Kidney International, Vol. 68 (2005), pp. 2497–2507

Transcriptional response in the unaffected kidney after contralateral hydronephrosis or nephrectomy

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Transcriptional response in the unaffected kidney after contralateral hydronephrosis or nephrectomy.

Background. Unilateral loss of kidney function is followed by compensatory contralateral growth. The early, genome-wide transcriptional response of the untouched kidney to unilateral ureteral obstruction (UUO) or unilateral nephrectomy is unknown.

Methods. Twelve adult male Sprague-Dawley rats were subjected to UUO and twelve rats to unilateral nephrectomy. At time points 12, 24, and 72 hours after insult four rats each were sacrificed and the contralateral kidney harvested for genome-wide gene expression analysis, transcription factor analysis, and histomorphology.

Results. Microarray studies revealed that the majority of differentially expressed transcripts were suppressed in UUO and unilateral nephrectomy compared to control kidneys. The function of these suppressed genes is predominantly growth inhibition and apoptosis suggesting a net pro-hypertrophic response. Insulin-like growth factor-2 (IGF-2)–binding protein was one of the few activated genes. We observed a distinctly different molecular signature between UUO and unilateral nephrectomy at the three time points investigated. The early response in UUO rats suggests a counterbalance to the nonfiltering kidney by activation of transport pathways such as the aquaporins. Unilateral nephrectomy kidneys, on the other hand, respond immediately to contralateral nephrectomy by activation of cell cycle regulators such as the cyclin family. Several genes with weakly defined function were found to be associated with either UUO or unilateral nephrectomy. Transcription factor analysis of the identified transcripts suggests common regulation at least of some of these genes. All kidneys showed normal histology.

Conclusion. Release of growth inhibition by nephrectomy leads to immediate cell cycle activation after unilateral nephrectomy, whereas UUO kidneys counterbalance filtration failure by activation of several transporters.

Unilateral nephrectomy is followed by cellular growth of the contralateral remaining kidney resulting in an early reconstitution of the glomerular filtration rate (GFR) [1, 2]. However, little is known about the factors influencing the compensatory increase of renal function and tissue in the remaining kidney.

Previous work by Halliburton and Thomson [3] showed that synthesis of RNA and DNA starts already 12 hours after nephrectomy and remains in elevated state until sufficient compensatory growth occurred. Several factors, including endogenous hormones, and growth factors like insulin-like growth factor-1 (IGF-1) were found to influence compensatory renal growth [4, 5]. After initially transient proliferation, cellular hypertrophy is the main compensatory response. Tubule cells increase in volume and protein content and almost no proliferation occurs after few days [6–8]. The finding of hypertrophy being the predominant response to contralateral loss of function is supported by the nearly unchanged mitotic index in the remaining kidney after unilateral nephrectomy. Moskowitz and Liu [9] pointed out that compensatory renal growth is negatively controlled. Proximal tubule and peritubular endothelial cells are the main area of hypertrophic growth [10, 11]. It has been shown that the hypertrophic growth response is mediated in connection to a cell cycle–dependent mechanism [12].

Although older studies revealed that transient hydronephrosis does not alter metabolism and organ function in the affected kidney, little is known about the response of the contralateral kidney [13]. It has been reported that unilateral ureteral obstruction (UUO) is associated with contralateral hyperplasia, while another group demonstrated compensatory hypertrophy [14, 15].

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Key words: kidney, compensatory growth, gene expression, microarray.

Received for publication March 25, 2005
and in revised form May 13, 2005, and June 17, 2005
Accepted for publication July 30, 2005

Since no clear target genes and metabolic pathways are known that may be involved in the immediate regulation of the compensatory growth response, we sought to elucidate the molecular regulation of this process by using an exploratory approach. Genome-wide gene expression analysis was used in rat models of UUO and unilateral nephrectomy to identify potential differences between UUO and unilateral nephrectomy in the contralateral kidney and to compare these expression profiles to normal rat control kidneys.

METHODS

Animal model

Twenty-four adult male Sprague-Dawly rats (304 ± 22 g) were assigned randomly into two groups of 12 rats each. Before surgery rats were anesthetized with pentobarbital 60 mg/kg intraperitoneally and maintained on a temperature-regulated operating table. Twelve animals underwent unilateral nephrectomy of the right kidney after the adrenal gland has been dissected free and was left in place. On the remaining twelve rats of the other group, ureteral ligation of the right kidney was performed (UUO). Twelve kidneys of sham-operated adult male Sprague-Dawly rats served as control. The postoperative two-layer wound closure was sealed with Vicryl 3.0. After 12, 24, and 72 hours the rats of both intervention groups were sacrificed and the remaining kidney was explanted under sterile conditions. Creatinine was measured from blood samples taken prior kidney explantation. The kidney was split longitudinally, one part was immediately minced and submerged into RNAlater™ (Ambion, Austin, TX, USA) to prevent RNA degradation, the other was subjected to paraffin embedding [16]. The animal study was approved by the Austrian Federal Ministry for Science, Section for Genetic Techniques and Laboratory Animals (GZ 66.009/164-BrGt/2003 and GZ 66.009/250-BrGt/2003).

Sample preparation

Isolation of total RNA from homogenized kidneys was performed by using Trizol® (Invitrogen Corp., Carlsbad, CA, USA), mRNA was separated by Oligotex® mRNA Midi Kit (Qiagen, Hilden, Germany). Pooled mRNA that was isolated from sham-operated rat kidneys was used as reference RNA. Quality of the isolated total RNA was checked by gel electrophoresis and with Agilent Bioanalyzer and RNA6000 LabChip® Kit (Agilent, Palo Alto, CA, USA). Sample and reference mRNA were labeled in a two-step procedure with CyScribe cDNA postlabeling kit (Amersham Pharmacia Biotech, Buckinghamshire, UK). Sample preparation and hybridization was performed in random order. Detailed protocols can be found on our website (*http://www.meduniwien.ac.at/nephrogene*).

Microarray hybridization and scanning

Mouse cDNA microarrays holding 39958 cDNA features were obtained from the Stanford University Functional Genomics core facility. It has been shown elsewhere that cross species hybridization of genes on microarrays is feasible [17]. The experiment procedure was performed as described previously [18]. Labeled samples were hybridized in Corning hybridization chambers (Corning Inc., Corning, NY, USA) overnight in a water bath (14 hours, 65°C). Arrays were scanned with a GenePix 4100A scanner (Axon Instruments, Union City, CA, USA). Image griding and calculation of spot intensity was performed using GenePix Pro 4.1 software.

Image-, grid-, and results files are stored at the Stanford microarray database (*http://genome-www4.stanford.edu/MicroArray/SMD/*) [19]. Information on samples, material, experimental set-up, and procedures follows MIAME guidelines (*http://www.mged.org*) to be found at *http://www.meduniwien.ac.at/nephrogene* [20]. All experimental, statistical and bioinformatics procedures were performed in Vienna.

Data processing and statistical analysis

The data sets consist of 39958 cDNA features, of which 20769 are clones with Unigene symbol and nontrivial Unigene title, 7538 have a Unigene symbol but a trivial Unigene title, 3333 are clones with no Unigene symbol and nontrivial Unigene title, and 7185 have no Unigene symbol and trivial Unigene title [21]. Only spots with intensities 1.2-fold over background in both channels 1 and 2 were used. In a first preprocessing step, a quality filter was applied on the data set by considering only genes and expressed sequence tags (ESTs) with at least 80% of valid entries in the 24 array experiments. The remaining missing values (3.9% of the whole data set) were substituted applying a k-nearest-neighbor algorithm, setting the number of neighbors k to 10 [22]. To focus on differentially regulated genes only those with at least 2 values greater or equal an absolute log2 R/G value of 1 depicting a twofold up- or down-regulation between sample RNA and reference RNA remained in the data set. No correction for a putative batch bias was necessary because only one array batch was used in the whole analysis for all 24 arrays. Ending up with a full data set consisting of 6763 genes and ESTs, a further preprocessing step was performed. In each of the two groups (UUO and unilateral nephrectomy) at every time point (12, 24, and 72 hours) outliers were removed to obtain a SD value less than 1 for the particular gene [23].

The resulting genes were annotated with respect to their molecular function, biologic processes, and cellular

Table 1. Body weight and serum creatinine 12, 24, and 72 hours after unilateral ureteral obstruction (UUO) and unilateral nephrectomy

	Body weight *g*			Serum creatinine *mg/dL*		
	12 hours	24 hours	72 hours	12 hours	24 hours	72 hours
Unilateral nephrectomy	304 ± 12	293 ± 7	293 ± 10	0.65 ± 0.1	0.72 ± 0.08	0.6 ± 0.06
UUO	332 ± 21	302 ± 21	304 ± 17	0.71 ± 0.06	0.61 ± 0.05	0.62 ± 0.06

Data are mean ± SD.

locations using gene ontology terms from the Gene Ontology Consortium [24]. The SOURCE tool from the Stanford Genomics Facility was used to associate gene ontology terms for the genes of interest [25].

An unsupervised hierarchical clustering algorithm was applied for graphic representation of the differentially expressed genes [26]. The Pearson *r* correlation coefficient was used as distance measure in the resulting dendrograms and average linkage was used as linkage rule in the cluster algorithm. Cluster analysis was performed with the MultiExperiment Viewer (MeV) developed at The Institute for Genomic Research (TIGR) [27].

Data were analyzed using SAS version 9.1.3 (SAS Institute, Cary, NC, USA). Changes in body weight and creatinine over time were analyzed by linear regression analysis using dummy variables for the time points. An unpaired *t* statistic of log-transformed expression values was used to evaluate differences between UUO and unilateral nephrectomy. No adjustment for multiple testing was performed, because small differences in some transcript values might be functionally important as well and thus should not be missed. Data are given as mean ± SD, a $P < 0.001$ was considered statistically significant.

Promoter/transcription factor binding site (TFBS) analysis

Promoter analysis was conducted on the resulting sets of differentially expressed genes comparing the UUO and the unilateral nephrectomy group. Gene symbols could be assigned to 33 unique genes out of the 66 cDNA features with 23 genes up-regulated in the UUO group and 10 genes up-regulated in the unilateral nephrectomy group.

The oPOSSUM database, a repository of transcription factor binding sites in the regulatory regions of genes, was used to find orthologous sequences as well as transcription factors in the set of investigated genes [28]. Utilizing the JASPAR database as repository for known TFBS the sequences ranging from −5000 to +5000 bases with respect to the transcription start site (TSS) of the target genes were scanned for putative TFBS [29]. To reduce the number of false positive hits, only those present in the mouse as well as in the orthologous human sequence were reported. To find these highly conserved regulatory regions (phylogenetic footprints) between the human and mouse genome the top 10% of conserved regions with a minimum conservation level of 70% were analyzed for TFBS. Of the 23 genes up-regulated in the UUO group 13 had an orthologous human mouse gene pair and could be further analyzed. In the unilateral nephrectomy group, seven out of the ten genes remained for further analysis.

Histomorphology

For histologic analysis 2 μm sections of formalin (4%, neutrally buffered)-fixed paraffin embedded rat kidneys were stained with hematoxylin and eosin and periodic acid-Schiff (PAS). A semiquantitative scoring of vascular and tubulointerstitial lesions was performed by H.R. who was blinded for the type of intervention.

RESULTS

Kidney function after UUO and unilateral nephrectomy

Serum creatinine values and body weight of UUO and unilateral nephrectomy animals at 12, 24, and 72 hours after insult are depicted in Table 1. Creatinine and body weight were unchanged over time in both groups.

Transcripts that are differentially regulated in both, UUO and unilateral nephrectomy, compared to control rat reference kidneys

Mean sector and printing plate analysis of variances (ANOVAs) r^2 were between 2×10^{-2} and 6×10^{-2} in the microarray experiments suggesting no dependence of results on spatial location or printing plate. The vast majority of all features that were more than twofold differentially expressed in both UUO and unilateral nephrectomy vs. control kidney were suppressed at each of the three time points (Table 2) (Web Fig. 1). Only three out of the 101 sequences were more than twofold up-regulated. Among the three is IGF-2 binding protein 1. Interestingly, the majority of suppressed genes are inhibitors of growth, suggesting that compensatory growth after UUO and unilateral nephrectomy is mediated by silencing of antigrowth transcripts. The finding that transcripts involved in protein degradation such as the ubiquitin pathway are suppressed as well, points into the same direction. Furthermore, transcription factors responsible for growth inhibition such as "inhibitor of growth family, member 4," were suppressed as well. Additionally, proapoptotic transcripts of enzymes such as granzyme A are suppressed suggesting an indirect stimulation of growth by a reduction of proapoptotic stimuli. Finally, the anti-inflammatory response is mediated by suppression of members of the complement cascade.

Table 2. Functional roles of differentially regulated genes between unilateral ureteral obstruction (UUO) and unilateral nephrectomy versus control at all three time points 12, 24, and 72 hours

Accession number	Gene name	Gene symbol	Mean expression UUO and unilateral nephrectomy at 12, 24, and 72 hours
Apoptosis			
AA138788	Granzyme A	Gzma	−1.46
Cell cycle			
BG065626	V-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)	Erbb3	−1.45
BG063022	Yamaguchi sarcoma viral (v-yes-1) oncogene homolog	Lyn	−1.41
BG064871	Lung carcinoma myc-related oncogene 1	Lmyc1	−1.23
Cytoskeleton			
BG066664	CDNA sequence BC032204	BC032204	−1.69
BG067147	Dynamin binding protein	Dnmbp	−1.26
Immune response			
BG066643	Complement component 4 binding protein	C4bp	−1.82
BG063898	Complement receptor-related protein	Crry	−1.44
Ion binding			
AU040159	RIKEN cDNA 4732418C07 gene	4732418C07Rik	−2.08
BG064367	Ectonucleoside triphosphate diphosphohydrolase 1	Entpd1	−1.74
BG066642	Cysteine and histidine-rich domain (CHORD)-containing, zinc-binding protein 1	Chordc1	−1.65
BG076243	Reticulocalbin 3, EF-hand calcium binding domain	Rcn3	−1.59
Membrane			
BG074161	RIKEN cDNA 2310075C12 gene	2310075C12Rik	−1.56
BG065276	Podocalyxin-like	Podxl	−1.55
BG076282	Discoidin, CUB and LCCL domain containing 1	Dcbld1	−1.53
Metabolism			
BG063893	SH3-binding kinase	Sbk	−1.84
BG063527	RAB geranylgeranyl transferase, b subunit	Rabggtb	−1.73
BG067167	Exonuclease 1	Exo1	−1.60
BG064371	Galactose-4-epimerase, UDP	Gale	−1.52
BG065124	Calcium activated nucleotidase 1	Cant1	−1.50
BG064066	**Phosphatidylinositol-4-phosphate 5-kinase, type 1 gamma	Pip5k1c	−1.33
BG063506	Maternal embryonic leucine zipper kinase	Melk	−1.20
Protein modification			
BG066359	Ubiquitin specific protease 1	Usp1	−1.99
BG070661	Procollagen, type IV, alpha 3 (Goodpasture antigen) binding protein	Col4a3bp	−1.93
BG066408	Dual specificity phosphatase 19	Dusp19	−1.76
BG066990	SUMO/sentrin specific protease 6	Senp6	−1.73
BG063225	Deltex 2 homolog (Drosophila)	Dtx2	−1.43
Receptor			
BG065542	Platelet derived growth factor receptor, alpha polypeptide	Pdgfra	−1.30
Signal transduction			
BG065571	CD97 antigen	Cd97	−1.77
BG063279	ADP-ribosylation factor-like 5	Arl5	−1.65
BG067803	Rap guanine nucleotide exchange factor (GEF) 5	Rapgef5	−1.43
Transcription			
BG064651	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta	Nfkbiz	−2.26
BG066487	Musculin	Msc	−2.17
C79450	Inhibitor of growth family, member 4	Ing4	−2.05
C85178	Zinc finger protein 68	Zfp68	−1.71
BG065165	Thymopoietin	Tmpo	−1.65
BG065915	Ets variant gene 6 (TEL oncogene)	Etv6	−1.58
BG066421	Necdin	Ndn	−1.57
C76795	Spi-C transcription factor (Spi-1/PU.1 related)	Spic	−1.44
Translation			
BG063554	Eukaryotic translation initiation factor 1A	Eif1a	−1.24
Transport			
BG066295	Transmembrane channel-like gene family 6	Tmc6	−1.99
BG066452	Trafficking protein particle complex 6B	Trappc6b	−1.99
C79033	Solute carrier family 34 (sodium phosphate), member 2	Slc34a2	−1.81
BG063793	Nucleoporin 93	Nup93	−1.58
BG066080	Solute carrier family 25 (mitochondrial carrier, phosphate carrier), member 3	Slc25a3	−1.50
BG068652	Karyopherin (importin) alpha 3	Kpna3	−1.45
BG066078	Syntaxin 3	Stx3	−1.31
Others			
BG066902	RIKEN cDNA C530030P08 gene	Fbxw11	1.52
BG063699	RAD1 homolog (S. pombe)	Rad1	1.44
BG068632	Insulin-like growth factor 2, binding protein 1	Igf2bp1	1.16

Continued

Table 2. continued

Accession number	Gene name	Gene symbol	Mean expression UUO and unilateral nephrectomy at 12, 24, and 72 hours
BG063823	Ubiquitin specific protease 8	Usp8	−2.53
BG075681	Transcribed locus		−2.18
BG066367	DNA segment, Chr 11, ERATO Doi 707, expressed	D11Ertd707e	−2.15
BG064115	**CDNA sequence BC046331	BC046331	−2.07
BG064336	DNA2 DNA replication helicase 2-like (yeast)	Dna2l	−1.99
BG063272	RIKEN cDNA 5730472N09 gene	5730472N09Rik	−1.92
BG066509	Pogo transposable element with KRAB domain	D1Ertd251e	−1.90
BG064669	Calmodulin regulated spectrin-associated protein 1	Camsap1	−1.85
BG063782	SMAD-specific E3 ubiquitin protein ligase 2	Smurf2	−1.84
BG065749			−1.77
BG066407			−1.75
BG064708	F-box and leucine-rich repeat protein 18	Fbxl18	−1.75
BG066389	A kinase (PRKA) anchor protein (yotiao) 9	Akap9	−1.73
BG064116	Axotrophin	Axot	−1.71
BG064705	RIKEN cDNA D630050G16 gene	D630050G16Rik	−1.70
BG066333			−1.67
BG063062	DNA segment, Chr 11, ERATO Doi 497, expressed	D11Ertd497e	−1.66
BG064402	RIKEN cDNA 1110034B05 gene	1110034B05Rik	−1.63
BG070987	Gene model 1, (NCBI)		−1.61
BG064464	RIKEN cDNA 2600005C20 gene	2600005C20Rik	−1.58
BG064647	YEATS domain containing 2	Sumo2	−1.57
BG066405	Limb region 1	Lmbr1	−1.57
BG064347	Myeloid/lymphoid or mixed lineage-leukemia translocation to 6 homolog (*Drosophila*)	AI315037	−1.56
BG064685	Queuine tRNA-ribosyltransferase domain containing 1	Qtrtd1	−1.56
C77431	RIKEN cDNA 4933412H03 gene	4933412H03Rik	−1.56
BG063954	Translin	Tsn	−1.55
AA409791	Transcribed locus		−1.51
BG065101	Expressed sequence AW320013	1700052N19Rik	−1.51
BG063963	RIKEN cDNA 1500032H18 gene	1500032H18Rik	−1.50
BG065887			−1.50
	IW:598		−1.49
BG070155	CGG triplet repeat binding protein 1	Cggbp1	−1.49
BG065100	RIKEN cDNA 1110001C20 gene	1110001C20Rik	−1.46
BG065037	Transmembrane protein 2	Tmem2	−1.45
BG063791	Mindbomb homolog 1 (Drosophila)	Mib1	−1.44
BG067204	Expressed sequence C85492	C85492	−1.42
BG066510	RIKEN cDNA 2410003B16 gene	2410003B16Rik	−1.41
BG065143	Zinc finger protein 94	Zfp94	−1.39
BG067950	RIKEN cDNA 4933428G09 gene	4933428G09Rik	−1.38
BG064636	Phosphatidylinositol glycan, class S	Pigs	−1.33
BG066303	Expressed sequence AI450241	AI450241	−1.28
BG064169	RuvB-like protein 1	Ruvbl1	−1.27
BG067623	Transcribed locus		−1.26
AW537825	RIKEN cDNA 2610033H07 gene	2610033H07Rik	−1.26
BG063302	Expressed sequence AA408865	AA408865	−1.25
BG066505	Ring finger protein 20	Rnf20	−1.25
BG063884	RIKEN cDNA B230205O20 gene	Wdr42a	−1.20

The majority of transcripts were suppressed in UUO and unilateral nephrectomy compared to control. Numbers are log2 of expression values, resulting in negative values for suppressed sequences and positive values for up-regulated features.

All these observations fit to the hypothesis that compensatory renal growth in a situation of contralateral insult is mediated by suppression of growth inhibitors belonging to the main gene ontology terms of apoptosis, metabolism, protein degradation, and inflammation.

Analysis of the regulation of transcripts that are differentially regulated at all three time points in UUO and unilateral nephrectomy separately changed the picture somehow (Web Table 1). Still the vast majority of transcripts was suppressed in UUO and unilateral nephrectomy compared to control kidney, but some active growth regulators such as mitogen-activated protein kinases (MAPK) or IGF-2 could also be found overexpressed.

Focusing on the sequential changes of transcripts over the three time points in each group UUO and unilateral nephrectomy separately, the picture became rather complex. Still more transcripts were suppressed than overexpressed, but the complexity of regulation of compensatory growth became apparent. The hundreds of more than twofold regulated genes and ESTs are displayed in Web Table 1 for all time points in each group.

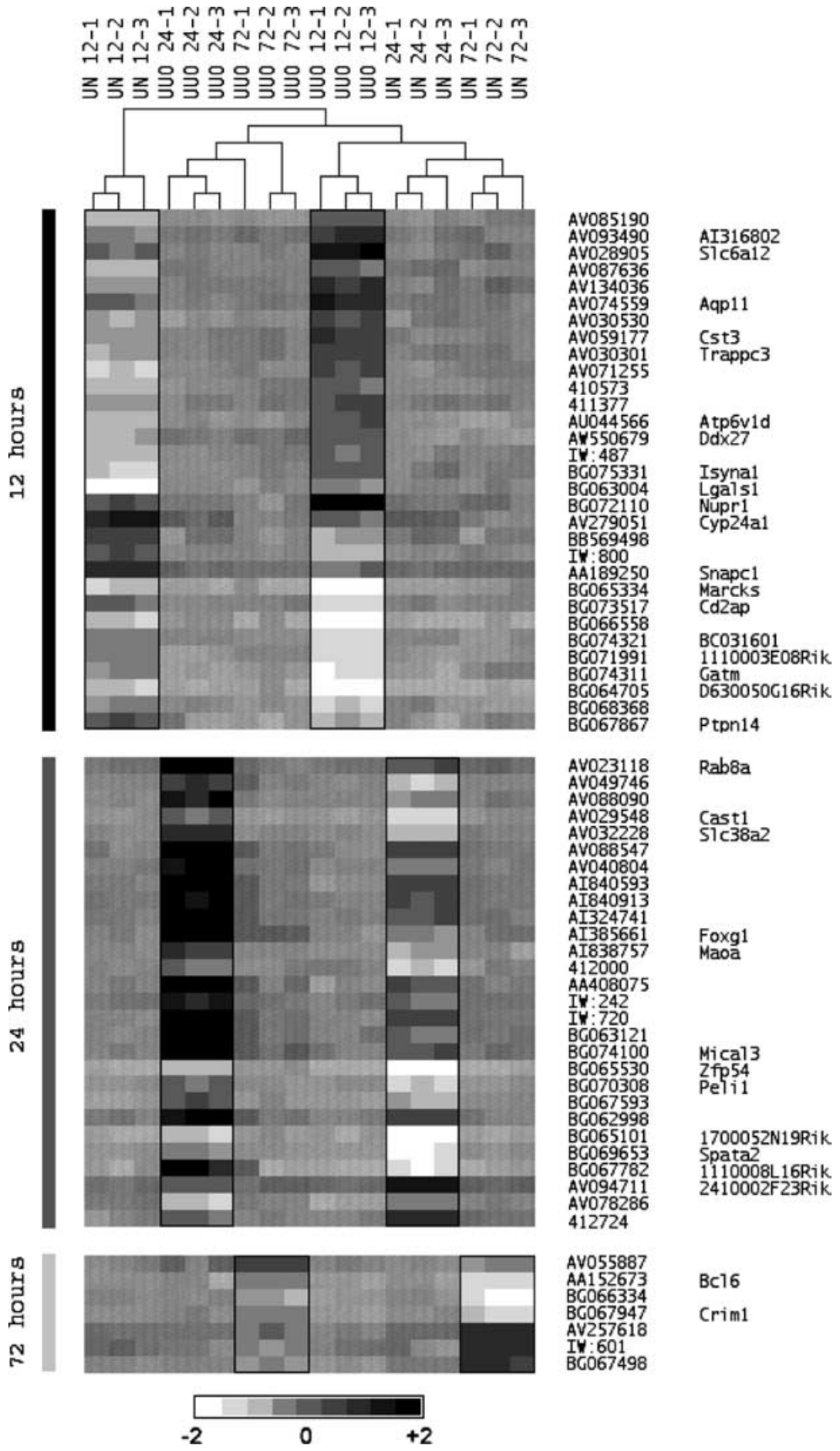
UN 12-1
UN 12-2
UN 12-3
UUO 24-1
UUO 24-2
UUO 24-3
UUO 72-1
UUO 72-2
UUO 72-3
UUO 12-1
UUO 12-2
UUO 12-3
UN 24-1
UN 24-2
UN 24-3
UN 72-1
UN 72-2
UN 72-3
12 hours
24 hours
72 hours
AV085190
AV093490 AI316802
AV028905 Slc6a12
AV087636
AV134036
AV074559 Aqp11
AV030530
AV059177 Cst3
AV030301 Trappc3
AV071255
410573
411377
AU044566 Atp6v1d
AW550679 Ddx27
IW:487
BG075331 Isyna1
BG063004 Lgals1
BG072110 Nupr1
AV279051 Cyp24a1
BB569498
IW:800
AA189250 Snapc1
BG065334 Marcks
BG073517 Cd2ap
BG066558
BG074321 BC031601
BG071991 1110003E08Rik
BG074311 Gatm
BG064705 D630050G16Rik
BG068368
BG067867 Ptpn14
AV023118 Rab8a
AV049746
AV088090
AV029548 Cast1
AV032228 Slc38a2
AV088547
AV040804
AI840593
AI840913
AI324741
AI385661 Foxg1
AI838757 Maoa
412000
AA408075
IW:242
IW:720
BG063121
BG074100 Mical3
BG065530 Zfp54
BG070308 Peli1
BG067593
BG062998
BG065101 1700052N19Rik
BG069653 Spata2
BG067782 1110008L16Rik
AV094711 2410002F23Rik
AV078286
412724
AV055887
AA152673 Bcl6
BG066334
BG067947 Crim1
AV257618
IW:601
BG067498
-2
0
+2

Fig. 1.

Transcripts that are differentially regulated in UUO vs. unilateral nephrectomy

Transcripts that are differentially regulated in UUO and unilateral nephrectomy at each of the three time points are displayed in Web Table 2. Only one transcript could be identified that separated UUO and unilateral nephrectomy in all three time points. At 12 hours genes that were more than twofold up-regulated in UUO vs. unilateral nephrectomy belong to the main functional groups of transporters, metabolism, and membranes (Table 3) (Fig. 1). Interestingly, genes that are involved in urine concentration such as aquaporins or the solute carrier family were abundantly expressed in UUO compared to unilateral nephrectomy. On the other hand, genes belonging to the cell cycle regulatory framework such as cyclins were more abundantly expressed at 12 hours in unilateral nephrectomy compared to UUO (Web Table 2) (Fig. 1). At 24 hours, members of the transport gene ontology group such as "solute carriers" were more than twofold up-regulated in UUO compared to unilateral nephrectomy. In unilateral nephrectomy kidneys, however, only one cyclin and some few metabolism and membrane genes were stronger expressed than in the UUO rat kidneys (Web Table 2). At 72 hours after the contralateral insult, UUO kidneys showed still up-regulation of solute carriers compared to unilateral nephrectomy. Furthermore, genes belonging to the main gene ontology terms of apoptosis, metabolism, protein modification, and some others were abundantly expressed in UUO vs. unilateral nephrectomy. In unilateral nephrectomy vs. UUO, however, only some membrane and metabolism genes were abundantly expressed.

The complexity of the response in a healthy kidney to contralateral injury can be appreciated by the many different transcription patterns between UUO and unilateral nephrectomy at the different time points investigated. No clear single hierarchical pathway could be identified that separated the compensatory response in the ipsilateral kidney after contralateral UUO or unilateral nephrectomy.

Transcription factor analysis

Seven transcription factors with a significantly higher number of binding sites compared to the reference data set of all orthologous human/mouse gene pairs in the oPOSSUM data base could be identified in the set of 23 genes up-regulated in the UUO group and are listed in Web Table 3. Four of the seven transcription factors belong to the FORKHEAD class of transcription factors, namely HFH-1 (Foxq1), HFH-2 (Foxd3), HFH-3 (Foxi1), and HNF-3beta (Foxa2). E4BP4 (Nfil3) and Chop-cEBP (Cebpa) are members of the bZIP class and Irf-1 (Irf1) is a member of the TRP-CLUSTER of transcription factors. A graphic representation of the genes holding binding sites for at least one of the transcription factors can be seen in Web Figure 2. Among the target genes are transporters like Aqp11 and Slc38a2 as well as genes involved in the immune system such as Bcl6 and Spata2.

No transcription factors with significantly higher numbers of binding sites in comparison to the reference dataset could be obtained when analyzing the 10 genes up-regulated in the unilateral nephrectomy group.

Histomorphology

By light microscopy renal histomorphology appeared entirely normal in all treatment groups. Kidneys from UUO or unilateral nephrectomy animals could not be discriminated from sham-operated control animals by histomorphologic criteria. In particular, there were no morphologic signs of tubular injury in any of the specimen.

DISCUSSION

In the present study we showed that contralateral uninephrectomy or ureteral obstruction cause a dramatic suppression of genes belonging to growth inhibition in the ipsilateral, unaffected kidney within the first 3 days after injury. Only few genes were overexpressed during that time frame compared to control rat kidneys. Besides the fact that gene expression profiles are different in UUO and unilateral nephrectomy compared to control rat kidneys, we also found distinctly different molecular signatures in UUO compared to unilateral nephrectomy kidneys. UUO was associated with an activation of genes belonging to the ontologies of transport, whereas unilateral nephrectomy caused more activation of genes involved in cell cycle and metabolism. The exact pathophysiology behind the different response to UUO and unilateral nephrectomy remains to be determined however. The variety of differentially regulated transcripts belonging to various gene ontologies in UUO and unilateral nephrectomy kidneys suggests a complex regulation of the different responses. Possible pathophysiologic mechanisms causing the observed molecular differences

Fig. 1. Expression profiles of differentially regulated genes at three time points (12, 24, and 72 hours) between unilateral ureteral obstruction (UUO) and unilateral nephrectomy. White color represents suppressed genes/sequences compared to control and black color abundantly expressed features over control. The unsupervised hierarchical cluster algorithm groups kidneys according to the similarity in their molecular signature next to each other (1 to 3). Unilateral nephrectomy kidneys from the same time point as well as UUO kidneys from the same time group together. Shaded areas represent other time points as the time indicated on the left. A heat map colored figure is available at: http://www.meduniwien.ac.at/nephrogene/data.

Table 3. Twofold or 0.5-fold differential regulation of genes in unilateral ureteral obstruction (UUO) versus unilateral nephrectomy

Accession number	Gene name	Gene symbol	Mean expression UUO	Mean expression unilateral nephrectomy
12 hours				
Cytoskeleton				
BG067867	Protein tyrosine phosphatase, nonreceptor type 14	Ptpn14	−0.90	0.31
Membrane				
BG065334	Myristoylated alanine rich protein kinase C substrate	Marcks	−2.53	−1.08
Metabolism				
BG075331	Myoinositol 1-phosphate synthase A1	Isyna1	0.20	−1.26
AV279051	Cytochrome P450, family 24, subfamily a, polypeptide 1	Cyp24a1	0.04	1.29
Transport				
AU044566	ATPase, H+ transporting, V1 subunit D	Atp6v1d	0.24	−0.96
AV030301	Trafficking protein particle complex 3	Trappc3	0.56	−0.70
AV074559	Aquaporin 11	Aqp11	1.16	0.03
AV028905	Solute carrier family 6 (neurotransmitter transporter, betaine/GABA), member 12	Slc6a12	1.67	0.09
BG074311	Glycine amidinotransferase (L-arginine:glycine amidinotransferase)	Gatm	−1.56	−0.28
Others				
BG063004	Lectin, galactose binding, soluble 1	Lgals1	−0.38	−1.77
AV071255			0.43	−1.22
AV085190			0.22	−1.13
	410573		0.02	−1.05
	IW:478		0.11	−1.02
AV087636			0.06	−0.97
AW550679	DEAD (Asp-Glu-Ala-Asp) box polypeptide 27	Ddx27	0.31	−0.85
AV030530			0.38	−0.66
AV134036			0.81	−0.65
	411377		0.44	−0.60
AV059177	Cystatin C	Cst3	0.70	−0.51
AV093490	Expressed sequence AI316802	AI316802	0.86	−0.32
BG072110	Nuclear protein 1	Nupr1	1.68	0.42
BG066558			−2.84	−1.21
BG064705	RIKEN cDNA D630050G16 gene	D630050G16Rik	−2.49	−1.17
BG074321	CDNA sequence BC031601	BC031601	−1.37	−0.25
BG068368			−1.29	−0.20
BG071991	RIKEN cDNA 1110003E08 gene	1110003E08Rik	−1.41	−0.20
BG073517	CD2-associated protein	Cd2ap	−1.31	0.01
	IW:800		−1.12	0.31
BB569498			−0.66	0.56
AA189250	Small nuclear RNA activating complex, polypeptide 1	Snapc1	−0.12	0.91
24 hours cell cycle				
AI385661	RIKEN cDNA 3110039M20 gene	Foxg1	1.98	−0.33
Metabolism				
AI838757	Monoamine oxidase A	Maoa	0.68	−0.81
BG074100	Flavoprotein oxidoreductase MICAL3	Mical3	1.92	0.29
Transport				
AV023118	RAB8A, member RAS oncogene family	Rab8a	1.75	0.42
Others				
BG065530	Zinc finger protein 54	Zfp54	−0.94	−2.70
BG065101	Expressed sequence AW320013	1700052N19Rik	−1.14	−2.32
BG067782	RIKEN cDNA 1110008L16 gene	1110008L16Rik	1.49	−1.68
BG069653	Spermatogenesis associated 2	Spata2	−0.37	−1.65
AV029548	O-acetyltransferase	Cast1	0.09	−1.37
	412000		−0.19	−1.21
BG070308	Pellino 1	Peli1	−0.02	−1.18
AV049746			0.84	−1.12
AV032228	Solute carrier family 38, member 2	Slc38a2	1.02	−0.95
BG067593			0.34	−0.87
AV088090			1.43	−0.42
	IW:242		1.25	−0.13
AV040804			1.78	−0.11
BG063121			2.05	0.01
AA408075			2.34	0.43
AI324741	Similar to Stefin homolog		1.90	0.47
AV088547			2.20	0.48
AI840913			1.78	0.54

Continued

Table 3. continued

Accession number	Gene name	Gene symbol	Mean expression UUO	Mean expression unilateral nephrectomy
AI840593			1.89	0.59
BG062998			1.66	0.60
	IW:720		2.29	0.60
AV078286			−1.25	−0.12
	412724		−0.02	1.04
AV094711	RIKEN cDNA 2410002F23 gene	2410002F23Rik	0.32	1.34
72 hours Apoptosis				
AA152673	B-cell leukemia/lymphoma 6	Bcl6	−0.20	−1.51
Others				
BG066334	Hypothetical LOC380894		−0.74	−1.76
BG067947	Cysteine-rich motor neuron 1	Crim1	−0.22	−1.34
AV055887			0.65	−0.44
	IW:601		−0.27	0.84
BG067498			−0.45	0.87
AV257618			0.02	1.06

All differences achieve a P value of < 0.001, no adjustment for multiple testing was performed. Numbers are log2 of expression values, resulting in positive values for abundant expression and negative values for suppression in UUO and unilateral nephrectomy.

in responses to UUO and unilateral nephrectomy include altered renal blood flow, different demands of solute excretion, or neuroendocrine crosstalk between kidneys. It has been reported by Moskowitz et al [30] that increased vasoconstriction in the renal cortex is among the early events of compensatory hypertrophy after unilateral nephrectomy. No such data, however, are available for the UUO model. It may further be speculated that hydronephrotic but perfused kidneys may release messengers into the blood stream causing contralateral activation of potent solute carriers or vice versa that loss of a growth inhibitor by unilateral nephrectomy cause immediate hypertrophy.

The question of ipsilateral response to contralateral nephrectomy has been studied previously. Liu and Preisig [12] studied compensatory renal hypertophy within the first 10 days after contralateral uninephrectomy in rats. The authors were primarily interested whether the compensatory growth was cell cycle dependent and used 5-bromo-2′-deoxyuridine (BrdU) incorporation as marker of hyperplasia while the protein to DNA content of proximal tubules was used as marker of hypertrophy. According to our data the authors found that compensatory growth after unilateral nephrectomy was caused by hypertrophy and was cell cycle–dependent. The authors found a late G_1 arrest caused by cyclin D expression, but insufficient activation of cyclin E, which is usually required for the cellular transition into the synthesis phase.

Mulroney and Pesce [31], on the other hand, showed that compensatory hypertrophy after uninephrectomy in adult male rats is growth hormone dependent but independent of IGF-1. In juvenile rats, however, the authors postulated the vice versa. We identified IGF-2 binding protein as one of the very few early continuously up-regulated genes in both unilateral nephrectomy and UUO. What that means in terms of regulation of early compensatory growth by the IGF axis is unclear, given the complex interplay of the IGF system. The IGFs comprise an intricate regulation system with two growth factors, cell-surface receptors, six high-affinity IGF binding proteins and proteases, as well as other IGF binding protein interacting molecules.

Besides expression of active growth regulators such as IGF we observed suppressed protein degradation and inhibition of metabolism after contralateral injury. It has been stated elsewhere that suppression of protein degradation by regulation of ubiquitin ligases is mandatory in compensatory hypertrophy of adult kidneys [32]. We have shown in the present paper that several ubiquitin proteases are suppressed at various time points in both groups suggesting a key regulatory principle in compensatory growth after both types of unilateral injury.

Besides the known responses to uninephrectomy little is known about transcriptional regulation of compensatory growth after UUO. We found mainly transcripts belonging to the superfamilies of transporter and membranes as well as metabolism abundantly expressed compared to the unilateral nephrectomy group. One might speculate that the nonfiltering but perfused kidney may send some signal to the unaffected kidney causing transcription of potent solute carriers and genes responsible for fluid regulation. Examples are the solute carrier families 6, 7, 9, 25, 35, and 38 and aquaporins. It is impossible, however, to speculate on the precise pathophysiology of these early events since many covariates such as antiduretic hormone levels and metabolism remain unknown. Morishita et al [33] used knockout mice to elucidate the physiological role of aquaporin 11. Aquaporin 11–deficient mice showed abnormal tubule cell proliferation and impaired basement membrane remodeling

leading to severe disturbances in tubule fluid transport. The vital importance of aquaporin 11 can be appreciated by the fact that most of the mice died before weaning.

The identification of common transcription factors in some of the differentially regulated genes between UUO and unilateral nephrectomy suggests coregulation of these transcripts. Overdier and colleagues showed that HFH-3 (Foxi1) is expressed in the distal tubules of embryonic and adult mouse kidneys. In their work they list Na/K-ATPase, Na/H and anion exchangers, E-cadherin, and mineralocorticoid receptor genes as target genes of the transcription factor HFH-3 (Foxi1) [34]. Another gene with a binding site for the HFH-3 (Foxi1) transcription factor in the regulatory region and expressed in renal tissue is the guanylyl cyclase/natriuretic peptide receptor-A gene (Npr1), as reported by Garg et al [35]. Braam et al [36] report coordinated changes in genes of the hepatocyte nuclear factor 3 family (NHF3, HNF3A, HNF3B, and HNF3G), and the interferon regulatory factors IRF1 and IRF5 in proximal tubule cells in response to angiotensin II.

Three of the seven transcription factors had at least one cDNA clone spotted on the microarray but only one (HFH-1) passed the preprocessing steps and could be further analyzed. The other two (Chop-cEBP and Irf-1) were excluded from analysis due to very low expression levels. HFH-1 (Foxq1) was moderately higher expressed in the UUO group as compared to the unilateral nephrectomy group. As previously reported by Moscowitz et al [30], the coordinate expression of genes involved in compensatory hypertrophy is mediated by the differential regulation of both, growth inducing and repressing transcription factors. Among these transcription factors was cyclin D which was also identified as being up-regulated 12 hours after unilateral nephrectomy compared to sham in our data. Other genes that were identified as relevant for compensatory growth by Moskowitz et al such as actin, heat shock proteins, and Na/K-ATPases were also found to be activated after unilateral nephrectomy in our analysis (Web Table 1).

CONCLUSION

We have shown that unilateral nephrectomy or hydronephrosis cause a set of transcriptional modifications in the unaffected contralateral kidney which is regulated at least in part by a set of few transcription factors. The response to UUO is fundamentally different than to unilateral nephrectomy. UUO is more associated with molecular signatures of solute and fluid transport as well as metabolism and membrane transport, whereas unilateral nephrectomy leads to an immediate hypertrophic response caused by cell cycle regulators such as cyclins and by suppression of growth inhibitors. Based on our exploratory data deductive studies may be designed that seek to elucidate the exact pathophysiologic differences of compensatory growth after UUO or unilateral nephrectomy.

ACKNOWLEDGMENTS

Financial support for this study was provided by the Austrian Science Fund (FWF P-15679 to R.O. and Erwin Schrödinger J2415-B11 to P.H.) as well as the National Institute of Health (NIH grant R21-DK63127 to T.W.M.). Web figures and web tables are publicly available through the web site *http://www.meduniwien.ac.at/nephrogene*

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