Roter Faden für das Manuskript

Transcriptional analysis of genes and pathways involved in renal organ protection in response to preconditioning

1. We used an accepted ischemia reperfusion model from the scientific literature and were able to show statistically significant differences in kidney function parameters (BUN, s-creatinine) and a trend to a better survival in response to hypoxic preconditioning and caloric restriction.
2. Furthermore we found statistically significant differences in histological damage. A pathologist with expertise in kidney diseases examined slides in a blinded fashion??
3. After sequencing healthy kidneys from control mice and mice treated with hypoxic preconditioning and caloric restriction we found an overlap of 51 genes, which were significantly regulated in the same direction in all conditions. The cut-off used was p adj < 0,05 and no foldchange cut-off.
4. In GO-term and pathway analysis we found overlapping terms:
    a. The KEGG pathway mmu0860: Porphyrin and chlorophyll metabolism
        i. The number of genes involved in this pathway was 10 / 9 / 4 for CR2, CR4 and HP, respectively.
        ii. Genes involved in all three conditions were Hmox1, Gusb
        iii. This pathway appeared consistently also under different cut-off conditions (padj <0,1, FC > 1,5, FPKM 39,81 / padj <0,1, FC > 1,5, no FPKM / padj <0,05, no FC, no FPKM)
        iv. Analysis of the gene list for above mentioned cut-off (padj < 0,05, no FC) with the ClueGO tool resulted in
            1. Porphyrin and chlorophyll metabolism
            2. Primary bile acid biosynthesis

            being the only significantly overlapping terms between CR2, CR4 und HP (p-value, enrichement …?!)
    b. For PQR
        i. GO-Terms for Biological process: cofactor metabolism, oxidation-reduction process, pigment metabolism, tetrapyrrole metabolism, transmembrane transport, vitamin metabolism
        ii. GO-Terms for Cellular component: cell fraction, endoplasmic reticulum, insoluble fraction, membrane fraction, microbody, microsome, mitochondrial matrix, mitochondrion, peroxisome, vesicular fraction
        iii. GO-Terms for Molecular function: cofactor binding, electron carrier activity, exopeptidase activity, heme binding, ion binding, iron ion binding, tetrapyrrole binding
5. The data acquired could be confirmed in a qPCR experiment on kidneys from the same conditions mentioned in 1).
6. In the qPCR data no organ overlap with liver and kidney could be shown.
    a. The lacking overlap between tissues might be at least partially due to technically problems (tissue specific pPCR / RT inhibition, small number of replicates for each group)
    b. Although not statistically significant 4 of the genes were regulated in the same direction in all tissues and conditions. (Up-regulated: Hao2, Slc7a12; Down-regulated: Cyp4a12a, Kif20b)
        i. A nonspecific CYP inhibitor, miconazole, inhibited 20-HETE production during ischemia–reperfusion and produced a profound reduction in myocardial infarct size (expressed as a percent of the area at risk) (19.6±1.7% [control], 8.4±2.5% [0.96 mg/kg miconazole] [DOG MODEL; application intra coronary]

Nithipatikom, K., Gross, E. R., Endsley, M. P., Moore, J. M., Isbell, M. A., Falck, J. R., et al. (2004). Inhibition of Cytochrome P450ω-Hydroxylase. Circulation.

7. FUNCTIONAL GENE RELEVANCE
   The extend of gene regulation (foldchange) for the genes we identified correlates with the outcome of the animals (survival, weight loss/gain, creatinine & urea values, recovery score, …)??
8. We found genes upregulated in control kidneys 24h after ischemia reperfusion injury to largely overlap with the 51 genes identified in response to preconditioning.

ClueGo settings und log-file

Selection Citeria:
Statistical Test Used = Enrichment/Depletion (Two-sided hypergeometric test)
Correction Method Used = Bonferroni step down
Min GO Level = 7
Max GO Level = 15
All GO Levels = false
Cluster #1
Sample File Name = File selection: ManuallyAddedOrModifiedIDs
Number of Genes = 2
Get All Genes = false
Min Percentage = 8.0
Get All Percentage = false

Cluster #2
Sample File Name = File selection: ManuallyAddedOrModifiedIDs
Number of Genes = 2
Get All Genes = false
Min Percentage = 8.0
Get All Percentage = false

Cluster #3
Sample File Name = File selection: ManuallyAddedOrModifiedIDs
Number of Genes = 2
Get All Genes = false
Min Percentage = 8.0
Get All Percentage = false

Combine Clusters With 'Or' = false
Percentage for a Cluster to be Significant = 60.0
GO Fusion = true
GO Group = true
Kappa Score Threshold = 0.4
Over View Term = SmallestPValue
Group By Kappa Statistics = true
Initial Group Size = 2
Sharing Group Percentage = 50.0

ClueGO Log:
All Results were created with ClueGO v2.1.3
Identifier types used: null
Evidence Codes used: [All]
#Genes in KEGG_26.08.2014 : 7563
#Genes in GO_MolecularFunction_22.08.2014_15h04 : 22852
#Genes in GO_CellularComponent_22.08.2014_15h04 : 23267
#Genes in GO_BiologicalProcess_22.08.2014_15h04 : 23333

#All unique Genes: 24053
Total # of Genes from Cluster#1 2171, with 313 (14,42%) missing!
Total # of Genes from Cluster#2 2121, with 306 (14,43%) missing!
Total # of Genes from Cluster#3 81, with 10 (12,35%) missing!
#All Genes found from initial Cluster#1 (1858.0): 1847.0 (99,41%)
#All Genes found from initial Cluster#2 (1815.0): 1804.0 (99,39%)
#All Genes found from initial Cluster#3 (71.0): 71.0 (100,0%)
#All Genes found from 3 initial Cluster(s) (3744.0): 2465 (65,84%)
#Genes found from all Clusters after selection: 67 (1,79%)