

Supplementary Materials and Methods

Cell culture and clonogenesis assay

HepG2, Hep3B, Huh-6, Huh-7(1), BC1, BC2, B16A2(2), HCT116 and HT29 cell lines were cultured as described(3). HepaRG cells were expanded as liver progenitors and differentiated to hepatocytes as described(4). Clonogenesis assays were performed as described by transfection of SFRP1 or SFRP5 cDNA plasmids(1, 3) with Lipofectamine Plus (Invitrogen). Cells were plated in triplicates on 100-mm tissue culture dishes and incubated for 14 days with G418. After hematoxylin staining, colonies (seen as dark spots) were digitized using a video camera. Image analysis was performed using ImageJ (NIH).

Nucleic acid extraction from human tissues and from in vitro cultures

RNA was extracted from 10 μ m cryosections obtained from frozen tissue blocks. Once tissues were placed on the specimen head of a Leica 3050S cryostat and trimmed to obtain a level and histologically controlled representative surface (hematoxylin or toluidine blue extemporaneous staining), the first two 5 μ m sections were set aside as permanent histological controls. Then, 10 μ m cryosections were obtained and immediately lysed in extraction buffer. Before removing the disk containing the specimen from the head, two 5 μ m cryosections were set aside again as permanent histological controls. Lead and rear permanent histological controls were formalin-fixed, HE-stained in a Leica ST5020 routine stainer and representativity was assessed by two independent observers (BT & OM). Non-representative samples were removed from the RNA and DNA extraction process. Representative samples were extracted with NucleoSpin® RNA II (Macherey-Nagel)

kit, as recommended by the manufacturer. Quality control of the extracted nucleic acids was performed by dosing the eluents with a NanoDrop spectrophotometer and by checking 28S/18S ratios by gel electrophoresis. For in vitro cultured cell layers, total RNA was purified with an RNeasy kit (Qiagen). Extracted RNA quality control was done as for human tissues.

Real-time PCR

Reverse-transcribed cDNA was obtained with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to manufacturer's instructions. Expression of relevant genes was measured by quantitative real-time PCR using the StepOnePlus™ System or the ABI prism 7900HT, power SYBR Green PCR master mix or Taqman probe-based assays (Applied Biosystems), according to the experiments. See Supplementary Table 1 for real-time PCR primers and TaqMan probes. Quantitative analyses of PCR data were conducted by the $2^{-\Delta\Delta C_t}$ method using housekeeping gene Ct values for normalization.

Tissue microarray (TMA) and immunohistochemistry scoring of human tissues

Selection of tissue blocs for TMA construction: The whole set of archival HE-stained sections from formalin-fixed, paraffin-embedded tissue blocks from each one of the 67 available HCC patients and from five histologically normal livers were reviewed (Nikon 80i microscope) to ensure that the selected paraffin bloc was the mirror lesion of the frozen sample. A region of interest (ROI, 1 cm in diameter) was labeled with a permanent marker to precisely localize the punch zone.

TMA construction, immunohistochemistry and signal scoring: TMA design and construction were done with a MiniCore3 tissue arrayer equipped with a MiniCore control station and TMADesigner2 tissue array design software (Alphelys, Plaisir, France). ROIs were punched in triplicate from the 67 available paraffin blocs from HCCs and from five histologically normal liver controls. Punch size was set at 1000 µm in diameter. HCC samples were randomly assigned to either one of two 132-punch receiver paraffin tissue blocks. Randomization was performed with a “*rand*” function in Microsoft Excel. The five histologically normal liver controls were included in both array blocs #1 and #2 to control for signal and background intensities. Five-µm microtome sections were processed for immunohistochemistry with a Discovery XT from Ventana Medical Systems (Roche) research slide staining system. See Supplementary Table 2 for antibodies used. Stained slides were converted into high-resolution digital data with a NanoZoomer digital slide scanner (Hamamatsu, Massy, France). Digital slides were viewed using NDP.view software (Hamamatsu) in a Dell S2415H 24-Inch Screen LED-Lit Monitor. The signal was independently read by two observers (RD and OM) who were blinded to any information. Discrepancies were resolved by consensus reading. A four-point scale (0-1-2-3-4) denoting increasing signal intensity was used.

Supplementary Figure legends:

Supplementary Fig. 1. (A) SFRP5 mRNA expression data extracted from the GSE49910 microarray meta-analysis dataset consisting of 745 primary cell samples analyzed with Affymetrix Human Genome U133 Plus 2.0 expression arrays. Normalized background-corrected intensities(5) of mRNA expression were filtered to reveal the cell lines expressing the highest mRNA levels of SFRP5 in at least duplicate samples. As SFRP5 was expressed at low levels, filtering was done using the median as cutoff. Cell lines below the defined threshold but originally used as controls of at least one cell line above the cutoff value were included for comparative purposes. Threshold lines for each gene are indicated (-----). (B) Generation of hepatocyte-like cells from fibroblast-derived undifferentiated iPS cells, as described(6). SFRP5 is shown in human foreskin fibroblasts; foreskin fibroblast-derived iPS cells; iPS cells undergoing hepatocyte differentiation; human fetal liver and primary human hepatocytes

Supplementary Fig. 2. (A) Expression of the five SFRP family members in normal liver (n=7) by real-time PCR. RNA expression values were calculated by the $\Delta\Delta C_t$ method using the 18S housekeeping gene as an internal standard. (B) Raw threshold cycle (C_t) values from real-time PCR experiments for the indicated SFRPs in seven normal human liver samples. Real-time PCR assays were performed in duplicate. For each SFRP gene, the mean \pm SD of C_t s are indicated, as well as the variation coefficient (VC). 18S expression values used for normalization are also shown. (C) Histogram showing relative C_t values from (A). Statistical significance of the C_t differences between the SFRP genes was calculated by Kruskal-Wallis ANOVA. (D)

Kruskal-Wallis post-hoc test showing P values for inter-gene differences. Statistically significant values are indicated in red.

Supplementary Fig. 3. Restoring SFRP1 and SFRP5 expression decreases clonogenesis in liver cancer. (A and B) Real-time-PCR-assessed mRNA expression of SFRP1 (A) and SFRP5 (B) in the liver cancer cell lines HepaRG, Hep3B, BC1, BC2, Huh-6, B16A2, HepG2 and Huh-7 and the colon cancer cell lines HT29 and HCT116. SFRP1 and 5 mRNA expressions are dramatically lower in liver and colon cancer cell lines than in normal liver tissue. (C) Huh-7 human hepatocellular carcinoma cells (WT β -catenin) stably transfected with the indicated expression vectors were seeded at low density and selected for 14 d with G418. After paraformaldehyde fixation and hematoxylin staining, colonies (seen as dark spots) were digitized using a video camera. (D) Clonogenesis was assessed with ImageJ (NIH). Histograms show colony size and number in cells transfected with SFRPs or empty vector.

Supplementary Fig. 4. Expression of the indicated genes in 82 **HCCs**; **C**, 23 histologically normal liver controls; **NT**, 66 matching non-tumor livers and **FNH**, 11 focal nodular hyperplasias. Charts show mean \pm confidence intervals. P = Non parametric ANOVA. Asterisks, Kruskal-Wallis post-hoc test: *, $P<0.05$; **, $P<0.01$ and ***, $P<0.001$, comparing SFRP1 and SFRP5 mRNA levels in **HCC** versus the other groups and FZD1, FZD7, COL1A1 and WNT3 mRNA levels in **C** versus the other groups.

Supplementary Fig. 5. Relative mRNA levels for SFRP2 (A), DKK1 (B), ACTA2 (C), WNT2 (D) and LAMC1 (E) assessed by real-time PCR in 82 **HCCs**; **C**, 23 histological normal liver controls; **NT**, 66 matching non-tumor livers and **FNH**, 11 focal nodular hyperplasias. Charts show mean \pm confidence intervals. Non parametric ANOVA and post-hoc tests denote the statistical inter-group differences. Significant differences are highlighted in red in the tables below each chart, when applied. For DKK1, there is no significant difference between the four groups taken together. However, HCCs are significantly higher than controls by Mann-Whitney U test, as shown in (B).

Supplementary Table legends:

Supplementary Table 1: Sybr Green primers and TaqMan probes.

Supplementary Table 2: Primary antibodies.

Supplementary Table 3: Description of the HCC patient cohort from Rennes University Hospital.

Supplementary Table 4: Unsupervised Gene Set Enrichment Analysis (GSEA) report showing the top 20% signatures from the Broad Institute Gene Set Database matching with the mesenchymal-lineage committed human hepatocellular carcinoma cell line 3SP(7) (GSE26391). MsigDB, Molecular signature Data Base; *SIZE*, number of genes included in each signature; NES, normalized enrichment score. Molecular signature family names are indicated on the *left*.

Supplementary Table 5: (A) Spearman rank-order correlation coefficients between the indicated genes in histologically normal liver samples, focal nodular hyperplasias (FNH), hepatocellular carcinomas (HCC) and matching non-tumor liver samples. The number of samples analyzed in each group is indicated. Cells show the correlation coefficient (R) between genes. Asterisks denote statistical significance: *, <0.05; **, <0.01; ***, <0.001. (B) Spearman rank-order correlation coefficients in 47 HCCs from an independent, previously described sample collection(8). Gene and protein expression as well as MMP2 enzymatic activity were assessed as described(8). Statistically significant values were highlighted in red. The false discovery rate was adjusted by the Benjamini-Hochberg procedure for multiple comparisons. *NS*, non-significant.

Supplementary Table 6: Description of the publically available HCC patient cohort from Roessler et al.(9). *NA*, not available. Staging and scores are indicated in increasing order.

Supplementary Table 7: Clinical variables associated with high Recurrence Index (RI) in a public mRNA expression dataset including tumor tissue (GSE14520) and clinical annotations (Supplementary Table 6) from 247 patients with HCC treated by surgical resection(9). Using the centile 0.75 of this index (RI>62), the 247-patient dataset was split into high (RI>62) and low (RI<62) risk groups. The *Metastasis Signature*(9) is a 161-gene risk classifier enabling prediction of tumor relapse in early-stage HCC patients. *NA*, number of patients for whom data were not available for the specified variable. *P* values were corrected by the Benjamini-Hochberg procedure for multiple tests.

Supplementary Table 8: Clinical variables predicting bad patient outcome are associated with the indicated genes in a public mRNA expression dataset including tumor tissue (GSE14520) and clinical annotations (Supplementary Table 6) from 247 patients with HCC treated by surgical resection(9). Preprocessed mRNA expression values were split into high and low expression groups according to the 0.5 centile, as indicated. The numbers (and %) of patients falling within the ≥ 0.5 centile and the < 0.5 centile groups are indicated. *P* values were corrected by the Benjamini-Hochberg procedure for multiple tests.

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