Supplementary material: Detailed microarray experiment and data analysis procedure.

Experimental design

Two independent gene chip analysis have been performed: (i) a time course analysis to identify the effect of a trans-aortic constriction (TAC) on the left ventricle and (ii) a comparison of TAC effect between wild-type and mice harboring a ventricular-restricted knock-out of the gp130 cytokine-receptor (gp130CKO, Hirota et al., 1999). For the first analysis, the time point 0 hour (non operated), 1 h, 5 h, 20 h, 4 days and 14 days were investigated. Sham operated mice were analyzed for the early time points (1 h. 5 h and 20 h). In the second analysis, wild-type, knock-out and respective sham-operated animals were compared 4 days after TAC. For each experimental group, 4-5 animals were operated and 2 were chosen for DNA chip analysis according to the level of pressure overload (surgically created trans-aortic gradient should be higher than 45 mmHg), the activation of signaling pathways or the lack of STAT3 activation in the case of gp130CKO, and the development of similar hypertrophic responses (Supplementary data Table IV and V).

Samples used, extract preparation and labeling and Hybridization procedures and parameters

Total RNA integrity from mouse left ventricle after TAC was assessed by capillary electrophoresis and spectrophotometry. Microarray analysis was performed as described in detail at http://www.genomics.uci.edu/DNA_array_core/Affymetrix.html. Briefly, cRNA was prepared from 8µg of total RNA, hybridized to MG-U74Av2 Affymetrix oligonucleotide arrays, scanned, and analyzed according to Affymetrix (Santa Clara, CA) protocols.

Measurement data and specifications

Scanned image files were visually inspected for artifacts and normalized to make comparisons across GeneChips using Affymetrix Microarray Suite software 5.0 (AMS 5.0). Detailed protocols for data extraction from Affymetrix oligonucleotide arrays and documentation on the sensitivity and quantitative aspects of the method have been described (Lipshutz et al., 1999).

Data Analysis

After combining AMS 5.0 Signals and Detection Call from each microarray, gene filtering was performed. For the acute phase (1 hour, 5 hours and 20 hours after TAC), Probe Sets were compared with data from corresponding sham mice. For the late phase, (4 days and 14 days after TAC), Probe Sets were compared with data from untreated mice. To be filtered, a Probe Set had to follow three criteria for at least one time point: (i) a 2-fold change cut off for the mean value, (ii) a difference between mean value and corresponding control mean value greater than 300, (iii) a Present call by AMS 5.0 in the 2 duplicate arrays from either banded or control.

In order to compare the gene expression data from gp130 heart restricted knock-out and wild-type mice, we first identified the Probe Sets showing a regulation after banding in wild-type or knock-out animals using the criteria listed above. Considering these Probe Sets affected by TAC, we then filtered the genes differentially regulated between wild-type and knock-out animals using the following criteria: (i) a 1.5-fold change cut off for the mean value, (ii) a difference between mean value and corresponding control mean value greater than 300, (iii) a Present call by AMS 5.0 in the 2 duplicate arrays from either banded or control.

Although these criteria were somewhat arbitrary, they served as an effective mean to identify a small group of genes with consistent differential expression. Cluster 2.11 and TreeView 1.5 were used to cluster and visualize the filtered data (Eisen et al., 1998). Pearson correlation metric (uncentered) and average linkage clustering on log transformed and normalized data was used. Hierarchical clustering analysis of arrays (before gene filtering) shows that the variability between time points is bigger than the variability between duplicates (data not shown).

References

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