**Materials and Methods:**

**Human tissues**
Use of human tissue samples was approved by the local ethics committee, and informed consent was obtained from all patients. For all three cell types, material was prepared from three different individuals, respectively.

**HSC and PSC isolation and culture**
HSC and PSC isolation and culture was done as previously described (1; 2). For HSC isolation, small tissue blocks (100 - 150 mg) were obtained from liver cirrhosis patients undergoing liver transplantation. For PSC isolation, tissue blocks were obtained from chronic pancreatitis (2 patients) or tumor-free fibrotic resection margins (1 patient) during pancreatectomy. The tissue blocks were cut (0.5-1 mm³) and seeded in 10 cm² uncoated culture wells (6 plate, 3-5 pieces/well) in the presence of 10% fetal calf serum in a 1:1 (v:v) mixture of Dulbecco’s modification of Eagle’s medium (DMEM) with Ham’s F12 medium. L-glutamine (2 mmol/l), penicillin/streptomycin and amphotericine were freshly added. Tissue blocks were cultured at 37°C in a 5% CO₂-air humidified atmosphere. Eighteen hours after seeding, culture medium was changed and 24 hours later the small tissue blocks were transferred to new culture plates. The stellate cells grew out in high number and purity from the tissue blocks one to three days later. The small tissue blocks were removed after 2-3 weeks. After reaching confluence, monolayers were trypsinized and passaged 1:3. Purity of the cells was assessed by morphology and cytofilament stainings of alpha-smooth-muscle actin, vimentin and desmin. Cell populations between passage 4 and 6 with 80 % confluence were used for this study.

**Skin fibroblast isolation and culture**
Primary skin fibroblasts were isolated by the outgrowth method using foreskin tissues from children. Full-thickness foreskin tissues were cut into small pieces of 0.5 ~ 1 mm³ using a razor blade under sterile conditions. The pieces were placed in 6-well plates (5~10 pieces/well) in 4 ml DMEM/Ham’s F-12 supplemented with 10% FCS. The plates were incubated under standard culture conditions (humidified 5% CO₂, 37°C). Medium was changed after 12 h. After additional 12 h, the small tissue blocks were transferred to new culture plates and allowed to settle and adhere to the bottom of the plates. During the next 3-5 days, cells grew out from the tissue blocks and formed a confluent layer. After reaching
confluence, monolayers were trypsinized and passaged 1:3. Cell populations between passage 4 and 6 with 80% confluence were used for this study.

**Microarrays**

The Human Genome Oligo-Set-Version 2.0 (Operon, Germany) representing 21,329 genes in the form of optimized 70-mer oligonucleotides was spotted onto GAPSII Slides (Corning, USA) using a OmniGrid Microarrayer (GeneMachines, San Carlos, USA), equipped with Stealth SMP3 Micro Spotting Pins (Telechem, Sunnyvale, CA, USA) at the Chip-Facility of the University of Ulm. Printing concentration of the oligos was 40µM in 3xSSC, 1.5M Betain. Information about each oligo and its representative gene is available online at http://www.operon.com/arrays/omad.php.

Oligonucleotides were immobilized on the slides by 15 min incubation at 80°C, followed by irradiation with UV light at 254 nm with an energy output of 120mJ/cm² in a Stratalinker Model 2400 UV illuminator (Stratagene).

**RNA amplification, labeling and hybridization**

In order to obtain sufficient amounts of RNA for hybridization, RNA was linearly amplified using the MessageAMP™ aRNA Kit (Ambion, Woodward, Austin, USA) according to the manufacturers instructions. For each linear amplification procedure 5-50 ng of purified total RNA was in vitro transcribed using 3.75 mM UTP and 3.75 mM 5-(3-aminoallyl)-UTP. The quality and quantity of the total and amplified RNA samples was determined with a 2100 Bioanalyzer (Agilent Technologies, Paolo Alto, California, USA). Each sample was amplified and hybridized in duplicate.

For each microarray hybridisation 700 ng of amplified aminoallyl RNA was resuspended in 4.5 µl 0.15 M sodium carbonate buffer (pH 9.0) and coupled with 4.5 µl Cy5 monoreactive dye (Amersham Biosciences, Uppsala, Sweden) prepared in 45 µl DMSO for 1 h at room temperature in the dark. The reaction was quenched by adding 4.5 µl 4 M hydroxylamine and incubation for 15 min at 25°C in the dark. Uncoupled dyes were removed by RNeasy-Kit (Qiagen, Hilden, Germany) and the final volume adjusted to 30 µl.

Reference cDNA was reverse transcribed from 10 µg of universal human reference RNA (Stratagene, La Jolla, CA) using Superscript III Reverse Transcriptase (Invitrogen, Carlsbad, CA), oligo-d(T)-primers and dTTP/oligoallyl-dUTP at a ratio of 1:2 according to the manufacturers instructions. The aminoallyl cDNA was coupled with 4.5 µl Cy3 monoreactive dye (Amersham Biosciences, Uppsala, Sweden) and purified as described above.
Following pre-incubation for 1 h at 42°C in prehybridization buffer (3x SSC, 0.25% SDS, 1% BSA), slides were denatured at 75°C for 1 min. 30 µl each of the labeled experimental and reference samples were hybridized for 14-18 h at 37°C in hybridization buffer (3x SSC, 0.25% SDS, 0.3 µg/µl poly dA, 0.5 µg/µl yeast tRNA, 1 µg/µl salmon sperm DNA) using a GeneTac hybridization chamber (Genomic Solutions, Cambridgeshire, United Kingdom). Slides were washed three times in 2x SSC, 5% formamide, 0.1% Tween 20 (pH 7.0) at 37°C, once in 1x PBS (pH 7.4), 0.05% Tween 20 (pH 7.0) at 25°C, once in 1x PBS (pH 7.4), 0.1% Tween 20 (pH 7.0) at 25°C for 3 min and once in 0.5x PBS (pH 7.4), 0.05% Tween 20 (pH 7.0) at 25°C for 5 min in the dark. Slides were dried in a centrifuge by spinning for 5 min at 1250 x g.

Image and Data analysis

Hybridization signals were visualized using a dual laser scanner (Axon 4000B) and analyzed with GenePix Pro 4.0 imaging software (Axon Instruments, Union City, CA). On visual inspection, spots of insufficient quality were excluded from further analysis. For the purposes of this study, we analyzed the signal intensities from the Cy5 channel only to avoid loss of data points due to low signal intensities in the reference channel. To correct for differences between the microarray slides and for gradients within a slide, a block normalisation was performed. Following local background correction, signal intensities were normalized to the average of medians of all spots within individual 4-by-4 subarray blocks on each slide. Blocknormalized expression values of all individual hybridizations are available as part of the supplementary data.

Print-tip LOESS-normalized ratios of experimental and common reference samples obtained with the LIMMA software package (3) for comparison with external data sets are available as part of the supplementary data.

Genes were defined as differentially expressed between two sets of samples if 1) the mean normalized expression value exceeded 0.5 in at least in one of the two sample sets; 2) the difference between the mean normalized expression values was at least two-fold between the sample sets; and 3) a two-sided T-test yielded a p-value of p<0.05. Lists of genes differentially expressed between the different sample sets are available as part of the supplementary data.

To further analyze the biological function of differentially expressed genes, the GoMiner package (4) was used to organize lists of genes for biological interpretation in the context of the Gene Ontology (GO) data base. The list of differentially expressed genes was compared
with the list of all genes exceeding a minimal expression threshold (normalized expression value greater 0.5 in at least one of the sample sets) to identify GO terms statistically significantly overrepresented among the differentially expressed genes. Since the tree format provided by GoMiner to visualize the results of the analysis is not suited to display the overlap of genes in different GO categories resulting from the association of genes with multiple GO terms, we have developed a new software tool termed 'VennMaster' (http://www.informatik.uni-ulm.de/ni/mitarbeiter/HKestler/vennm) which facilitates visualization of associations between GO categories based on the evaluation of genes mutually represented in different categories. VennMaster displays GO categories in the form of a Venn diagram, where each category is displayed as a polygon sized according to the number of differentially expressed genes contained within the category, and polygons overlap proportionally if the represented categories share differentially expressed genes.

Unsupervised hierarchical cluster analysis of expression profiles was performed using the ‘Cluster’ and ‘TreeView’ software tools (5).

**Quantitative real-time PCR analysis**

The same RNA preparations that were used for expression profiling were used for validation of the expression data by real time PCR. cDNA was reverse transcribed from 5 µg of total RNA using Superscript III Reverse Transcriptase (Invitrogen, Carlsbad, CA) and random decamers according to the manufacturers instructions. Quantitative real time PCR analyses using the comparative C<sub>T</sub> method were performed on an ABI PRISM 7700 Sequence Detector System using the SYBR Green PCR Master Mix kit (Perkin Elmer, Applied Biosystems, Wellesley, MA, USA) according to the manufacturers instructions. Following initial incubation at 50 °C for 2 min and 10 min at 95 °C, amplification was performed for 40 cycles at 95 °C for 15 sec and 60 °C for 1 min. Specific primer pairs were determined with the PrimerExpress® program (Applied Biosystems). The human cyclophilin A gene (RefSeq ID NM_021130) was used as the internal standard.
References


