1. ARRAY DESIGN DESCRIPTION

A brief description of the array design, feature location, information on the cDNA collection and the spotting protocols can be found on the producer´s website at
http://www.microarray.org/sfgf/jsp/home.jsp

Protocols for the pre-hybridisation procedures (post-processing) of the arrays can be downloaded from our website at http://www.microarray.at

2. EXPERIMENT DESCRIPTION

2.1. Experimental design

2.1.1.Laboratory, authors, contact

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2.1.2. Type of experiment

We obtained gene-expression profiles of microdissected renal-tubule cells from patients with proteinuric nephropathies. Based on the renal function during follow-up, the patients were divided in stable (n=14) and progressive (n=7) subjects (table 1).

2.1.3. Experiment factors

The cells of interest were laser-capture microdissected from frozen sections from archived kidney biopsy material.

2.1.4. Microarray hybdridizations

Initially all samples were processed as technical duplicates (2 x 21 arrays); due to a large number of signal-negative spots several arrays were excluded leaving 36 arrays for analysis. The samples P2, P6, P7, S10, S13 and S14 were analysed as individual arrays, all other samples were analysed after combination of duplicate arrays.

2.1.5. Reference

Stratagene Universal Human Reference RNA was used as hybridization reference.

2.1.6. Quality control

To test for reproducibility we calculated the intra-array variability of the duplicate arrays. Duplicate arrays were combined before statistical analysis where applicable.

2.2. Samples used, extract preperation, amplification and labelling

2.2.1. Bio-source properties

Organism: Homo sapiens. Patient and control characteristics can be found in the manuscript and on our website (http://www.microarray.at).

2.2.2. Biomaterial manipulations, amplification and labelling protocol

Frozen kidney biopsies were stained for alkaline phospatase, then the tubule cells were lasercapture microdissected using the PixCell II™ Laser Capture Microdissection System and CapSure™ LCM Caps. RNA was isolated using Pico Pure™ RNA Isolation Kit (all Arcturus, Mountain View, CA). We performed two rounds of linear RNA amplification using RiboAmp™ RNA Amplification Kit (Arcturus, Mountain View, CA). Reference RNA was as well amplified

twice. Protocols for RNA amplification, RNA labelling, hybridization and washing of microarrays can be downloaded from our website (http://www.microarray.at).

2.3. Hybridization procedures and parameters

S … Stable patients

P … Progressive patients

Experiment Name user - defined	**Array-ID** batch, number (Stanford)	**bar code** on array (Stanford)	**optical control** 1=ok, 2=moderate, 3=bad
P1-1	shfr156	12897396	1
P1-2	shfd63	12794749	1
P2-1	shfd176	12794521	1
P3-1	shfr140	12897213	1
P3-2	shfd103	12794723	1
P4-1	shfd145	12789555	1
P4-2	shfd105	12794605	1
P5-1	shfr146	12898156	1
P5-2	shfr137	12897216	1
P6-1	shfd102	12794724	1-2
P7-1	shfd104b	12794606	1-2
S1-1	shep145a	12667919	2
S1-2	shep218	12663957	1
S2-1	shep169	12667937	2
S2-2	shep66	12667815	1
S3-1	shfa144	12795928	1-2
S3-2	shep246	12664345	1
S4-1	shep11a	12664308	2
S4-2	shep249	12664348	1
S5-1	shep120	12667740	1
S5-2	shep250	12664349	1-2
S6-1	shep119	12667741	1
S6-2	shfr23	12921608	1
S7-1	shep168	12667936	1
S7-2	shep221	12663960	1
S8-1	shep217	12663956	1-2
S8-2	shep35	12667836	1
S9-1	shep220	12663959	1
S9-2	shep144	12667921	1
S10-2	shep70	12668212	1
S11-1	shep141	12667924	1-2
S11-2	shep36	12667835	1-2
S12-1	shep167	12667935	1
S12-2	shep69	12668210	1
S13-1	shep13	12664313	1
S14-1	shfr138	12897215	1-2

Experiment Name	SMD-Array-color-tool	
user - defined	Sector-ANOVA R-squared values	Printing-Plate-ANOVA R-squared values
P1-1	0,022	0,164
P1-2	0,012	0,201
P2-1	0,013	0,176
P3-1	0,022	0,080
P3-2	0,012	0,119
P4-1	0,009	0,114
P4-2	0,022	0,121
P5-1	0,032	0,136
P5-2	0,026	0,110
P6-1	0,028	0,167
P7-1	0,009	0,193
S1-1	0,014	0,186
S1-2	0,008	0,188
S2-1	0,057	0,154
S2-2	0,008	0,170
S3-1	0,015	0,091
S3-2	0,016	0,135
S4-1	0,046	0,054
S4-2	0,005	0,033
S5-1	0,008	0,161
S5-2	0,024	0,137
S6-1	0,011	0,116
S6-2	0,027	0,084
S7-1	0,020	0,178
S7-2	0,003	0,169
S8-1	0,010	0,196
S8-2	0,007	0,214
S9-1	0,008	0,201
S9-2	0,011	0,196
S10-2	0,016	0,181
S11-1	0,014	0,180
S11-2	0,014	0,148
S12-1	0,006	0,142
S12-2	0,056	0,158
S13-1	0,008	0,178
S14-1	0,053	0,128

Experiment Name	GenePix Quality Report					
user - defined	Median signal-to-background		Mean of median background		Median signal-to-noise	
	635	532	635	532	635	532
P1-1	3,8	3,0	64	74	6,1	9,1
P1-2	3,9	2,7	66	88	7,4	8,1
P2-1	2,7	2,0	142	182	4,9	4,1
P3-1	3,1	2,0	108	123	6,7	4,8
P3-2	3,7	2,9	126	145	7,7	8,7
P4-1	2,7	2,2	115	127	5,0	5,2
P4-2	3,3	2,3	99	124	7,8	5,6
P5-1	2,4	2,4	202	110	4,2	6,7
P5-2	2,3	2,1	164	135	2,8	5,0
P6-1	3,8	3,3	434	360	9,0	12,5
P7-1	3,3	2,8	272	371	5,8	6,7
S1-1	3,8	4,0	133	113	5,8	9,3
S1-2	5,1	4,7	132	131	9,9	13,9
S2-1	4,6	3,7	138	142	9,9	10,5
S2-2	6,0	4,4	103	111	13,5	13,9
S3-1	4,1	2,3	123	158	7,2	4,7
S3-2	4,2	3,6	155	129	10,5	10,0
S4-1	7,4	3,4	84	128	16,8	8,0
S4-2	8,1	4,0	79	151	15,2	9,9
S5-1	4,2	3,7	144	118	9,3	10,7
S5-2	3,0	3,9	186	123	6,1	11,5
S6-1	9,7	7,2	106	127	24,2	26,1
S6-2	2,5	1,8	112	136	5,2	4,6
S7-1	5,2	5,8	164	131	10,8	16,3
S7-2	3,1	4,3	198	115	5,9	12,1
S8-1	4,1	3,5	123	120	7,9	8,7
S8-2	8,9	5,9	115	127	26,7	16,3
S9-1	2,7	3,5	259	140	5,3	9,1
S9-2	2,8	3,5	249	142	5,0	9,9
S10-2	3,7	4,5	191	144	6,4	16,0
S11-1	3,4	3,9	136	113	5,5	9,6
S11-2	2,4	3,7	243	127	4,1	11,1
S12-1	3,7	4,1	182	142	8,5	13,6
S12-2	6,2	5,5	136	133	14,7	18,1
S13-1	2,7	3,7	185	116	4,5	10,5
S14-1	2,1	1,7	315	382	2,9	4,0

2.4. Measurement data and specification of data processing

2.4.1. Raw data description

Scan hardware: GenePix 4000 B (Axon Instruments, Union City, CA)

Scan software: GenePix Pro 4.1 (Axon Instruments, Union City, CA)

Raw data can be found in the data section of our website (http://www.microarray.at).

2.4.2. Background subtraction

The “local feature” algorithm was used for individual background subtraction.

2.4.3. Image analysis and quantitation

Image gridding and calculation of spot intensity was performed with GenePix Pro 4.1 software

2.4.4. Normalized and summarized data

Normalization was done through the default computed normalization by SMD (at http://genomewww5.stanford.edu/help/results_normalization.shtml). For data retrieval the log2 red/green normalized ratio was used. Signals with a signal intensity in the red channel (patient sample) < 2.5 fold over background were excluded from analysis. Genes with values in less than 80 % of the samples were excluded from analysis. Those two filter options resulted in a gene count of 19921 cDNA clones remaining in the analysis dataset. A two-sample t-test ($p < 0.05$) and a two-fold-change criterion were used to identify differentially expressed genes when comparing both patient cohorts.