Supporting methods

Surgical procedures and treatments

Animal experiments were carried out according to the CEE directives for animal experimentation (decree 2001-131; 'J.O.' 06/02/01).

Adenoviruses

All viruses (Ad-DsR, Ad-PV-NES-DsR, Ad-βGal) were obtained using standard procedures (1,2) and stored at -80°C in PBS/7% glycerol. Stocks of adenoviral particles were quantified as viral particles (VP) by spectrophotometry (OD 260/280 nm) and as pfu by plaque assay. VP/pfu ratios were in the range of 30–50. All experiments were run with at least two independent preparations of each virus.

Immunohistochemistry

Kupffer cells were detected on liver sections using the ED2 antibody (*BMA Biomedicals*) and an Alexa 488 conjugated secondary antibody. A monoclonal antibody (clone M6, *Alexis Biochemicals*) was used to detect the hepatocyte marker cytokeratin 18 (CK18), with an Alexa 633 conjugated secondary antibody. Triple histofluorescence experiments were performed to detect DsRed (λ_{ex}=550nm, λ_{em}=580nm), ED2 (λ_{ex}=488nm, λ_{em}=510nm) and CK18 (λ_{ex}=633nm, λ_{em}=665nm) on a Nikon EZ-C1 confocal microscope. Fluorescence from far red was artificially converted into blue for easier analysis.

Supporting Figure legends

Supporting Figure S1.

Western blot analysis of PV expression in different organs 4 days after Ad-PV-NES-DsR or Ad-DsR portal injection (25 µg protein/lane). Native PV was detected at 12 KDa in rat leg (EDL) muscle. Fusion protein PV-NES-DsR was found at 42 KDa.

Supporting Figure S2. Early cytokine transcription after PH is altered in PV-NES expressing rats.

A.-B. Quantitative PCR analysis of cytokine mRNA (IL6, IL10, IL1β, TNFα) expression 1 hour after PH. **Insert** in panel A: plasma IL6 after PH in DsR and PV-NES-DsR infected rats. **C.** Double histochemistry on liver sections at the time of PH in a PV-NES-DsR rat. ED2 immunostaining (green) and DsR fluorescence were detected, and independent images were merged. The majority of ED2-positive Kupffer cells did not express PV-NES-DsR at the time of PH. **D and E.** Confocal microscopy analysis of ED2-labelled liver sections in a PV-NES-DsR rat. Some periportal ED2-positive Kupffer cells (green; λ_{ex}=488nm, λ_{em}=510nm) harbored DsRed-positive intracellular material (red; λ_{ex}=550nm, λ_{em}=580nm) **(D)**. CK18 immunolabeling (blue; λ_{ex}=633nm, λ_{em}=665nm) revealed hepatocyte engulfment by ED2-positive periportal Kupffer cells **(E)**. z series (20 xy sections), xz and xy sections taken as indicated (white dotted line) on the corresponding xy compilation are shown. Bar: 5 µm. H: hepatocyte; s: sinusoid. **C-E.** One representative image is shown (n=4 in each experiment).

Cytokines play an early role in mediating liver regeneration. We observed that PV-NES-DsR rats exhibited impaired cytokine (particularly IL-6) mRNA induction 1 hr after PH, as compared with control rats. Plasma IL-6 concentration also was reduced in PV-NES-

DsR as compared with DsR rats, although this difference reached statistical significance only at H24 after PH. Because cytokines are mainly produced by Kupffer cells after PH (3), we looked at PV-NES-DsR expression in those cells at the time of PH. We show that most Kupffer cells did not express PV-NES-DsR at this time. A minority of periportal Kupffer cells exhibited DsR fluorescence in PV-NES-DsR as well as in DsR rats, suggesting that those cells may have marginally expressed the transgene and/or, more probably, engulfed Ad-infected (DsR positive) hepatocytes. Thus, intracellular calcium buffering with potential effects on cytokine gene induction (4), although in a small fraction of Kupffer cells cannot be ruled out. Other indirect mechanisms related with hepatocyte calcium buffering may also be involved.

Supporting Figure S3. Comparison of rats infected with Ad-DsR and Ad-PV-NES-DsR.

Rats injected with Ad-PV-NES-DsR or Ad-DsR were comparable at the time of PH in terms of initial liver weight, transgene expression (DsRed fluorescence), systemic inflammation (plasma ALT, IL6, TNFα), and liver inflammatory components (IL1, IL6, IL10 and TNFα mRNAs).

A. Oil Red O staining on liver sections 24 hours after sham surgery or PH. Steatosis was similar in the two rat groups. One section for each Ad-infected rat group is shown, representative of 5 experiments. **B.** Initial (before PH) liver weight was not significantly different in the two rat groups. **C.** ALT activity before and after PH. **D**. Cytokine mRNA induction in pre-PH livers (relative to non-infected livers) were similar in DsR and PV-NES-DsR expressing rats. CV: central vein. PS: portal space.

Supporting Figure S4. PV-NES expression alters cytosolic calcium signals in Hela cells. Forty-eight hours after Ad-PV-NES-DsR adenovirus infection, Hela cells were loaded with Fura2, then analyzed in videomicroscopy for their responses to ATP as a calcium-mobilizing agonist. Calcium responses in cells expressing high levels of DsR (panel α) and low levels of DsR (panel β). Trace representative of 34 (high DsRed) and 28 (low DsRed) cells in 4 experiments.

Supporting Figure S5. Apoptosis after PH in PV-NES-DsR and DsR expressing rats. Terminal deoxynucleotidyl transferase (dTd)-mediated deoxyuridine triphosphate (dUTP) nick end-labeling (TUNEL-AP) assay was performed on frozen sections. Sections were counterstained with hematoxylin. All positive cells in each section (X160) were counted. No significant difference was found between the two rat groups.

Supporting Figure S6. Ad PV-NES-DsR inhibits HGF-induced ERK phosphorylation in SkHep cells. A. Western blot analysis of phospho-ERK1/2 and total ERK1/2. A representative immunoblot is shown. C=Control cells, stimulated (C^+) or not (C^-) with HGF (100 ng/ml). Phospho-ERK1/2 was found to be similar in non-stimulated uninfected and Ad-infected cells. **B.** Band ratio (phospho/total) in each condition, expressed as a % of C^- cells (n=3 experiments in each cell group).

Supporting Figure S7. Bile acids and glucose in the plasma of DsR and PV-NES rats. As hepatocyte calcium signals modulate bile flow and glucose metabolism (36), we measured bile acids and glucose in the plasma of DsR and PV-NES rats, but did not find any significant difference between the two groups. **A.** Blood glucose. The hypoglycemia observed in the hours after PH, reported by others (5), was similar in the two rat groups. **B.**

Plasma total bile acids. The cholestasis observed after PH, also reported by others (6) was similar in the two rat groups.

Supporting references

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Supporting Table 1

Gene	Sequence	Temperature (°C)	Number of cycles	Amplicon length (bp)
Rat cyclin A	FW 5'AGT GCC GCT GTC TCT TTA CC 3' RV: 5'CAT AGC ATG GGG TGA TTC AA 3'	60	40	90
Rat cyclin D1	FW 5'GCA CAA CGC ACT TTC TTT CC 3' RV 5'TCC AGA AGG GCT TCA ATC TG3'	60	40	85
Rat cyclin E	FW :5' CTG AGA GAT GAG CAC TTT CTG C 3' RV : 5' TGT CTC TCT GTG GAG CTT ATA GAC TT 3'	60	40	114
Rat Cdk2	5' GCA TCT TTG CCG AAA TGG T 3' 5' GAT CCG GAA GAG TTG GTC AAT 3'	60	40	74
18S	FW 5' GTA ACC CGT TGA ACC CCA TT3' RV 5'CCA TCC AAT CGG TAC TAG CG 3'	60	40	151
GAPDH	Fw 5' TCCCTCAAGATTGTCAGCAA 3' Rv 5' AGATCCACAACGGATACATT 3'	60	40	308
Rat cfos	Fw 5' CACTCCCAGCTGCACTACCTA T 3' Rv 5' GCAAGCTCAGTGAGTCAGAG 3'	60	40	134
Rat cjun	Fw 5' CCGGCTAGAGGAAAAAGTGA 3' Rv 5' TGAGTTGGCACCCACTGTTA 3'	60	40	134
Rat TNF-α	Fw 5' TGATCGGTCCCAACAAGGAG 3' Rv 5' TGATGAGAGGGAGCCCATTT 3'	60	40	51
Rat IL-1β	Fw 5' AGACAGCTGCACTGCAGGCT 3' Rv 5' CAGCACGAGGCATTTTTGTT 3'	60	40	51
Rat IL-6	Fw 5' AGACTTCCAGCCAGTTGCCT 3' Rv 5' GGCAGTGGCTGTCAACAACA 3'	60	40	52

***Reverse transcriptase- polymerase chain reaction (RT-PCR) and Quantitative PCR*:**

Total RNA was extracted using Trireagent (Sigma), according to the manufacturer's recommendations. Complementary DNA (cDNA) was prepared by reverse transcription of 1 µg of total RNA using superscript II enzyme and random primers (Invitrogen). Reverse-

transcribed mRNA (cDNA) was treated with DNAse (Quiagen) according to the manufacturer's recommendations and amplified by PCR in the presence of independent sense and antisense primers. Primers and PCR conditions are described in the above table. Amplification products were separated by gel electrophoresis (2% agarose) and visualised by ethidium bromide staining. For quantitative PCR, cDNAs were amplified using SYBR green PCR kit (Bio-Rad) and Chromo 4 Real-Time detector (Bio-Rad) and normalized to levels of actin and GAPDH using Opticon Monitor 3 software (Bio-Rad).