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The Circulating IGF System in Hepatocellular Carcinoma: The Impact of Liver Status and Treatment

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ABSTRACT

Background: Previous studies have demonstrated an elevated IGF-II mRNA expression and protein levels in tumors and blood from patients with hepatocellular carcinoma (HCC), hereby suggesting a role of IGF-II as a pathogenic marker of HCC. We hypothesized that in HCC, an increased IGF-II secretion would translate into an elevated circulating IGF bioactivity, which would normalize following treatment.
Methods: Patients with HCC (n = 39) were studied before and after radio-frequency ablation and/or transarterial chemo-embolization. Baseline data were compared to healthy subjects (n = 150) and patients with liver cirrhosis (n = 41). Serum levels of IGF ligands and IGF binding proteins (IGFBPs) were determined using gold standard methods as well as novel assays and compared to liver function tests and HCC treatment status.
Results: At baseline, HCC patients differed from cirrhosis patients and healthy controls regarding IGF-I (29 [23–37] vs. 12 [7–19] vs. 109 [103–116] μg/l), IGF-II (254 [224–288] vs. 118 [102–137] vs. 545 [525–566] μg/l) and IGF bioactivity (0.53 [0.41–0.68] vs. 0.29 [0.24–0.34] vs. 1.43 [1.33–1.53] μg/l) (mean [95% confidence interval], all age-adjusted $P < 0.001$). All variables but IGFBP-2 were strongly associated with liver status (MELD score), and accordingly, differences were either attenuated or disappeared when adjusted for MELD score. There was no effect of treatment on any IGF variables.
Conclusions: The marked differences in IGF and IGFBP levels between patients with HCC, liver cirrhosis and healthy subjects are mainly explained by variations in liver status. Therefore, this study questions the clinical utility of circulating IGF variables as markers of HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is a common disease on a global scale and associated with chronic viral hepatitis, exposure to dietary aflatoxin B1, and liver cirrhosis [1]. Irrespective of etiology, the common trait in HCC consists of an imbalance between pro-apoptotic and anti-apoptotic signaling. Anti-apoptotic signaling in hepatocytes is maintained through the PI3-K/Akt and Ras/Raf/MEK/MAPK pathways [2], two major downstream targets of the IGF-I receptor (IGF-IR) [3]. As both IGF-I and IGF-II activate the IGF-IR, both growth factors may influence hepatocyte survival and enrich hepatocellular cancer stem cell populations [4].

The physiology, pathophysiology, and clinical utility of IGF-II have recently been reviewed [5]. Excess IGF-II stimulates cancer cell proliferation and increases cancer cell survival both in vitro and in vivo [6], including in HCC cells [7]. IGF-II is essential for intrauterine growth and placental development. Within the first weeks after birth, there is a shift from potent fetal promoters to a weaker adult promoter, resulting in a very low expression of the *igf2* gene in the normal adult liver [8]. By contrast, an elevated *igf2* gene expression has been demonstrated in neoplastic tissues from patients with HCC and in animal models of HCC [8], and this over-expression is linked to reactivation of the fetal promoters and deactivation of the adult promoter [9]. Indeed, reducing IGF-II mRNA levels with anti-sense oligonucleotides resulted in decreased cell proliferation in human hepatoma cell lines with high IGF-II secretion [10]. At the molecular level hepatitis B virus (HBV) and hepatitis C virus (HCV) increase the production of IGF-II, thus promoting growth and viability of malignant cells [8].

Several clinical studies have demonstrated elevated IGF-II levels (mRNA and protein) in both tumors and blood from HCC patients, hereby suggesting a role of IGF-II as a marker of HCC [11–15]. By contrast, others have found sparse evidence of an association between IGF-II and HCC [16]. The reasons for this discrepancy have not been thoroughly investigated. Furthermore, it remains to be clarified whether serum IGF-II may be used as a biomarker of the response to HCC treatment.

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We hypothesized that IGF-II would be elevated in HCC and normalize with radio-frequency ablation (RFA) or transarterial chemo-embolization (TACE). IGF-II exerts its biological effects via interaction with the IGF-IR [3], and accordingly, we found it of interest to investigate the ability of serum from HCC patients to activate the IGF-IR under physiological conditions. To this end, an IGF-IR kinase receptor activation (KIRA) assay, developed and validated in our laboratory [17], was applied to compare serum obtained from well-characterized patients with HCC, patients with liver cirrhosis and healthy control subjects. This measure of IGF bioactivity was compared with measurements of IGF-I, IGF-II, and pro-IGF-II, using size exclusion gel chromatography at low pH, which is the gold standard method to eliminate IGFBP interference [18]. Finally, we assessed the impact of the marked reduction in tumor burden following RFA and TACE of HCC tumors on the circulating IGF system.

2. Materials and Methods

2.1. Patient Characteristics

The HCC patients included in this study were referred to the Department of Hepatology and Gastroenterology, Aarhus University Hospital, Denmark, from December 2007 through October 2010. The Department is a tertiary treatment center for the Western part of Denmark with a population of 2 million inhabitants. Patients were diagnosed, staged and allocated to treatment according to international guidelines for management of HCC [19,20] independently of study participation. This study focused on patients eligible for RFA or TACE. In total 39 consecutive patients were included, of which 11 were Barcelona-Clínic Liver Cancer (BCLC) stage A and 28 were BCLC stage B. None of the patients had lymph node spread or distant metastases. Treatment consisted of RFA (n = 7), TACE (n = 20), combination of RFA and TACE (n = 10), or TACE followed by sorafenib (n = 2). Patients were followed for up to 12 weeks after treatment with visits at baseline (i.e. the day before the first treatment), week 1, week 4, and week 10–12. Investigations included blood samples, medical imaging and physical examination with assessment of general performance and liver function tests. The majority of patients (n = 29) completed 12 weeks, 6 completed 4 weeks, and the remaining 4 patients completed the baseline visit only. Eighteen patients completed 4 or 12 weeks but omitted the visit at week 1 for practical reasons. The patients were diagnosed a median of 0.6 months before inclusion (range 0 to 13.9 months). Study exclusion criteria were portal vein thrombosis, previous cancer, current cancer apart from HCC, and impaired kidney function (creatinine >150 μmol/l). Some clinical data on the HCC cohort has recently been described in a publication focusing on vitamin B12 and its binding proteins [21].

A group of healthy control subjects (n = 150) as well as a group of patients with decompensated liver cirrhosis (n = 41) were included in order to describe the impact of liver status on IGF variables in our setting. The healthy control subjects ranged from 20 to 70 years of age and were evenly distributed with 15 women and 15 men in each decade [22]. The patients with liver cirrhosis and without HCC were originally described by Jeyaratnaganthan et al. [23]. This group is referred to as "LC" throughout. Although the IGF variables were described previously, all samples from the LC cohort were re-measured in conjunction with the present study to ascertain comparability to the HCC cohort. A description of the study participants is given in Table 1. Liver cirrhosis was primarily caused by excessive intake of alcohol in both the HCC cohort and the LC cohort (13/29 and 22/41, respectively), and other causes were non-alcoholic liver cirrhosis (7/29 and 16/41, respectively), or ascribed to other specific etiologies. HCV was present in 6/39 HCC patients and in 0/41 LC patients. None were HBV positive. For HCC patients and LC patients, liver status was established by Child-Pugh Score and model of end-stage liver disease (MELD) score [24]. The MELD score was originally described as a tool to prioritize among patients waiting for a donor liver transplant, but it is often used in a wider context as an estimate of liver status.

All participants gave their written informed consent prior to inclusion. The study was approved by the local ethics committee (ID number: 20070156) and conducted in accordance with the declaration of Helsinki.

2.2. IGF Bioactivity Assay

The kinase receptor activation (KIRA) assay is designed to measure the ability of a given sample to phosphorylate the IGF-IR in cultured cells. We use human embryonic kidney cells (HEK 293) transfected with cDNA encoding the full-length human IGF-IR gene. These cells are cultured and stimulated and their phosphorylated IGF-IRs assayed as previously described by Chen et al. [17] with modifications [25]. In brief, the cells are stimulated with serum diluted 1:10 in Krebs Ringer buffer for 15 min at 37 °C. Then the samples are quickly removed, and the cells lysed. In the next step, the crude cell lysates are assayed for phosphorylated IGF-IRs by a time-resolved immunofluorometric assay (TR-IFMA) based on commercial antibodies. The signals from unknown samples are read against a serial dilution of IGF-I (WHO reference preparation 02/254, obtained from National Institute for Biological

Table 1
Study cohort.

	HCC (all)	HCC (no cirr.)	HCC (with cirr.)	LC (no HCC)	Healthy controls
No. of patients	39	10	29	41	150
Age (years)	66.4 (63.3–69.5)	70.8 (62.1–79.5)	64.9 (62.1–67.7)	53.4 (50.8–55.9)†	44.0 (41.6–46.4)‡
Age range (years)	41–85	41–84	48–85	37–69	20–70
Sex (female/male)	7/32	5/5	2/27*	12/29	75/75‡
Body Mass Index (kg/m^2)	27.1 (25.8–28.4)	26.9 (24.5–29.3)	27.2 (25.7–28.8)*	25.0 (23.3–26.6)†	23.9 (23.4–24.4)‡
Child-Pugh group (A/B/C)	30/9/0	10/0/0	20/9/0*	0/12/29†	
MELD score	9.5 (8.5–10.6)	7.5 (6.4–8.9)	10.3 (9.0–11.5)*	17.8 (15.9–19.7)†	
Ascites (yes/no)	8/31	1/9	7/22*	41/0†	
					Reference interval
INR (units)	1.2 (1.1–1.3)	1.1 (0.9–1.2)	1.2 (1.1–1.3)*	1.6 (1.5–1.7)†	<1.2
Albumin (μmol/l)	545 (468–635)	635 (600–672)	516 (422–632)	343 (320–368)†	540–680
Bilirubin (μmol/l)	17.2 (14.6–20.3)	12.4 (8.9–17.3)	19.3 (16.2–22.9)*	70.0 (51.9–94.3)†	5–25
ALT (units/l)	45.6 (36.6–56.8)	46.7 (26.5–82.2)	45.2 (35.9–56.9)	36.9 (29.8–45.7)	10–70[a]
ALKP (units/l)	126 (107–149)	110 (84–144)	132 (108–163)	266 (211–335)†	35–105

Numbers are given as median and 95% confidence interval or number of participants in each category. ALT: alanine transaminase; and ALKP: alkaline phosphatase. For BMI, values for 2 HCC patients and 6 cirrhosis patients were missing.

† $P \le 0.007$ compared to all HCC patients.

‡ $P \le 0.003$ compared to all HCC patients.

* $P = 0.05$ or less compared to HCC patients without cirrhosis.

[a] Reference interval for men only. The corresponding interval in women is 10-45 units/l. In men, the reference interval for women is 10–45 units/l.