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Fetal- and Tumor-specific Regulation of Growth Hormone Receptor Messenger RNA Expression in Human Liver[1]

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Abstract

Eight different 5′-untranslated region variants of the human growth hormone receptor (hGHR) mRNA have been identified in adult liver (V1–V8). We have compared the expression of two of these variants (V1 and V3) in several human fetal and postnatal tissues (including liver) as well as in hepatoblastomas (HBs) and hepatocellular carcinomas (HCCs). Using reverse transcription-PCR assays, followed by Southern blotting to confirm the specificity of the amplified fragments, we found that V3 was expressed in all fetal and postnatal liver (n = 13 fetal and 5 postnatal), kidney (n = 4 fetal and 4 postnatal), lung (n = 4 fetal and 2 postnatal), intestine (n = 8 fetal and 4 postnatal), skeletal muscle (n = 1 fetal and 1 postnatal), and adrenal (n = 1 fetal and 1 postnatal) samples. In contrast, V1 was expressed only in postnatal liver. We then screened for V1 and V3 in HBs (n = 17, ages 6–36 months, including 5 with paired normal liver), and HCCs (n = 4, ages 50–75 years, with paired normal liver). V1 was undetectable in 15 of 17 HBs, including all HBs paired with (V1-expressing) normal liver; the absence of V1 did not correlate with patient age, sex, HB subtype, ± chemotherapy, exon 3-retaining and -deficient hGHR mRNA isoform pattern, or loss of heterozygosity at 11p, 1p, and 1q. The four HCCs showed marked (>20-fold; n = 2) or complete (n = 2) suppression of V1 as compared to paired normal liver. V3 was expressed in all HBs, HCCs, and paired normal livers. Interestingly, V3, but not V1, was detected in two Wilms' tumor and paired normal kidney specimens. Our findings suggest that, in the human, there is tissue-, fetal- and tumor-specific regulation of V1 hGHR mRNA.

Introduction

HB[3] is the most frequent hepatic tumor in children, accounting for more than 50% of all pediatric liver malignancies (reviewed in Refs. 1 and 2). Based on histopathological studies, HB is classified as an "embryonal tumor of childhood," along with Wilms' tumor and medulloblastoma. Although most cases are sporadic, HB has been associated with the Beckwith-Wiedemann syndrome and familial adenomatous polyposis (1, 2). Molecular genetic analyses have shown a LOH at 11p, 1p, or 1q in approximately one-third of informative cases (1–4). HCC is strongly correlated with liver cirrhosis and the presence of hepatitis B viral antigens and is frequently associated with LOH at the type-II insulin-like growth factor receptor locus on chromosome 6q (1). However, the etiologies of both HB and HCC remain poorly understood.

The hGHR is encoded by exons 2–10 of the *hGHR* gene on chromosome 5 (5). hGHR mRNA has been detected in all fetal and postnatal tissues examined to date (6, 7). Two isoforms of the mRNA coding region have been identified: exon 3 can be retained or deleted (6, 7). In several species, growth hormone receptor mRNA has been characterized by heterogeneity in its 5′UTR (8–11), suggesting complex transcriptional regulation and/or extensive alternative splicing upstream of the translation initiation codon of the precursor mRNA transcripts. In the human, eight different 5′UTR variants (V1-V8) diverging 12-bp upstream from the translation initiation codon have been isolated from an adult liver cDNA library (8). Interestingly, the ovine and rat V1 homologues seem to be liver-specific and developmentally regulated: V1 is expressed in their respective postnatal, but not fetal, livers (9, 10). Given the data from the animal studies, we hypothesized that the human V1 transcript would be liver-specific and under developmental control. We also speculated that V1 mRNA expression might be absent in the embryonal/fetal HBs and altered in adult HCCs. Therefore, in the present study, we determined the tissue specificity of the V1 transcript, characterized its ontogeny in human liver, and investigated its expression pattern in HB and HCC. V3 was examined in parallel as a positive control because preliminary data had shown that it is widely expressed in human tissues. Our data suggest that, in the human, there is tissue-, fetal- and tumor-specific regulation of V1 mRNA.

Materials and Methods

Tissues. Tissues from human fetuses (n = 21; 10–20 wks FA) were obtained at the time of therapeutic abortion; specimens from premature newborns (n = 2; 25 and 30 wks FA) and postnatal patients (n = 38; ages 1 week-80 years) were obtained at the time of surgery or within 4–10 h after death. All tissues were flash-frozen in dry-ice/acetone and were stored immediately at −70°C for RNA extraction. Fetal age was determined by foot length (6). Protocols for obtaining human tissues were approved by local ethics committees, and informed consent was obtained in all cases.

RT-PCR/Southern Blot. Total RNA was isolated using either the acid-guanidium-phenol-chloroform (12) or guanidine thiocyanate/CsCl gradient (13) method. For each reaction, 5 μg of total RNA were reverse transcribed for 1 h at 48°C in the presence of 2.5 units of avian myeloblastosis virus reverse transcriptase (Life Technologies, Inc., Gaithersburg, MD), 80 units of RNAsin (Promega, Madison, WI), 0.6 μM of a specific antisense primer (1A; Fig. 1; 5′-AGG TAT CCA GAT GGA GGT AAA CG-3′), 0.48 mM deoxyribonucleotides (Pharmacia/Biotech, Baie D'Urfe, Quebec, Canada), 10 mM $MgCl_2$, 10 mM DTT, 100 mM Tris-HCl (pH 8.3), and 50 mM KCl. Before the RT reaction, the RNA was heated at 70°C for 5 min to disrupt any secondary structure. Then 6 μl of RT product were amplified for 25 cycles in the presence of 2.5 units of *Taq* DNA polymerase (Life Technologies, Inc.), 0.5 mM deoxyribonucleotides, 0.25 μM of hGHR sense [1S (5′-AGA TTG AGA ATG ACT GAT TTG GGA G-3′) or 3S (5′-GGA GAC CTT GGA AGG GAC AGA G-3′)] and

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[3] The abbreviations used are: HB, hepatoblastoma; hGHR, human growth hormone receptor; HCC, hepatocellular carcinoma; 5′UTR, 5′ untranslated region; wks FA, weeks of fetal age; LOH, loss of heterozygosity; RT, reverse transcription; SSPE, saline-sodium phosphate-EDTA.

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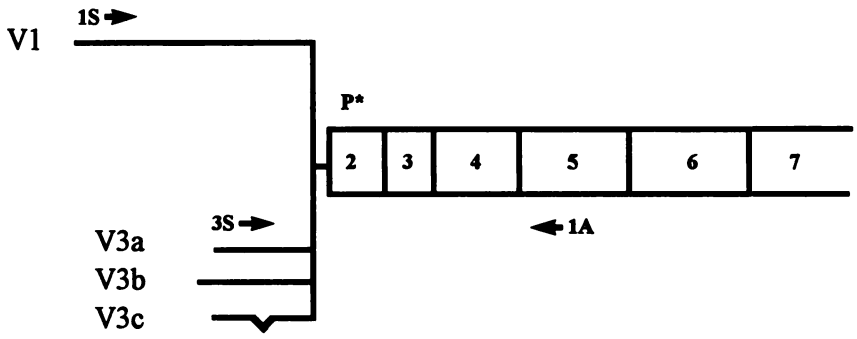

Fig. 1. RT-PCR/Southern blot strategy. *V1, V3a, V3b,* and *V3c* hGHR cDNA transcripts are shown. Exons, *numbered boxes*; 5'UTRs, *solid lines*. The approximate locations of the sense (*S*) and antisense (*A*) primers and the nested hybridization probe (*P**) are indicated. Primer 1A was used in the RT reactions. Single-stranded V1 and V3 cDNA transcripts were amplified using primer sets 1S/1A and 3S/1A, respectively. The lengths of the expected V1 and V3 (with or without the exon 3 deletion) PCR fragments are listed. The precise nucleotide sequences of the oligonucleotides are given in "Materials and Methods."

	PCR Product sizes (base pairs)	
	Exon 3 Retaining	Exon 3 Deleted
V1	671	605
V3a/V3b	502	436
V3c	409	343

antisense (1A) primers (Fig. 1), 3 mM $MgCl_2$, 20 mM Tris-HCl (pH 8.4), and 50 mM KCl. Before the PCR reaction, the RT product was heated for 5 min at 95–98°C to denature the avian myeloblastosis virus reverse transcriptase. The first cycle consisted of 3-min denaturation at 92°C, 1-min annealing at 61°C, and 3-min elongation at 72°C. Subsequent cycles consisted of 30-s denaturation at 92°C, 1-min annealing at 61°C, and 1.5-min elongation at 72°C. The reaction was terminated with a final elongation of 5 min at 72°C. PCR assays testing for expression of V1 transcripts were carried out in the presence of 224 fmol of internal standard. The internal standard was constructed using the method of Jin *et al.* (14).

Amplified hGHR cDNA fragments were electrophoresed through 2% agarose gels and were then transferred to 0.45-μm positively charged nylon membranes (Schleicher & Schuell, Keene, NH) by capillary action with 10 × SSPE. Before blotting, gels were denatured by soaking twice for 15 min in 0.5 N NaOH/1.5 M NaCl and were then neutralized by soaking twice more for 15 min in 1 M Tris-HCl (pH 7.4)/1.5 M NaCl. The nucleic acids were immobilized onto the membranes by exposing the blots to UV light (254–312 nm) for 5 min.

Blots were prehybridized for 3–5 h at 42°C in the presence of 6 × SSPE, 1% SDS, 10 × Denhardt's solution, and 0.15 mg/ml of denatured salmon sperm DNA (Sigma Chemical Co., St. Louis, MO). The volumes of the prehybridization and hybridization solutions were 100 $\mu l/cm^2$ of nylon membrane. Hybridization occurred overnight at 65°C. The hybridization solution contained 6 × SSPE, 1% SDS, 0.1 mg/ml of denatured salmon sperm DNA, and 26.3 nCi of probe/cm^2 of nylon membrane. After hybridization, blots were washed twice for 10 min at 65°C with 6 × SSPE/1% SDS and then were washed once more for 10 min at 60°C with 2 × SSPE/1% SDS. Bands were visualized by autoradiography (1–5 days of exposure) and were quantified using the Fuji Bioimager (Fuji Medical Systems USA, Stamford, CT). For quantitative V1 hGHR analysis, a ratio of the radioactivity intensity of each sample PCR band to that of its internal standard PCR fragment was calculated; initial studies had shown that under the specified RT-PCR conditions, amplification of the sample and internal standard cDNA fragments was still in an exponential phase. Subsequent comparisons were made for each paired set of normal and tumor liver specimens to determine differences in relative levels of expression.

Approximately 150 nmol of the nested oligonucleotide probe (P*; Fig. 1; 5'-CTG CTG TTG ACC TTG GCA CTG GC-3') were end-labeled and then were purified through a Sephadex G-50 medium (Pharmacia/Biotech) column. The end-labeling reaction occurred at 37°C for 1 h in the presence of 2.5 μCi/μl of γ-[^{32}P]ATP (ICN Pharmaceuticals Canada Ltd., Montreal, Quebec, Canada), 20 units of T_4 polynucleotide kinase (Life Technologies, Inc.), 70 mM Tris-HCl (pH 7.6), and 10 mM $MgCl_2$, 100 mM KCl, and 1 mM 2-mercaptoethanol.

Results

A RT-PCR/Southern blot approach (Fig. 1) was used to screen fetal and postnatal human tissues for the presence of V1 and V3. Because previous studies have shown that human adult liver expresses both V1 and V3 mRNA transcripts (8), total RNA from an adult liver sample served as a positive control in all RT-PCR assays. A water blank served as a negative RT-PCR control. Whenever possible, samples were run in parallel without reverse transcriptase to rule out sample contamination with cDNA; limited amounts of sample RNA precluded a nonreverse transcriptase test for all specimens. After each set of RT reactions, two aliquots from each RT product were taken: one was tested for V1, the other for V3. Because we anticipated that V1 transcripts would be undetectable or low in all specimens other than postnatal human normal liver, we synthesized a V1 cDNA construct with an internal deletion and added it to each PCR reaction tube as a positive internal control for amplification. It should also be noted that the antisense primer was designed to permit the detection of both exon 3-retaining and -deleted hGHR mRNA isoforms (Fig. 1).

Previous studies have identified three V3 subvariants (V3a, V3b, and V3c), which are thought to arise from alternative splicing of a single precursor mRNA transcript (8). However, our V3-specific sense PCR primer (3S; Fig. 1) cannot differentiate between V3a and V3b cDNA isoforms. All three V3 subvariants are hereafter collectively referred to as V3.

V3, but not V1, was detected in fetal and postnatal kidney [n = 4 fetal (14–16 wks FA) and 4 postnatal (ages 2–62 years)], lung [n = 4 fetal (13.8–19 wks FA) and 2 postnatal (ages 69 and 80 years)], intestine [n = 8 fetal (11.7–20 wks FA) and 4 postnatal (ages 40–64 years)], skeletal muscle [n = 1 fetal (18 wks FA) and 1 postnatal (age 1 week)], and adrenal [n = 1 fetal (13.3 wks FA) and 1 postnatal (age 29 years)] (data not shown). Only the control postnatal liver was V1-positive, suggesting that V1 mRNA expression is liver-specific. The absence of V1 in specimens other than the control adult liver cannot be attributed to failed RT or PCR reactions because, in each

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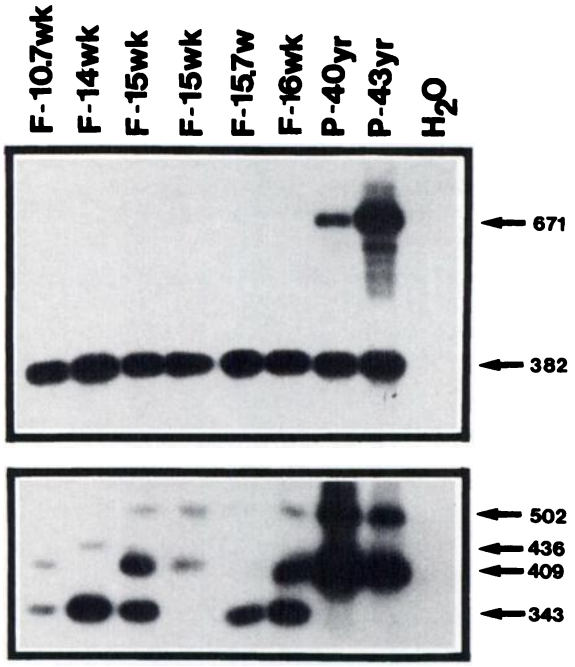

Fig. 2. Representative Southern blots illustrating the ontogeny of V1 (*upper panel*) and V3 (*lower panel*) hGHR mRNA transcripts in liver. The donor age of fetal (*F*; in wks FA) and postnatal (*P*; in years) samples is given above each lane. H_2O served as a negative RT-PCR control. *Right*, sizes of the expected PCR fragments (in bp). For details on the expected bands, refer to Fig. 1. Three different V1 and V3 hGHR mRNA expression profiles are illustrated in the *upper* and *lower panels*, respectively, based on whether the specimens express the exon 3-retaining isoform (*15 wk, 40 yr, 43 yr*), the exon 3-deleted isoform (*14 wk, 15.7 wk*) or a combination of the two isoforms (*10.7 wk, 15 wk, 16 wk*). Although not all of the expected bands are clearly visible on this autoradiograph (24-h exposure), they were all apparent after a 72-h exposure. The 382-bp band (*upper panel*) corresponds to the V1 internal PCR control. The band below the expected 671-bp V1 PCR fragment in sample *P-43yr* is an amplification artifact and was not reproducible.

case, V3 transcripts and V1 internal cDNA controls were readily observed (data not shown).

We then delineated the ontogeny of V1 and V3 in human liver. All 13 fetal specimens (10–30 wks FA) had undetectable levels of V1, whereas V1 was readily observed in transplant donor livers (n = 5, ages 11–62 years; Fig. 2 and data not shown). Both fetal and postnatal samples expressed V3 (Fig. 2 and data not shown). The V1 internal PCR control was detected in all cases (Fig. 2 and data not shown). Interestingly, exon 3-retaining and -deleted mRNA transcripts were found to contain both V1 and V3 leader sequences (Figs. 2 and 3 and data not shown).

Next, we screened for V1 and V3 in a large series of HBs (n = 17, ages 6–36 months, including 5 with paired normal liver). Although V3 was present in all samples, V1 was undetectable in 15 of 17 HBs, including the 5 HBs paired with V1-expressing normal liver (Fig. 3 and Table 1). The V1 internal PCR control fragment was detected in all V1 assays (Fig. 3 and data not shown). The absence of V1 did not correlate with patient age, sex, HB subtype, ± chemotherapy, exon 3-retaining and -deficient hGHR mRNA isoform pattern, or LOH at 11p, 1p, and 1q (Table 1). The two HBs that expressed V1 were predominantly of an embryonic phenotype; neither showed unique or variant histopathological features.

Adult HCCs (n = 4; ages 50–75 years) and their paired normal livers were also tested for V1 and V3 expression. Semiquantitative V1 RT-PCR analysis was performed in duplicate. In each case, the ratio of the intensity of the test PCR band to that of the internal standard PCR fragment was calculated, and V1 expression was compared between the tumor and its paired normal specimen. The four HCCs showed marked (>20-fold, n = 2) or complete (n = 2) suppression of V1 as compared to paired normal liver (Fig. 3 and Table 1). The V1 internal standard PCR fragment was detected in all V1 RT-PCR assays (Fig. 3 and data not shown). V3 was expressed in all HCCs and paired normal livers (Fig. 3 and Table 1).

Finally, to determine whether altered V1 expression occurred in a second embryonal type of tumor, we tested for V1 and V3 expression in two Wilms' tumor specimens (ages 2 and 4 years) and their paired normal kidneys. V3, but not V1, was present in both normal and tumor kidney tissues (data not shown). Amplification of the V1 internal standard was observed in all V1 RT-PCR assays (data not shown).

Discussion

In the present study, we found that V3 transcripts are widely expressed in human fetal and postnatal tissues. In contrast, V1 mRNA regulation is tissue- and developmental-specific; as we hypothesized, based on studies in the ovine and rat (9, 10), V1 expression seems to be limited to the postnatal liver. In all three species, these V1 transcripts are first detectable around the time of birth. The ovine V1 is absent in 80-day fetal liver but is present in 14-day postnatal hepatocytes, by RNase protection assay (9). The rat liver homologue (GHR1) appears during the fourth postnatal week, using Northern blot analysis (10). Our RT-PCR investigation shows that V1 is absent in two premature (25 and 30 wks FA) newborn hepatic tissues but is present in five postnatal infant livers (ages 9–36 months).

We conclude that V1 mRNA is either down-regulated in fetal liver or up-regulated in postnatal liver. Transcription of the ovine and rat V1 homologues is regulated by a specific promoter on the *GHR* gene (9, 10). Although we cannot exclude the possibility of alternative splicing as the regulatory mechanism for V1 synthesis in the human,

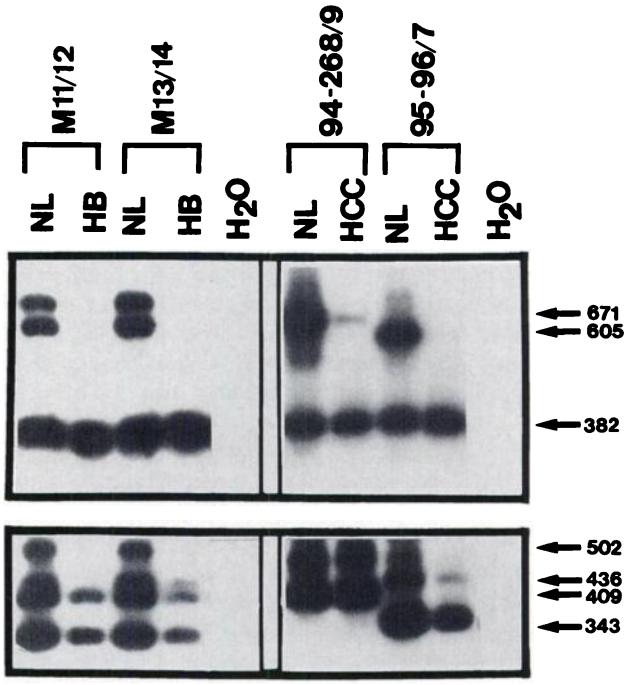

Fig. 3. Representative Southern blots demonstrating the expression profiles of V1 (*upper panel*) and V3 (*lower panel*) hGHR mRNA transcripts in HBs and HCCs. The results from two sets of HBs and HCCs with their paired normal liver (*NL*) are shown. H_2O served as a negative RT-PCR control. *Right*, sizes of the expected PCR fragments (in bp). For details on the expected bands, refer to Fig. 1. Three different V1 and V3 hGHR mRNA expression profiles are illustrated in the *upper* and *lower panels*, respectively, based on whether the specimens express the exon 3-retaining isoform (*94–268/9*), the exon 3-deleted isoform (*95–96/7*), or a combination of the two isoforms (*M11/12, M13/14*). Although not all of the expected bands are clearly visible on this autoradiograph (24-h exposure), they were all apparent after a 72-h exposure. The 382-bp band (*upper panel*) corresponds to the V1 internal PCR control.

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Table 1 *Summary of clinical and experimental data*

Sample	Age[a]	Sex	Tumor type[b]	± Chemo	LOH 11p[c,d]	LOH 1p[d]	LOH 1q[d]	hGHR exon 3 mRNA	hGHR V1 mRNA	hGHR V3 mRNA
R378	21	M	HB; mixed	+	−	+	+	3+	−	+
R656	11	F	HB; epithelial	−	−	−	−	3+/3−	−	+
R682	8	F	HB; mixed	−	−	−	−	3+	−	+
R683	13	M	HB; epithelial	−	−	−	−	3+/3−	−	+
R684	12	M	HB; epithelial	−	−	−	−	3+	−	+
R685	27	F	HB; epithelial	−	+	+	−	3+/3−	−	+
R704	27	F	HB; epithelial	−	ND	ND	−	3+	−	+
R366	11	F	HB; epithelial	−	−	−	−	3+/3−	−	+
R371	8	F	HB; mixed	−	+	−	−	3+/3−	−	+
U62	6	M	HB; epithelial	−	+	−	−	3+/3−	−	+
R365	6	F	HB; epithelial	+	ND	ND	ND	3+	+	+
R707	34	F	HB; epithelial	−	+	−	−	3+	+	+
R331	9	F	HB; mixed	+	−	+	−	3+	−	+
R332	9	F	NL to R331	+	NA	NA	NA	3+	+	+
R374	12	M	HB; mixed	−	−	−	−	3+	−	+
R375	12	M	NL to R374	−	NA	NA	NA	3+	+	+
M12	13	M	HB; epithelial	−	ND	ND	ND	3+/3−	−	+
M11	13	M	NL to M12	−	NA	NA	NA	3+/3−	+	+
M14	20	M	HB; epithelial	+	ND	ND	ND	3+/3−	−	+
M13	20	M	NL to M14	+	NA	NA	NA	3+/3−	+	+
M16	36	M	HB; epithelial	−	ND	ND	ND	3+	−	+
M15	36	M	NL to M16	−	NA	NA	NA	3+	+	+
94–269	75	M	HCC	−	ND	ND	ND	3+	(+)	+
94–268	75	M	NL to 94–269	−	NA	NA	NA	3+	+	+
95–97	53	M	HCC	−	ND	ND	ND	3−	(+)	+
95–96	53	M	NL to 95–97	−	NA	NA	NA	3−	+	+
CO81P	50	F	HCC	−	ND	ND	ND	3+/3−	−	+
CO82P	50	F	NL to CO81P	−	NA	NA	NA	3+/3−	+	+
1416C	52	F	HCC	−	ND	ND	ND	3+/3−	−	+
1416AB	52	F	NL to 1416C	−	NA	NA	NA	3+/3−	+	+

[a] Donor age of HB specimens is given in months, donor age of HCC samples is given in years.
[b] All major HB subtypes are represented: epithelial (fetal, embryonal, fetal/embryonal, macrotrabecular, anaplastic) and mixed (epithelial/mesenchymal ± teratoid).
[c] Ref. 4.
[d] Ref. 3; F, female; M, male; NL, normal liver; NA, not applicable; ND, not done; 3+, exon 3-retaining hGHR mRNA; 3−, exon 3-deleted mRNA; +, detected; −, not detected; (+), V1 levels were > 20-fold decreased compared to those of normal paired liver. All four HCC specimens stained negative for hepatitis B viral antigen.

we can speculate (based on the sheep and rat studies) that V1 expression is controlled by a specific promoter on the *hGHR* gene as well. If this is the case, then around the time of birth there must be suppression of V1-promoter repressing factor(s) and/or the turning on of transcriptional activating factor(s). Before exploring these possibilities, the V1 promoter for the *hGHR* gene will have to be mapped and sequenced.

The biological significance of the V1 and V3 leader sequences remains unknown. Complex 5′UTR structures have been shown to modify mRNA translation initiation rates (15). Interestingly, there is a 6-fold increase in total hGHR mRNA concentration[4] and a 3-fold increase in human growth hormone binding[5] from the fetal to postnatal liver. Therefore, one question to pursue is whether the turning on of V1 mRNA in postnatal liver accounts for these ontogenic changes.

Interestingly, V1, but not V3, transcripts were absent in 15 of 17 HBs, including all 5 HB specimens with paired normal (V1-expressing) liver. In addition, we tested four adult HCCs and observed either marked ($n = 2$, >20-fold) or complete ($n = 2$) suppression of V1, but not V3, as compared to paired normal liver. Thus, we have strong evidence in support of our hypothesis that the lack of V1 mRNA is a marker of human liver differentiation, and that the absence or suppression of V1 expression reflects the continued "fetal" state of hepatic tumors.

Most striking is the "universality" of V1 regulation in HBs. All except 2 of the 17 HBs tested showed a lack V1 expression regardless of patient age, sex, HB subtype (all major subtypes were examined), ± prior chemotherapy, exon 3- retaining and -deficient hGHR mRNA isoform pattern, or LOH at 11p, 1p, and 1q. These data suggest that the absence of V1 mRNA is a molecular phenotypical marker of HB.

No other HB marker has been described with such a high level of correlation. For example, α-fetoprotein is detected in only 60% of HB patients, and β-human chorionic gonadotropin is detected in 3% (1, 2). There is little evidence of a role for any single chromosomal abnormality or p53 mutation (1, 2). Thirty-two HBs (some of which were tested for V1 and V3 expression in the present study) were examined for LOH on chromosome 1 because deletions on both 1p and 1q are common in human malignancies: 7 tumors had LOH on 1p; 7 tumors showed LOH on 1q; and 3 tumors had LOH on both arms (4). LOH at 11p has been reported for only one-third of all informative HB cases (1–3), and loss of imprinting at the insulin-like growth factor II gene locus has been reported for one-eighth (16, 17). Thus, the lack of V1 expression that we observed in 88% of HBs in the present study seems to be the most important association reported to date.

We have suggested previously that expression of the exon 3-deleted hGHR mRNA, either alone or with the exon 3-retaining transcript, is predominant before 20 wks FA (6). Therefore, it was interesting to examine whether, like early- to mid-gestational human fetal liver, the fetal/embryonal HB preferentially expresses the exon 3-deficient mRNA. All 5 HBs with paired normal tissue expressed the same exon 3 isoform pattern as their respective adjacent normal liver, and 8 of 17 HB specimens tested showed expression of the exon 3-deleted mRNA. Because these results are midway between the early- to mid-gestational (61%) and postnatal (20%) frequency data obtained previously (6), no conclusion can be drawn.

The mechanism for synthesis of the exon 3-retaining and -deleted hGHR mRNA isoforms is not understood. Because 5′UTR sequences

[4] G. Zogopoulos and C. G. Goodyer, unpublished observations.
[5] R. M. Figueiredo and C. G. Goodyer, unpublished observations.

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may control alternative splicing at the most 5′ end of the precursor mRNA (18), it was of interest to assess whether V1 or V3 associate preferentially with either the exon 3-retaining or -deficient transcripts. In agreement with the findings of Esposito *et al.* (19), our data demonstrate that this is not the case: both exon 3-retaining and -deleted mRNA isoforms contain V1 and V3 leader sequences.

We conclude that, in the human, there is tissue-, fetal- and tumor-specific regulation of the V1 hGHR mRNA transcript. We speculate that an inability to activate V1 expression occurs concomitantly with HB tumorigenesis. Furthermore, V1 suppression in HCC may be a measure of the degree of dedifferentiation to the embryonal/fetal stage that the HCC tumor cells have undergone. Whether V1 dysregulation is a cause or effect of the tumor state remains unknown. We hypothesize that, in the human (as in the ovine and rat), transcription of hGHR mRNAs containing the V1 leader sequence is driven by a specific V1 promoter, and that, in the hepatic tumor state, there is "fetal" control of the transcription factor(s) regulating this promoter.

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