Growth Hormone & IGF Research 20 (2010) 345–355

Contents lists available at ScienceDirect

Growth Hormone & IGF Research

journal homepage: www.elsevier.com/locate/ghir

Identification of differentially expressed genes in the kidneys of growth hormone transgenic mice

K.T. Coschigano [a,b,*], A.N. Wetzel [b,1], N. Obichere [a], A. Sharma [a], S. Lee [a], R. Rasch [c], M.M. Guigneaux [d], A. Flyvbjerg [e], T.G. Wood [d], J.J. Kopchick [a,b]

[a] Department of Biomedical Sciences, College of Osteopathic Medicine, 228 Irvine Hall, Ohio University, Athens, OH 45701, USA
[b] Edison Biotechnology Institute, Ohio University, Athens, OH 45701, USA
[c] Department of Cell Biology, Institute of Anatomy, University of Aarhus and Electron Microscopy Laboratory, Aarhus University Hospital, Skejby, Denmark
[d] Sealy Center for Molecular Medicine and Department of Biochemistry & Molecular Biology, University of Texas Medical Branch, Galveston, TX 77555, USA
[e] Medical Research Laboratories, Clinical Institute & Medical Department M (Diabetes & Endocrinology), Aarhus University Hospital, Skejby, Denmark

ARTICLE INFO

Article history:
Received 17 September 2009
received in revised form 7 June 2010
accepted 9 June 2010
Available online 23 July 2010

Keywords:
Growth hormone transgenic mice
Immunoglobulins
Kidney
cDNA subtraction
Microarray
Real-time RT/PCR
Kidney androgen-regulated protein
Inflammation
Chemokine (C–C motif) ligand 2/monocyte chemotactic protein-1

ABSTRACT

Objective: Bovine growth hormone (bGH) transgenic mice develop severe kidney damage. This damage may be due, at least in part, to changes in gene expression. Identification of genes with altered expression in the bGH kidney may identify mechanisms leading to damage in this system that may also be relevant to other models of kidney damage.

Design: cDNA subtraction libraries, northern blot analyses, microarray analyses and real-time reverse transcription polymerase chain reaction (RT/PCR) assays were used to identify and verify specific genes exhibiting differential RNA expression between kidneys of bGH mice and their non-transgenic (NT) littermates.

Results: Immunoglobulins were the vast majority of genes identified by the cDNA subtractions and the microarray analyses as being up-regulated in bGH. Several glycoprotein genes and inflammation-related genes also showed increased RNA expression in the bGH kidney. In contrast, only a few genes were identified as being significantly down-regulated in the bGH kidney. The most notable decrease in RNA expression was for the gene encoding kidney androgen-regulated protein.

Conclusions: A number of genes were identified as being differentially expressed in the bGH kidney. Inclusion of two groups, immunoglobulins and inflammation-related genes, suggests a role of the immune system in bGH kidney damage.

1. Introduction

A multitude of studies have led to the conclusion that GH plays a role in the progression of kidney damage, in the presence or absence of diabetes. Involvement of GH, either directly or through its effector molecule IGF-I, in the early stages of diabetic nephropathy was suggested in two cross-sectional studies in human type I diabetic patients. In one study, a positive correlation was found between serum IGF-I and glomerular filtration rate [1]. In another study, a strong positive correlation between urinary IGF-I and kidney volume and between urinary IGF-I, urinary GH, and microalbuminuria was seen [2]. Direct evidence for a role of GH in nephropathy came from studies of transgenic mice chronically expressing GH, growth hormone releasing factor (GHRF), or IGF-I [3,4]. In the absence of diabetes, significant glomerular enlargement was seen in all three strains of mice. However, mesangial proliferation followed by progressive glomerulosclerosis was found in the GH and GHRF mice but not the IGF-I mice. The GH transgenic mice progressed to end stage renal disease, resulting in premature death [5]. Progressive increases of mRNA levels of collagen IV α1, laminin B1, tenascin, transforming growth factor (TGF)-β1 and platelet-derived growth factor (PDGF)-B were also seen in the GH transgenic mice [6].

Additional studies suggested that suppression of the GH signaling pathway can reverse or prevent diabetic kidney complications. Initial evidence came from studies demonstrating the protective effect of hypopituitarism against glomerular basement membrane thickening and the normalization of glomerular basement membrane and mesangial changes caused by diabetes [7,8]. Studies of transgenic mice that express a GH receptor antagonist demonstrated protection from glomerulosclerosis during streptozotocin (STZ) induced type I diabetes, even though blood glucose and glycated hemoglobin levels were high [9–11]. A molecular analysis of these diabetic transgenic mice revealed normal mRNA and protein levels of glomerular type IV

* Corresponding author. Department of Biomedical Sciences, College of Osteopathic Medicine, 228 Irvine Hall, Ohio University, Athens, OH 45701, USA. Tel.: +1 740 593 2196; fax: +1 740 597 2778.
E-mail address: coschigk@ohio.edu (K.T. Coschigano).
[1] Current address: Department of Veterinary Preventive Medicine, The Ohio State University, Sisson Hall A101, A109, 1920 Coffey Road, Columbus, OH 43210, USA.

1096-6374/$ – see front matter © 2010 Growth Hormone Research Society. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.ghir.2010.06.001

collagen and laminin B1, in contrast to the elevated levels seen in diabetic non-transgenic controls [11,12]. It has been shown that STZ-diabetic as well as nonobese diabetic (NOD) mice have elevated levels of circulating GH and low levels of IGF-I, similar to the situation seen in human diabetic patients, suggesting that mice may serve as a good model for diabetes studies [13,14]. In a test of therapeutic efficacy for the GH antagonist, administration of the GH antagonist to STZ-diabetic and nonobese diabetic (NOD) mice did not alter the already elevated serum GH levels and reduced serum IGF-I levels seen in the untreated diabetic control mice, but it did reduce the kidney weight, IGF-I accumulation, glomerular volume, and urinary albumin excretion (UAE), indicating a therapeutic role for GH antagonist [13,14]. Finally, in a system where GH signaling is completely abolished due to disruption of the GH receptor gene, the kidneys of these mice also were protected against STZ-diabetes induced glomerular hypertrophy and glomerulosclerosis [15].

GH acts through its receptor to initiate a series of signaling cascades that ultimately lead to changes in gene expression [16]. While a number of GH-regulated genes have been identified, genes relating GH to kidney damage are still largely unknown. Utilizing the fact that bGH mice develop progressive glomerulosclerosis with increasing age, we chose them as a model system for identifying genes involved in kidney damage. We created cDNA subtraction libraries from kidney RNA of bGH and NT littermates at different ages and validated differential expression of specific clones by northern blot analysis. We also performed microarray analyses comparing gene expression in the kidneys of bGH and NT littermates. Finally, we compared the levels of select gene expression by real-time RT/PCR analysis. Here we identify genes that appear to be differentially expressed in the kidneys of bGH mice as compared to their NT littermates. Consideration of the list of differentially expressed genes suggests involvement of the immune system in the bGH kidney damage.

2. Materials and methods

2.1. Animals

The mice used in this study express a bovine GH cDNA under the control of a mouse metallothionein transcriptional regulatory element. Most of the experiments used mice with a mixed C57Bl6J and SJL background as in previous studies [17–19]. The mice used for the microarray study were a pure C57Bl6J genetic background [20]. All transgenic mice were compared to NT controls of the same genetic background. Transgenic males were bred with non-transgenic female littermates in the mouse facility of Ohio University's Edison Biotechnology Institute (EBI). Animals are only accepted into the facility from commercial specific-pathogen-free (SPF) suppliers or from other facilities that have a proven record of clean health. EBI facilities are serology tested for a standard panel of murine viruses four times a year. Necropsy, histology and parasitology are performed twice a year. Mice were housed in microisolator cages, maintained on a 14h light/10h dark cycle and allowed ad libitum access to water and standard rodent chow (Prolab RMH 3000, PMI Nutrition International, Inc., Brentwood, NJ). Genotypes of progeny were confirmed by PCR analysis of genomic DNA obtained by a tail clip using the same method as described for bGH antagonist (GHA) mice [21].

At 2 months, 5 months, or 12 months of age female NT and bGH transgenic mice ($n=3$ for each genotype and age) were weighed and urine collected for a 2 hour period and then stored at −80°C for albumin and creatinine analyses. The following day the mice were sacrificed by cervical dislocation and trunk blood collected following immediate decapitation. Whole blood was centrifuged at 7000 ×g for 10 min at 4 °C and serum stored at −80°C for IGF-I and IGFBP analyses. The right kidney was removed and snap-frozen in liquid nitrogen for later isolation of total RNA for cDNA subtraction library creation ($n=1$ for each genotype and age) and northern analyses. The left kidney was removed, weighed, and a transverse section of the kidney containing the papilla placed in fixative (0.1 M cacodylic acid, 1% glutaraldehyde, and 2% paraformaldehyde) for glomerular analyses by microscopy. Multiple tissues of two additional 12 month old NT females and one additional 12 month old bGH female were collected for subsequent RNA isolation and northern analyses. Kidneys from additional mice at each age also were collected for RNA isolation and northern analyses. Kidneys from 11 month old female bGH and NT mice were obtained for total RNA extraction and use in the microarray ($n=3$ for each genotype) and real-time RT/PCR analyses ($n=5$ for bGH and 6 for NT).

The RNA used for Fig. 6 was from an unrelated diabetes study using female NT mice. At 2 months of age, the mice received a single intraperitoneal injection of saline or 155 mg streptozotocin (STZ)/kg body weight. Diabetic mice and age-matched saline controls were sacrificed 10 weeks after the onset of diabetes (blood glucose>300 mg/dl) and the right kidney removed and snap-frozen in liquid nitrogen for later isolation of total RNA. RNA samples from two saline-injected (non-diabetic; ND) and two STZ-induced diabetic (DB) mice were analyzed in the current study.

Protocols were approved by the Ohio University Institutional Animal Care and Use Committee and followed federal, state, and local guidelines.

2.2. Glomerular and mesangial measurements

Glomerular volume for the 2 month, 5 month and 12 month old mice ($n=3$ for each genotype and age) was measured as previously described [13]. Fixed tissue was embedded in Technovit 7100 (Heracus Kulzer GMBH, Wehrheim, Germany), 2 μm thick sections were cut on a rotation microtome, and sections were stained with peroidic acid-Schiff (PAS) and hematoxylin. Profile areas were traced using a computer-assisted morphometric unit (Image Tool: University of Texas Health Science Center, San Antonio, TX). Glomerular cross-sectional area (A_G) was determined as the average area of all of the glomeruli visualized in the section (a range of 33–56 glomeruli; tuft omitting the proximal tubular tissue within Bowman's capsule). Glomerular tuft volume (V_G) was calculated as $V_G=\beta/\kappa\times(A_G)^{3/2}$, where $\beta=1.38$, which is the shape coefficient for spheres (the idealized shape of glomeruli), and $\kappa=1.1$, which is a size distribution coefficient. The mesangial region was measured by point counting of the PAS-stained area as a fraction of the thuft area by applying a grid where about 20 points fell on the PAS-stained area in each of about 25 randomly selected glomerular profiles.

2.3. Urinary albumin excretion and serum IGF-I and IGFBP measurements

Urinary albumin concentrations for the 2 month, 5 month and 12 month old mice ($n=3$ for each genotype and age) were determined by radioimmunoassay using rat albumin antibody and standards as previously described [13]. Semilog linearity of mouse urine and rat albumin (in the standard) was found at multiple dilutions, indicating antigen similarity between mouse albumin and rat albumin. Urinary volume output was normalized by urinary creatinine concentration measured using an automated technique adapted from the method of Jaffé [22].

Serum IGF-I levels were measured, after extraction with acid-ethanol, by radioimmunoassay (RIA) using a polyclonal rabbit antibody (Nichols Institute Diagnostics, San Capistrano, CA) and recombinant human IGF-I as standard (Amersham International) as previously described [13]. Intra- and interassay coefficients of variation were <5% and <10%, respectively.

Serum IGFBP-1, -2, -3, and -4 levels were assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and