Systemic AAV10.COMP-Ang1 rescues renal glomeruli and pancreatic islets in type 2 diabetic mice

Mi Tian[1,3], M.D., Lara S. Carroll[2], Ph.D., Li Tang[1], M.D., Ph.D., Hironori Uehara[2], Ph.D., Christof Westenfelder[1], M.D., Balamurali K. Ambati[2], M.D., Ph.D., Yufeng Huang[1], M.D., Ph.D.*

[1]Department of Internal Medicine, Division of Nephrology, University of Utah, Salt Lake City, UT, USA; [2]Department of Ophthalmology and Visual Science, John A. Moran Eye Center, University of Utah, Salt Lake City, UT, USA. [3]Division of Nephrology, Department of Internal Medicine, Shengjing Hospital, China Medical University, Shenyang 110004, China.

*Address correspondence to: Dr. Yufeng Huang, Division of Nephrology, Department of Internal Medicine, University of Utah School of Medicine, Wintrobe Rm 403, 26 N Medical Dr, Salt Lake City, UT 84132, USA, Phone: 801-585-0581, Fax: 801-213-2563, Email: Yufeng.huang@hsc.utah.edu.

Running title: COMP-Ang1 and diabetic visceropathies

Online Supplement

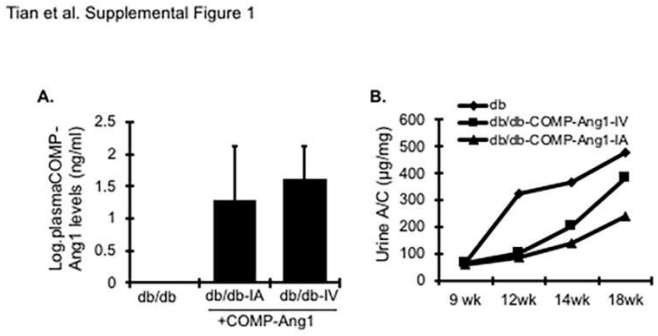
Methods

Procedures of left carotid artery injection and direct renal delivery of AAVrh10.GFP/AAVrh10.COMP-Ang1 to normal or db/db mice.

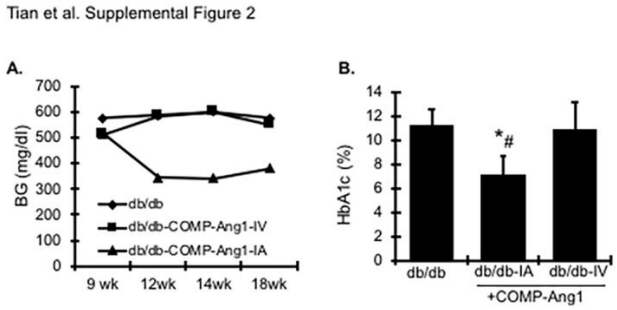
Endotoxin-free and sterile delivery of AAVrh10.COMP-Ang1, AAVrg10.GFP or PBS control was used for all injections as described in the experimental design. The aseptic procedures of left intra-carotid artery injection were carried out in mice under deep anesthesia. After mice were in deep anesthesia as determined by no jerking when their toes were pinched, the neck hair was shaved, cleaned with Betadine Scrub followed by an alcohol rinse. A sticky-backed drapeis applied to the area and a longitudinal incision was made in sterile instruments on the neck area. After exposing the left carotid sheath, the common, internal, and external carotid are dissected, the external carotid and the distal part of the common carotid were ligated with sterile 6-0 absorbable suture. A microclamp was used to clamp the proximal part of the common carotid artery before using PE10 tubing to catheterize the vessel leading to the aorta. The PE tubing was connected with a three-way stopcock via a 30 G needle. AAVrh10.COMP-Ang1, or AAVrh10.GFP in 250 µl PBS or PBS only in 1ml syringe were injected directly downward into the common carotid artery to the aorta over the course of 10 seconds. After injection, the proximal part of the common carotid artery was ligated with sterile 6-0 absorbable suture. The area was cleaned with gauze containing antimicrobial agent Amerse Germicide (Convatec, St. Louis, MO) or Betadine Scrub. The flank incision was sutured closed. The direct renal intracortex infusion of AAVrh10.GFP was performed to place a subcutaneous osmotic mini-pump delivering AAVrh10.GFP. Briefly, the kidney was exposed from the flank region and a catheter was placed in the renal cortex, approximately 2.0mm underneath the surface, and secured using Vetbond glue; the other end of the catheter was connected to the osmotic mini-pump delivering AAVrh10.GFP. After each procedure animals were removed to a warming table

until they waked up. Buprenex (0.05mg/kg) was given subcutaneously twice a day, for 72 hours. Animals were monitored for discomfort, agitation, etc.

Figures



Supplemental Figure 1. (**A**) Plasma COMP-Ang1 levels determined at end of study (18 weeks of age) by using specific ELISA method for COMP-Ang1 described previously (23). Similar elevated circulating COMP-Ang1 levels were observed in db/db mice receiving AAvrh10.COMP-Ang1 plasmid via either intraarterial injection (db/db-COMP-Ang1-IA, n=5) or intravenous injection (db/db-COMP-Ang1-IV, n=6) at age of 18 weeks. No COMP-Ang 1 protein was detected in untreated db/db mice at age of 18 weeks (db/db, n=6). (**B**) Progression of urinary albumin excretion in db/db mice from 9 wks of age to 18 weeks of age determined by urinary albumin/ creatinine (A/C) ratio (UAR), measured by the DC2000+ microalbumin reagent kit (Bayer Healthcare). The levels of UAR were continuously elevated in db/db mice between 9 weeks to 18 weeks of age in untreated db/db mice, which was consistent with our previous reports in this model. Treatment with AAVrh10.COMP-Ang1 via either intraarterial injection or intravenous injection reduced the progression of UAR levels. However, reduction of urinary A/C ratio was significantly enhanced in db/db mice injected via the carotid artery.



Supplemental Figure 2. Blood glucose (BG) levels (A) and blood glycosylated hemoglobin (HbA1C) levels (B, at end of study) of db/db mice determined in tail blood samples using a blood glucose meter (Glucometer Elite XL, Bayer Healthcare, Elkhart, IN, USA) and the DC 2000+ HbA1c kit (Bayer Healthcare) respectively. Untreated diabetic db/db mice (db/db, n=6) kept hyperglycemia through the whole experiment period. Treatment with a single dose of AAVrh10.COMP-Ang1 via the tail vein injection (db/db+COMP-Ang1-IV, n=6) had no effect on blood glucose levels and HbA1c levels in db/db mice. However, treatment with a single dose of AArh10.COMP-Ang1 via the carotid arterial injection (db/db+COMP-Ang1-IA, n=5) reduced the blood glucose levels in db/db mice started 2 weeks after injection and led to significant reduction in HbA1C levels at 8 weeks after injection. * vs. db/db, $P<0.05$; # vs. db/db+COMP-Ang1-IV, $P<0.05$.