stimulation of HC MC4R is associated with a modulation of extracellular signal–regulated kinase and signal transducer and activator of transcription 3 pathways regulating liver regeneration.

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Introduction

Melanocortin receptor 4 (MC4R), a G-protein–coupled receptor, plays a pivotal role in the regulation of energy homeostasis and anti-inflammation [1]. MC4R is mechanistically regulated by α-melanocyte-stimulating hormone (α-MSH, MC4R agonist) and agouti-related peptide (AgRP, MC4R inverse agonist), both of which are derived from proopiomelanocortin [1]. MC4R is conventionally considered to be expressed in the central nervous system and exerts its biological effects to peripheral organs [2]. However, recent studies identified the expression of MC4R in liver and adipose tissues as well [3,4].

Genes involved in direct energy generating processes, such as oxidative phosphorylation, electron transport, and adenosine triphosphate synthesis, were found upregulated in both liver and adipose tissue of α-MSH-treated pigs homozygously expressing missense mutations in MC4R [4]. Interestingly, in mice, central administration of a MC4R antagonist (SHU9119) or AgRP robustly increased feeding behavior, indicating that antagonism of MC4R is an important orexigenic signal [5]. Knockout of MC4R and proopiomelanocortin [6] or overexpression of AgRP [7] led to the same obese phenotype in mice. A similar genetic pattern was also observed in humans with mutations in MC4R [8]. These reports highlighted the crucial effects of MC4R in energy metabolism.

It was reported that α-MSH prevented lipopolysaccharide-induced hepatic inflammation by inhibiting production of chemokines, which then modulated the infiltration of inflammatory cells [9]. Moreover, activation of MC4R attenuated cerebral, myocardial, testicular and renal inflammation and ischemia–reperfusion injury through regulating extracellular signal–related kinase (ERK) 1/2, tumor necrosis factor α (TNF-α) and so forth, consequently trigging repair pathway [10–13]. Interestingly, gene expression of all MCR subtypes was recently discovered in rat liver, and the level of MC4R was most dramatically increased during acute phase response [3]. However, the precise mechanism of how MC4R exerts anti-inflammatory effects and interacts with other biochemical processes is not well defined yet.

Liver regeneration after partial hepatectomy (PH) is mostly dependent on the replication of hepatocytes (HCs), which are completely differentiated and normally quiescent cells, and do not rely on the activation of a compartment of hepatic stem cells [14]. Tremendous genes are involved in liver regeneration, and the essential circuitry required for the process could be categorized into three networks: metabolic (e.g., Heparin-binding epidermal growth factor and amphiregulin), cytokines (e.g., interleukin 6 and TNF-α), and growth factors (e.g., hepatocyte growth factor, and epidermal growth factor) [15].

Based on the best of present knowledge, MC4R is involved in metabolic and inflammatory processes, so we hypothesized that MC4R could play an important role in the proliferation of liver cells after PH. The present study investigates the MC4R expression pattern in regenerating rat liver after 2/3 PH and its interaction with other signaling pathways being involved in regulation of liver regeneration.

Materials and methods

Chemical reagents

The following materials were used in this study: Moloney murine leukemia virus reverse transcriptase, Promega, Mannheim, Germany; SYBRGreen master mix and stepOne software, Applied Biosystems, Darmstadt, Germany; Complete Protease Inhibitor Cocktail Tablets, Roche, Mannheim, Germany; Hybond enhanced chemiluminescent (ECL) nitrocellulose membranes; Amersham Biosciences, Buckinghamshire, UK. ECL solutions A and B Western blotting protocol, GE Healthcare (Braunschweig, Germany); Film processor machine, Konica SRX-101A, medical film processor; 4, 6-diamidino-2-phenylindole was from Molecular Probes Europe BV, Leiden, The Netherlands; Goat and rabbit serum, Dako, Glostrup, Denmark, and α-MSH, Tocris Bioscience, MO.

Animals and experimental design

Male Wistar rats, purchased from Harlan-Winkelmann, Germany (body weight 180–200 g), were used for the experiments and were housed at a room temperature of 22°C–24°C and a relative humidity of ~65% with a 12-h light–dark cycle for 1 wk for allowance of acclimatization before the start of the experiments. The animals were kept on standard laboratory chow until 12 h before surgery and fresh tap water *ad libitum* till the beginning of the experiments. All experiments were conducted in accordance with the German legislation on protection of animals and the NIH Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council) and were approved by the local governmental ethical committee.

2/3 PH or sham (SH) operation was performed under ether anesthesia by midventral laparotomy, ligation of the median anterior and left lateral hepatic lobes separately with a silk suture, and complete excision of ligated lobes. SH operations consisted of a midventral laparotomy of similar extent, gentle manipulation of the liver, followed by surgical closure of the abdominal wall similar to PH-operated rats.

Rats were sacrificed at 0, 2, 4, 8, 16, 24, 48, 72 h and 1 wk after PH or SH (three rats per time point). Livers were snap frozen in liquid nitrogen and stored at −80°C until use.

Isolation of HCs and treatment with α-MSH

Rat primary HCs were isolated from healthy, non-hepatectomized rats according to the method previously described [16]. The isolated cells were exposed to 10 μg/mL α-MSH in M199 culture medium with 1.5% antibiotics. HCs were then harvested in all *in vitro* experiments at 1, 6, and 24 h after treatment for protein study. Saline-treated cultured HCs served as negative controls at all studied time points. The entire experiment of HCs isolation and Western blots were independently repeated three times.

Isolation of total RNA and real-time polymerase chain reaction

Total RNA was isolated from rat livers and converted into complementary DNA by reverse transcriptase for real-time