Supplemental data to article

Steroid donor pretreatment to prevent postischemic renal allograft failure: a randomised, controlled trial

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Genomics and proteomics

Methods

Bioinformatic work flow

The microarray dataset consisted of 41,421 cDNA features. 41,025 of those held a UniGene Cluster ID, 396 were expressed sequence tags (ESTs) not assigned to a UniGene Cluster. Mean sector and printing plate ANOVA r2-values of the microarray experiments were on average 4.5x10-2 and 3.1x10-2 respectively, suggesting no dependency of results on spatial location or plate printing procedures. In a first pre-processing step a quality filter was applied on the dataset by considering only genes and ESTs with spot intensities of 1.5-fold over background in either channel 1 or 2 in the array experiments, yielding 34,599 cDNA features. The remaining missing data points were substituted applying a k-nearest-neighbor algorithm, where the number of neighbors, k, was set to 10 (45). No correction for a putative batch bias was necessary because only one array batch was used in the whole analysis for all arrays. We used the significance analysis of microarrays (SAM) to determine significant differentially expressed genes (DEGs) between steroid and placebo treatment (46). The number of permutations was set to one hundred and genes with a fold change over 2 and a delta value over 1.2 were assigned as DEGs resulting in a false discovery rate (median) of 0.47%. DEGs were hierarchically clustered and graphically represented using the MultiExperiment Viewer (MeV) developed at The Institute for Genomic Research (TIGR) (47). The Cosine correlation and complete linkage were used as distance measure and linkage rule in the hierarchical cluster algorithm, respectively (47, 48).

DEGs were furthermore analyzed with respect to their molecular functions, associated biological processes, and cellular locations using gene ontology terms (GO-Terms) as provided by the Gene Ontology Consortium (49). The SOURCE tool from the Stanford Genomics Facility was used for linking GO-Terms to the genes of interest (50). Functional grouping of genes was based on GO-Terms, PANTHER (Protein ANalysis THrough Evolutionary Relationships) ontologies, and information derived from the protein data retrieval system iHOP (Information Hyperlinked over Proteins) (51, 52).

Regulatory network analysis

To determine the interaction of DEGs providing an indication of potential functional interactions, human protein-protein interactions (PPIs) as provided by the Online Predicted Human Interaction Database (OPHID) were retrieved.(53) All differentially expressed genes with a fold change over 2 were considered in this network analysis. A protein-protein interaction network was generated using the nearest neighbour expansion method as proposed by Chen et al. (54). ProteoLens (http://bio.informatics.iupui.edu/proteolens/) was used for graphical representation of the network.

Results

Genes Differentially Expressed Between Steroid and Placebo

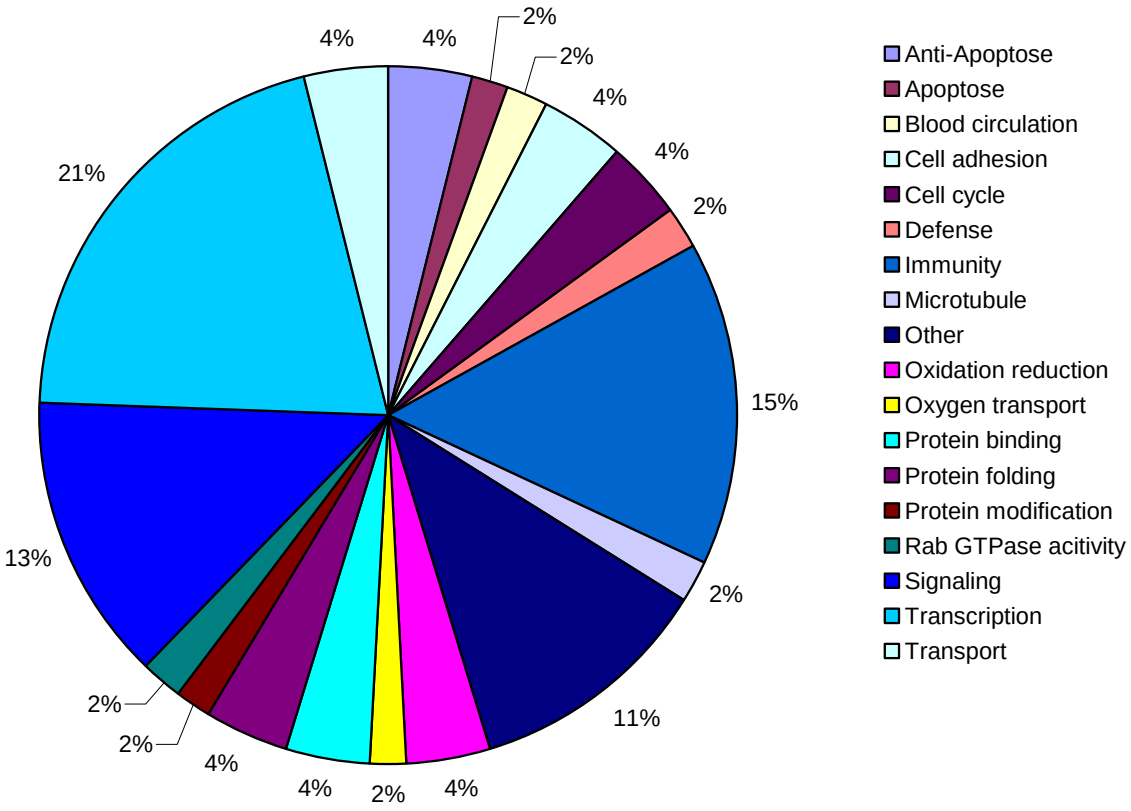
In total 52 features were identified as significant differentially expressed when comparing the gene expression profiles of zero-hour kidney biopsies between steroid and placebo treatment with a fold change over 2. These features represent forty-six unique genes, resulting in thirty-nine down-regulated and seven up-regulated genes in the steroid group (figure 1). Unsupervised hierarchical clustering of the 40 samples stemming from thirty-three deceased donors showed a separation of steroid and placebo treatment based on the gene expression profiles. According to Gene Ontology (GO) classification up-regulated transcripts belong mainly to immunity response, transcription and signaling indicating suppression of inflammation in the graft by steroid treatment (Appendix Table 1 and Appendix Figure 1).

Appendix Table 1. Significant differentially expressed genes between steroid and placebo treatment with the corresponding Gene Ontology (GO) term and reference identifying this gene as a corticosteroid target listed by fold change.

Accession No.	Symbol	Name	fold change	GO Classification	Regulated by corticosteroid treatment
W86653	FKBP5	FK506 binding protein 5	4.88	Protein folding	Hubler 2004 (38), Woodruff 2007 (44)
AA877253	RNF186	Ring finger protein 186	4.12	Protein binding	
AA775091	TSC22D3	TSC22 domain family, member 3	3.03	Transcription	Woodruff 2007 (44)
AI668637	PLN	Phospholamban	2.54	Blood circulation	McTiernan 1997 (42)
AA910933	SLC25A45	Solute carrier family 25, member 45	2.50	Transport	
AA971563	SGSM3	Small G protein signaling modulator 3	2.50	Protein binding	
AA490088	SUSD3	Sushi domain containing 3	2.24		
N30976	CYP24A1	Cytochrome P450, family 24, subfamily A, polypeptide 1	-2.01	Oxidation reduction	
H13469	TUSC3	Tumor suppressor candidate 3	-2.03	Protein modification	
R25377	DEK	DEK oncogene (DNA binding)	-2.04	Transcription	
AA194983	TNFRSF11B	Tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)	-2.08	Apoptose	Makrygiannakis 2006 (41)
AI003775	LOC387763	Hypothetical LOC387763	-2.08		
AI922872	SOCS3	Suppressor of cytokine signaling 3	-2.09	Anti-Apoptose	Paul 2000 (43)
AA461108	EFNB2	Ephrin-B2	-2.10	Signaling	
AA700471	MYO10	Myosin X	-2.10	Signaling	
AA865469	TUBA1A	Tubulin, alpha 1a	-2.12	Microtubule	

Accession No.	Symbol	Name	fold change	GO Classification	Regulated by corticosteroid treatment
H70774	HIST1H2BG	Histone cluster 1, H2bg	-2.12	Defense	
AI831083	DPYSL3	Dihydropyrimidinase-like 3	-2.12	Signaling	
AA425102	CCL2	Chemokine (C-C motif) ligand 2	-2.12	Immunity	Ansari 2007 (33), Lund 2007 (40), Ishmael 2008 (39)
T72915	SOCS3	Suppressor of cytokine signaling 3	-2.13	Anti-Apoptose	Paul 2000 (43)
AA706968	ZWINT	ZW10 interactor antisense	-2.16	Cell cycle	
N63988	IFIT2	Interferon-induced protein with tetratricopeptide repeats 2	-2.17	Immunity	
AA057170	ADAMTS1	ADAM metallopeptidase with thrombospondin type 1 motif, 1	-2.19	Signaling	
H70961	NFKBIZ	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta	-2.19	Immunity	Ishmael 2008 (39)
AA454810	TACSTD2	Tumor-associated calcium signal transducer 2	-2.21	Signaling	
AA165664	UGCG	UDP-glucose ceramide glucosyltransferase	-2.23		
AA598526	HIF1A	Hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	-2.28	Transcription	Hata 2008 (37)
W42723	CXCL1	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	-2.37	Immunity	Ishmael 2008 (39)
AA485373	TMEM49	Transmembrane protein 49	-2.38		
AA457138	FZD8	Frizzled homolog 8 (Drosophila)	-2.38	Signaling	
AA026120	BHLHB2	Basic helix-loop-helix domain containing, class B, 2	-2.40	Transcription	
T97782	SLC2A14	Solute carrier family 2 (facilitated glucose transporter), member 14	-2.41	Transport	
AA424804	RCBTB2	Regulator of chromosome condensation (RCC1) and BTB (POZ) domain containing protein 2	-2.42		
AI262129	POSTN	periostin, oseoblast specific factor	-2.44	Cell adhesion	Woodruff 2007 (44)
AA456366	JUNB	Jun B proto-oncogene	-2.45	Transcription	Hansson 2008 (36)
AA457705	IER3	Immediate early response 3	-2.47	Immunity	

Accession No.	Symbol	Name	fold change	GO Classification	Regulated by corticosteroid treatment
AA486533	EGR1	Early growth response 1	-2.51	Transcription	Ishmael 2008 (39)
AA496359	IER2	Immediate early response 2	-2.51		
AA446120	ADM	Adrenomedullin	-2.56	Signaling	de Kruif 2008 (35)
AA723035	ZFP36L1	Zinc finger protein 36, C3H type-like 1	-2.58	Transcription	
AA425102	CCL2	Chemokine (C-C motif) ligand 2	-2.59	Immunity	Ansari 2007 (33), Lund 2007 (40), Ishmael 2008 (39)
N94468	JUNB	Jun B proto-oncogene	-2.60	Transcription	Hansson 2008 (36)
AA495759	ZNF652	Zinc finger protein 652	-2.65	Transcription	
W47003	HIF1A	Hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	-2.73	Transcription	Hata 2008 (37)
AA877213	CYP24A1	Cytochrome P450, family 24, subfamily A, polypeptide 1	-2.75	Oxidation reduction	
T77817	CCL2	Chemokine (C-C motif) ligand 2	-2.80	Immunity	Ansari 2007 (33), Lund 2007 (40), Ishmael 2008 (39)
R20750	FOS	V-fos FBJ murine osteosarcoma viral oncogene homolog	-3.09	Immunity	Chen 2008 (34)
AI927438	HBB	Hemoglobin, beta	-3.22	Oxygen transport	Woodruff 2007 (44)
AI628353	TBC1D9	TBC1 domain family, member 9 (with GRAM domain)	-4.76	Rab GTPase acitivity	
AI383157	NRXN2	Neurexin 2	-4.85	Cell adhesion	
AA481758	DNAJB1	DnaJ (Hsp40) homolog, subfamily B, member 1	-4.95	Protein folding	
AI095024	SNAPC2	Small nuclear RNA activating complex, polypeptide 2, 45kDa	-5.12	Transcription	

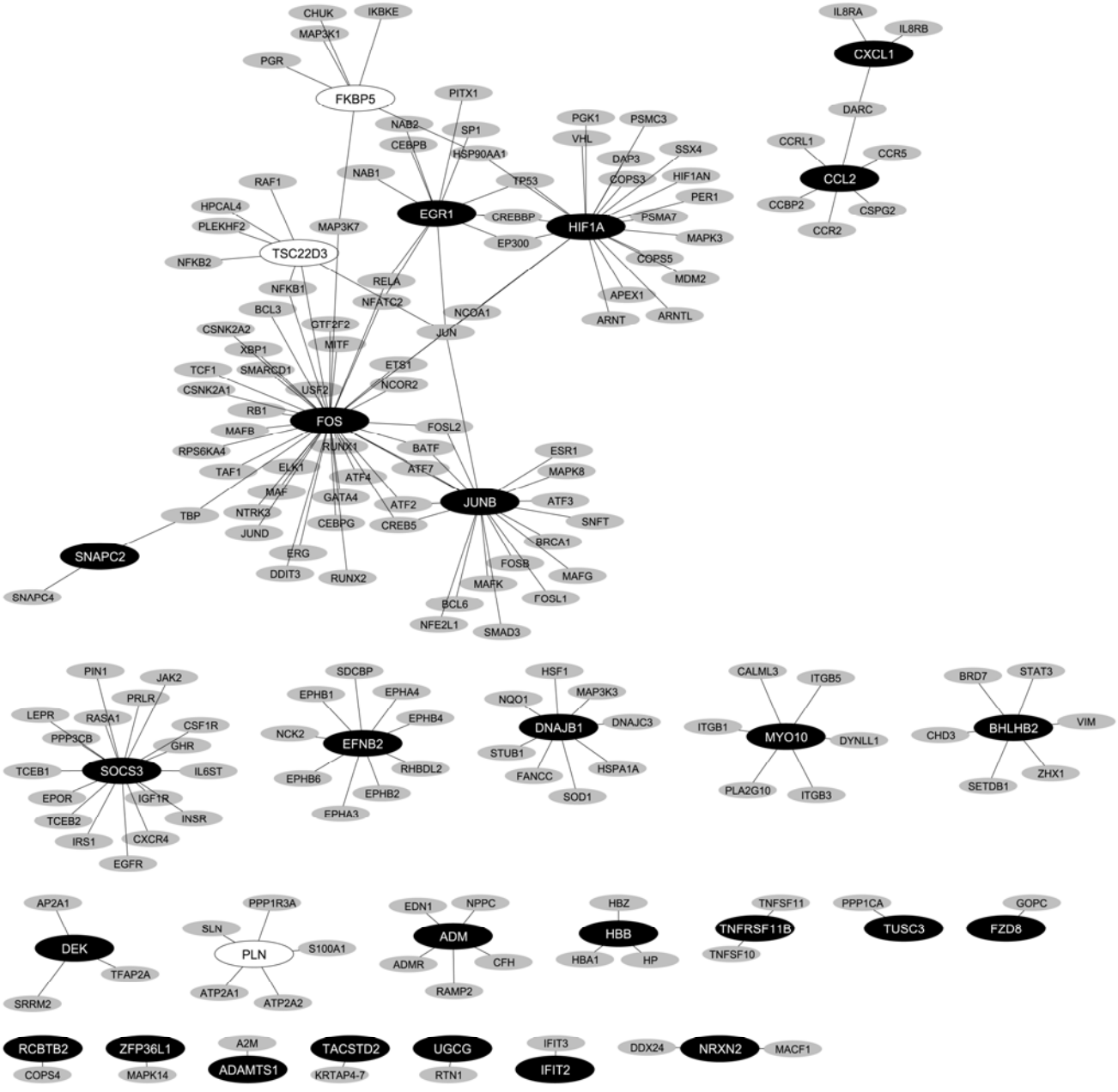


Appendix Figure 1. Gene Ontology (GO) classification of the differentially expressed genes (DEGs) with a fold change over two.

Interactome Analysis

Twenty-eight genes (25 DEGs down-regulated and 3 DEGs up-regulated in steroid group) of the 46 significantly DEGs (39 down-regulated and 7 up-regulated) have at least one interacting partner according to Online Predicted Human Interaction Database. The initial list of 28 genes could therefore be extended, thus including all interacting protein forming the respective interaction network. The resulting interaction graph gave 193 nodes and 187 edges (Appendix Figure 2). Seven (five DEGs down-regulated and two DEGs up-regulated) of the 28 genes derived from expression analysis could be detected in the largest subnetwork. FOS (V-fos FBJ murine osteosarcoma viral oncogene homolog), JUNB (Jun B proto-oncogene), EGR1 (Early growth response 1), HIF1A (Hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor) and SNAPC2 (Small nuclear RNA activating complex, polypeptide 2, 45kDa) are down-regulated genes and FKBP5 (FK506 binding protein 5) and TSC22D3 (TSC22 domain family, member 3) are up-regulated genes in the largest subnetwork. Also two chemokines are down-regulated by steroid treatment and are connected over only one interaction partner. CCL2

(Chemokine (C-C motif) ligand 2) and CXCL1 (Chemokine (C-X-C motif) ligand 1) play important roles in the chemokine mediated signaling pathway and thus in the inflammation cascade.



Appendix Figure 2. Protein-protein interaction network of significantly DEGs with a fold change over two, respectively. Black nodes (25 DEGs) indicate down-regulated genes and white nodes (three DEGs) up-regulated genes with corticosteroid use. Gray nodes represent proteins identified by the nearest neighbour expansion method.