Insulin-Like Growth Factor 2 and Incidence of Liver Cancer in a Nested Case–Control Study

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ABSTRACT

Background: Insulin-like growth factor (IGF)2 is a potent mitogen. To elucidate the relationship between IGF2 and risk of tumorigenesis, we analyzed associations between serum levels of IGF2 and incidence of liver cancer in a prospective case–control study nested in the Japan Collaborative Cohort study.

Methods: A baseline survey was conducted from 1988 using blood samples from 39,242 subjects. Those who had been diagnosed with liver cancer by 1997 were regarded as cases. For each case, we randomly selected two or three controls matched for sex, age, and residential area. Conditional logistic regression was used to estimate ORs for cancer incidence associated with IGF2.

Results: This analysis included 86 cases and 294 controls. Low IGF2 was associated with risk of future liver cancer (P_{trend} <0.001). After controlling for alcohol intake, body mass index, smoking, hepatitis viral infection, IGF1, and IGF-binding protein-3, participants with low IGF2 displayed a higher risk of liver cancer ($P_{trend} < 0.001$). Individuals in quintiles 2 to 5 showed lower risk compared with quintile 1 (OR range, 0.05–0.16). In both sexes and in both nonelderly and elderly groups, subjects in the lowest quintiles showed higher risks of liver cancer. Limiting subjects to those followed for 3 years, low IGF2 was associated with cancer risk ($P_{trend} < 0.001$).

Conclusions: Our findings suggest that low serum IGF2 level, especially below 460 ng/mL, is related to future risk of liver cancer.

Impact: Our findings highlight this important biomarker for further analysis in large prospective cohorts and pooled investigation with other cohorts.

Introduction

In the vast majority of human malignancies, including liver cancer, progression of the cancer requires signals from growth factor receptors in addition to carcinogenesis (1, 2). The insulin-like growth factor (IGF) system, including ligands IGF1 and IGF2 and the receptor (type-1 IGF receptor, IGF1R), is one axis providing such signal (2–4). IGFs bind to IGF1R and then activate multiple downstream signal pathways, modulated in multiple ways under a homeostatic state (5, 6). Hepatocyte-synthesized IGF2 evokes endocrine activity, whereas extrahepatic tissue-synthesized IGF2 exerts autocrine/paracrine effects. *IGF2* gene transcription is initiated from a liver-specific P1 promoter in adult liver, but three other ubiquitous P2–P4 promoters remain active in adult peripheral tissues (7). In most tissues, *IGF2* transcription is controlled by genomic imprinting that restricts expression to the paternal allele. Receptor activation is controlled by the amount of active (free form) IGFs, which is regulated by six IGF-binding proteins (IGFBP1 to IGFBP6) and the type-2 IGF receptor (IGF2R; refs. 5, 8). In serum, most IGFs are in an inactive form as complexes with IGFBPs. Of the six IGFBPs, IGFBP3 shows the highest concentrations in serum. IGF2R can bind IGF2 and promote endocytosis and lysosomal degradation of IGF2. Compared with IGF1, however, the functions and roles of IGF2 in carcinogenesis have not been elucidated in detail.

IGF1R expression is induced under both physiologic and pathologic conditions, in hepatocytes as well as in hepatocellular carcinoma (HCC) cells (9). HCC cells have demonstrated increased *IGF2* expression (10). Upregulation of IGF2 is an early event in hepatocarcinogenesis, prior to the appearance of morphologically distinct dysplasia. Elevated transcript levels of focal *IGF2* indicate increased risks of both cholangiocellular and hepatocellular carcinomas (11). *IGF2* was one of 31 genes upregulated in hepatitis B viral-induced HCC (12). On the other hand, serum levels of IGF2 were lower in the patients with HCC than in healthy controls (13). IGF2 and IGF2R genes polymorphisms and their combinations were associated with the risk of HCC (13, 14).

Elevated serum total IGF1 concentrations or free IGF1 levels (as represented by the molar ratio of IGF1 to IGFBP3) are associated with increased risks of colon, breast, and prostate cancers (15–17). In addition, low serum levels of total IGFBP3 or free IGFBP3 (as estimated by the molar difference, i.e., IGFBP3 − IGF1) enhance the risk of malignant neoplasms, including liver cancer (17–19). However, insufficient information has been accumulated regarding the relationship between serum IGF2 levels and incidences of neoplasms, including liver cancer. Although several relationships between IGFs and risks of site-specific carcinomas were reported from the Japan Collaborative Cohort (JACC) study (18, 20–22), the relationship between incidence of liver cancer and IGF2 levels has not been clarified. We thus assessed

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this relationship in a case–control study nested in a prospective JACC cohort.

Materials and Methods

Study population and serum samples

We conducted a nested case-control study as part of the JACC study, which evaluated cancer risk associated with lifestyle factors. Details of JACC study have been described previously (23–25). In brief, a baseline survey was conducted from 1988 to 1990, when 110,585 apparently healthy individuals (age, 40–79 years) who had received a general health checkup were enrolled as a basic cohort population from 45 communities throughout Japan. All participants completed a questionnaire that included information about demographic characteristics, lifestyle factors, and medical histories. About 35% of cohort subjects (39,242 in total) voluntarily provided serum samples, which were kept at −80°C until needed for biochemical assays.

Written informed consent was obtained from each participant by having the study participants sign the cover of the questionnaire in the majority of study areas. However, it was obtained at the group level in a few areas because the concept of informed consent was not popularized during the 1980s in Japan. In that case, the municipality head gave the consent to participation representing the participants living in that area. This study was conducted in accordance with International Ethical Guidelines for Biomedical Research Involving Human Subjects and Declaration of Helsinki. This study was approved by the human ethics review committees at Hokkaido University.

Follow-up, identification of malignant tumors, and control selection

In 24 of the 45 studied communities, the incidence of malignant neoplasms was followed (25). Participants were followed from the baseline survey. Subjects with any malignant tumor history at baseline were excluded. Subjects who moved away from the original community were treated as dropouts from this study, because deaths after moves could not be detected in the follow-up system of this study. The occurrence of liver cancer was confirmed in population-based tumor registries or by reviewing the records of local major hospitals. We defined liver cancer as C22 (malignant neoplasm of the liver and intrahepatic bile ducts) according to the 10th revision of the International Statistical Classification of Diseases and Related Health Problems (http://www.who.int/classifications/icd/en/). Participants diagnosed with liver cancer by 1997 were regarded as cases in this nested case-control study. For each case, we randomly selected three controls matched for age, sex, and residential area, with fewer than three control subjects selected in some cases depending on this selection criterion (26). This analysis involved 86 cases, including 71 cases with C22.0 (HCC), 6 with C22.1 (intrahepatic bile duct carcinoma), 2 with C22.7 (other specified carcinoma of liver), and 7 with C22.9 (malignant neoplasm of liver, not specified as primary or secondary), and 249 control subjects.

Biochemical assays of serum samples

Serum concentrations of IGF2, IGF1, and IGFBP3 were measured at a single laboratory (SRL) using immune-radiometric assays with commercially available kits (Daiichi Radioisotope Laboratories) by trained staff who were blinded to case/control status in 1999 and 2000. Details of assays for serum levels of IGF2, IGF1, and IGFBP3 have been described previously (27). Hepatitis B virus surface antigen and hepatitis C virus antibody (third generation) were measured in the SRL laboratory (28).

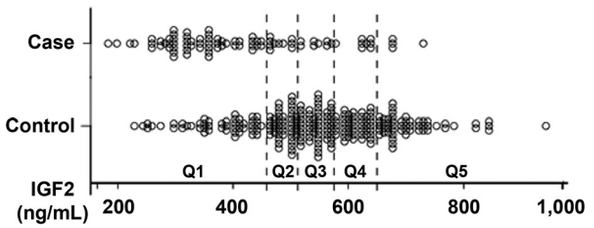

Figure 1.
Serum concentration of IGF2 in cases and controls. IGF2 levels in cases were lower than those in controls. The number of cases in the first quintile was the most of all quintiles.

Statistical analysis

Proportions and mean values in baseline characteristics between cases and controls were compared using *t*-tests or chi-squared tests. Results are presented as mean ± SD. Values of $P < 0.05$ were taken to indicate statistical significance. Serum concentrations were divided into quintiles based on the distribution of serum concentrations in all control participants, with the first quintile used as a reference. IGF2 quintile values for quintiles 1 to 5 were <460, 460–520, 521–580, 581–650, and >650 ng/mL, respectively (**Fig. 1**). IGF1 quintile values for quintiles 1 to 5 were <67, 67–98, 99–120, 121–150, and >150 ng/mL, respectively. IGFBP3 quintile values for quintiles 1 to 5 were <2.17, 2.17–2.59, 2.60–2.99, 3.00–3.49, and >3.49 μg/mL, respectively.

Using conditional logistic regression, ORs for the incidence of liver cancers associated with serum levels of IGF2 were assessed. ORs were controlled for body mass index (BMI, calculated as weight in kilograms divided by the square of the height in meters and categorized as <18.5, 18.5–24.9, 25.0–29.9, ≥30.0 kg/m^2, or missing), alcohol consumption (never, former, or current drinker, or missing), and cigarette smoking status (never, former, or current smoker, or missing), and hepatitis viral infection. ORs were also adjusted for alcohol consumption, BMI, smoking status, hepatitis viral infection, IGF1, and IGFBP3. ORs for the incidence of liver cancer associated with serum IGF2 were also assessed by both age and gender differences. To exclude effects of latent cancer on IGF2, we analyzed cases that were diagnosed at least 3 years after baseline examination. The statistical significance of trends across exposure quintiles was evaluated by including ordinal terms for each quintile of serum level and entering the variable as a continuous term in this model. All *P* values and 95% confidence intervals (CI) presented were based on two-sided tests.

Results

Table 1 shows baseline characteristics of the case and control groups. No significant differences in height, weight, BMI, or smoking habits were evident between groups. For alcohol consumption, the percentage of never drinkers was higher in the control group than in the case group. In terms of hepatitis viral infection, positive was higher percentages in cases than in controls. Mean serum levels of both IGF2 (**Fig. 1**) and IGFBP3 were significantly lower in cases than controls. Mean serum concentration of IGF1 tended to be lower in cases than controls, but was not significantly different.

Serum concentration of IGF2 was inversely associated with the risk of liver cancer ($P_{trend} < 0.001$, **Table 2**). Moreover, subjects in quintiles 2 to 5 displayed lower risks compared with quintile 1 (OR range, 0.04–0.10). These results remained unchanged after adjusting for BMI, alcohol consumption, smoking habit, and hepatitis virus infection. After additionally controlling for IGF1 and IGFBP3, the results were

Table 1. Selected baseline characteristics of case and control groups.

	Cases	Controls	*P* value
Number of subjects	86	249	
Age (mean ± SD)	64.9 ± 7.1	64.1 ± 6.7	0.330
Male (*n*)	50 (58.0%)	146 (58.6%)	1.000[a]
Height (cm; mean ± SD)	156.8 ± 8.9	156.1 ± 8.1	0.481
Weight (kg; mean ± SD)	55.6 ± 9.2	54.3 ± 7.8	0.231
BMI (kg/m^2; mean ± SD)	22.6 ± 3.0	22.3 ± 2.6	0.460
Cigarette smoking (*n*)			0.586[a]
Never	29 (33.7%)	65 (26.1%)	
Past	17 (19.8%)	59 (23.7%)	
Current	35 (40.7%)	109 (43.8%)	
Alcohol intake (*n*)			0.015[a,b]
Never	35 (40.7%)	127 (51.0%)	
Past	13 (15.1%)	12 (4.8%)	
Current	35 (40.7%)	101 (40.6%)	
Hepatitis viral infection (*n*)			<0.001[a,b]
Negative	29 (33.7%)	206 (82.7%)	
Positive	57 (66.3%)	42 (16.9%)	
IGF2 (ng/mL; mean ± SD) total	416.3 ± 128.7	549.7 ± 123.5	<0.001[b]
Male	379.2 ± 121.9	530.5 ± 119.0	<0.001[b]
Female	467.8 ± 121.4	576.9 ± 125.2	<0.001[b]
IGF1 (ng/mL; mean ± SD) total	96.7 ± 50.4	108.9 ± 52.9	0.061
Male	95.0 ± 43.8	112.4 ± 48.4	0.026[b]
Female	98.9 ± 59.0	104.1 ± 58.6	0.651
IGFBP3 (μg/mL; mean ± SD) total	2.23 ± 0.80	2.83 ± 0.82	<0.001[b]
Male	2.04 ± 0.73	2.76 ± 0.82	<0.001[b]
Female	2.48 ± 0.83	2.94 ± 0.81	0.004[b]

[a]Chi-squared test.
[b]$P < 0.05$.

still the same. Moreover, participants in quintiles 2 to 5 exhibited lower risks compared with quintile 1 (ORs were 0.14, 0.16, 0.15, and 0.05, with 95% CIs of 0.04–0.52, 0.03–0.79, 0.03–0.86, and 0.01–0.35, respectively). The results were supported by a cubic spline curve (Supplementary Fig. S1), indicating almost linear association between IGF2 and log-odds for the incidence.

To investigate interactions with sex, we calculated ORs in subgroups (**Table 3**). In male subjects, serum IGF2 level correlated inversely with risk of liver cancer ($P_{trend} < 0.001$). Individuals in quintiles 2 to 5 displayed lower risk compared with quintile 1 (OR range, 0.03–0.08). Results were again unchanged after controlling for BMI, alcohol intake, tobacco smoking status, and hepatitis virus infection ($P_{trend} = 0.004$). After additional adjustments for IGF1 and IGFBP3, low serum IGF2 tended to be related to higher risk of liver cancer, but that relationship was not significant ($P_{trend} = 0.091$). Quintiles 2 and 5 of IGF2 showed lower risks of liver cancer (OR = 0. 02 and 0.01; 95% CI, 0.00–0.30 and 0.00–0.32, respectively).

Among female participants, serum IGF2 level was inversely related to the risk of liver cancer ($P_{trend} < 0.001$; **Table 3**). Subjects in quintiles 2 to 5 showed a lower risk compared with quintile 1 (OR = 0. 22, 0.07, 0.09, and 0.05; 95% CI = 0.05–0.93, 0.02–0.36, 0.02–0.41, and 0.01–0.27, respectively). After adjusting for BMI, drinking, smoking, and hepatitis virus infection, the results remained the same ($P_{trend} = 0.003$). After additionally controlling for IGF1 and IGFBP3, almost the same results were detected ($P_{trend} = 0.028$).

To assess interactions with age, ORs were again analyzed in subgroups (**Table 4**). Among nonelderly participants (population <65 years old), low serum levels of IGF2 correlated with increased risk of liver cancer ($P_{trend} < 0.001$). Subjects in quintiles 2 to 5 displayed lower risks compared with quintile 1 (OR = 0. 03, 0.01, 0.01, and 0.01; 95% CI, 0.00–0.25, 0.00–0.11, 0.00–0.16, and 0.00–0.09, respectively). After controlling for BMI, drinking, smoking, and hepatitis viral infection, the same results were detected ($P_{trend} = 0.002$). After additional adjustments for IGF1 and IGFBP3, low IGF2 levels tended

Table 2. ORs and 95% CIs for liver cancers with reference to serum concentrations of IGF2.

	Quintile					
	1 (reference)	2	3	4	5	P_{trend}
IGF2 (ng/mL, range)	<460	460–520	521–580	581–650	>650	
No. of case/control	60/54	8/49	7/50	7/47	4/49	
OR (95% CI)	1	0.10 (0.04–0.27)	0.08 (0.03–0.23)	0.08 (0.03–0.24)	0.04 (0.01–0.14)	<0.001[a]
OR adjusted 1 (95% CI)	1	0.14 (0.04–0.45)	0.10 (0.03–0.39)	0.10 (0.03–0.36)	0.05 (0.01–0.24)	<0.001[a]
OR adjusted 2 (95% CI)	1	0.14 (0.04–0.52)	0.16 (0.03–0.79)	0.15 (0.03–0.86)	0.05 (0.01–0.35)	<0.001[a]

Note: Adjusted 1, adjusted for alcohol intake, BMI, cigarette smoking, and hepatitis viral infection. Adjusted 2, adjusted for alcohol intake, BMI, cigarette smoking, hepatitis viral infection, IGF1, and IGFBP3.
[a]$P < 0.05$.

Table 3. ORs and 95% CIs for liver cancers with reference to serum concentrations of IGF2 among gender subgroups.

		Quintile					
		1 (reference)	2	3	4	5	P_{trend}
	IGF2 (ng/mL, range)	<460	460–520	521–580	581–650	>650	
Male	No. of case/control	39/37	4/37	3/24	3/25	1/23	
	OR (95% CI)	1	0.06 (0.02–0.24)	0.08 (0.02–0.38)	0.08 (0.02–0.35)	0.03 (0.00–0.23)	<0.001[a]
	OR adjusted 1 (95% CI)	1	0.07 (0.01–0.40)	0.16 (0.02–1.09)	0.07 (0.01–0.64)	0.03 (0.00–0.46)	0.004[a]
	OR adjusted 2 (95% CI)	1	0.02 (0.00–0.30)	0.32 (0.02–5.05)	0.07 (0.00–2.02)	0.01 (0.00–0.32)	0.091
Female	No. of case/control	21/17	4/12	4/26	4/22	3/26	
	OR (95% CI)	1	0.22 (0.05–0.93)	0.07 (0.02–0.36)	0.09 (0.02–0.41)	0.05 (0.01–0.27)	<0.001[a]
	OR adjusted 1 (95% CI)	1	0.36 (0.06–2.33)	0.09 (0.01–0.60)	0.13 (0.02–0.78)	0.07 (0.01–0.51)	0.003[a]
	OR adjusted 2 (95% CI)	1	0.60 (0.07–5.30)	0.10 (0.01–1.15)	0.18 (0.02–2.02)	0.07 (0.00–1.12)	0.028[a]

Note: Adjusted 1, adjusted for alcohol intake, BMI, cigarette smoking, and hepatitis viral infection. Adjusted 2, adjusted for alcohol intake, BMI, cigarette smoking, hepatitis viral infection, IGF1, and IGFBP3.
[a]$P < 0.05$.

Table 4. ORs and 95% CIs for liver cancers with reference to serum concentrations of IGF2 among age subgroups.

		Quintile					
		1 (reference)	2	3	4	5	P_{trend}
	IGF2 (ng/mL, range)	<460	460–520	521–580	581–650	>650	
<65 years old	No. of case/control	32/20	5/30	1/26	4/26	2/25	
	OR (95% CI)	1	0.03 (0.00–0.25)	0.01 (0.00–0.11)	0.01 (0.00–0.16)	0.01 (0.00–0.09)	<0.001[a]
	OR adjusted 1 (95% CI)	1	0.02 (0.00–0.39)	0.00 (0.00–0.07)	0.00 (0.00–0.14)	0.00 (0.00–0.19)	0.002[a]
	OR adjusted 2 (95% CI)	1	0.03 (0.00–1.95)	0.01 (0.00–0.95)	0.01 (0.00–2.17)	0.00 (0.00–1.52)	0.075
≥65 years old	No. of case/control	28/34	3/19	6/24	3/21	2/24	
	OR (95% CI)	1	0.17 (0.04–0.66)	0.22 (0.07–0.74)	0.16 (0.04–0.63)	0.09 (0.02–0.44)	<0.001[a]
	OR adjusted 1 (95% CI)	1	0.24 (0.05–1.16)	0.41 (0.09–1.84)	0.23 (0.04–1.22)	0.11 (0.02–0.68)	0.010[a]
	OR adjusted 2 (95% CI)	1	0.05 (0.00–0.63)	0.72 (0.06–8.87)	0.20 (0.01–3.13)	0.01 (0.00–0.53)	0.026[a]

Note: Adjusted 1, adjusted for alcohol intake, BMI, cigarette smoking, and hepatitis viral infection. Adjusted 2, adjusted for alcohol intake, BMI, cigarette smoking, hepatitis viral infection, IGF1, and IGFBP3.
[a]$P < 0.05$.

to show higher risks of liver cancer without statistical significance ($P_{trend} = 0.075$).

In elderly individuals (≥65 years old), low IGF2 level was related to future risk of liver cancer ($P_{trend} < 0.001$, **Table 4**). Participants in quintiles 2–5 had lower risks compared with quintile 1 (OR = 0. 17, 0.22, 0.16, and 0.09; 95% CI, 0.04–0.66, 0.07–0.74, 0.04–0.63, and 0.02–0.44, respectively). After several adjustments, the same results were detected ($P_{trend} = 0.010$–0.026).

To exclude possible effects of latent cancers on IGF2 levels, we then limited the analysis to 73 cases, which were diagnosed at least 3 years after baseline survey, and 210 controls (**Table 5**). Low serum IGF2 levels were related to increased risk of liver cancer ($P_{trend} < 0.001$). Subjects in quintiles 2–5 had lower risk compared with quintile 1 (OR = 0. 13, 0. 10, 0.11, and 0.05; 95% CI, 0.04–0.35, 0.03–0.28, 0.04–0.30, and 0.01–0.19, respectively). After several adjustments, the same results were seen ($P_{trend} < 0.001$–0.025).

Discussion

High serum concentration of IGF1 and low serum concentration of IGFBP3 are risk factors for several site-specific cancers (15–17). We reported that both total IGFBP3 and free IGFBP3, estimated as the molar difference between IGFBP3 and IGF1 (i.e., free IGFBP3 = IGFBP3 − IGF1), were associated with decreased risk of liver cancer, with free IGFBP3 showing a stronger relationship than total IGFBP3 (18). However, the relationship between serum concentration

Table 5. ORs and 95% CIs for liver cancers followed over 3 years with reference to serum concentrations of IGF2.

	Quintile					
	1 (reference)	2	3	4	5	P_{trend}
IGF2 (ng/mL, range)	<460	460–520	521–580	581–650	>650	
No. of case/control	49/47	7/38	6/42	7/41	4/42	
OR (95% CI)	1	0.13 (0.04–0.35)	0.10 (0.03–0.28)	0.11 (0.04–0.30)	0.05 (0.01–0.19)	<0.001[a]
OR adjusted 1 (95% CI)	1	0.18 (0.06–0.60)	0.13 (0.03–0.46)	0.12 (0.03–0.44)	0.07 (0.02–0.32)	<0.001[a]
OR adjusted 2 (95% CI)	1	0.19 (0.05–0.74)	0.19 (0.04–0.96)	0.20 (0.04–1.14)	0.07 (0.01–0.52)	0.025[a]

Note: Adjusted 1, adjusted for alcohol intake, BMI, cigarette smoking, and hepatitis viral infection. Adjusted 2, adjusted for alcohol intake, BMI, cigarette smoking, hepatitis viral infection, IGF1, and IGFBP3.
[a]$P < 0.05$.

of IGF2 and the risk of cancers has seldom been reported. Compared with IGF1, the pathological effects of IGF2 on hepatocarcinogenesis have not been studied in detail.

This study revealed that lower serum concentration of IGF2, especially concentrations <460 ng/mL, was related to future risk of liver cancer. Those findings were unchanged after adjusting for BMI, drinking, smoking, and hepatitis virus infections. Moreover, the results showed the same tendency even when analyses were performed in subgroups by both sex and age. Limiting subjects to those followed for over 3 years, the lowest quintile showed the highest risk of liver tumors. These results seem inconsistent with the fact that IGF2 is known as a potent mitogen similar to IGF1. However, these results were similar to another report that serum IGF2 levels of HCC were lower than those of healthy controls (13).

One reason why subjects in the lowest quintile of IGF2 showed a higher risk of liver cancer may be that serum IGF2 has been reported to be low with poor nutritional status and in several chronic diseases, as follows. IGF2 is downregulated during starvation, possibly as a measure to avoid hypoglycemia (29). IGF2 was low in patients with hypothyroidism (30), and both IGF1 and IGF2 levels were low in patients with liver cirrhosis (31, 32). Almost 80% of patients with cirrhosis showed IGF2 levels below normal range (31). Serum concentrations of IGF2 correlated negatively with severity of liver cirrhosis (Child score), whereas levels of IGF1 did not.

A second possibility is that low IGF2 might suggest the possibility of future weight gain. In a prospective study of subjects with normal glucose tolerance, low serum IGF2 level predicted future weight gain and obesity (33). Compared with quintile 1 for IGF2 (<400 ng/mL), quintiles 2 to 5 showed lower risk of future weight gain (OR = 0.36–0.49). In another prospective study, low serum concentrations of IGF2 predicted weight gain in normal-weight participants with type 2 diabetes (34). Subjects in the lowest IGF2 quintile in this study may thus have gained weight before hepatocarcinogenesis. However, we could not confirm this, since both weight and IGF2 concentration were recorded only once, at the beginning of the study.

A third possibility is that low IGF2 levels may stimulate expressions of both IGF1 and IGF1R before hepatocarcinogenesis. In *IGF2*-knockout mice, IGF1 expression was upregulated (35). When the amounts of ligands were reduced, increased levels of receptors may be induced under homeostatic conditions by feedback mechanisms.

IGF1R expression is induced in both hepatocytes and HCC cells (9). Although levels of both IGFs were low in patients with liver cirrhosis (31, 32), high focal levels of *IGF2* RNA were found in hepatocytes of patients with hepatitis virus-associated cirrhosis. Compared with noncirrhotic livers, all cirrhotic specimens showed reduced hepatocellular expression of IGF2R protein. Downregulation of IGF2R and upregulation of IGF2 in hepatocytes seem to be early events in hepatocarcinogenesis (11). *IGF2* gene promoters differ between the liver and peripheral tissues (7). Moreover, loss of imprinting of *IGF2* reportedly leads to IGF2 overexpression during carcinogenesis (29). Local concentrations of IGF2 in pre- and transforming hepatocytes might thus be higher than estimated from serum IGF2 levels.

Several limitations must also be considered. First, serum concentrations of IGFs and IGFBP3 were assayed at only a single time point, in the baseline survey. We therefore did not investigate chronologic changes in association with carcinogenesis. Another limitation was that some data regarding drinking, smoking, and BMI were missing from the JACC study, as self-administered questionnaires were used (23–25). A third limitation was that we assessed IGFs and IGFBP3, but not other IGFBPs or receptors. In addition, there was the possibility of false-positive results due to multiple tests.

In conclusion, low-serum IGF2 might be associated with future risk of malignant liver tumors. The cut-off value of IGF2 related to the risk of liver cancer might be defined as <460 ng/mL.

Authors' Disclosures

No disclosures were reported.

Authors' Contributions

Y. Adachi: Investigation, methodology, writing–original draft, project administration. **M. Nojima:** Data curation, formal analysis, investigation. **M. Mori:** Supervision, an original member of the JACC study. **R. Himori:** Investigation, writing–review and editing. **T. Kubo:** Investigation, writing–review and editing. **N. Akutsu:** Validation, writing–review and editing. **Y. Lin:** Supervision, writing–review and editing. **Y. Kurozawa:** Writing–review and editing, an original member of the JACC study. **K. Wakai:** Supervision, writing–review and editing, an original member of the JACC study. **A. Tamakoshi:** Resources, supervision, writing–review and editing, an original member of the JACC study.

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References

1. Baserga R. Oncogenes and the strategy of growth factors. Cell 1994;79:927–30.
2. Adachi Y, Yamamoto H, Ohashi H, Endo T, Carbone DP, Imai K, et al. A candidate targeting molecule of insulin-like growth factor-I receptor for gastrointestinal cancers. World J Gastroenterol 2010;16:5779–89.
3. Adachi Y, Yamamoto H, Imsumran A, Oka T, Oki M, Nosho K, et al. Insulin-like growth factor-I receptor as a candidate for a novel molecular target in the gastrointestinal cancers. Dig Endosc 2006;18:245–51.
4. Yee D. A tale of two receptors: insulin and insulin-like growth factor signaling in cancer. Clin Cancer Res 2015;21:667–9.
5. Foulstone E, Prince S, Zaccheo O, Burns JL, Harper J, Jacobs C, et al. Insulin-like growth factor ligands, receptors, and binding proteins in cancer. J Pathol 2005;205:145–53.
6. Gu F, Schumacher FR, Canzian F, Allen NE, Albanes D, Berg CD, et al. Eighteen insulin-like growth factor pathway genes, circulating levels of IGF-I and its binding protein, and risk of prostate and breast cancer. Cancer Epidemiol Biomarkers Prev 2010;19:2877–87.
7. Kasprzak A, Adamek A. Insulin-like growth factor 2 (IGF2) signaling in colorectal cancer-from basic research to potential clinical applications. Int J Mol Sci 2019;20:4915.

8. Firth SM, Baxter RC. Cellular actions of the insulin-like growth factor binding proteins. Endocr Rev 2002;23:824–54.
9. Caro JF, Poulos J, Ittoop O, Pories WJ, Flickinger EG, Sinha MK. Insulin-like growth factor I binding in hepatocytes from human liver, human hepatoma, and normal, regenerating, and fetal rat liver. J Clin Invest 1988;81:976–81.
10. Sohda T, Iwata K, Soejima H, Kamimura S, Shijo H, Yun K. In situ detection of insulin-like growth factor II (IGF2) and H19 gene expression in hepatocellular carcinoma. J Hum Genet 1998;43:49–53.
11. Sedlaczek N, Hasilik A, Neuhaus P, Schuppan D, Herbst H. Focal overexpression of insulin-like growth factor 2 by hepatocytes and cholangiocytes in viral liver cirrhosis. Br J Cancer 2003;88:733–9.
12. Couvert P, Carrié A, Pariès J, Vaysse J, Miroglio A, Kerjean A, et al. Liver insulin-like growth factor 2 methylation in hepatitis C virus cirrhosis and further occurrence of hepatocellular carcinoma. World J Gastroenterol 2008;14:5419–27.
13. Rashad NM, El-Shal AS, Abd Elbary EH, Abo Warda MH, Hegazy O. Impact of insulin-like growth factor 2, insulin-like growth factor receptor 2, insulin receptor substrate 2 genes polymorphisms on susceptibility and clinicopathological features of hepatocellular carcinoma. Cytokine 2014;68:50–8.
14. Weng CJ, Hsieh YH, Tsai CM, Chu YH, Ueng KC, Liu YF, et al. Relationship of insulin-like growth factors system gene polymorphisms with the susceptibility and pathological development of hepatocellular carcinoma. Ann Surg Oncol 2010;17:1808–15.
15. Chan JM, Stampfer MJ, Giovannucci E, Gann PH, Ma J, Wilkinson P, et al. Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. Science 1998;279:563–6.
16. Hankinson SE, Willett WC, Colditz GA, Hunter DJ, Michaud DS, Deroo B, et al. Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. Lancet 1998;351:1393–6.
17. Ma J, Pollak MN, Giovannucci E, Chan JM, Tao Y, Hennekens CH, et al. Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3. J Natl Cancer Inst 1999;91:620–5.
18. Adachi Y, Nojima M, Mori M, Matsunaga Y, Akutsu N, Sasaki S, et al. Insulin-like growth factor-related components and the risk of liver cancer in a nested case-control study. Tumour Biol 2016;37:15125–32.
19. Adachi Y, Nojima M, Mori M, Yamashita K, Yamano HO, Nakase H, et al. Insulin-like growth factor-1, IGF binding protein-3, and the risk of esophageal cancer in a nested case-control study. World J Gastroenterol 2017;23:3488–95.
20. Wakai K, Ito Y, Suzuki K, Tamakoshi A, Seki N, Ando M, et al. Serum insulin-like growth factors, insulin-like growth factor-binding protein-3, and risk of lung cancer death: a case-control study nested in the Japan Collaborative Cohort (JACC) Study. Jpn J Cancer Res 2002;93:1279–86.
21. Lin Y, Tamakoshi A, Kikuchi S, Yagyu K, Obata Y, Ishibashi T, et al. Serum insulin-like growth factor-I, insulin-like growth factor binding protein-3, and the risk of pancreatic cancer death. Int J Cancer 2004;110:584–8.
22. Sakauchi F, Nojima M, Mori M, Wakai K, Suzuki S, Tamakoshi A, et al. Serum insulin-like growth factors I and II, insulin-like growth factor binding protein-3 and risk of breast cancer in the Japan Collaborative Cohort study. Asian Pac J Cancer Prev 2009;10:51–5.
23. Ohno Y, Tamakoshi A, Group JS. Japan collaborative cohort study for evaluation of cancer risk sponsored by monbusho (JACC study). J Epidemiol 2001;11:144–50.
24. Tamakoshi A, Yoshimura T, Inaba Y, Ito Y, Watanabe Y, Fukuda K, et al. Profile of the JACC study. J Epidemiol 2005;15:S4–8.
25. Tamakoshi A, Ozasa K, Fujino Y, Suzuki K, Sakata K, Mori M, et al. Cohort profile of the Japan Collaborative Cohort Study at final follow-up. J Epidemiol 2013;23:227–32.
26. Tamakoshi A, Suzuki K, Ito Y, Watanabe Y, Inaba Y, Tajima K, et al. Selection of cases and controls for the nested case-control study within the Japan Collaborative Cohort Study: the First-wave. Asian Pac J Cancer Prev 2009;10:1–5.
27. Ito Y, Nakachi K, Imai K, Hashimoto S, Watanabe Y, Inaba Y, et al. Stability of frozen serum levels of insulin-like growth factor-I, insulin-like growth factor-II, insulin-like growth factor binding protein-3, transforming growth factor beta, soluble Fas, and superoxide dismutase activity for the JACC study. J Epidemiol 2005;15:S67–73.
28. Wakai K, Kurozawa Y, Shibata A, Fujita Y, Kotani K, Ogimoto I, et al. Liver cancer risk, coffee, and hepatitis C virus infection: a nested case-control study in Japan. Br J Cancer 2007;97:426–8.
29. Livingstone C. IGF2 and cancer. Endocr Relat Cancer 2013;20:R321–39.
30. Tada H, Watanabe Y, Futakuchi Y, Amino N. Change in serum concentration of insulin-like growth factor II (IGF-II) in patients with thyroid disease. Endocr J 1994;41:541–5.
31. Nikolic JA, Todorovic V, Bozic M, Tosic L, Bulajic M, Alempijevic T, et al. Serum insulin-like growth factor (IGF)-II is more closely associated with liver dysfunction than is IGF-I in patients with cirrhosis. Clin Chim Acta 2000;294:169–77.
32. Aleem E, Elshayeb A, Elhabachi N, Mansour AR, Gowily A, Hela A. Serum IGFBP-3 is a more effective predictor than IGF-1 and IGF-2 for the development of hepatocellular carcinoma in patients with chronic HCV infection. Oncol Lett 2012;3:704–12.
33. Sandhu MS, Gibson JM, Heald AH, Dunger DB, Wareham NJ. Low circulating IGF-II concentrations predict weight gain and obesity in humans. Diabetes 2003;52:1403–8.
34. Heald AH, Kärvestedt L, Anderson SG, McLaughlin J, Knowles A, Wong L, et al. Low insulin-like growth factor-II levels predict weight gain in normal weight subjects with type 2 diabetes. Am J Med 2006;119:167.
35. Silva D, Venihaki M, Guo WH, Lopez MF. Igf2 deficiency results in delayed lung development at the end of gestation. Endocrinology 2006;147:5584–91.