Development and characterization of a humanized GLP-1 receptor mouse model for translational drug development

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Injectable peptide-based GLP-1 receptor (GLP1R) agonists have shown remarkable therapeutic success in the treatment of type 2 diabetes and obesity. In comparison, small-molecule GLP1R agonists may further improve patient compliance and bioavailability through oral administration. In contrast to peptides, non-peptide ligands make fewer receptor contacts and amino acid differences across species may therefore significantly impact receptor binding and functional effects of small molecule GLP1R ligands. Animal models expressing the human GLP1R may therefore better enable in vivo characterization of small-molecule GLP1R agonists and translate preclinical pharmacodynamics to the clinic. We therefore developed a humanized GLP1R (hGLP1R) mouse model using CRISPR-Cas9 gene-editing technology. The present study aimed to phenotype the hGLP1R mouse as compared to wild-type mice.

Methods

Studies were performed in 15-24 week-old chow-fed male hGLP1R mice. Wildtype (WT, C57BL/6NJ strain) and littermate mice served as controls. In food intake studies, mice were fasted for 9h before lights off, then dosed with vehicle (PO), semaglutide (10 nmol/kg, SC) or orforglipron (3 mg/kg, PO). For IPGTT, mice were fasted for 6h before receiving a glucose challenge (2 mg/kg, IP), then dosed with vehicle (PO), semaglutide (10 nmol/kg, SC) or orforglipron (1 mg/kg, PO). For histology, animals were perfused with PBS-NBF, and the pancreas and brains were stained with anti-mGLP1R (#ab218532, Abcam) and anti-hGLP1R antibody (Mab3F52-s, DSHB).

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1 The humanized GLP1R mouse model

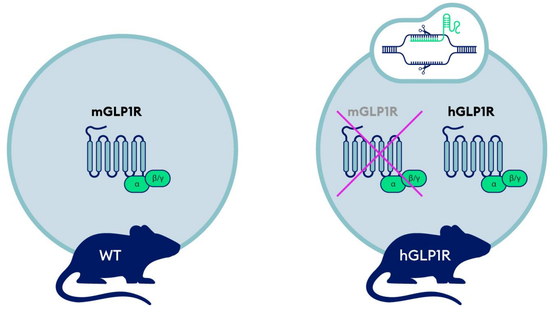

Figure 1. Humanized GLP-1 receptor mouse model generation. The humanized GLP1R mouse model was generated on a C57BL/6NJ background using CRISPR-Cas9 technology, replacing the endogenous murine GLP-1 receptor (mGLP1R) with the human receptor (hGLP1R) at whole-body level.

2 Human GLP-1 receptor expression in the brain and pancreas of hGLP1R mice

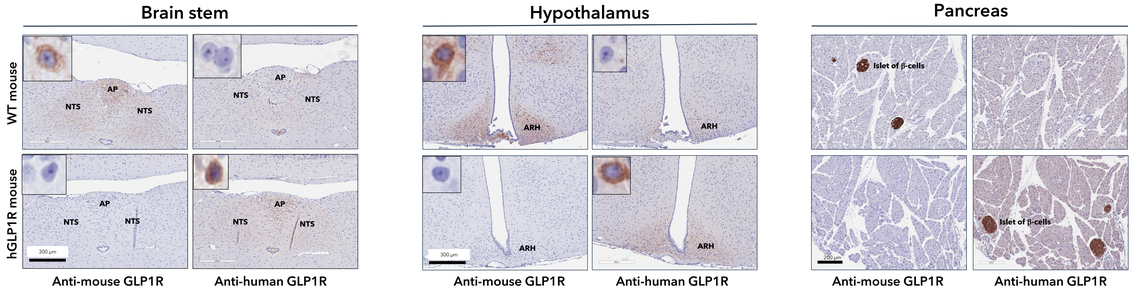

Figure 2. The humanized GLP-1 receptor mouse expresses only the hGLP1R receptor. Anti-mouse and anti-human GLP1R antibodies were used to profile GLP1R expression in the brain stem (AP, area postrema; NTS (nucleus of the solitary tract), hypothalamus (ARH, arcuate hypothalamic nucleus) and pancreas of hGLP1R mice and littermate wild-type (WT) control mice.

3 Comparable efficacy of semaglutide and orforglipron on gross metabolic parameters in hGLP1R mice

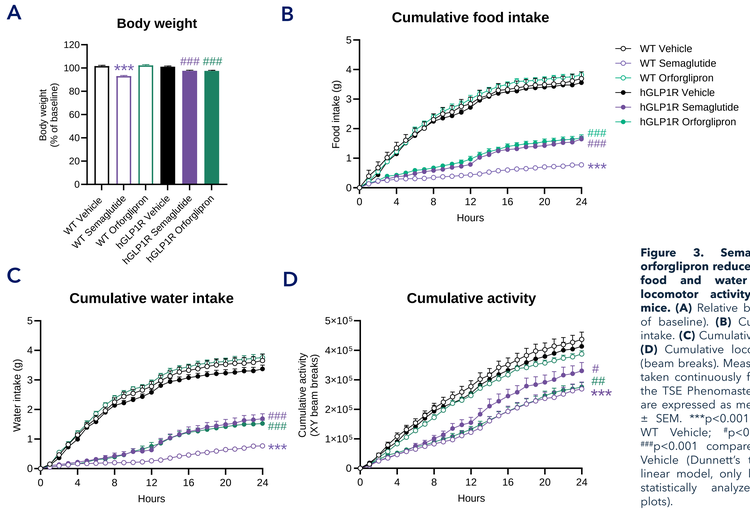

Figure 3. Semaglutide and orforglipron reduce body weight, food and water intake and locomotor activity in hGLP1R mice. (A) Relative body weight (% of baseline). **(B)** Cumulative food intake. **(C)** Cumulative water intake. **(D)** Cumulative locomotor activity (beam breaks). Measurements were taken continuously for 24 hours in the TSE Phenomaster system. Data are expressed as mean of n=10-12 ± SEM. ***p<0.001 compared to WT Vehicle; #p<0.05, ##p<0.01, ###p<0.001 compared to hGLP1R Vehicle (Dunnett's test one-factor linear model, only last data point statistically analyzed in profile plots).

4 Comparable efficacy of semaglutide and orforglipron on glycemic control in hGLP1R mice

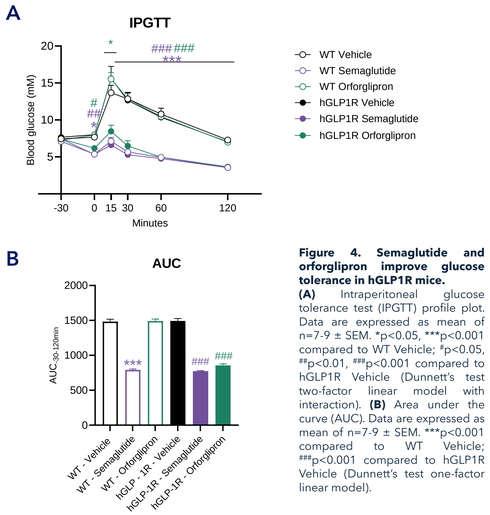

Figure 4. Semaglutide and orforglipron improve glucose tolerance in hGLP1R mice. **(A)** Intraperitoneal glucose tolerance test (IPGTT) profile plot. Data are expressed as mean of n=7-9 ± SEM. *p<0.05, ***p<0.001 compared to WT Vehicle; #p<0.05, ##p<0.01, ###p<0.001 compared to hGLP1R Vehicle (Dunnett's test two-factor linear model with interaction). **(B)** Area under the curve (AUC). Data are expressed as mean of n=7-9 ± SEM. ***p<0.001 compared to WT Vehicle; ###p<0.001 compared to hGLP1R Vehicle (Dunnett's test one-factor linear model).

Conclusion

- A novel hGLP1R mouse model was generated using CRISPR-Cas9 gene-editing technology
- Immunohistochemical analyses confirmed whole-body replacement of endogenous mGLP1R with hGLP1R
- Peptide (semaglutide) and non-peptide (orforglipron) GLP1R agonists have comparable efficacy on metabolic and glycemic endpoints in hGLP1R mice
- As expected, orforglipron showed no effect on metabolic and glycemic parameters in wild-type controls
- The Gubra hGLP1R mouse is applicable for characterizing non-peptide GLP1R agonists targeting obesity and associated metabolic disorders