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Sex- and age-dependent effects of Gpr30 genetic deletion on the metabolic and cardiovascular profiles of diet-induced obese mice

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ABSTRACT

The G protein-coupled receptor 30 (GPR30) has been claimed as an estrogen receptor. However, the literature reports controversial findings and the physiological function of GPR30 is not fully understood yet. Consistent with studies assigning a role of GPR30 in the cardiovascular and metabolic systems, GPR30 expression has been reported in small arterial vessels, pancreas and chief gastric cells of the stomach. Therefore, we hypothesized a role of GPR30 in the onset and progression of cardiovascular and metabolic diseases. In order to test our hypothesis, we investigated the effects of a high-fat diet on the metabolic and cardiovascular profiles of Gpr30-deficient mice (GPR30-lacZ mice). We found that GPR30-lacZ female, rather than male, mice had significant lower levels of HDL along with an increase in fat liver accumulation as compared to control mice. However, two indicators of cardiac performance assessed by echocardiography, ejection fraction and fractional shortening were both decreased in an age-dependent manner only in Gpr30-lacZ male mice. Collectively our results point to a potential role of Gpr30 in preserving lipid metabolism and cardiac function in a sex- and age-dependent fashion.

1. Introduction

In the late 1990s the G protein-coupled receptor 30 (GPR30) was independently cloned by four different groups (Carmeci et al., 1997; O'Dowd et al., 1998; Owman et al., 1996; Takada et al., 1997). In a gene expression analysis of breast cancer SkBr3 cells, estrogen was proposed to function as a GPR30 ligand, leading to the activation of the Erk-1/-2 kinases via epidermal growth factor receptor (EGFR) (Filardo, 2002; Filardo et al., 2000, 2002, 2007). Revankar et al. (2005) used estrogen fluorescent derivatives to show that GPR30 was localized in the endoplasmic reticulum. However, both GPR30 intracellular and plasma membrane localizations have been reported (Filardo et al., 2007; Funakoshi et al., 2006; Matsuda et al., 2008; Otto et al., 2008; Sanden et al., 2011; Tica et al., 2011). Indeed, the G1 and G15 compounds have been identified as GPR30-agonist and antagonist respectively, and G1 has been reported to specifically reproduce estrogen effects in several cases (Bologa et al., 2006; Dennis et al., 2009). Upon estrogen stimulation, Gpr30 signaling has been associated with adenylyl cyclase, ERK and phosphatidylinositol 3-kinase (PI3K) activation as well as intracellular calcium mobilization (Prossnitz and Barton, 2009). Gpr30 is widely recognized as a new estrogen receptor (GPR30 was renamed into GPER1: G protein-coupled estrogen receptor 1), however the literature reports controversial results concerning the effective nature of GPR30 ligand and its signaling capabilities (Levin, 2009; Otto et al., 2008, 2009; Razandi et al., 2003). In order to elucidate the physiological function of GPR30 four different Gpr30 knockout mouse models have been generated by various groups (Isensee et al., 2009; Martensson et al., 2009; Otto et al., 2009; Wang et al., 2008). So far, the approaches have been disparate and often the results are conflicting (Langer et al., submitted for publication; Mizukami, 2010). However, an involvement of GPR30 in the regulation of vascular tone, blood pressure and cardiac function seems to emerge from several studies (Haas et al., 2009; Jessup et al., 2010; Takada et al., 1997) as well as a role of GPR30 in metabolism regulation (Ford et al., 2011; Haas et al., 2009; Martensson et al., 2009). Consistently, through analyzing a mutant

Abbreviations: AoVel, aortic blood velocity; ALAT, alanine aminotransferase; ALP, alkaline phosphatase; ASAT, aspartate aminotransferase; EF, ejection fraction; FS, fractional shortening; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein-cholesterol; LDL, low-density lipoprotein-cholesterol; TGs, triglycerides; wt, wild-type.

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mouse model that harbors a β-galactosidase (lacZ) reporter (Gpr30-lacZ) within the Gpr30 locus leading to a partial deletion of the Gpr30 coding sequence, Isensee and co-workers found Gpr30 mainly expressed in the microvasculature endothelium (a sensitive target of pathologies connected to diabetes). Additionally, a high Gpr30 expression was reported in the pancreas and stomach (Isensee et al., 2009). Therefore, we hypothesized a role for GPR30 in the onset and progression of cardiovascular and metabolic diseases. We evaluated the effects of the genetic deletion of Gpr30 in a diet-induced obesity (DIO) mouse model, through the assessment of metabolic and cardiovascular parameters for which blood chemistry, histological and hemodynamic studies (i.e. echocardiography) were performed and combined in a longitudinal study. Our results provide evidence of a sex- and age-dependent role of GPR30 in lipid metabolism and cardiac function.

2. Materials and methods

2.1. Animals

The Gpr30 KO mouse model (Gpr30-lacZ) used in the present study was the same as described by Isensee et al. (2009). Briefly, Gpr30-LacZ mice were generated by Deltagen (San Carlos, USA) through a homologous recombination approach in 129/Sv ES cells. A LacZ-Neomycin cassette was used to disrupt the open reading frame (ORF) in exon 3 of the Gpr30 gene. The insertion deleted 349 bp of the ORF encoding the first two transmembrane domains and the first intracellular loop. Male chimeras were mated to C57BL/6 mice and F1 heterozygous males backcrossed to C57BL/6 for six generations.

Experiments were performed using 6 month old male and female homozygous mutant knockout mice, as well as sex- and age-matched control littermates. Briefly, animals were fed with the following diet for 20 weeks: high-fat diet (HFD): rodent purified diet, w/ 60% energy from fat, 60 kcal percent fat, blue; control diet (CD): rodent purified diet, w/ 10% energy from fat, 10 kcal percent fat, yellow (BCMIPS, London, UK). Experiments in baseline were performed on 6 month old mice. The feeding protocol was started when mice were 7 months old. Other experiments were performed after 4 (age: 8 months) and 20 weeks (age: 12 months) of HFD. All animal procedures were performed in compliance with the German animal welfare law and previous authorization of the "Landesamt für Arbeitsschutz, Gesundheitsschutz und technische Sicherheit Berlin (LAGetSi)" (District Government of Berlin).

2.2. Blood chemistry

Mouse blood samples were obtained using a retro-orbital puncture under isoflurane anesthesia (Isoflurane, Abbott, Wiesbaden, Germany). Blood was drained using a sterile and heparinized end-to-end capillary in sterile tubes provided with Li-Heparin. Samples were immediately stored at 4 °C and subsequently centrifuged at 4000 rpm for 10 min. Plasma supernatant was carefully transferred into new tubes and stored at −20 °C. Analysis of samples was performed using an Olympus AU 400 analyzer (Synlab laboratories, Berlin, Germany). The following parameters were analyzed: ALAT, albumin, ALP, ASAT, bilirubin, GGT, HDL, LDL, and TGs.

2.3. Histology

Animals were sacrificed and liver samples were submerged in a fixative solution (4% paraformaldehyde in PBS) at 4 °C overnight, washed in PBS for 12 h at 4 °C, and finally dehydrated and paraffin embedded. Sections were stained by the standard hematoxylin and eosin (H&E) method. All tissues were examined by light microscopy. Quantitative histology was performed using the ImageJ software. In order to measure liver fat extension, at least five images from three different sections per animal were analyzed. On average, each group contained 4 animals and altogether 480 images were analyzed and compared.

2.4. Liver triglyceride quantification

A liver triglyceride assay was conducted as reported by Foryst-Ludwig et al. (2008). Briefly, liver tissues were homogenized in liquid nitrogen and treated with ice-cold chloroform/methanol/water mixture (2:1:0.8) for 2 min. After centrifugation, the aqueous layer was removed, chloroform layer decanted, and the mixture evaporated at 70 °C. The residues were dissolved in isopropanol, and assessed for triglyceride content using an enzymatic-calorimetric test according to the manufacturer's instructions (Diagnostic System, Holzheim, Germany).

2.5. Echocardiography

Echocardiography was performed using a Vevo 7700 imaging system connected to a Vevo anesthesia system (VisualSonics Inc., Toronto, Canada). Mice were anesthetized using isoflurane (isoflurane 2%, oxygen 98%), and laid supine fixed to a platform for analysis through surgical tape stripes. Body temperature was monitored via a rectal thermometer and maintained at 36–38 °C using a heating pad and a medical lamp. All hairs were removed from the chest using a chemical hair remover. Scanning was performed using an ultrasonic gel opportunely pre-warmed and spread on the mouse chest. All images and calculations were obtained as described by Zhou et al. (2004).

2.6. Statistical analysis

Statistical analysis was performed using the SPSS software. Experimental groups were compared using the statistical procedure analysis of the variance (one-way ANOVA) and/or a Student's t-test. One-way ANOVA was followed by post-hoc analysis for multiple comparisons (Tukey or Tamhane's T2 test). Hypothesis of equality of means rejected with a p-value < 0.05. Age-dependent effects on cardiovascular and metabolic parameters analyzed in this study were evaluated using a paired-samples t-test comparing the means between two related experimental groups. Values in figures and tables are expressed as means ± standard deviation.

3. Results

3.1. Gpr30-lacZ mice fed with HFD were indistinguishable by weight, body mass and glucose tolerance from their wt littermates

Metabolic and hemodynamic studies were performed on Gpr30-lacZ sex and age-matched control mice fed with a HFD for 20 weeks.

In a previous publication we have already reported the results of body weight, body mass composition and glucose tolerance measurements (Isensee et al., 2009). Briefly, body weight and body mass composition assessed by nuclear magnetic resonance did not reveal any genotype-dependent difference between HFD and CD groups. In order to detect potential differences in glucose clearance efficiency between homozygous mutant and control mice, intra-peritoneal glucose tolerance test (IPGTT) was performed. However, no statistically significant differences between Gpr30-lacZ and control mice were found. Additionally we did not observe genotype-dependent differences in plasma glucose levels during HFD feeding (unpublished data in Supplemental Fig. A of this study).

3.2. Plasma lipid profiling revealed reduced HDL levels in Gpr30-lacZ females fed with HFD

Plasma lipid profiles for both Gpr30-lacZ and wt mice were assessed after 4 and 20 weeks of HFD. After 4 weeks of HFD no significant genotype-dependent differences were found among groups (Table 1).