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Gene expression profiling reveals signatures characterizing histologic subtypes of hepatoblastoma and global deregulation in cell growth and survival pathways

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Summary

Hepatoblastoma is the most common malignant tumor of the liver of children worldwide. Histologically, hepatoblastomas show marked variation in the type and proportion of epithelial (fetal, embryonal, or small cell) and mesenchymal components with differing prognosis and response to therapy. The pure fetal–type hepatoblastoma, presenting as stage 1 and resectable, has the best prognosis, whereas the small cell histology has been associated with unfavorable outcome. Using gene expression profiling, we demonstrate that in addition to *Wnt* pathway deregulation, cell growth and survival pathways are also globally deregulated in hepatoblastomas. Furthermore, the different histologic subtypes are characterized by specific gene expression and pathway signatures that give insight into the degree of molecular heterogeneity that is present among these tumors. Although *Wnt* signaling pathway upregulation is common to all histologic types of hepatoblastoma, this pathway is even more significantly deregulated in aggressive hepatoblastomas. In addition, deregulation of MAPK signaling pathway and antiapoptotic signaling is preferentially upregulated in aggressive epithelial hepatoblastomas with a small cell component. The gene expression signatures reported here provide possible prognostic and diagnostic markers as well as therapeutic targets for this disease.

Keywords

Gene expression profiling; Fetal; Small cell and embryonal hepatoblastoma; Liver tumors; Pediatric

1. Introduction

Hepatoblastoma (HB) is the most common malignant tumor of the liver of children worldwide [1] and despite its rarity has been treated with some success by the combined efforts of several national and international consortia [2]. Nevertheless, some children die because of early and widespread dissemination and resistance to usually effective chemotherapy [3]. Histologically, HBs show marked variation in the type and proportion of epithelial (fetal, embryonal, or small cell) and mesenchymal components, with differing prognosis and response to therapy [3]. The stage 1 pure fetal (PF)–type HB can be cured by surgery alone, whereas small cell

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undifferentiated histology has been associated with unfavorable outcome regardless of resectability [4].

The occurrence of HB in the context of familial syndromes such as Beckwidth-Wiedemann syndrome and familial adenomatous polyposis with APC mutations indicates the multiplicity of specific signaling pathways in the development of HB. Although *APC* mutations have been infrequent in sporadic HB, recent studies have identified *β*-catenin mutations and *met* deregulation in childhood HB, thus implicating the *wnt* and *MAPK* signaling pathways in the biology of these tumors [5]. Paradoxically, the elevation of *wnt* antagonists has also been described in HB [6] which partly represents a negative feedback response resulting from *β-catenin* mutations and constitutive activation of the canonical *Wnt* pathway [6].

In a recent report, Luo et al [7] compared HB with hepatocellular carcinomas (HCC) and identified upregulation of expression of *MIG6, TGFb1, DLK1,* and *IGF2* in HB. These genes were differentially expressed between HB and HCC and did not separate HB histologic subtypes. A difference in expression of *claudin 1* and *claudin 2*, which are epithelial tight junction proteins, has been reported between fetal and embryonal HB [8].

Given the widespread deregulation of the *Wnt–β-catenin* pathway in multiple histologic subtypes, we hypothesized that the prognostic differences associated with histologic subtypes of HB may be explained by perturbation of other pathways and genes. To dissect the full spectrum of genetic changes, we carried out gene expression profiling on a panel of HB using the Affymetrix platform (Affymetrix, Santa Clara, CA). We found that, in addition to *Wnt*, cell growth and survival pathways are also globally deregulated in HB. Furthermore, the different subtypes were characterized by specific gene expression and pathway signatures that give us insight into the extensive heterogeneity that is characteristic of this tumor. These same genes provide powerful prognostic and diagnostic markers as well as possible therapeutic targets for this disease.

2. Materials and methods

2.1. Hepatoblastoma samples and RNA preparation

After obtaining institutional review board approval, 13 primary HBs were analyzed in the study, including 8 that are exclusively epithelial (2 PF, 3 fetal/embryonal, 3 fetal/embryonal/small cell), and 5 with mixed epithelial (fetal/embryonal) and mesenchymal components. Frozen and paraffin-embedded tumor tissues were obtained from the Cooperative Human Tissue Network (Biopathology Center, Columbus, OH), and tumors were seen in consultation at Texas Children's Hospital, Department of Pathology. Three separate sets of pooled fetal liver (FL) and normal adult liver (NL) mRNA (BD Clontech, Mountain View, CA) were used as reference controls. For validation studies, 34 cases of HB (including 12 of the 13 primary HBs used for gene profiling) were used for quantitative real-time reverse transcription-polymerase chain reaction (Qrt-RT-PCR) analysis

2.2. RNA Extraction, quantitation, and analysis

Total RNA was isolated from frozen tissue sections and areas of tumor previously confirmed by histologic examination using RNAqueous Micro Kit (Ambion, Austin, TX #1927) followed by Dnase treatment and inactivation. RNA concentration was analyzed using a NanoDrop ND-1000 Spectrophotometer. To evaluate the integrity of RNA in the samples after quantitation of concentration, an appropriate dilution of total RNA was analyzed using the Agilent 2100 Bioanalyzer and the RNA 6000 Nano LabChip kit.

2.3. Quantitative real-time reverse transcription-PCR

This procedure and the sequences of the primers used are detailed in Lopez-Terrada et al [9]. Simply, expression of 17 WNT target genes (DKK, IGF2, AXIN2, SOX9, MMP7, BetaTRCP, bone morphogenetic protein 4, CMYC, Cyclin D1, EGFR, Gpr49, ITF2/TCF4, MET, NKD, NLK, and UPAR) and 2 Notch pathway genes (DLK, Hes1) was analyzed in 32 HB cases by Qrt-RT-PCR using SYBR Green. Results were normalized using normal liver controls.

2.3.1. Gene profiling and quality analysis—Commercially available high-density oligonucleotide microarrays HG_U133A and U133 plus (Affymetrix) were used for this study. Preparation of cRNA, hybridization, scanning, and image analysis of the arrays were done according to the manufacturers' protocols. Briefly, 5 μg of total RNA was used to generate cRNA probes and combined with a mixture of control cRNAs (made from bacterial genes BioB, BioC, BioDN, and CreX) before hybridization. All GeneChip images were visually inspected for irregularities. The raw median signal for 17 (13 HBs, 3 pooled FL, and 1 pooled NL) of the arrays (75 ± 41) and the median percentage of genes present (55 ± 4.03) indicated the high overall quality of the assays.

2.3.2. Data analysis—Raw images (.dat files) from Affymetrix GeneChip scanner were processed with dChip 2006 software (http://biosun1.harvard.edu/complab/dchip/). The raw signal of individual probes for the 17 arrays was normalized against the chip with median raw signal intensity and is based on a set of probes called an "invariant set" that consists of points from nondifferentially expressed genes. After normalization, the expression values of each gene in all samples were computed using a perfect match–only model followed by outlier detection algorithm. A gene was identified as present (P call) when a *P* value of less than .05 is obtained for the probe set. The tumor and reference samples analyzed with the 2 chips sets U133A and U133 plus were merged using the U133A gene info list. They were then scaled to have the same median array. Expression values were normalized using the same "invariant set" normalization method [10]. HB3 was excluded from further analysis because the percentage of genes present on the chip analysis was lower than 50%. Unsupervised hierarchical clustering was done with dChip 2006.

2.3.2.1. Identification of differentially expressed genes: With the dChip 2006 software, differentially expressed genes greater than 1.5-fold, with intensity difference greater than 100 units, were obtained by comparing the entire group of HB tumors with the triplicate samples of pooled FL. With the use of the same criteria, subsets of tumors classified as PF, epithelial (fetal/embryonal), mixed epithelial and mesenchymal, and epithelial (fetal/embryonal) with small cell components were independently compared with FL. The list of genes differentially expressed between the FL and all HBs was used for hierarchical clustering analysis using the Euclidean distance approach for distance metric and the centroid method for linkage. Gene ordering was done by cluster tightness. The *P* value for calling a significant cluster was .001.

2.3.2.2. Analysis of signaling pathway changes and alterations: The pattern of alteration of signaling pathways was determined using the Web-based Intelligent Systems and Bioinformatics software (http://vortex.cs.wayne.edu/) [11–15] and Ingenuity Pathway Analysis software (https://analysis.ingenuity.com/). These software applications allow a determination of the relative functional significance of molecules present on the differentially expressed gene list. Using the list of differentially expressed genes, they construct functional profiles (using gene ontology terms) including biochemical function, biological process, cellular role, cellular component, and molecular function. They also highlight statistically significant cellular functions (at $P < .05$), which allows a better understanding of the biological phenomenon present in the set of tumors analyzed.

3. Results

The expression profiles of ~22 000 transcripts were analyzed in a panel of HBs using the Affymetrix U133A gene list. Twelve arrays with P call greater than 65% were selected for the analyses. Three pooled FL and one pooled NL were used as controls.

3.1. Fetal liver versus all histologic types of HB

Comparison of FL with all HBs showed a total of 942 differentially expressed genes. This gene list was used for unsupervised hierarchical clustering to see whether HB can be stratified into subgroups based on gene expression signatures. The control FL and NL clustered together in the dendrogram are shown in Fig. 1. Epithelial HB with small cell components and 4 of 5 mixed epithelial and mesenchymal HB clustered together into 2 respective groups. The PF HB and epithelial (fetal + embryonal) HB were randomly clustered between the mixed epithelial and mesenchymal tumors and FL.

Pathway analysis of the differentially expressed genes between FL and all HB revealed the deregulation of a number of cell signaling pathways. *Wnt* signaling (Fig. 2C), cell cycle (Fig. 2B), adipocytokine signaling, *TGF β* (Fig. 2D), *PPAR* signaling, and extracellular matrix–receptor interaction pathways were significantly upregulated ($P < .05$) (Fig. 2A). The apoptosis pathway was significantly down-regulated ($P = .05$) (Fig. 2F). In this comparison, the gene expression profiles of HB overlapped with the gene expression profiles of melanoma, small cell lung cancer, and prostate cancer (Fig. 2E and G).

3.1.1. *Wnt* signaling pathway genes—The *Wnt* pathway was the most significantly upregulated pathway in all HB (Table 1). The *Wnt* ligand *Wnt5a* and molecules directly involved in the cell cycle including *Cyclin D1 (CCND1)* involved in cell proliferation were induced, as were some genes in the noncanonical *Wnt* pathway such as *DAAM1* and *ROCK2*. *Wnt* antagonists *dickkopf homolog 1, 2, and 4 (DKK1, 2, and 4) and WNT inhibitory factor 1,* which are also target genes of the Wnt pathway, were also induced (Fig. 2C). As most HBs have a constitutively active *β-catenin* protein, upstream antagonists are likely to have no influence.

3.1.2. Cell cycle genes—Cell cycle genes *CCND1*, *CDC14 cell division cycle 1 homolog B,* and *RBL2* are upregulated. In contrast, many cell cycle genes were downregulated. These include *cell division cycle 20 homolog*, *mini chromosome maintenance complex component 7*, *polo-like kinase 1*, *pituitary tumor–transforming 1*, *RBL2*, *ring-box 1*, *TGF β1*, and *tyrosine 3-monooxygenase/tryptophan (YWHAH)* (Fig. 2B).

3.1.3. *TGF* signaling pathway genes—Upregulated genes include *bone morphogenetic protein 4, chordin, follistatin, retinoblastoma-like 2 (p130), ROCK2,* and *SMAD3*. *TGF β1* and *ring-box1* were downregulated (Fig. 2D).

3.1.4. Apoptosis—Proapoptotic genes downregulated include *BCL2-like 1 (BCL2L1)* and *protein kinase cAMP-dependent regulatory type II*. In contrast, a number of antiapoptotic and prosurvival genes are upregulated. These include *BIRC2, CASP8,* and *FADD-like apoptosis regulator (CFLAR), PIK3R1, PPP3CA, tumor necrosis factor receptor superfamily member (TNFRSF10B),* and *tumor necrosis factor (ligand) superfamily member (TNFSF10)* (Fig. 2F).

3.1.5. Cancer-related gene expression—Some of the differentially regulated genes recapitulate the pattern of gene alterations seen in a number of malignant tumors such as small cell lung cancer, melanoma, and prostate cancer. Notable among these genes is the upregulation of the antiapoptotic gene *baculoviral IAP repeat-containing 2 (BIRC2)* and prosurvival gene

phosphoinositide-3-kinase regulatory subunit 1 (p85). In contrast, proapoptotic gene *BCL2L1* is downregulated (Fig. 5). Cell cycle–related genes *cyclin-dependent kinase 2 (cdk2)* and *cyclin E1* are down-regulated. Extracellular matrix proteins *laminin α 3 and 4* and *laminin gamma 1* are upregulated. In addition, *phosphatase and tensin homolog* is also upregulated, a feature shared with small cell lung cancer and prostate cancer. Proliferation enhancing genes including *cAMP responsive element binding protein 3–like 2*, *v-erb-b2 erythroblastic leukemia viral oncogene (ERBB2)*, *glycogen synthase kinase 3 β*, and *insulin-like growth factor 1* are also upregulated (Fig. 2E and G).

3.2. Fetal liver or PF HB versus histologic subtypes of HB

A significant number of genes involving multiple pathways were differentially expressed between fetal liver versus PF HB (1260 genes; Fig. 3A), fetal liver versus epithelial HB (fetal and embryonal) (1135 genes; Fig. 3B), fetal liver versus mixed epithelial (fetal/embryonal) and mesenchymal HB (824 genes; Fig. 3C), and fetal liver versus epithelial HB with fetal, embryonal, and small cell components (622 genes; Fig. 3D).

Similarly, a comparison of PF HB with other histologic subtypes showed a number of differentially expressed genes as follows: PF HB versus epithelial HB with fetal and embryonal components—239 genes; PF HB versus mixed epithelial (fetal, embryonal) and mesenchymal HB—439 genes; PF HB versus epithelial HB with fetal, embryonal, and small cell components —3075 genes (Fig. 4A and B). Many of the same pathways including cell cycle, *Wnt*, *TGF β* signaling pathway, adherens junction, and antiapoptosis were significantly differentially deregulated between PF liver and the more aggressive subtypes containing a small cell component. Notably, MAPK signaling pathway was found to be differentially upregulated between PF HB and epithelial HB tumors with a small cell component (Table 2).

3.3. Comparison of the differentially expressed genes between fetal liver and HB subtypes

The differentially expressed gene lists obtained from a comparison of fetal liver versus all histologic types of HB, versus PF HB or epithelial (fetal/embryonal) HB or mixed epithelial (fetal/embryonal) and mesenchymal HB or epithelial (fetal/embryonal/small cell) HB were subjected to analysis with the ingenuity software (https://analysis.ingenuity.com/) to determine the extent of divergence, if any, in the biofunctional classification of the differentially expressed genes between all HBs as well as subtypes of HB and fetal liver. The analysis shows similar patterns of significant upregulation of cancer-related genes, cell death regulatory genes, and cell cycle control genes in all histologic groups of HB. Although all groups show significant upregulation of cellular growth and proliferation genes, there is a significantly lower level of expression of cellular growth and proliferation genes in PF HB. Cell cycle genes *cyclin D3* and *cyclin E1* were significantly downregulated relative to fetal liver. In addition, organismal survival genes were significantly upregulated in epithelial HB with fetal/embryonal and/or small cell components in contrast to PF HB in which the expression of those genes did not reach statistical significance (Fig. 5).

3.4. Validation of differential expression of *Wnt* and *Notch* pathway genes

The level of expression of Wnt signaling and Notch pathway genes was examined in a larger subset of 34 HBs by quantitative real-time RT-PCR. This validation subset also included the 12 HBs used for the gene expression profiling studies. The result of analysis of these genes is presented in a related manuscript by Lopez-Terrada et al [9].

3.5. Chromosomal localization of upregulated genes in HB

The chromosomal localization of genes that are differentially expressed between fetal liver and all the HB was determined. The analysis shows localization of these genes in all chromosomes

with no preferential localization in a specific subset of chromosomes (Fig. 6A). In addition, the chromosomal localization of genes differentially expressed between PF and epithelial tumors with fetal and embryonal components (Fig. 6B), mixed epithelial and mesenchymal components (Fig. 6C), and epithelial tumors with small cell component (Fig. 6D) was also determined, respectively. There were significant differences in the chromosomal distribution of the differentially expressed genes between PF and epithelial HB with fetal and embryonal components. The chromosomes with significant differential concentration of these genes were chromosomes 4, 8, 9, 10, 13, 14, 15, 18, 20, 21, and 22. Similarly, genes differentially expressed between PF HB and mixed epithelial and mesenchymal HBs were significantly more concentrated on chromosomes 13, 18, and 21. Epithelial HB with a small cell component showed a high density of differentially expressed genes when compared with PF HB, but there was no specific predilection for a specific subset of chromosomes.

3.6. Conclusion

HBs with well-differentiated PF epithelial morphology comprise about 5% of tumors. When associated with low mitotic activity, they represent the best prognostic histologic subtype of HB and if amenable to surgical resection (stage I) are cured without chemotherapy [4]. This contrasts with all other HB subtypes that require cisplatin/doxorubicin-based chemotherapy as well as surgical resection and in some cases liver transplantation [16].

Gene expression profiling studies in HBs are few, with only a few differentially expressed genes with prognostic significance having been identified, including increased expression of the *polo-like kinase 1* gene in association with aggressive phenotype and poor prognosis [7, 17]. In a recent study, IGF2, fibronectin, *DLK1*, *TGFb1*, *MALAT1*, and *MIG6* were found to be overexpressed in 7 HBs compared with HCC [7].

In this study, we have analyzed the gene expression profile of different histologic HB subtypes associated with prognosis. We note that canonical *Wnt* signaling is upregulated in all HBs, with the most significant induction in the more aggressive tumors (Table 1). For example, Cyclin D1 (a marker of *Wnt* activation) is significantly more upregulated than in PF HB with better prognosis. Cyclin D3 and E1 were in fact found to be downregulated in PF HB. As has been previously reported [18], we also observed upregulation of *Wnt* pathway antagonists such as *DKK1* in all HBs. As these antagonists act upstream of *CCND1* in the *Wnt* pathway and their expression is also upregulated by *Wnt* pathway activation, we suggest therefore that their upregulation may only serve as markers of *Wnt* pathway activation.

Similarly, although cell cycle genes are significantly upregulated in the combined group of all HBs, the PF subtype showed relatively very low levels of expression of cell cycle genes. These levels were even significantly lower than seen in fetal liver (probably because of the large proportion of proliferating hematopoietic precursors from extramedullary hemopoiesis that is normally present in fetal liver), a finding consistent with the reported low proliferative activity in fetal HB (Table 2).

A comparison of fetal HB with fetal liver showed an upregulation of antiapoptotic pathways including the upregulation of *CFLAR* and downregulation of proapoptotic genes like *BCL2L1*. Epithelial tumors containing not only fetal but also embryonal and small cell components do not only show upregulated antiapoptotic genes but they also have a set of organismal prosurvival genes such as the *PIK3R1*, *PPP3CA*, *tumor necrosis factor receptor superfamily member (TNFRSF10B)*, and *tumor necrosis factor (ligand) superfamily member (TNFSF10)* highly upregulated as well and at levels significantly higher than in PF HBs (Fig. 4).

A noteworthy observation in this study is the finding of significant upregulation of MAPK signaling pathway genes in epithelial tumors with small cell component when compared with PF HB. This suggests that the aggressive phenotype seen in these tumors may be partly related to the activation of the MAPK pathway. Notable upregulated components of this pathway include *epidermal growth factor receptor (erythroblastic leukemia viral [verb-b] oncogene homolog, avian) (EGFR), fibroblast growth factor 1 (acidic), fibroblast growth factor 14, fibroblast growth factor 23,* and *transforming growth factor β receptor 1 (TGFB R1). EGFR* and *TGFB R1* have been reported as upregulated in HB [7,19]. We find a specific relationship between aggressive histology and the upregulation of the expression of these genes. These findings provide insight into the differential role of *Wnt* signaling, apoptotic/organismal survival, cell cycle genes, and MAPK pathway activation between PF HB and epithelial HB with small cell component. As EGFR is also a target of canonical *Wnt* pathway activation, its upregulation provides an important link between canonical Wnt activation and upregulation of the MAPK pathway in aggressive HB.

Mixed epithelial and mesenchymal HBs share the expression profiles of epithelial tumors containing fetal and embryonal components only. In addition, they show a significant upregulation of genes in the extracellular matrix–receptor interaction pathway including CD47 molecule, laminin α 4, laminin β 1, secreted phosphoprotein 1 (bone osteopontin; SPP1), and synaptic vesicle glycoprotein 2A (SPR1), which is consistent with the presence of mesenchymal differentiation in these tumors.

A number of chromosomal events including copy gains of portions or entire components of chromosomes 2, 8, 14, 19, 20, and X [2,20–32] have been described by us and others. In addition, recurring aberrations of 1q12-q21 and 2q [22,27–29] have also been reported. A comparison of the differentially expressed genes between fetal liver and all the HBs shows a nonselective distribution of genes in all chromosomes in a proportion consistent with the relative size of each chromosome. The chromosomal distribution of genes that are differentially expressed between PF liver and epithelial HB with fetal and embryonal components showed significant differential concentration on chromosomes 4, 8, 9, 10, 13, 14, 15, 18, 20, 21, and 22. In addition, there are a much larger number of genes that are upregulated in the epithelial tumors containing either the embryonal or small cell components, with the differentially expressed genes from tumors having a small cell component showing the greatest density of genes per chromosome. This observation is intuitive and is consistent with greater perturbation of global gene expression in tumors with a small cell component.

In summary, we demonstrate a progressive perturbation of critical signaling pathways responsible for growth, cell proliferation, and organismal survival in HBs. Although *Wnt* signaling pathway upregulation is common to all histologic types of HB, this pathway is even more significantly induced in aggressive HBs. In addition, induction of MAPK signaling pathway and antiapoptotic signaling is preferentially seen in aggressive epithelial HBs with a small cell component.

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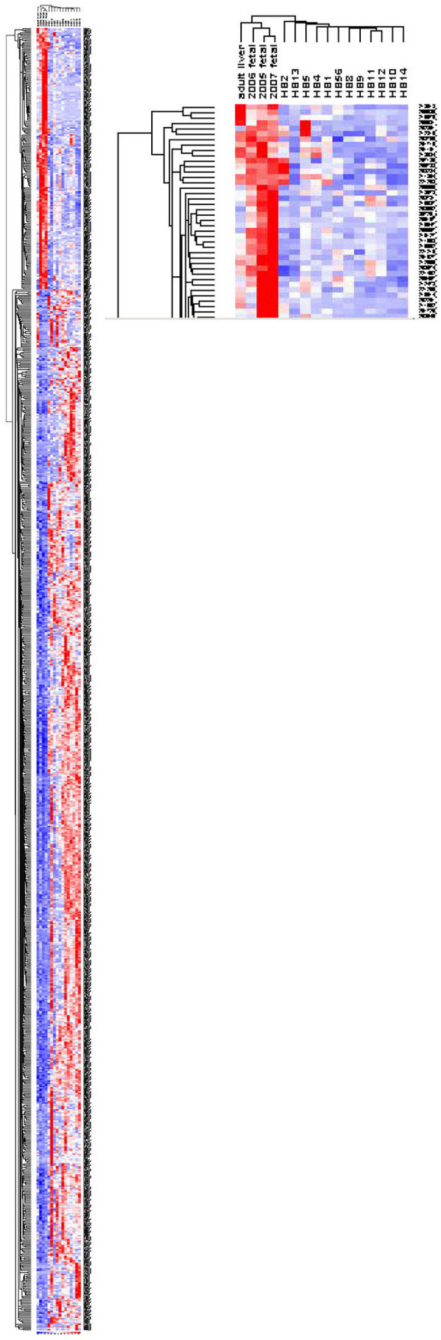

Fig. 1.

Unsupervised hierarchical clustering analysis produced separate groupings for normal liver tissues (fetal liver and adult liver), 4 of 5 mixed epithelial and mesenchymal HBs (HB9, 10, 11, and 12) and epithelial HB with small cell component (HB4 and 5). Pure fetal HB (HB13 and 56) and epithelial tumors with only fetal and embryonal components (HB1, 8, and 14) are randomly distributed.

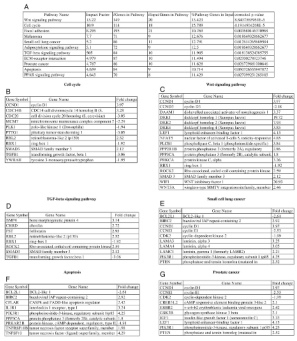

Fig. 2.

Analysis of genes differentially expressed between fetal liver and all HBs shows significant activation of signaling pathways including (A) wnt signaling, cell cycle, etc. The fold change for genes which are components of the (B) cell cycle, (C) wnt signaling pathway, (D) TGF β signaling pathway, and (F) apoptosis, as well as deregulated genes shared with other specific malignancies such as (E) small cell lung cancer and (G) prostate cancer, is shown.

Fetal Liver vs Pure fetal HB

A

Pathway Name	Impact Factor	Corrected p-value
Cell cycle	12.447	1.0910261553E-5
Prostate cancer	6.722	0.003227448217892
Wnt signaling pathway	6.001	0.010562672731135
Melanoma	5.818	0.050046212677767
Renal cell carcinoma	5.611	0.024272059006404
Adipocytokine signaling pathway	5.214	0.021975461880607
Thyroid cancer	4.322	0.037784385836888

Fetal liver vs Epithelial (Fetal + Embryonal) HB

B

Pathway Name	Impact Factor	corrected p-value
Cell cycle	13.948	2.859844785E-6
TGF-beta signaling pathway	7.59	0.00225274605802
Wnt signaling pathway	7.193	0.004574859618242
Pancreatic cancer	5.419	0.019059002305523
Adherens junction	4.984	0.025166871928585

Fetal Liver vs Mixed Epithelial and Mesenchymal HB

C

Pathway Name	Impact Factor	corrected p-value
Wnt signaling pathway	13.54	1.586305088 08E-4
Melanoma	8.268	0.008669479845177
Focal adhesion	7.508	0.004290618320707
Colorectal cancer	6.794	0.003512747418379
Cell cycle	6.506	0.003239347626046
thyroid cancer	5.669	0.021226046383472
ECM-receptor interaction	5.659	0.010267225983245
Prostate cancer	5.448	0.01207737100297
Small cell lung cancer	4.992	0.01411838981802
Melanogenesis	4.916	0.045499618094107
Basal cell carcinoma	4.539	0.031699458971814
Endometrial cancer	4.189	0.049069797450401

Fetal Liver vs Epithelial (Fetal + Embryonal + Small cell) HB

D

Pathway Name	Impact Factor	corrected p-value
Cell cycle	20.782	2.724808E-9
Circadian rhythm	11.966	0.009925912897377
TGF-beta signaling pathway	7.319	0.006427118894395
Chronic myeloid leukemia	6.935	0.004163433539015
Wnt signaling pathway	6.792	0.003994342971435
Melanoma	6.56	0.008228684822052
Complement and coagulation cascades	6.119	0.022185484849355
Pancreatic cancer	5.991	0.011493552875276
Ubiquitin mediated proteolysis	5.839	0.006622358174813
Thyroid cancer	5.414	0.009494593333264
Adherens junction	5.307	0.01450530140189
Apoptosis	5.209	0.022184405305865
Glioma	4.973	0.045423417137902

Fig. 3.

A–D show genes differentially expressed between fetal liver and other histologic subtypes of HB, the association of these genes with the deregulation of specific pathways, and genes shared with other specific malignancies. The *P* values reflect the degree of “significance” of perturbation of specific pathways such as wnt and cell genes which correlate with aggressiveness of histologic subtypes.

A

Pathway Name	Impact Factor	corrected p-value
Prostate cancer	13.233	5.642389819E-6
MAPK signaling pathway	8.445	8.84172591885E-4
Regulationof actin cytoskeleton	6.642	0.005104530943268
Melanoma	6.085	0.005893360861044
Dorso-ventral axis formation	5.848	0.009540439109097
Endometrial cancer	5.541	0.019232713825431
Glioma	5.389	0.014191931924717
Chronic myeloid leukemia	5.294	0.017538829274449
Renal cell carcinoma	5.249	0.028377646683544
Colorectal cancer	4.493	0.031752790054533
Non-small cell lung cancer	4.377	0.046844407480892
Notch signaling pathway	4.347	0.030821552252144

Pathway Genes Details - MAPK signaling pathway - KEGG

B

Gene Symbol	Gene Name	Fold change
ACVR1 B	activin A receptor, type IB	2.7
AKT3	v-akt murine thymoma viral oncogene homolog 3 (protein kinase B, gamma)	-4.25
CACNA1I	calciu m channel, voltage-dependent, T type, alpha 1I subuni t	2.06
CACNA1S	calcium channel, voltage-dependent, L type, alpha 1S subunit	1.93
CACNB1	calciu m channel, voltage -dependent, beta 1 subunit	-2.47
CACNB2	calcium channel, voltage-dependent, beta 2 subunit	-2.75
CACNG1	calcium channel, voltage-dependent, gamma subunit 1	2.09
CACNG4	calcium channel, voltage-dependent, gamma subunit 4	2.01
CACNG5	calcium channel, voltage-dependent, gamma subunit 5	14.83
CD14	CD14 molecule	3.41
CRKL	v-crk sarcoma virus CT10 oncogene homolog (avian)-like	2.09
DUSP10	dual specificity phosphatase 10	-2.47
DUSP4	dual specificity phosphatase 4	-2.63
EGFR	epidermal growth factor receptor (enythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)	1.76
ELK1	ELK1 member of ETS oncogene family	2.02
FASLG	Fas ligand (TNF superfamily, member 6)	3.31
FGF1	fibroblast growth factor 1 (acidic)	2.65
FGF14	fibroblast growth factor 14	2.71
FGF17	fibroblast growth factor 17	1.98
FGF22	fibroblast growth factor 22	-3.05
FGF23	fibroblast growth factor 23	2.02
FLNA	filamin A, alpha (actin binding protein 280)	1.86
GADD45B	growth arrest and DNA-damage-inducible, beta	-2.81
GADD45G	growth arrest and DNA-damage-inducible, gamma	-3.16
GNA12	guanine nucleotide binding protein (G protein) alpha 12	2.0
IKBKB	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta	-2.86
IKBKG	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma	2.01
IL1B	interleukin 1, beta	2.81
IL1R2	interleukin 1 receptor, type II	1.83
KRAS	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	-3.02
MAP2K3	mitogen-activated protein kinase kinase 3	2.41
MAP2K5	mitogen-activated protein kinase kinase 5	1.99
MAP2K7	mitogen-activated protein kinase kinase 7	-2.28
MAP3K14	mitogen-activated protein kinase kinase kinase 14	3.01
MAP3K3	mitogen-activated protein kinase kinase kinase 3	2.97
MAP3K7IP2	mitogen-activated protein kinase kinase kinase 7 interacting protein 2	-5.03
MAP4K3	mitogen-activated protein kinase kinase kinase kinase 3	-1.74
MAPK1	mitogen-activated protein kinase 1	1.69
MAPK7	mitogen-activated protein kinase 7	2.34
MAPKAPK3	mitogen-activated protein kinase-activated protein kinase 3	-2.62
MOS	v-mos Moloney murine sarcoma viral oncogene homolog	3.37
MRAS	muscle RAS oncogene homolog.	2.34
NF1	neurofibromin 1 (neurofibromatosis, von Recklinghausen disease, Watson disease)	2.87
NR4A1	nuclear receptor subfamily 4, group A, member 1	2.49
NTRK2	neurotrophic tyrosine kinase, receptor, type 2	1.94
PAK2	p21 (CDKN1 A)-activated kinase 2	-16.49
PDGFA	platelet-derived growth factor alpha polypeptide	-2.78
PDGFB	platelet-derived growth factor beta polypeptide (simian sarcoma viral (v-sis) oncogene homolog)	-2.29
PDGFRA	platelet-derived growth factor receptor, alpha polypeptide	-2.71
PDGFRB	platelet-derived growth factor receptor, beta polypeptide	2.95
PLA2G12A	phospholipase A2, group XIIA	-1.72
PLA2G2A	phospholipase A2, group IIA (platelets, synovial fluid)	5.92
PLA2G2D	phospholipase A2, group IID	2.03
PPP3CC	protein phosphatase 3 (formerly 2B), catalytic subunit, gamma isoform	-2.46
PRKACA	protein kinase, cAMP-dependent, catalytic, alpha	2.86
PTPN7	protein tyrosine phosphatase, non-receptor type 7	2.46
RAP1B	RAP1B, member of RAS onc ogene family	-1.87
RASGRF1	Ras protein-specific guanine nucleotide-releasing factor 1	3.18
RASGRP2	RAS guanyl releasing Protein 2 (calcium and DAG-regulated)	2.33
RPS6KA5	ribosomal protein S6 kinase, 90kDa, polypeptide 5	-2.55
SRF	serum response fac tor (c-fos serum response element-binding transcription factor)	2.52
STK4	serine/threonine kinase 4	-13.2
STMN1	stathmin 1/oncoprotein 18	-4.21
TAOK3	TAO kinase 3	-2.89
TGFBR1	transforming growth factor, beta r eceptor I (activin A receptor ty pe II-like kinase, 53kDa)	3.16

Fig. 4.

Analysis of genes differentially expressed between PF HB and epithelial HB with small cell component shows (A) significant perturbation of MAPK signaling pathway among others. MAPK signaling pathway genes and their fold change are shown in (B).

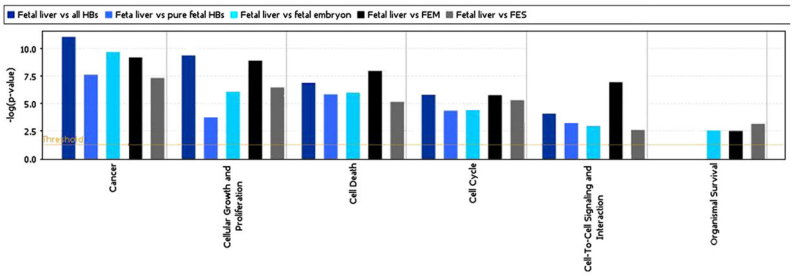

Fig. 5.

Bar chart illiustrates the differential perturbation of genes involved in specific biofunctions including cancer related genes, cell growth and proliferation, cell death, cell cycle, and organismal survival when each of the histologic subtypes are compared with normal fetal liver. It also shows a significant differential upregulation of organismal survival genes only in the epithelial HBs with embryonal and/or small cell components.

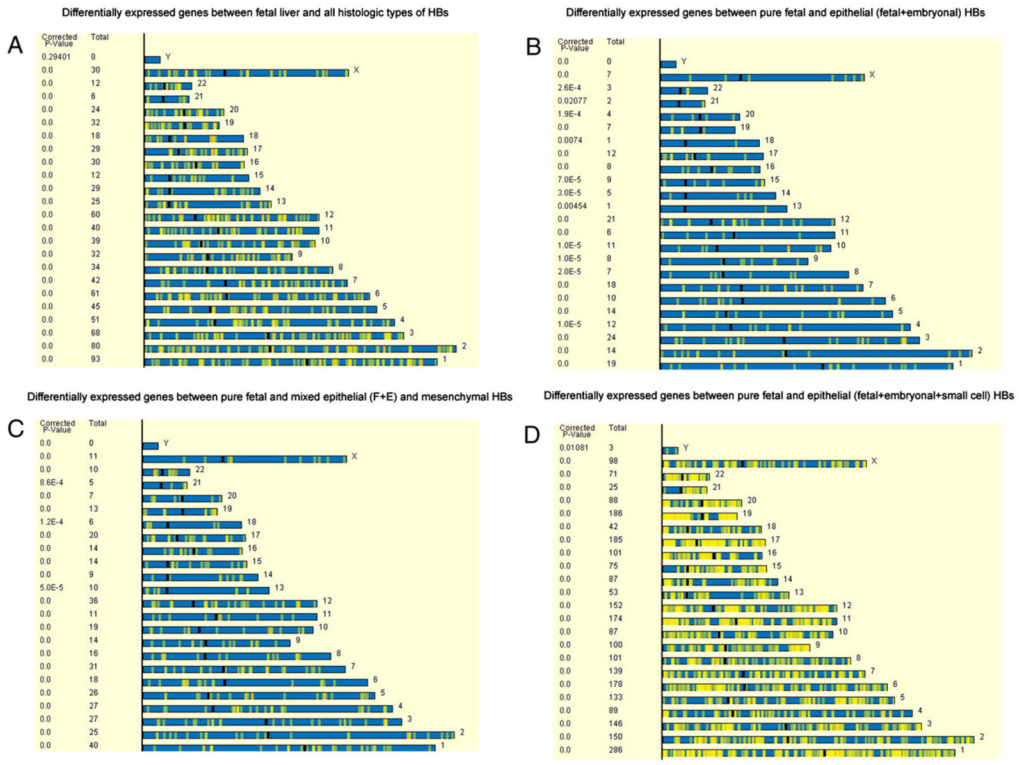

Fig. 6.

Chromosomal localization of genes differentially expressed between (A) FL versus all HBs, (B) PF HB versus epithelial HBs with embryonal component, (C) PF HB versus mixed epithelial and mesenchymal HB, and (D) PF versus epithelial HB with a small cell component. Note a high density of differentially expressed genes between PF and epithelial HB with a small cell component.

Table 1

Histologic classification of HB tumors analyzed

HB no.	Histologic type	Affymetrix microarray chip	Group
HB1	Epithelial HB, fetal and embryonal	U133 A	2
HB2	Mixed epithelial and mesenchymal HB	U133 A	3
HB3	Epithelial HB, fetal, embryonal, and small cell	U133 A	4
HB4	Epithelial HB, fetal, embryonal, and small cell	U133 A	4
HB5	Epithelial HB, fetal, embryonal, and small cell	U133 A	4
HB8	Epithelial HB, fetal and embryonal (postchemotherapy)	U133 plus 2	2
HB9	Mixed epithelial (fetal and embryonal) and mesenchymal	U133 plus 2	3
HB10	Mixed epithelial (fetal and embryonal) and mesenchymal	U133 plus 2	3
HB11	Mixed epithelial (fetal and embryonal) and mesenchymal	U133 plus 2	3
HB12	Mixed epithelial (fetal and embryonal) and mesenchymal	U133 plus 2	3
HB13	Epithelial, HB, PF	U133 plus 2	1
HB14	Epithelial, HB, fetal and embryonal	U133 plus 2	2
HB56	Epithelial, HB, PF	U133 plus 2	1
2005	Fetal liver U133 A	U133 A	Ref control
2006	Fetal liver U133 plus 2	U133 plus 2	Ref control
2007	Fetal liver U133 plus 2	U133 plus 2	Ref control

Table 2

A comparison of the *P* values associated with pathway perturbation for (A) cell cycle, (B) wnt signaling pathway, (C) MAPK signaling pathway, and (D) Notch signaling pathway among compared groups of HB tumors and FL or PF HB

A. Wnt signaling pathway	
Fetal liver *vs* all HBs	*P* = 8.8E-5
Fetal liver *vs* PF HB	*P* = .01
Fetal liver *vs* fetal and embryonal HB	*P* = .005
Fetal liver *vs* mixed epithelial (fetal, embryonal) and mesenchymal HB	*P* = 1.5E-4
Fetal liver *vs* fetal, embryonal, and small cell	*P* = .004
Pure fetal HB *vs* fetal and embryonal HB	*P* > .05
Pure fetal HB *vs* mixed epithelial (fetal, embryonal) and mesenchymal HB	*P* > .05
Pure fetal HB *vs* fetal, embryonal, and small cell HB	*P* > .05
B. Cell cycle	
Fetal liver *vs* all HBs	*P* = 4.19E-5
Fetal liver *vs* PF HB	*P* = 1.09E-5
Fetal liver *vs* fetal and embryonal HB	*P* = 2.86E-6
Fetal liver *vs* mixed epithelial (fetal, embryonal) and mesenchymal HB	*P* = 3.0E-3
Fetal liver vs fetal, embryonal, and small cell	*P* = 2.7E-9
C. MAPK signaling pathway	
Fetal liver *vs* all HBs	*P* > .05
Fetal liver *vs* PF HB	*P* > .05
Fetal liver *vs* fetal and embryonal HB	*P* > .05
Fetal liver *vs* mixed epithelial (fetal, embryonal) and mesenchymal HB	*P* > .05
Fetal liver *vs* fetal, embryonal, and small cell	*P* > .05
Pure fetal HB *vs* fetal and embryonal HB	*P* > .05
Pure fetal HB *vs* mixed epithelial (fetal, embryonal) and mesenchymal HB	*P* > .05
Pure fetal HB *vs* fetal, embryonal, and small cell HB	*P* = 8.8E-4
D. Notch signaling pathway	
Fetal liver *vs* all HBs	*P* > .05
Fetal liver *vs* PF HB	*P* > .05
Fetal liver *vs* fetal and embryonal HB	*P* > .05
Fetal liver *vs* mixed epithelial (fetal, embryonal) and mesenchymal HB	*P* > .05
Fetal liver *vs* fetal, embryonal, and small cell	*P* > .05
Pure fetal HB *vs* fetal and embryonal HB	*P* > .05
Pure fetal HB *vs* mixed epithelial (fetal, embryonal) and mesenchymal HB	*P* > .05
Pure fetal HB *vs* fetal, embryonal, and small cell HB	*P* = 3.0E-2