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Zonal Gene Expression in Mouse Liver Resembles Expression Patterns of Ha-ras and β-Catenin mutated hepatomas

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Running title: Zonal gene expression in liver

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Abbreviations: GS, glutamine synthetase; Cyp, Cytochrome P450; AhR, aryl hydrocarbon receptor; CAR, constitutive androstane receptor

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Abstract

Hepatocytes of the periportal and perivenous zones of the liver lobule differ in their levels and activities of various enzymes and other proteins. We have recently suggested that β-Catenin- and Ras-dependent signaling pathways play an important role in the regulation of perivenous and periportal gene expression profiles. This hypothesis was primarily based on similarities in zonal differences in gene expression of hepatocytes from normal liver with gene expression patterns of liver tumors: several proteins and mRNAs preferentially expressed in periportal hepatocytes were often overexpressed in *Ha-ras* mutated mouse liver tumors while perivenous markers were overexpressed in *Ctnnb1* (encoding β-Catenin) mutated tumors. We have now extended this work by use of data from 2 previously conducted microarray analyses aimed to analyze (i) global gene expression patterns of *Ha-ras* and *Ctnnb1* mutated mouse liver tumors and (ii) transcriptome differences between periportal and perivenous mouse hepatocytes. By comparison of the datasets, 134 genes or expressed sequences were identified that were present in both datasets. Gene expression patterns in perivenous hepatocytes and *Ctnnb1* mutated hepatoma cells were strongly correlated: 96.5% of the genes present in both datasets were regulated in the same direction. In analogy, expression of 74.1% of the genes deregulated in *Ha-ras* mutated tumors was correlated with the respective expression patterns in periportal hepatocytes. These findings favour the hypothesis that gene expression patterns in periportal and perivenous hepatocytes are regulated, at least in part, by Ras- and β-Catenin-dependent signaling pathways.

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Based on the location of the blood vessels, the terminal branches of the portal and the hepatic (central) veins, and the direction of the blood flow, the individual liver lobule can be subdivided into an upstream “periportal” and a downstream “perivenous” (pericentral) region. Hepatocytes located in either of the two regions have different, often complementary functions, as indicated by differences in the content and activity of several metabolic key enzymes (for review see ((Jungermann and Katz, 1989; Gebhardt, 1992)). This so-called ‘zonation’ of metabolism in the liver is also of particular impact on the metabolism of pharmaceuticals and xenobiotics, since most of the enzymes involved in the metabolism of such compounds show distinct differences in basal as well as inducible expression between perivenous and periportal hepatocytes, with the main detoxification enzymes of both phase I and phase II of xenobiotic metabolism being located preferentially in perivenous hepatocytes (for reviews on zonation of xenobiotic metabolism see (Jungermann and Katz, 1989; Oinonen and Lindros, 1998)). As a consequence, various exogenous compounds lead to a preferential damage of hepatocytes located in either the perivenous or periportal area of the liver lobule (for review see (Lindros, 1997)).

We have recently developed a hypothesis to explain the mechanisms that govern zonal differences in gene expression in liver (Hailfinger et al., 2006). According to our hypothesis, two opposing signals, each gradual in nature, dictate the gene expression patterns of hepatocytes within the different areas of the liver lobule. We postulated that a β-Catenin-activating signal which is probably delivered by endothelial cells of the central veins, and a second opposing signal, presumably generated by blood-borne molecules activating a Ras-dependent signaling pathway, trigger perivenous- and periportal-specific mRNA expression profiles. This concept was deduced from the observation of striking similarities in the expression of selected “marker” genes in *Ctnnb1* (encoding β-Catenin) and *Ha-ras* mutated hepatoma cells with their corresponding expression patterns in perivenous and periportal hepatocytes, respectively. We have now extended our previous study and compared the transcriptome of periportal and perivenous hepatocyte subpopulations with that of *Ha-ras* and *Ctnnb1* mutated mouse liver tumors.

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Methods:

Datasets on global mRNA expression patterns in mouse liver tumors mutated in either *Ha-ras* or *Ctnnb1* and datasets on global mRNA expression patterns in perivenous and periportal mouse hepatocytes were available to us from previous experiments conducted in our laboratory (Stahl et al., 2005a; Braeuning et al., 2006). In these studies, gene expression profiles had been analyzed by use of the Affymetrix GeneChip MOE-430A (Affymetrix, Santa Clara, CA, USA) containing approximately 22,600 probe sets including more than 14,000 well-characterized mouse genes. Since the criteria for statistical analysis of expression data varied in the two studies, we re-evaluated the tumor datasets using as discriminators a $|\log_2$ expression ratio$| \geq 1$ (equivalent to ≥ 2-fold change) and a threshold of 0.1 for the false discovery rate adjusted *p*-values to allow a direct comparison with our data on zonal differences in gene expression in normal liver (for further details see (Stahl et al., 2005a; Stahl et al., 2005b; Braeuning et al., 2006)). The former cutoff was chosen because expression differences smaller than 2-fold are very difficult to detect by quantitative RT-PCR, which was used in the previous studies for verification of the microarray results. The latter was chosen to keep the expected proportion of false-positives below 10%.

Results:

We have recently determined by microarray analysis the RNA expression profiles of mouse liver tumors harboring activating mutations in either *Ha-ras* or *Ctnnb1* (Stahl et al., 2005a). In that study, liver tumors of the 2 genotypes were found to differ strikingly with respect to their gene expression patterns. More recently, we performed a microarray analysis of periportal and perivenous subpopulations of mouse hepatocytes enriched by combined digitonin/collagenase perfusion (Braeuning et al., 2006). In this latter study, 198 genes or expressed sequences (corresponding to 243 probe sets) were identified that demonstrated a ≥ 2-fold difference in expression between hepatocytes from the 2 different zones of the liver at a threshold of 0.1 for the false discovery rate adjusted *p*-values. Since more stringent criteria for statistical significance had been applied in our tumor microarray study, we now reanalyzed the tumor datasets using the same cutoffs as in our study on gene expression in perivenous and periportal hepatocyte subpopulations. Under these conditions, 777 (*Ctnnb1*) and 1063 (*Ha-ras*) probe sets were identified representing transcripts that differed in expression from normal liver (for illustration see Figure 1). We then used the tumor datasets for a comparison with the gene expression profiles in perivenous and periportal hepatocytes.

Upon overlay of the datasets, we found that 158 of the 243 probe sets (corresponding to 134 of 198 genes or expressed sequences) identified as differentially regulated in the zonation experiment were also present in either one of the two tumor datasets (see Figure 1). A total of 55 probe sets complied with the condition: zonation of expression of the respective transcripts in normal liver AND deregulation in *Ctnnb1* but not *Ha-ras* mutated tumors. The analogous number for alterations in *Ha-ras* but not *Ctnnb1* mutated tumors was 64. Zonation in normal liver AND deregulation in both tumor types was indicated by 39 probe sets. Therefore, 94 (55+39) and 103 (64+39) probe sets were identified for which their transcripts show zonal expression in normal liver AND alterations in *Ctnnb1* or *Ha-ras* mutated tumors, respectively.

The tumor vs. normal liver expression ratios of the 94 transcripts that showed alterations in *Ctnnb1* mutated tumors and zone-specific expression were then plotted against

their log expression ratios in perivenous versus periportal hepatocytes (Figure 2A). Each dot represents one probe set. Dots in the upper right gray area indicate probe sets that were (i) up-regulated in *Ctnnb1* mutated tumors and (ii) showed higher expression in perivenous than in periportal hepatocytes. In analogy, dots in the lower left area represent probe sets down-regulated in *Ctnnb1* mutated tumor cells with lower expression in perivenous than in periportal hepatocytes. Since several genes were represented by more than one probe set on the microarray, the numbers downsize from 94 to 85, if genes and expressed sequences are regarded instead of probe sets (for a detailed list see Supplementary Table 1). Of these 85 genes, 82 (96.5%) were regulated in the same direction, and only 3 genes (representing the 4 probe sets in the white areas of the plot in Figure 2A) showed alterations in opposite directions.

An analogous comparison was performed with gene expression data from *Ha-ras* mutated tumors, but for the sake of clearness, the y-axis was inversed now showing expression ratios of periportal versus perivenous hepatocytes (Figure 2B). The 103 probe sets that indicated deregulated genes and expressed sequences in *Ha-ras* mutated tumors which also demonstrated significant zonal expression in normal liver correspond to again 85 different transcripts (for a detailed list see Supplementary Table 2). Of these, 63 (74.1%) showed unidirectional changes in their expression in periportal hepatocytes and *Ha-ras* mutated tumors. Taken together, these results indicate that the gene expression patterns in the perivenous hepatocytes are highly correlated with those in *Ctnnb1* mutated hepatoma cells, whereas the expression patterns of periportal hepatocytes show some correlation with those of Ha-ras mutated hepatoma cells.

In the Supplementary Tables 1 and 2, genes are categorized according to the presumed function of their proteins. Since we were interested to know whether the above described concordance between gene expression in periportal and perivenous hepatocytes and *Ha-ras* and *Ctnnb1* mutated tumors would differ between functional categories, we performed gene expression profile comparisons for selected subsets of categorized genes as shown in Figure 3. Within these categories we found up to 100% of concordance of gene

expression between perivenous hepatocytes and *Ctnnb1* mutated tumors and between periportal hepatocytes and *Ha-ras* mutated tumors, respectively. However, certain genes with preferential periportal expression, belonging to the categories xenobiotic metabolism and transport, were decreased in *Ha-ras* mutated tumors, thus breaking the rule (for a list of these genes see Supplementary Table 2).

With 24 members (22 metabolic enzymes plus two nuclear receptors mediating the induction of xenobiotic metabolizing enzymes, i.e. AhR and CAR), the genes associated to xenobiotic metabolism constitute the largest group of functionally related genes that exhibit significant expression differences between hepatocytes from the two zones of the liver with 19 of them showing a preferentially perivenous zonation and 5 being mainly expressed in periportal hepatocytes (Braeuning et al., 2006). As shown in Table 1, 9 of these 24 genes were deregulated in both *Ctnnb1* and *Ha-ras* mutated tumors, 13 genes were present in one of the tumor datasets, whereas only 2 genes were not aberrantly expressed in either of the two tumor genotypes. Upon comparison of expression profiles it becomes apparent that most “perivenous” genes of both phase I and phase II of xenobiotic metabolism are overexpressed in *Ctnnb1* mutated tumor tissue (12 of 19 genes). On the other hand, in *Ha-ras* mutated hepatoma cells the majority of the “perivenous” genes is downregulated (11 of 19) with only one exception breaking the rule. Looking at the few genes with periportal localization, a general downregulation in *Ctnnb1* mutated tumors (3 of 5) is found. *Ha-ras* mutated tumors also express lower levels of mRNAs for periportal enzymes (3 of 5), again with one gene being regulated in the opposite direction. Thus, most of the periportally localized genes of the xenobiotic metabolism fail to show a correlation between *Ha-ras* mutated tumors and periportal hepatocytes.

Discussion:

Recently, we have developed a hypothesis to explain zonal heterogeneity in gene expression in murine liver (Hailfinger et al., 2006). We postulated the existence of two diametrically opposing signals, each gradual in nature, one activating β-Catenin-dependent and the other activating Ras-dependent downstream targets, which together determine periportal- and perivenous-specific hepatocyte differentiation. This hypothesis was primarily based on the finding that expression patterns of selected “marker” proteins/mRNAs in mouse liver tumors with activating mutations in *Ctnnb1* or *Ha-ras* were very similar to those of perivenous and periportal hepatocyte populations, respectively. The results from our present investigation on global gene expression profiles considerably extend our previous findings and give further support in favour of our recent hypothesis.

Our idea that zonal-specific gene expression in liver is triggered by external stimuli and is not intrinsically defined in periportal and perivenous hepatocytes, respectively, is supported by cell culture experiments, where hepatocytes are removed from their physiological environment such as the blood stream and neighboring endothelial cells. Under these conditions, striking differences in expression of zonated marker proteins such as GS or Cyp1a begin to alleviate already a few hours after perfusion (own unpublished observation). Furthermore, addition of serum to hepatocyte cultures strongly attenuates expression and inducibility of perivenous markers (unpublished observation), suggesting that a yet unidentified, probably Ras activating serum factor may play a role. Further support for our hypothesis comes from the finding that the expression of perivenous marker mRNAs is clearly inducible in periportal hepatocyte subpopulations *in vivo* and *in vitro* by activation of β-Catenin signaling ((Cadoret et al., 2002; Benhamouche et al., 2006); and own unpublished observations).

There is good evidence for the idea that the “perivenous phenotype” of hepatocytes is mediated via signaling through β-Catenin, since, e.g., expression of an activated form of β-Catenin in transgenic mice results in positive staining for the perivenous marker GS in most hepatocytes within the liver lobule (Cadoret et al., 2002) whereas GS and various CYP

isoforms are not expressed in perivenous hepatocytes from mice with a liver-specific conditional knockout of β-Catenin (Sekine et al., 2006). Additionally, the tumor suppressor APC (adenomatous polyposis coli), an important regulator of β-Catenin signaling, was established as the "zonation keeper" in liver (Benhamouche et al., 2006). Activation of β-Catenin in perivenous hepatocytes may be triggered by endothelial cells of the central veins which may deliver Wnt molecules activating upstream receptors within the Wnt/β-Catenin pathway. Endothelial cells play a decisive role in promotion of hepatic specification during the early stages of liver organogenesis (Matsumoto et al., 2001). Hepatocytes co-cultured with cells of the endothelial-like line RL-ET-14 express GS when neighboring the endothelial cells (Gebhardt et al., 1998) and GS expression could be inhibited in this system by silencing of β-Catenin expression by small-interfering RNA (Kruithof-de Julio et al., 2005).

β-Catenin signaling also seems to be an important regulator of xenobiotic metabolism in liver, firstly, since expression of various CYP isoforms is absent in perivenous hepatocytes from mice with a liver-specific knockout of β-Catenin (Sekine et al., 2006), secondly, since activation of β-Catenin signaling is able to induce the expression of several cytochrome P450 isoforms in cultured primary mouse hepatocytes (Hailfinger et al., 2006), and thirdly, since mutational activation of *Ctnnb1* in liver tumors is able to induce an expression profile of xenobiotic metabolizing enzymes which is highly correlated to that of perivenous hepatocytes (Table 1; see also (Loeppen et al., 2005)). On the other hand, in *Ha-ras* mutated tumor cells we observed a general downregulation of "perivenous" mRNAs encoding xenobiotic metabolism-related enzymes. This is in line with the observation that an activated version of Ha-ras downregulates AhR function and Cyp1a expression in mammary carcinoma cells and keratinocytes (Reiners et al., 1997). Often, enzymes which were upregulated in *Ctnnb1* mutated tumors were downregulated in *Ha-ras* mutated hepatomas, suggesting an antagonism of both pathways in the regulation of gene expression. However, since some "periportal" mRNAs were also downregulated in *Ha-ras* mutated liver tumors, additional signaling cascades different from the β-Catenin or Ha-ras pathway seem to be involved in the regulation of these genes. A candidate signal transducer mediating the "periportal"

phenotype of xenobiotic metabolizing enzymes may be growth hormone, since it has been demonstrated to determine the preferential periportal localization of Cyp2c7 in rat liver (Oinonen et al., 2000).

In summary, our present data clearly demonstrate that the patterns of gene expression in perivenous hepatocytes strongly resemble those of *Ctnnb1*-mutated hepatoma cells with activated β-Catenin, while the gene expression patterns of periportal hepatocytes resemble those of *Ha-ras* mutated hepatoma cells. There is convincing evidence from this and previous studies by our and other groups (Cadoret et al., 2002; Loeppen et al., 2002; Benhamouche et al., 2006; Sekine et al., 2006) that β-Catenin signaling plays a decisive role in mediating the perivenous phenotype of hepatocytes but further studies are required to identify the nature and the signal transduction mechanisms of the opposing factor(s) that appear to dictate the periportal hepatocytes phenotype.

References:

Benhamouche S, Decaens T, Godard C, Chambrey R, Rickman DS, Moinard C, Vasseur-Cognet M, Kuo CJ, Kahn A, Perret C and Colnot S (2006) Apc tumor suppressor gene is the "zonation-keeper" of mouse liver. *Dev Cell* **10:**759-770.

Braeuning A, Ittrich C, Köhle C, Hailfinger S, Bonin M, Buchmann A and Schwarz M (2006) Differential gene expression in periportal and perivenous mouse hepatocytes. *FEBS J* **273:**5051-5061.

Cadoret A, Ovejero C, Terris B, Souil E, Levy L, Lamers WH, Kitajewski J, Kahn A and Perret C (2002) New targets of beta-catenin signaling in the liver are involved in the glutamine metabolism. *Oncogene* **21:**8293-8301.

Gebhardt R (1992) Metabolic zonation of the liver: regulation and implications for liver function. *Pharmacol Ther* **53:**275-354.

Gebhardt R, Schuler M and Schorner D (1998) The spontaneous induction of glutamine synthetase in pig hepatocytes cocultured with RL-ET-14 cells is completely inhibited by trijodothyronine and okadaic acid. *Biochem Biophys Res Commun* **246:**895-898.

Hailfinger S, Jaworski M, Braeuning A, Buchmann A and Schwarz M (2006) Zonal gene expression in murine liver: Lessons from tumors. *Hepatology* **43:**407-414.

Jungermann K and Katz N (1989) Functional specialization of different hepatocyte populations. *Physiol Rev* **69:**708-764.

Kruithof-de Julio M, Labruyere WT, Ruijter JM, Vermeulen JL, Stanulovic V, Stallen JM, Baldysiak-Figiel A, Gebhardt R, Lamers WH and Hakvoort TB (2005) The RL-ET-14 cell line mediates expression of glutamine synthetase through the upstream enhancer/promoter region. *J Hepatol* **43:**126-131.

Lindros KO (1997) Zonation of cytochrome P450 expression, drug metabolism and toxicity in liver. *Gen Pharmacol* **28:**191-196.

Loeppen S, Koehle C, Buchmann A and Schwarz M (2005) A beta-catenin-dependent pathway regulates expression of cytochrome P450 isoforms in mouse liver tumors. *Carcinogenesis* **26:**239-248.

Loeppen S, Schneider D, Gaunitz F, Gebhardt R, Kurek R, Buchmann A and Schwarz M (2002) Overexpression of glutamine synthetase is associated with beta-catenin-mutations in mouse liver tumors during promotion of hepatocarcinogenesis by phenobarbital. *Cancer Res* **62:**5685-5688.

Matsumoto K, Yoshitomi H, Rossant J and Zaret KS (2001) Liver organogenesis promoted by endothelial cells prior to vascular function. *Science* **294:**559-563.

Oinonen T and Lindros KO (1998) Zonation of hepatic cytochrome P-450 expression and regulation. *Biochem J* **329 (Pt 1):**17-35.

Oinonen T, Ronis M, Wigell T, Tohmo K, Badger T and Lindros KO (2000) Growth hormone-regulated periportal expression of CYP2C7 in rat liver. *Biochem Pharmacol* **59:**583-589.

Reiners JJ Jr., Jones CL, Hong N, Clift RE, Elferink C (1997) Downregulation of aryl hydrocarbon receptor function and cytochrome P450 1A1 induction by expression of Ha-ras oncogenes. *Mol Carcinog* **19:**91-100.

Sekine S, Lan BYA, Bedolli M, Feng S and Hebrok M (2006) Liver-specific loss of ß-catenin blocks glutamine synthetis pathway activity and cytochrome P450 expression in mice. *Hepatology* **43:**817-825.

Stahl S, Ittrich C, Marx-Stoelting P, Kohle C, Altug-Teber O, Riess O, Bonin M, Jobst J, Kaiser S, Buchmann A and Schwarz M (2005a) Genotype-phenotype relationships in hepatocellular tumors from mice and man. *Hepatology* **42:**353-361.

Stahl S, Ittrich C, Marx-Stoelting P, Kohle C, Ott T, Buchmann A and Schwarz M (2005b) Effect of the tumor promoter phenobarbital on the pattern of global gene expression in liver of connexin32-wild-type and connexin32-deficient mice. *Int J Cancer* **115:**861-869.

Footnotes:

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Figure legends:

Figure 1: Comparison of mRNA expression profiles of *Ctnnb1* and *Ha-ras* mutated liver tumors with those of perivenous and periportal hepatocytes. Microarray expression data of *Ctnnb1* or *Ha-ras* mutated tumors vs. normal liver (Stahl et al., 2005a; Braeuning et al., 2006) were re-evaluated using the discriminators $p \leq 0.1$ and $|\log_2 \text{expression ratio}| \geq 1$ for detection of significant alterations in gene expression. The resulting data were then compared with expression data of perivenous and periportal hepatocytes isolated from normal liver which had been analyzed by the same criteria (Braeuning et al., 2006). The total number of probe sets analyzed was 22,600. Numbers in the circles indicate probe sets with significant alterations in expression of their corresponding transcripts. Of the 243 (55+39+64+85) probe sets with differential expression between perivenous and periportal hepatocytes (zonated in normal liver), 158 (55+39+64) were also deregulated in either one or both of the tumor types.

Figure 2: Gene expression patterns of perivenous and periportal hepatocytes from normal liver correlate with those of *Ctnnb1* and *Ha-ras* mutated liver tumors. Using the microarray datasets and criteria of analysis described in Figure 1, differential gene expression in perivenous and periportal hepatocytes was correlated with expression changes in *Ctnnb1* or *Ha-ras* mutated liver tumors. The plots show $\log_2$ expression ratios of probe sets representing transcripts with significant alterations in both screens. **(A)** Gene expression ratios in *Ctnnb1* mutated versus normal liver (n.l.) plotted against expression ratios in perivenous versus periportal hepatocytes. **(B)** Gene expression ratios in *Ha-ras* mutated versus normal liver (n.l.) plotted against expression ratios in periportal versus perivenous hepatocytes. Dots in the gray areas indicate probe sets with unidirectional alterations.

Figure 3: Concordance of gene expression in *Ctnnb1* and *Ha-ras* mutated hepatoma cells with the expression of the respective genes in perivenous and periportal normal hepatocytes. For comparison, genes were categorized according to the function of their products, as

indicated. Numbers in the bars indicate the respective number of genes represented by the bar.

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Tables:

Table 1: Zonal-specific expression of xenobiotic metabolizing enzymes in the liver and correlation to *Ctnnb1* and *Ha-ras* mutated liver tumors. Significant (adjusted *p* value ≤ 0.1) up- or downregulation ≥ 2-fold is indicated by arrows; arrows in brackets indicate a difference in expression of < 2-fold. Perivenous zonation is highly correlated to overexpression in *Ctnnb1* mutated tumors and downregulation in *Ha-ras* mutated hepatomas.

Accession no	Gene	zonation	*Ctnnb1* mutated	*Ha-ras* mutated
NM_009997	cytochrome P450, family 2, subfamily a, polypeptide 4 /// cytochrome P450, family 2, subfamily a, polypeptide 5	perivenous	↑	↑
NM_010359	glutathione S-transferase, mu 3	perivenous	↑	---
NM_008183	glutathione S-transferase, mu 2	perivenous	↑	↓
NM_145603	carboxylesterase 2	perivenous	↑	---
NM_008184	glutathione S-transferase, mu 6	perivenous	↑	↓
NM_134144 /// NM_206537	cytochrome P450, family 2, subfamily c, polypeptide 50 /// cytochrome P450 family 2, subfamily c, polypeptide 54	perivenous	---	↓
NM_009993	cytochrome P450, family 1, subfamily a, polypeptide 2	perivenous	↑	↓
NM_028089	cytochrome P450, family 2, subfamily c, polypeptide 55	perivenous	↑	---
NM_007815	cytochrome P450, family 2, subfamily c, polypeptide 29	perivenous	---	---
NM_013809	cytochrome P450, family 2, subfamily g, polypeptide 1	perivenous	---	---
NM_008898	P450 (cytochrome) oxidoreductase	perivenous	↑	---
NM_021282	cytochrome P450, family 2, subfamily e, polypeptide 1	perivenous	---	↓
NM_009803	constitutive androstane receptor	perivenous	---	↓
NM_013464	aryl-hydrocarbon receptor	perivenous	↑	↓
NM_010002	cytochrome P450, family 2, subfamily c, polypeptide 38	perivenous	↑	↓
NM_010356	glutathione S-transferase, alpha 3	perivenous	(↑)	↓
NM_019878	sulfotransferase family 1B, member 1	perivenous	---	↓
NM_016771	sulfotransferase family 1D, member 1	perivenous	---	↓
NM_010358 /// XM_485676	glutathione S-transferase, mu 1 /// similar to Glutathione S-transferase Mu 1 (GST class-mu 1) (Glutathione S-transferase GT8.7) (pmGT10) (GST 1-1)	perivenous	↑	---
NM_008182	glutathione S-transferase, alpha 2 (Yc2)	periportal	---	↓
NM_020577	arsenic (+3 oxidation state) methyltransferase	periportal	↓	---
NM_007817	cytochrome P450, family 2, subfamily f, polypeptide 2	periportal	↓	↓
NM_020564	sulfotransferase family 5A, member 1	periportal	---	↓
NM_028270	aldehyde dehydrogenase 1 family, member B1	periportal	↓	↑

Figure 1

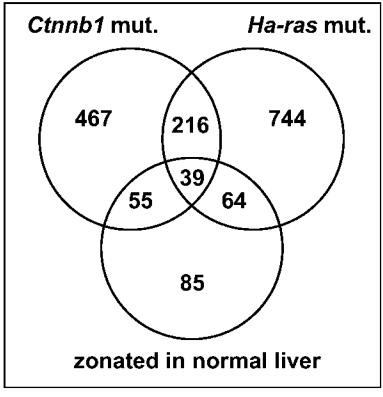

Ctnnb1 mut.
Ha-ras mut.
467
216
744
39
55
64
85
zonated in normal liver

Figure 2A

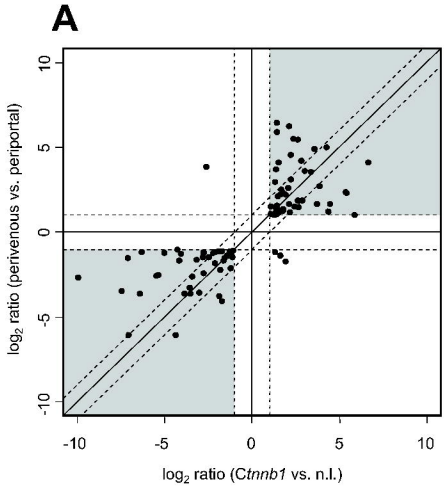

A
log2 ratio (perivenous vs. periportal)
log2 ratio (Ctnnb1 vs. n.l.)
10
5
0
-5
-10
-10
-5
0
5
10

Figure 2B

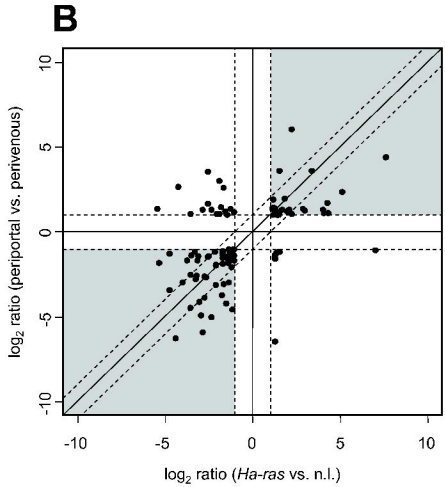
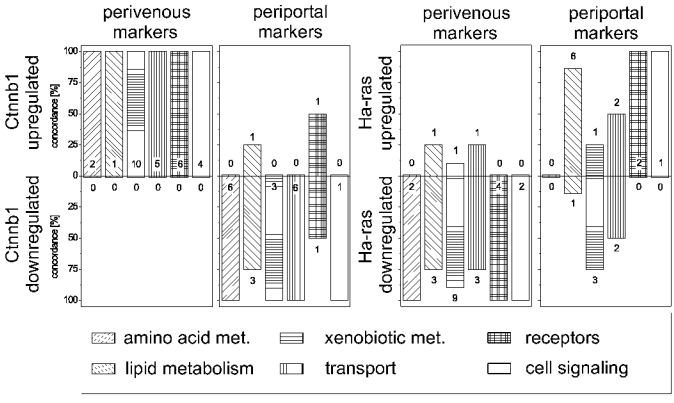

B
log2 ratio (periportal vs. perivenous)
log2 ratio (Ha-ras vs. n.l.)
10
5
0
-5
-10
-10
-5
0
5
10

Figure 3


perivenous markers
periportal markers
perivenous markers
periportal markers
Ctnnb1 upregulated
Ctnnb1 downregulated
Ha-ras upregulated
Ha-ras downregulated
concordance [%]
amino acid met.
xenobiotic met.
receptors
lipid metabolism
transport
cell signaling