Supplemental data to article

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

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Genomics and proteomics

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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://bioinformatics.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.

<table>
<thead>
<tr>
<th>Accession No.</th>
<th>Symbol</th>
<th>Name</th>
<th>fold change</th>
<th>GO Classification</th>
<th>Regulated by corticosteroid treatment</th>
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<tr>
<td>W86653</td>
<td>FKBP5</td>
<td>FK506 binding protein 5</td>
<td>4.88</td>
<td>Protein folding</td>
<td>Hubler 2004 (38), Woodruff 2007 (44)</td>
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<td>Ring finger protein 186</td>
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<td>Protein binding</td>
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<td>AA775091</td>
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<td>TSC22 domain family, member 3</td>
<td>3.03</td>
<td>Transcription</td>
<td>Woodruff 2007 (44)</td>
</tr>
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<td>AI686837</td>
<td>PLN</td>
<td>Phospholamban</td>
<td>2.54</td>
<td>Blood circulation</td>
<td>McTiernan 1997 (42)</td>
</tr>
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<td>AA910933</td>
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<td>Transport</td>
<td></td>
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<tr>
<td>AA971563</td>
<td>SGSM3</td>
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<td>2.50</td>
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<td>AA194983</td>
<td>TNFRSF11B</td>
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<td>Apoptose</td>
<td>Makrygiannakis 2006 (41)</td>
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<td>Accession No.</td>
<td>Symbol</td>
<td>Name</td>
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<td>GO Classification</td>
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<td>H70774</td>
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<td>Defense</td>
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<td>CCL2</td>
<td>Chemokine (C-C motif) ligand 2</td>
<td>-2.12</td>
<td>Immunity</td>
<td>Ansari 2007 (33), Lund 2007 (40), Ishmael 2008 (39)</td>
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<td>Suppressor of cytokine signaling 3</td>
<td>-2.13</td>
<td>Anti-Apoptose</td>
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<td>AA706968</td>
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<td>ZW10 interactor antisense</td>
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<td>N63988</td>
<td>IFIT2</td>
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<td>AA057170</td>
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<td>H70961</td>
<td>NFKBIZ</td>
<td>Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta</td>
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<td>Immunity</td>
<td>Ishmael 2008 (39)</td>
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<tr>
<td>AA445810</td>
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<td>Tumor-associated calcium signal transducer 2</td>
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<td>Signaling</td>
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<td>AA165664</td>
<td>UGCG</td>
<td>UDP-glucose ceramide glucosyltransferase</td>
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<tr>
<td>AA598526</td>
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<td>Immunity</td>
<td>Ishmael 2008 (39)</td>
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<td>AA485373</td>
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<td>Transmembrane protein 49</td>
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<td>AA457138</td>
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<td>Frizzled homolog 8 (Drosophila)</td>
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<td>AA026120</td>
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<td>AA424804</td>
<td>RCBTB2</td>
<td>Regulator of chromosome condensation (RCC1) and BTB (POZ) domain containing protein 2</td>
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<td>AI262129</td>
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<td>Cell adhesion</td>
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<td>Transcription</td>
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<td>IER3</td>
<td>Immediate early response 3</td>
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<td>Accession No.</td>
<td>Symbol</td>
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<td>AA486533</td>
<td>EGR1</td>
<td>Early growth response 1</td>
<td>-2.51</td>
<td>Transcription</td>
<td>Ishmael 2008 (39)</td>
</tr>
<tr>
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<td>IER2</td>
<td>Immediate early response 2</td>
<td>-2.51</td>
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<tr>
<td>AA446120</td>
<td>ADM</td>
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<td>Signaling</td>
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<td>AA723035</td>
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<td>-2.58</td>
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<td>-2.65</td>
<td>Transcription</td>
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<td>W47003</td>
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<td>-2.73</td>
<td>Transcription</td>
<td>Hata 2008 (37)</td>
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<td>AA877213</td>
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<td>-2.75</td>
<td>Oxidation reduction</td>
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<td>-2.80</td>
<td>Immunity</td>
<td></td>
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<tr>
<td>R20750</td>
<td>FOS</td>
<td>V-fos FBJ murine osteosarcoma viral oncogene homolog</td>
<td>-3.09</td>
<td>Immunity</td>
<td>Chen 2008 (34)</td>
</tr>
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<td>AI927438</td>
<td>HBB</td>
<td>Hemoglobin, beta</td>
<td>-3.22</td>
<td>Oxygen transport</td>
<td>Woodruff 2007 (44)</td>
</tr>
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<td>AI628353</td>
<td>TBC1D9</td>
<td>TBC1 domain family, member 9 (with GRAM domain)</td>
<td>-4.76</td>
<td>Rab GTPase activity</td>
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<tr>
<td>AI383157</td>
<td>NRXN2</td>
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<td>-4.85</td>
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<td>AA481758</td>
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<td>DnaJ (Hsp40) homolog, subfamily B, member 1</td>
<td>-4.95</td>
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<td></td>
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<tr>
<td>AI095024</td>
<td>SNACP2</td>
<td>Small nuclear RNA activating complex, polypeptide 2, 45kDa</td>
<td>-5.12</td>
<td>Transcription</td>
<td></td>
</tr>
</tbody>
</table>
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.