Regenerative response in the pig liver remnant varies with the degree of resection and rise in portal pressure

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Mortensen KE, Conley LN, Hedegaard J, Kalstad T, Sorensen P, Bendixen C, Revhaug A. Regenerative response in the pig liver remnant varies with the degree of resection and rise in portal pressure. Am J Physiol Gastrointest Liver Physiol 294: G819–G830, 2008. First published January 10, 2008; doi:10.1152/ajpgi.00179.2007.—After parenchymal loss, the liver regenerates restoring normal mass and metabolic function. Prevailing theories on triggering events leading to regeneration include humoral, metabolic, and flow-mediated mechanisms, the latter emphasizing the importance of shear stress mediated nitric oxide regulation. We aimed to investigate whether the grade of resection and hence the portal venous pressure and sinusoidal shear stress increase would be reflected in the gene expression profiles in the liver remnant by using a global porcine cDNA microarray chip with ~23,000 genes represented. Six pig livers were resected with 62% (low portal pressure resection) and 75% resection (high portal pressure resection) and sampled consecutively. Analysis of gene expression profiles in the regenerating liver over a time span of 1 min to 1 wk post-PHx. Common to all is the upregulation of genes associated with transport control; inflammatory/acute phase response; cytoskeletal and extracellular remodeling; regulation of cell cycle entry and progression, including cell cycle checkpoint genes; synthesis of constitutive plasma proteins; biosynthesis of critical cell components; and signal transduction. Genes regulating intermediary metabolism of drugs, lipids, sugars, amino acids, nucleotides, and steroids were commonly downregulated. Notably, several of these studies (5, 71) report an upregulation of genes inducing antiproliferative effects (RhoB, PAI-1, and Quiescin Q6) as early as 90 min after PHx, illustrating the dual nature of the regenerative response.

The aspects of changes in portal pressure and sinusoidal flow in the liver remnant have not been addressed in previous microarray experiments. Based on the shear stress theory (49, 53, 63, 64, 66, 73), we performed an analysis of gene expression profiles in consecutive biopsies from liver remnants after two different grades of resections: 62% resection [low portal venous pressure resection (LPVR)] and 75% resection [high portal venous pressure resection (HPVR)] with the primary aim of investigating whether the pressure and flow differences in these two sets of liver remnants would generate activation patterns corroborating the theory of shear stress induced initiation of the regenerative response. To this end, we utilized a novel porcine cDNA microarray chip with ~23,000 genes represented, encompassing the entire pig genome and enabling...
a more comprehensive investigation of the regenerative process compared with previous studies based on limited microarray platforms. In addition, as most previous regeneration models have been based on rodents, we chose to examine the process in the pig, as this species bears closer genetic and physiological resemblance to man.

MATERIALS AND METHODS

Six castrate sus scrofa domesticus were used for all experiments. The committee of the Norwegian Experimental Animal Board approved all protocols, which were conducted in compliance with the institutional animal care guidelines and the National Institute of Health’s Guide for the Care and Use of Laboratory Animals [DHHS Publication No. (NIH) 85-23, Revised 1985]. Anesthesia was induced and maintained as described previously from our laboratory (44). Operative procedures: after a midline laparotomy and placement of all catheters, the hepatic artery supplying segments II and III (left lateral lobe) together with the portal branch of these segments was ligated using an absorbable polyfilament suture on a large needle. Thereafter, the lobe was strangulated with a 0.5-cm wide cotton ribbon, removed, and weighed. Segments IV, V, and VIII were removed in a similar manner in the LPPR series leaving segments VI, VIII, and I in place (62% resection). In the HPPR series, the resection was continued and segments VI and VII were also removed (75% resection).

A 16-G central venous catheter (CVK, Secalon T) was placed in the left external jugular vein for administration of anesthesia and infusions. A 16-G CVK was placed in the left femoral artery for continuous arterial blood sampling and pressure monitoring. A 7-Fr 110-cm angiographic catheter (Cordis, Johnson&Johnson) was placed in the right hepatic vein just before entering the inferior caval vein for blood sampling. Correct placement was ensured by direct palpation and later controlled by postmortem dissection. A 4-Fr pediatric central venous catheter (Arrow International) was placed in the portal vein with the tip ~5 cm from the liver hilus for pressure monitoring and blood sampling.

A 12-mm flow probe (Medistim, Oslo, Norway) was placed around the portal vein. Calibrated transducers (Transpac 3, Abbott Critical Care Systems, Chicago, IL) were used for continuous pressure registration. The transducers were connected to an amplifier (Gould, 2800S). Pulsatile signals were displayed on a monitor, digitalized, and stored electronically (Advantech, Industrial Computer).

Biopsies were taken from the remaining segments VI and VII in the LPPR series and from segment I in the HPPR series and placed immediately in RNA Later (Ambion). The consecutive samples were taken some distance apart to avoid disturbance from the sampling procedure. Sampling time points were 1, 30, and 90 min and 3, 4, and 6 h after resection.

Blood extraction was performed before biopsy sampling. Samples were taken from the portal vein, femoral artery, and hepatic vein. IL-1, IL-6, TNF-α, TGF-α, TGF-β, and EGF were analyzed using ELISA (Quantikine, R&D Systems, and Searchlight Pierce Biotechnology), and aspartate aminotransferase, alanine aminotransferase, glutamyl transpeptidase, pyruvate, glucose, lactate, and alkaline phosphatase levels were quantified by calorimetric, ultraviolet-photometric, and HPLC analysis (Roche, PerkinElmer). Biochemical flux across the liver remnant was analyzed using linear mixed models in SPSS version 13, testing time (T), and group x time (GT) interaction. P values ≤0.05 were considered significant.

The microarray experiments were conducted as a common reference design using liver total RNA purified from an unrelated animal as the reference, allowing comparison of all the different samples to each other (the acute phase response due to the operation in itself could in theory affect gene expression in both groups of liver remnants; however, as the perioperative conditions were almost identical, we considered this effect to be equal for the two resection groups and chose not to use a sham as the reference). Total RNA was purified and aminally-labeled cDNA (aa-cDNA) was synthesized from 20 μg of total RNA. The reference sample was labeled with Alexa 488, each individual sample was labeled with Alexa 594, and the samples were combined pair wise and hybridized to the pig array DIAS_PIG_55K2, which consists of 26,879 PCR products amplified from unique cDNA clones. After hybridization, washing, and drying, the slides were scanned, and the median intensities were computed. Statistical analysis was carried out in the R computing environment using the Bioconductor package Linear Models for Microarray Analysis (Llima; Refs. 29, 69). The log2-transformed ratios of Alexa-594 to Alexa-488 were normalized within slide using printtip-loess and were analyzed to identify genes being significantly differentially expressed by time within treatment as well as between treatments. Time contrasts were formed referring to the sample taken at the 1-min time point. The genes found significantly expressed between at least two time points were further analyzed referring to Online Mendelian Inheritance in Man (OMIM; Ref. 2) and Gene Ontology (GO) to group the genes by function. More detailed descriptions of the microarray experiments are available at the National Center for Biotechnology Information’s Gene Expression Omnibus (GEO; Refs. 1, 7, 20) through the GEO series accession number GSE6860.

The data were analyzed within each resection group (LPPR and HPPR) in two ways. First, using top tables for each of the five time intervals (1 to 30 min, 1–90 min, 1 min to 3 h, 1 min to 4 h, and 1 min to 6 h post-PHx), we classified all genes into 14 functional groups by molecular function and biological process according to the GO and OMIM. Second, using K-means clustering, we defined 20 clusters in each group and selected from these 8 clusters of special interest on the basis of their nonuniform profiles for closer analysis with GO and OMIM. For direct comparison of the two resection groups, we investigated the genes that were expressed in both groups at each time point, selecting those that had a statistically different expression level in the two groups (herein termed “within time point contrasts”). We avoided using fold change cutoff values because this approach fails to take the uncertainty of variability into account (i.e., a gene may exhibit a 10-fold change and yet not be significant because of its variability) and has the potential of excluding genes of biological importance whose expression values are below the cutoff value.

For RT-PCR validation, sets of two primers and a probe for eight chosen target genes and one control gene (18S) were designed using the Primer Express software package (version 2.0; Applied Biosystems). To avoid genomic DNA contamination, the primers were designed to span exon boundaries. The probes for the target genes were labeled with either the fluorescent reporter VIC or SYBR Green. Five micrograms of total RNA of each sample were reverse transcribed using random hexamer primers and SuperScript II Reverse Transcriptase (Invitrogen). Real-time quantitative RT-PCR was performed on the ABI Prism 7900HT sequence detection system (Applied Biosystems) by monitoring the increase in fluorescence due to the binding of SYBR green or VIC to double-stranded DNA. The amplification conditions were as follows: 50°C for 2 min, 95°C for 10 min, and 40 cycles each of 95°C for 15 s and 60°C for 1 min. Each cDNA sample was run as technical triplicates. The standard curve method was used to calculate the relative mRNA levels. The quantity mean of the triplicate measurements was normalized against the 18S gene, and the mean of the biological replicates was calculated as well, yielding a single value for each timepoint. The profiles of these values were compared with the profiles of the microarray data.

RESULTS

General Results

Hemodynamics. Liver resection resulted in a gradual rise in portal venous pressure, reflecting an increase in resistance to
blood flow through the liver sinusoids as the blood flow per gram remaining liver tissue increases. Upon additional resection of segments VI and VII (from a 62 to 75% resection), the pressure rose precipitously; from a baseline average of 6.1 mmHg, the venous portal pressure rose to 8.2 mmHg in the low-pressure group (35% increase) and to 12 mmHg in the high-pressure resection group (110% increase), remaining relatively stable until experiment termination (Fig. 1). Similarly, the average portal blood flow per gram remaining liver tissue rose from ~1–2 ml/g in the LPPR group and to 5 ml/g in the HPPR group.

**Serum analysis.** We found a significant net absorption of alanine aminotransferase, glutamyl transpeptidase, and glucose across the liver remnant after resection in both LPPR and HPPR resection groups. This uptake increased over time, but there was no difference between the groups. A net discharge of aspartate aminotransferase and alkaline phosphatase was seen. This net discharge increased over time, and there were significant group × time interactions with a larger discharge seen in the HPPR group. No changes in pyruvate flux were observed.

No significant changes in IL-1, IL-6, TNF-α, and EGF were found. Beginning at 2 h post-PHx, an increase in serum levels of TGF-α from an average of 8.9 pg/ml (±4.6 SD) to 75.85 pg/ml (±14.9 SD) was observed in the LPPR group but not in the HPPR group (P = 0.011). Serum TGF-β rose significantly in both groups (P = 0.017), but there was no group difference.

**Microarray Results**

A total of 1,095 genes was differentially expressed at least once between two time points in the LPPR group and 1,010 genes in the HPPR group (the online version of this article contains supplemental data; see Tables S1 and S2). Of these, 174 genes were common to both groups. Approximately 50% of the common genes regulate intermediate metabolism or have an unknown function. Approximately 35% regulate transcription, signal transduction, translation, transport, protein metabolism, and oxidoreductase activity. The remaining 15% regulate cell cycle, apoptosis, cytoskeleton, acute-phase response, nucleic acid metabolism, and cell proliferation.

**Top-table analysis LPPR group.** Analysis of time trends among upregulated genes in the LPPR group revealed a predominance of expression of genes governing cell proliferation in the earlier time periods, whereas cell cycle and apoptosis genes and genes regulating translation tended to be expressed in later phases. Inflammation/acute-phase response genes were evenly expressed throughout the experiment. Analysis of time trends among the downregulated genes in the LPPR group revealed a relative increase in the downregulation of genes controlling lipid/hormone/amine/alcohol metabolism towards the end of the experiment. The expression of genes regulating transport and cell-cell signaling/signal transduction and cell adhesion remained relatively stable (Fig. 1). Similarly, the average portal blood flow per gram remaining liver tissue rose from ~1–2 ml/g in the LPPR group and to 5 ml/g in the HPPR group.

**Top-table analysis HPPR group.** We observed a gradual increase over time in the upregulation of the genes controlling protein metabolism, cell cycle and/or apoptosis, and translation. Genes regulating cell-cell signaling/signal transduction/cell adhesion and transport were gradually less upregulated throughout the experiment. Genes regulating lipid/hormone/amine/alcohol metabolism and oxidoreductase function were increasingly downregulated over time (Fig. 2.).

**K-means clustering.** All genes differentially expressed at least once between two time points were clustered into 20 groups (see Supplemental Figs. S3 and S4). When analyzing the chosen eight clusters in the HPPR and LPPR groups with GO and OMIM, we found the following similarities between the two resection groups (see Supplemental Figs. S1 and S2): 1) Genes regulating cell-cell signaling/signal transduction/cell adhesion and translation were up- and downregulated to similar degrees in both series. 2) With the exception the genes in cluster 13 in the LPPR series, we found genes regulating inflammation/acute-phase response to be marginally upregulated in both resection groups. 3) Genes regulating transport and cell proliferation/development were largely upregulated in both series. 4) Genes regulating lipid/hormone/amine/alcohol metabolism.

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**Fig. 1.** Portal venous pressure in the high portal venous pressure resection (HPPR) and low portal venous pressure resection (LPPR) series (mean pressure ±SD).
metabolism were generally downregulated in both series. 5) In general, more genes regulating protein metabolism were upregulated than downregulated in both series throughout the experiment. Genes regulating transcription, cell cycle and/or apoptosis, extracellular matrix/cytoskeleton, and nucleic acid metabolism were upregulated to a larger degree in the HPPR series.

**Genes Regulating Cell Cycle and Apoptosis**

The 26 differentially expressed genes regulating cell cycle and apoptosis are schematically presented in Table 1 and Fig. 3.

A dual response was seen during the first 30 min after PHx in the HPPR, series as the upregulation of PACS-2 would in theory result in caspase activation (68) and downregulation of CASP8 would modify caspase-dependent apoptosis (57).

Potential products of the upregulated genes in the LPPR series in the time interval 1–90 min organize the microtubuli apparatus (involved in cell division; KIF20A; Ref. 34), are involved in p53-dependent apoptosis and TGF-β-dependent apoptosis through interaction with Dapk1 (MIB1; Ref. 38, 40) and protection from serum starvation-induced apoptosis (MCTS1; Ref. 67).

Products of the five upregulated genes in the HPPR group in the same time interval could potentially inhibit DNA damage and cytolysis (NME1; Refs. 21, 43), counteract apoptosis (Bcl2; Ref. 35) and MAPK8IP2 (52) facilitate entrance into the G1-phase (UBE2C; Ref. 59), and mediate p53 dependent activation of NFκ-B through the RAF/MEK1(MAPK2K1)/p90(rsk) pathway (MAPK2K1; Ref. 62), which in turn could both protect or contribute to apoptosis (24). [Studying liver regeneration, Su et al. (71) found BCL-X, BCL-2-related protein A1 and bcl-3 upregulated after a PHx in mice and Locker et al. (48) found Bcl2 upregulated after PHx]. Downregulated in the HPPR group in this time period were two genes controlling apoptosis (CYCS; Ref. 47, and Casp7; Ref. 54) and one gene associated with cell cycle progression (HNRPK; Ref. 15).

Within 3 h, four genes were found upregulated in the HPPR group, whose products inhibit caspase-9-dependent apoptosis (PTMA; Ref. 39), regulate centrosome duplication in the G1 phase of the cell cycle (MPM1; Ref. 55), possibly function as a tumor suppressor (BCLAF; Ref. 41), and are found localized in the nucleoli G1 and G2 phase of the cell cycle (SBDSP; Ref. 6).

Within 4 h in the LPPR group, we found differentially expressed genes whose products regulate centrosome duplica-
tion in the G1 phase of the cell cycle (MPM1; Ref. 55) are involved in centrosome formation and mitosis (SCYL1; Ref. 42), and are found localized in the nucleoli G1 and G2 phase of the cell cycle (SBDSP; Ref. 6). In the HPPR group, we found NPM1 upregulated and other genes whose products regulate the centriole cycle and mitosis (CETN2; Ref. 46); promote the expression of the c-myc gene, abundantly found in hepatocellular carcinomas (NME2; Ref. 37); and potentially inhibit DNA damage and cytolysis (NME1; Ref. 21, 43).

Downregulated genes in this time period were only found in the HPPR group. The products of these genes are associated with complex formation with p53 in G0/G1 and S/G2/M phases of the cell cycle (MDM2; Ref. 25), regulate the homeostasis of liver cell number, and inhibit Fas-mediated cell death (FAIM2; Refs. 3, 70), and lead to G2/M cell cycle arrest and apoptosis (IGFBP5; Ref. 11). [Specifically addressing the liver regeneration, Desbarats and Newell (16) found that Fas engagement accelerates liver regeneration after PHx.]

Within 6 h post-PHX, the previously described genes NPM1 and PTMA were upregulated in the LPPR and HPPR series, respectively, whereas BTG2 was downregulated in the LPPR series. BTG2 belongs to a family of structurally related proteins that appear to have antiproliferative properties (61).

Genes Regulating NO Metabolism and Oxidative Stress

Differentially expressed genes regulating NO metabolism and oxidative stress are presented schematically in Table 2 and Fig. 4.

Heat shock factor-1 (HSF-1) expression was gradually upregulated in the HPPR series with a maximum value at 3 h post-PHX. HSF-1, a major heat stress-response factor, upregulates many other heat shock protein (HSP) genes, which in turn are cytoprotective and probably play a role in ischemic preconditioning due to their ATP-sparing effects. HSPs in turn have been found to inhibit the formation of the apoptosome complex through forming complexes with Apaf1 and cytochrome c, inhibiting the final activation of caspase-3 (78). HSP90 has also been found to play a central role in statin-induced angiogenesis (9) and activates endothelial nitric oxide synthase (eNOS) upon shear stress stimulation (27). HSF-1 also activates PKB/AKT, which in turn phosphorylates and activates eNOS (17).

Activation of eNOS is partially dependent on Ca\(^{2+}\) calmodulin that binds eNOS upon an increase in intracellular Ca\(^{2+}\) concentration. We found that this gene was upregulated in both resection series within 3 h post-PHX and that it stayed so until experiment termination.

In the HPPR series, we found the NOSIP transcript elevated at 3 h post-PHX. The activity of eNOS is regulated by its intracellular location and association with NOSIP. Dedio et al. (14) found possible eNOS activity inhibition by this protein and cellular translocation of eNOS from the caveolin-bound plasmalemmal location to the intracellular golgi apparatus.

Dimethylaminohydrolase (DDAH) reduces tumor asymmetric dimethylarginine in turn stimulating tumor growth and angiogenesis through increased NO levels and VEGF expression (45). In the present investigation, DDAH2 remained downregulated throughout the 6 h of observation post-PHX in both series. However, at 3 h, we observed a significant rise in the mRNA level in the HPPR series.
Oxygen radicals such as H$_2$O$_2$ and O$_2$ are established activators of the caspase cascade. Dimmeler et al. (18) found that shear stress mediated upregulation of SOD1 mRNA and protein levels and eNOS activity abrogated the activation of the TNF-α induced and oxidized low-density lipoprotein induced caspase activation in human umbilical vein endothelial cells. We found SOD1 consistently downregulated in both resection series, with the exception of an increased level at 3 h in the HPPR series.

The antioxidant catalase is well established as an apoptotic inhibitor (4) being a scavenger of hydrogen peroxide. Catalase was downregulated in both resection series within 3 h in the HPPR series and within 4 h in the LPPR series.

GSTP1 was downregulated within 6 h in the HPPR series. Henderson et al. (31) reported that in GSTP1-null mice the absence of the enzyme significantly enhanced the ability of the liver to maintain GSH homeostasis in the face of oxidative stress, suggesting that GSTP1 causes a higher level of oxidative stress in the hepatocytes by redox cycling. In the absence of GSTP1, the constitutive activation Jun N-terminal kinase results in activation of c-jun rendering both cytoprotective effects and apoptosis. Interestingly, Hojo et al. (36) found that laminar fluid shear stress inhibited H$_2$O$_2$-induced JNK activation and increased the GSH-to-GSSG ratio, and Hermann et al. (32) found that the combined inhibition of NO synthase and GSH biosynthesis completely reversed the protective effect of shear stress.

Table 2. Differentially expressed genes regulating NO metabolism and oxidative stress according to time intervals in the LPPR and HPPR series

<table>
<thead>
<tr>
<th>Contrast Group</th>
<th>1–30 min</th>
<th>1–90 min</th>
<th>1 min–3 h</th>
<th>1 min–4 h</th>
<th>1 min–6 h</th>
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<tr>
<td>LPPR</td>
<td>0</td>
<td>0</td>
<td>↑ SCAP2</td>
<td>↓ Catalase</td>
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<tr>
<td>HPPR</td>
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<td>↓ Catalase (−1.5)</td>
<td>↓ Catalase (−1.6)</td>
<td>↓ SOD1 (−1.3)</td>
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<td>↑ NOSIP (1.2)</td>
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Fold change differential expression is in parentheses.
suggesting that both NO synthase and the GSH redox cycle system control the apoptosis-suppressing effect of shear stress.

Src-associated protein 2 (SCAP2) was upregulated in both series after PHx but significantly more in the LPPR group at 3 h. In a bovine aortic endothelial cell culture, Davis et al. (13) found that shear stress induced a transient expression of eNOS and prolonged half-life of eNOS mRNA through interaction with c.Src. SCAP2 is phosphorylated by Src family kinases.

RT-PCR validation. A comparison of the RT-PCR and microarray profiles of eight selected genes is illustrated in Fig. 5. The profiles were congruent for NOSIP, SOD1, NOS3, and cFOS. The remaining gene profiles were only comparable over certain time points.

**DISCUSSION**

This study shows that the immediate regenerative response in the liver remnant, as quantified by gene expression, is influenced by the grade of resection and increase in portal pressure; 62% resection affects genes primarily regulating the cell cycle, whereas 75% resection affects genes primarily regulating apoptosis, NO metabolism, and oxidative stress.

With respect to genes controlling the cell cycle and apoptosis, we observed quantitative and qualitative differences between resections yielding LPPR (62% resection) and HPPR (75% resection). Of all the 26 genes differentially expressed, a major part (18 genes) was found in the HPPR group. Qualitatively, the genetic response after a high-grade resection seems to center around the regulation of apoptosis, inhibiting death-promoting pathways, particularly the caspase system, whereas after a lower grade resection we observe the differential regulation primarily of genes regulating the cell cycle and cytoskeletal framework. Specifically, we observed in the LPPR group that genes promoting progression through the G1 phase were upregulated and genes inhibiting progression were downregulated. In addition, genes associated with the microtubuli apparatus and centrosomes were found to be upregulated. Over time, it seems that apoptosis is downregulated during the earlier time points (within 90 min), whereas cell cycle progression and microtubuli/centrosome regulation are regulated somewhat later (90 min to 4 h post-PHX). From these results, it would seem that a higher grade of resection primarily results in an inhibition of the apoptotic apparatus, whereas a lower grade resection stimulates primarily G1-phase cell cycle progression.

With regard to genes controlling NO metabolism and oxidative stress, we also observed several patterns distinguishing the two resection groups. However, the potential role of NO in liver regeneration appears to be quite complex and necessitates more discussion here. NO has been shown to inhibit apoptosis of endothelial cells through the regulation of Bcl2 (8) and the caspase cascade (18, 19, 60). On the other hand, NO has shown both antiproliferative properties through the induction of p21 (30) and potential proliferative properties through the activation of activator protein-1 (56). Furthermore, other studies (65, 66) have illustrated that NOS inhibitors (N’G-nitro-L-arginine methyl ester) have blocked liver regeneration after PHx, while the addition of NO donors (SIN-1) has restored the process.
Fig. 5. Comparison of average relative mRNA levels (± SD) for 8 genes as measured by RT-PCR (●: Qty mean target/18S) and microarray (■: M-Log base 2).
NO may also have direct apoptotic effects by combining with superoxide $O^-$ forming peroxynitrite ONOO$^-$ leading to DNA damage and apoptosis (10). In the present study, a major part of genes regulating NO metabolism is found in the HPPR group (Table 2). Several genes regulating the activity of eNOS were found differentially expressed. NOSIP competes with caveolin-1 (recently found essential for liver regeneration; Ref. 23) in the binding of eNOS. Upon binding eNOS, NOSIP (upregulated in the HPPR group) translocates the enzyme from the plasmalemma to the Golgi apparatus and possibly the mitochondria, reflecting functional regulation by cellular compartmentalization after PHx. DDAH2 (upregulated in the HPPR group) regulates eNOS activity indirectly by its degradation of asymmetric dimethylarginine, which in turn converts eNOS to methyamine and citrulline. This could possibly reflect a very early physiological response in NO regulation and neovascularization in the liver remnant, as angiogenesis is central to neovascularization of regenerated hepatocyte islands in the regenerating liver (74). Genes encoding calcium calmodulin (essential for eNOS activity) were upregulated in both resection series, and HSF-1 (activating eNOS via PKB/AKT) was upregulated in the HPPR series, reflecting activation of eNOS in the liver remnants.

Interestingly, several genes, previously found activated in response to various cellular stresses, were found to be downregulated in our liver remnants (SOD1, catalase, and GSTP1). Taken together, this suggests that the cells in the liver remnants were under reduced oxidative stress over time. This is in contrast to Fausto’s metabolic theory of reactive oxygen species triggering the regenerative response after PHx (22). Dimmeler et al. (18) showed that laminar flow shear stress protects against oxidative stress by the upregulation of SO, and Hojo et al. (36) found that laminar fluid shear stress inhibited $H_2O_2$-induced JNK activation and increased GSH-to-GSSG ratio. Of the genes associated with redox cycling, all were differentially expressed in the HPPR, whereas only one (catalase) was found in the LPPR group. Taken together, this may suggest that the increased shear stress in the HPPR group decreases the level of oxidative stress within the endothelial and juxtaposed hepatocytes.

Functional classification of the differentially expressed genes with GO demonstrates the complexity of the immediate genetic response in the liver remnant after PHx, as genes representing almost all functional groups are differentially expressed at one time or another. This is not surprising, as the process of liver regeneration necessarily involves multiple metabolic pathways. However, we find some differences between the two resection groups when it comes to functional classification, as genes regulating transcription, cell cycle and/or apoptosis, extracellular matrix and/or cytoskeleton, and nucleic acid metabolism seem to be upregulated to a larger degree after a larger resection, tentatively due to a greater increase in portal pressure. Apart from this, our porcine study corroborates the findings (5, 26, 48, 71, 77) in previous microarray studies on liver regeneration in rodents that report the differential expression of genes in various functional groups some of which are upregulated and some of which are downregulated (see Table 3).

There are several important differences between the referred studies in Table 3 and the present study: our microarray analysis is based on a cDNA chip containing ~23,000 gene elements (vs. 2,500–6,000 gene elements), the biopsies are

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Table 3. Overview of functional classification of genes according to Gene Ontology and their expression at various time points in 5 reference studies using microarray analysis of gene expression in liver remnants after partial hepatectomy. Gray squares denote time points of mRNA extraction in the respective studies. Black lines denote time points in which genes in the different functional groups were collectively differentially expressed.
taken consecutively from the same animal (vs. different animals killed at different times), and the species studied is different (pig vs. rodent). In our opinion, using a global chip containing more gene elements gives a superior picture of the genome-wide expression in the liver remnant after PHx. Using a large animal model allowed us to sample consecutively from the same individual animal, which reduces the likelihood of individual animals distorting the overall picture should their profiles vary greatly from the mean. Furthermore, 7 of the 26 genes regulating cell cycle and/or apoptosis that we report in the present study have been previously reported in microarray studies of liver regeneration in rodents (5, 26, 48, 71, 77). The remaining 19 genes are mainly described in models of cell culture. When studying a highly coordinated process such as liver regeneration, we maintain that it is better to do so in an integrated biological system rather than in an isolated cell culture. Finally, as porcine genetics are closer to humans than rodents are, studying liver regeneration in a porcine model ought to be advantageous as far as translational science is concerned.

The RT-PCR validation of the microarray analysis was not entirely satisfactory. We suggest two explanations for this. First, the partial incompatibility may be explained by the fact that RT-PCR measures the abundance of a single transcript or transcript segment, whereas microarray data may result from hybridization of multiple transcript isoforms to a probe or probes on an array. Second, with comparisons between time-course experiments such as the present one, there will be noise not only due to biological variation in the individual animals as a response to the surgery itself but also due to the time perspective of when it happens, that is, the genetic response of some animals to an intervention may be slower or faster than others. These factors may increase the variability not only of the microarray data but also of the RT-PCR data. We believe that, although the microarray data are not fully substantiated by the RT-PCR validation, the genes found differentially expressed by microarray analysis in our study are nonetheless found coassociated within the biological context of the cell cycle and/or apoptosis, NO metabolism, and the redox state. In our opinion, this constellation is an argument advocating that the genes in fact are differentially expressed because it infers biological meaning and thus adds credibility to our conclusions.

A microarray experiment cannot specifically falsify or confirm a null hypothesis, as it is, in essence, a screening technique. However, we applied the method in this study to shed light on present regeneration theories of portal pressure and sinusoidal shear stress and ventured to hypothesize that the immediate regenerative response in the liver remnant after a liver resection would vary according to the level of resection because of the differences in portal pressure incurred by varying levels of resection. We believe that this study presents data suggesting that there are qualitative and quantitative differences in the regenerative response depending on the level of resection and that these differences may be caused by differences in portal and hence sinusoidal pressure and shear stress in the respective liver remnants.

Graded liver resection in the pig may serve as a model to investigate shear stress in the liver remnant. Refined in vivo models of shear stress in the liver need to be explored to investigate increased sinusoidal flow per se (without prior resection) to isolate the phenomenon of increased flow. In addition, further studies with a longer time frame need to be conducted investigating the possible physiological consequences (such as differences in mitosis, apoptosis, and liver mass restoration) of the differences in the regenerative response between high- and low-pressure resections observed in the present study.

GRANTS

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REFERENCES


