Plasma Insulin-Like Growth Factor-Binding Protein-2 Levels as Diagnostic and Prognostic Biomarker of Colorectal Cancer

Jyh-Ming Liou,* Chia-Tung Shun,* Jin-Tung Liang, Han-Mo Chiu, Mei-Jyh Chen, Chien–Chuan Chen, Hsiu-Po Wang, Ming-Shiang Wu, and Jaw-Town Lin

Departments of Internal Medicine (J.-M.L., H.-M.C., M.-J.C., C.-C.C., H.-P,W., M.-S.W., J.-T.L.), Pathology (C.-T.S.), Surgery (J.-T.L.), and Primary Care Medicine (M.-S.W.), National Taiwan University Hospital, College of Medicine, and Graduate Institute of Epidemiology (J.-M.L.), College of Public Health, National Taiwan University, Taipei 100, Taiwan; and Department of Internal Medicine (J.-T.L.), E-Da Hospital and I-Shou University, Kaohsiung County 840, Taiwan

Context: Overexpression of IGF-II and IGF-binding protein (IGFBP)-2 has been reported in several cancers.

Objective: We aimed to assess the roles of plasma IGF-II and IGFBP-2 levels as diagnostic and prognostic biomarkers and the impact of loss of imprinting (LOI) of IGF-II on the survival of colorectal cancer (CRC).

Design: We conducted a case control and prospective cohort study for diagnostic and prognostic values, respectively.

Patients and Setting: Plasma levels of IGF-II and IGFBP-2 were measured in 162 patients with CRC before surgery, in paired 15 patients after curative surgery, in 24 patients with advanced colon polyps, and in 114 healthy controls between 2003 and 2006 in National Taiwan University Hospital.

Results: The area under the curve values of using IGFBP-2 as a diagnostic marker for advanced colon polyp and CRC were 0.654 [95% confidence interval (CI) = 0.547–0.76; $P = 0.017$] and 0.815 (95% CI = 0.766–0.864; $P < 0.001$), respectively. The sensitivity and specificity for diagnosing CRC were 80.2 and 64%, respectively, if the cutoff value of IGFBP-2 was 377 ng/ml. In the multivariate Cox proportional hazards regression model, higher IGFBP-2 levels were associated with increased risk of mortality [hazard ratio (HR) = 2.46; $P = 0.017$], whereas higher IGF-II levels were associated with reduced risk of mortality (HR = 0.42; $P = 0.044$). LOI of IGF-II was associated with increased risk of mortality (HR = 7.91; $P = 0.014$) in patients with stage IV disease.

Conclusions: IGFBP-2 is a potential diagnostic and prognostic biomarker of CRC. LOI of IGF-II is significantly associated with poor prognosis in patients with stage IV disease. **(*J Clin Endocrinol Metab* 95: 1717–1725, 2010)**

Colorectal cancer (CRC) is an important cause of cancer mortality in Western countries, and its incidence and mortality rate are also increasing in many Asian countries (1, 2). Early detection by screening can lead to earlier diagnosis and improved survival. Fecal occult blood test, double-contrast enema, sigmoidoscopy, and colonoscopy or mixed strategies have been used in the screening of CRC (2, 3). Unfortunately, there are still no reliable serological biomarkers in CRC screening. IGF-II is an important autocrine and paracrine growth factor in the development of

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2009-2668 Received December 14, 2009. Accepted January 15, 2010.
First Published Online February 15, 2010
* J.-M.L. and C.-T.S. contributed equally to this work.

Abbreviations: AJCC, American Joint Committee on Cancer; AUC, area under the curve; BMI, body mass index; CEA, carcinoembryonic antigen; CI, confidence interval; CRC, colorectal cancer; HR, hazard ratio; IGFBP, IGF-binding protein; LOI, loss of imprinting; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic.

cancers. Overexpression of IGF-II was associated with increased susceptibility to colon carcinoma in mice (4). The serum levels of IGF-II were reported to be higher in patients with colonic adenoma and CRC than in healthy subjects (5–8). Furthermore, the combination of IGF-II and other biomarkers was shown to exhibit excellent sensitivity and specificity in distinguishing early ovarian cancer patients from healthy controls (9, 10).IGF-binding protein (IGFBP)-2 is one of the binding proteins that modulate the interactions of IGF ligands with IGF-I receptor. The serum levels of IGFBP-2 were elevated in patients with colorectal and ovarian cancers, with a trend from early to advanced disease (8, 11, 12). Taken together, levels of IGFBP-2 and IGF-II may serve as potential biomarkers for the detection of CRC.

In addition to their roles in the development of cancer, recent studies showed that IGF-II was required for the progression to advanced medulloblastoma in mice (13). Overexpression of IGF-II in tumor tissues was associated with more advanced disease and poor survival in prostate cancer, ovarian cancer, gastrointestinal stromal tumor, and CRC (14–17). Plasma IGF-II levels were higher in patients with more advanced CRC (18). However, whether higher plasma IGF-II levels are associated with survival in patients with CRC remains unknown. Overexpression of IGFBP-2 also plays an important role in the progression and invasion of glioma and ovarian cancer (19, 20). Elevated serum IGFBP-2 was associated with a higher risk of relapse after stem cell transplantation in childhood acute myelogenous leukemia and acute lymphoblastic leukemia and poor prognosis in ovarian cancer (12, 21, 22). However, the role of circulating IGFBP-2 levels on the survival of CRC patients has not been reported. Loss of imprinting (LOI), defined as aberrant expression of the normally silent maternally inherited allele, is an epigenetic mechanism that leads to the overexpression of IGF-II. LOI of IGF-II has been reported to be associated with increased susceptibility to CRC and more advanced disease in several cancers (23–26). However, whether LOI of IGF-II is associated with survival in patients with CRC has not been reported. Therefore, we aimed to assess the roles of plasma IGF-II and IGFBP-2 levels as diagnostic and prognostic biomarkers and the impact of LOI of IGF-II on survival in CRC.

Patients and Methods

Patients

To evaluate the role of IGFBP-2 and IGF-II as diagnostic biomarkers for CRC, a case control study was done. Pathologically documented CRC patients who underwent surgery in National Taiwan University Hospital between October 2003 and April 2006 were enrolled as case group. Subjects who did not have CRC as documented by colonoscopy or other malignancies were enrolled as control group. Patients with advanced colon polyps, defined as polyp size of 1 cm or bigger or contained villous components on histological examination, were also enrolled. To assess the roles of IGFBP-2, IGF-II, and LOI of IGF-II in the prediction of survival, a prospective cohort study was done. The demographic and clinicopathological data in patients with CRC were prospectively collected on enrollment, and they were observed until death or September 2009. Death was ascertained by the medical record in the hospital or reporting by family. Patients with the following conditions were excluded from this study: 1) patients who refused to give written informed consent for the collection and analysis of blood and surgical specimens, 2) patients with inflammatory bowel disease or with a known family history of familial adenomatous polyposis or hereditary nonpolyposis CRC, 3) patients who underwent concurrent chemoradiotherapy before surgery, 4) patient received surgery for recurrent CRC, and 5) patients who died of complications or other diseases during the same hospitalization of surgery. Poorly differentiated (high-grade) tumor was defined as less than 10% of tumor cells formed glands, and mucinous tumor was defined as those containing more than 50% extracellular mucin. The study protocol was approved by the Institutional Review Boards of National Taiwan University Hospital.

Measurement of plasma IGF-II and IGFBP-II levels

Blood samples were centrifuged, divided, stored in tubes coated with lithium heparin, and frozen at −70 C until use. Plasma IGF-II and IGFBP-2 levels were measured with the commercially available ELISA kit (Diagnostic Systems Laboratories, Webster, TX) following the manufacturer's instructions in the Taipei Lezen Laboratory Center. All assays were duplicated and were measured by one laboratory technician who was blind to the disease status and clinical outcomes. All samples were measured in duplicate, and subsets of samples were reassayed six times in every ELISA plate for quality control. The interassay and intraassay variabilities were 7.9 and 7.0% for IGF-II and were 5.7 and 2.3% for IGFBP-2, respectively.

Nucleic acid preparation and quantitative analysis of *IGF-II* imprinting status

The detailed methods used in the determination of IGF-II LOI status has been described in our previous study (26). In brief, genomic DNA from peripheral blood leukocytes and RNA from CRC tissue were extracted. The sequences of primer set II were: *IGF-II*/Af 5′-CCT TGG ACT TTG AGT CAA ATT-3′ and *IGF-II*/Br 5′-GGT CGT GCC AAT TAC ATT TCA-3′. The 293-bp PCR products of genomic DNA were classified as A/A, B/B, and A/B, and heterozygote (A/B) was considered informative for LOI analysis. The primer sequences of primer set I used in RT-PCR were *IGF-II*/Zf 5′-CCT CCG ACC GTG CTT CCG GAC-3′ and *IGF-II*/Er 5′-GGA TGG GAA TTG AGA TGT AAG-3′. The presence of both alleles A and B with a ratio less than 3-fold difference in expression between two alleles after *Apa*I digestion were considered as LOI of the *IGF-II* gene according to previous studies (27).

Statistical analysis

Demographic data were analyzed with χ^2 test or Fisher's exact test as appropriate. Continuous data were compared with

independent-sample Student's *t* test or by one-way ANOVA as appropriate. The differences in plasma levels of IGF-II and IGFBP-2 between cases and controls were compared using the nonparametric Mann-Whitney *U* test. The IGF-II and IGFBP-2 levels before and after surgery in paired samples were compared with the Wilcoxon signed ranks test. The sensitivity and specificity of the plasma levels of IGF-II and IGFBP-2 were analyzed with the receiver operating characteristic (ROC) curve. The survival curve (all-cause mortality) according to LOI status of IGF-II and plasma levels of IGF-II and IGFBP-2 were constructed using the Kaplan-Meier method and compared by the log-rank test. The Cox proportional hazards regression model were used to calculate the hazard ratios (HR) and 95% confidence interval (CI) in both univariate and multivariate analysis. All *P* values were two-tailed with 0.05 specified as statistical significance. All statistical analyses will be performed with statistical software (SPSS version 10.0 for Windows; SPSS Inc., Chicago, IL).

Results

IGFBP-2 and IGF-II levels among CRC patients at different stage and controls

Totally, 162 patients with CRC, 24 patients with advanced colon polyps, and 114 healthy controls were enrolled in this study. The IGF-II imprinting status was informative in 146 CRC patients. The distribution of age, gender, and body mass index (BMI) were not significantly different between the three groups, as shown in Table 1. The mean IGFBP-2 levels were significantly higher in CRC patients (1136.6 ng/ml) than in controls (371.1 ng/ml) ($P < 0.001$, Table 1). In contrast, the IGF-II levels were similar between CRC patients and controls (Table 1). The mean plasma IGF-II levels (mean difference, 117.3 ng/ml, 95% CI = −28.5–263 ng/ml) and IGFBP-2 levels (mean difference, 216.7 ng/ml; 95% CI = −110–544.3 ng/ml) were not significantly different according to the imprinting status of IGF-II. The plasma IGF-II levels correlated negatively with IGFBP-2 levels ($\beta = -0.155$; 95% CI = −0.428 to −0.067; $P = 0.007$).

In the stratified analysis by American Joint Committee on Cancer (AJCC) stage, the IGFBP-2 levels remained significantly higher in CRC patients at different stages than in normal controls (Fig. 1A). Besides, the IGFBP-2 levels were also significantly higher in patients with stage II, III, and IV diseases than in patients with stage I disease (Fig. 1A). The IGF-II levels were significantly higher in patients with stage II and III disease than in patients with stage I disease (Fig. 1B). In contrast, the IGF-II levels were lower in patients with advanced colon polyps (990.8 ng/ml) and stage I disease (944.5 ng/ml) than in normal controls (Fig. 1B).

Changes of IGFBP-2 and IGF-II levels before and after curative surgery

Postoperative plasma samples were available in 15 patients after curative surgery (mean 44.1 months, range 37–63 months). The IGFBP-2 levels were significantly reduced from 1029 ng/ml before surgery to 126 ng/ml after curative surgery ($P = 0.001$). In contrast, IGF-II levels increased from 1368 ng/ml before surgery to 1605 ng/ml after curative surgery, but the difference was not statistically significant ($P = 0.078$).

Plasma IGFBP-2 and IGF-II as diagnostic biomarkers for CRC

The ROC curves of using plasma IGFBP-2 and IGF-II values in the diagnosis of advanced colon polyp and CRC are shown in Fig. 2. The area under the curve (AUC) of using IGFBP-2 as diagnostic biomarker for advanced colon polyp was 0.654, with 95% CI ranging from 0.547–0.760 ($P = 0.018$, Fig. 2A). The AUC of using IGFBP-2 as a diagnostic biomarker for CRC was 0.815 (95% CI =

TABLE 1. Demographic data in patients with CRC and advanced colonic polyp and controls

	Control (n = 114)	Polyp (n = 24)	Cancer (n = 162)	*P* value
Gender (male/female)	63/51	13/11	93/69	0.916
Mean age, yr (SD)	63.14 (10.2)	60.5 (11.9)	65.4 (12.6)	0.078
BMI, mean (SD)	23.9 (3.31)	23.5 (3.7)	23.2 (3.37)	0.253
Diabetes mellitus	6	4	23	0.043
Cigarette smoking	14	3	46	0.037
IGFBP-2 level, mean (SD)	371.1 (342)	497.4 (297.4)	1136.6 (342)	<0.001
IGF-II level, mean (SD)	1301.69 (623.7)	990.8 (328.5)	1324.6 (454.2)	0.013
Distribution of cancer (proximal/distal)			41/121	
Differentiation (well, moderate/poor, mucinous)			148/14	
Lymph node metastasis			79	
T-stage (T1/T2/T3/T4)			13/26/103/20	
Liver metastasis			18	
Lymphatic invasion			78	
Venous invasion			50	
AJCC stage (I/II/III/IV)			13/70/58/21	

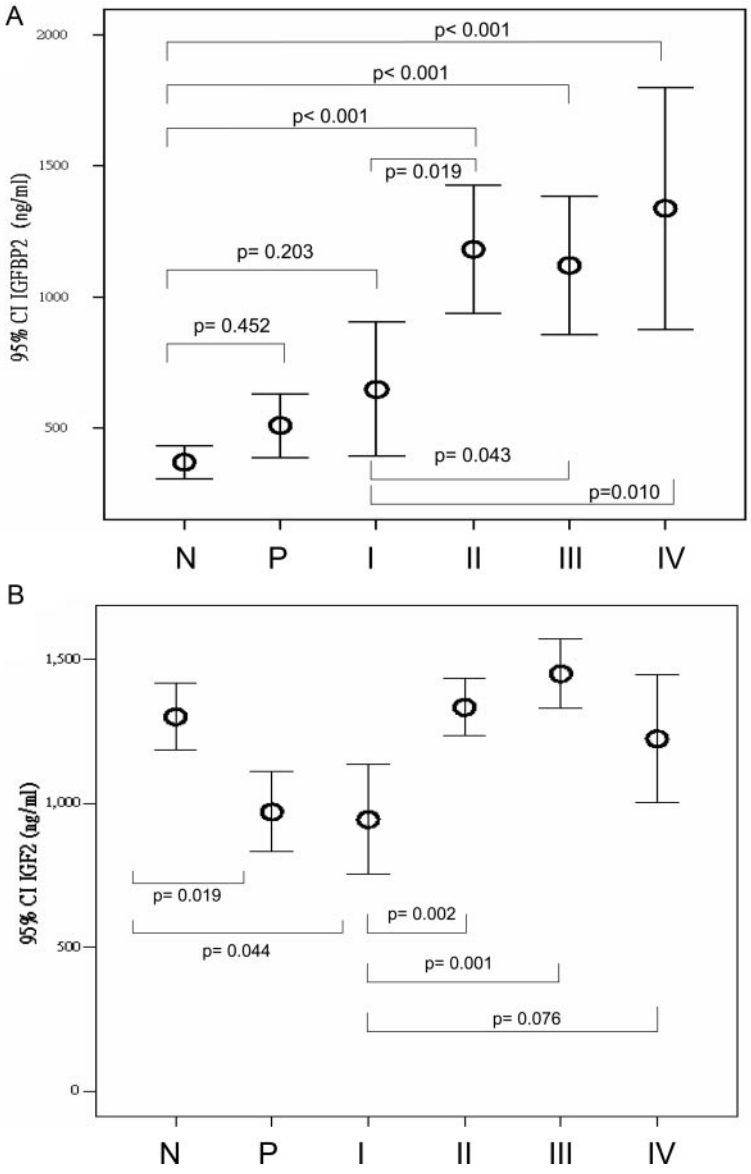

FIG. 1. Plasma levels of IGFBP-2 (A) and IGF-II (B) among patients with and without CRC and colon polyp. N, Normal controls; P, patients with advanced colon polyps; I, II, III, and IV represent patients with stage I, II, III, and IV CRC, respectively.

0.766–0.864; $P < 0.001$, Fig. 2B). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for diagnosing CRC were 80.2, 64, 76, and 69.5%, respectively, if the cutoff value of IGFBP-2 was 377 ng/ml. When further stratified according to AJCC stage, the AUC were 0.733 (95% CI = 0.605–0.861; $P = 0.006$) for stage I disease, 0.821 (95% CI = 0.761–0.882; $P < 0.001$) for stage II disease, 0.815 (95% CI = 0.748–0.882; $P < 0.001$) for stage III disease, and 0.845 (95% CI = 0.743–0.948; $P < 0.001$) for stage IV disease (Fig. 2, C–F). The sensitivity, specificity, PPV, and NPV for diagnosing advanced colon polyp were 70.8, 51.8, 23.6, and 89.4%, respectively, if the cutoff value of IGFBP-2 was 300 ng/ml. The sensitivity, specificity, PPV, and NPV for diagnosing advanced colon polyp were 50, 64, 22.6, and 85.9%, respectively, if the cutoff value of IGFBP-2 was 377 ng/ml.

In contrast, the role of plasma IGF-II levels was less helpful in the diagnosis of CRC. The AUC of using IGF-II as a diagnostic marker were 0.569 (95% CI = 0.499–0.638; $P = 0.052$) for CRC and 0.335 (95% CI = 0.221–0.450; $P = 0.011$) for advanced colon polyps. Carcinoembryonic antigen (CEA) also distinguished patients with CRC from healthy controls (AUC = 0.891; 95% CI = 0.848–0.934; $P < 0.001$). However, CEA was not helpful in the diagnosis of patients with stage I CRC (AUC = 0.609; 95% CI = 0.409–0.809; $P = 0.345$) and advanced colon polyps (AUC = 0.676; 95% CI = 0.476–0.876; $P = 0.052$).

Survival in CRC patients according to plasma IGFBP-2 and IGF-II levels and LOI status of IGF-II

The Kaplan-Meier survival curves according to plasma IGFBP-2 and IGF-2 levels are shown in Fig. 3. Patients in the highest tertile of plasma IGFBP-2 (>1160 ng/ml) and IGF-II (>1550 ng/ml) were defined as having higher IGFBP-2 and IGF-II levels, respectively. Patients with higher IGFBP-2 levels had significantly lower overall survival rate than patients with lower IGFBP-2 levels ($P = 0.001$, Fig. 3A). In contrast, patients with higher plasma IGF-II levels had significantly better survival rates than those with lower IGF-II levels ($P = 0.032$, Fig. 3B). In patients with stage IV disease, the presence of LOI of IGF-II was significantly associated with a worse overall survival ($P = 0.003$, Fig. 3D).

On Cox univariate proportional hazards analysis, we found that the presence of lymph node metastasis (HR = 5.0; $P < 0.001$), deeper invasion (HR = 4.13; $P = 0.018$), liver metastasis (HR = 8.73; $P < 0.001$), lymphatic invasion (HR = 2.94; $P = 0.003$), venous invasion (HR = 2.14; $P = 0.02$), poorly differentiated tumor (HR = 3.84; $P = 0.001$), more advanced stages (HR = 6.56; $P < 0.001$), and higher baseline IGFBP-2 levels (HR = 2.77; $P = 0.001$) were associated with increased risks of mortality (Table 2). In contrast, higher baseline IGF-II levels were associated with reduced risks of mortality (HR = 0.42; $P = 0.037$). After adjustment for age, gender, lymph node metastasis, depth of invasion, liver metastasis, lymphatic invasion, venous invasion, and BMI in the multivariate analysis, we found that patients with higher IGFBP-2 was

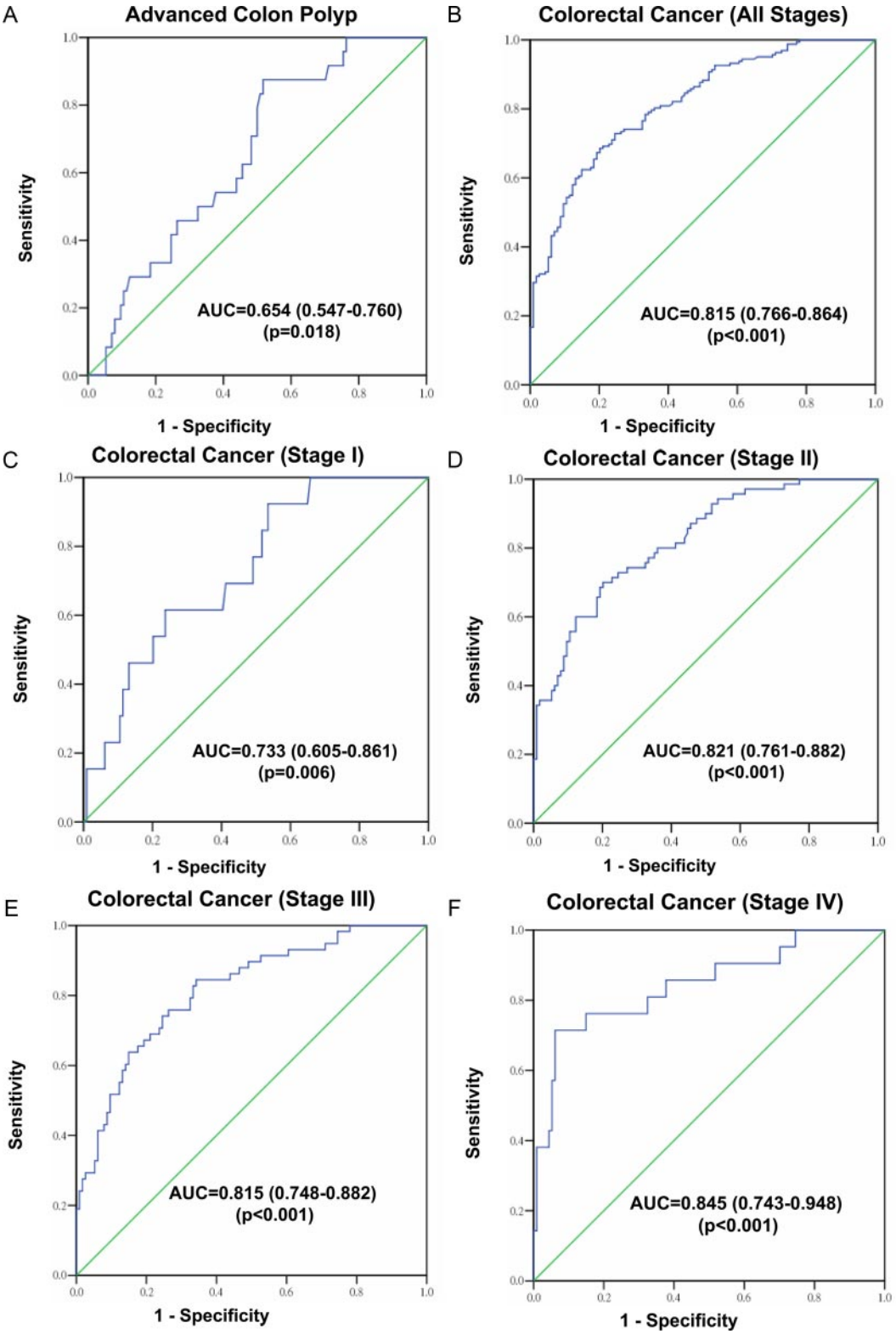

FIG. 2. ROC curves of using plasma IGFBP-2 levels in distinguishing advanced colon polyp and CRC, with AUC (95% CI) shown.

independently associated with increased risk of mortality (HR = 2.46; 95% CI = 1.18–5.16; $P = 0.017$) compared with those with lower IGFBP-2 levels (Table 3). In contrast, patients higher IGF-II levels were independently associated with reduced risk of mortality (HR = 0.42; 95% CI = 0.18–0.98; $P = 0.044$). The presence of LOI of IGF-II was associated with increased risk of mortality (HR = 7.91; $P = 0.014$) in patients with stage IV disease after adjustment for age, gender, and BMI.

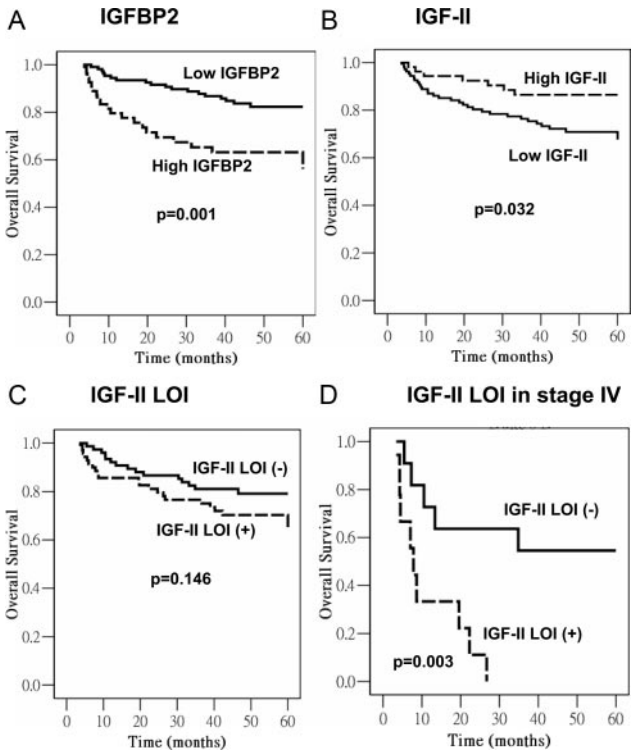

FIG. 3. The Kaplan-Meier survival curve of overall survival among CRC patients.

Discussion

In the present study, we have demonstrated that plasma IGFBP-2 levels can distinguish patients with CRC and advanced colon polyps from healthy subjects. Plasma IGFBP-2 levels were also significantly reduced after curative surgery for CRC. Besides, patients with higher plasma IGFBP-2 levels were independently associated with worse overall survival. Taken together, IGFBP-2 was a potential diagnostic and prognostic biomarker for CRC. In contrast, plasma IGF-II levels are not helpful in distinguishing CRC patients from controls. However, higher plasma IGF-II levels were associated with better overall survival in CRC patients. Interestingly, the presence of loss of imprinting of IGF-II in patients with stage IV disease was associated with a significantly worse overall survival.

TABLE 2. Univariate analysis for the prediction of mortality in CRC patients

	HR	95% CI	*P* value
Age, >60 *vs.* ≤60 yr	1.11	0.99–1.05	0.212
Gender, male *vs.* female	1.87	0.93–3.78	0.079
Lymph node metastasis, yes *vs.* no	5.01	2.30–10.93	<0.001
Depth of invasion, T3+T4 *vs.* T1+T2	4.13	1.27–13.43	0.018
Liver metastasis, yes *vs.* no	8.73	4.46–17.06	<0.001
Stage, III+IV *vs.* I+II	6.56	2.74–15.69	<0.001
Lymphatic invasion, yes *vs.* no	2.94	1.46–5.93	0.003
Venous invasion, yes *vs.* no	2.14	1.13–4.04	0.020
Differentiation, poor *vs.* moderate/well	3.84	1.76–8.39	0.001
IGFBP-2, >1160 *vs.* ≤1160 ng/ml	2.77	1.47–5.25	0.002
IGF-II, >1550 *vs.* ≤1550 ng/ml	0.42	0.18–0.95	0.037
IGF-II, LOI LOI (+) *vs.* LOI (–)	1.63	0.84–3.16	0.149
CEA, >5 *vs.* ≤5 ng/ml	3.92	1.51–10.16	0.005

TABLE 3. Multivariate Cox proportional hazards model analysis in the prediction of mortality in CRC patients

	HR	95% CI	*P* value
All[a]			
Lymph node metastasis, yes *vs.* no	3.65	1.45–9.19	0.006
Distant metastasis, yes *vs.* no	6.02	2.84–12.76	<0.001
IGFBP-2, >1160 *vs.* ≤1160 ng/ml	2.46	1.176–5.158	0.017
IGF-II, >1550 *vs.* ≤1550 ng/ml	0.42	0.18–0.98	0.044
CEA, >5 *vs.* ≤5 ng/ml	2.12	0.77–5.84	0.148
Stage IV[b]			
IGFBP-2, >1160 *vs.* ≤1160 ng/ml	5.09	1.21–21.37	0.026
LOI IGF-II, LOI (+) *vs.* LOI (−)	7.91	1.52–41.23	0.014

[a] Adjusted for age, gender, lymph node metastasis, depth of invasion, distant metastasis, lymphatic invasion, venous invasion, and BMI.

[b] Adjusted for age, gender, and BMI.

Elevated serum IGFBP-2 levels have been reported in patients with malignancies of the ovary, colon, prostate, and advanced solid tumor (6, 12, 28, 29). The result from our study was in agreement with previous studies that the plasma levels of IGFBP-2 are elevated in patients with CRC. We further demonstrated that elevated IGFBP-2 levels can distinguish patients with colon polyps and CRC from healthy controls. Although the sensitivity and specificity remained unsatisfactory for early CRC and colon polyps, a recent study showed that the combination of six biomarkers exhibit excellent sensitivity and specificity in distinguishing early ovarian cancer patients from healthy controls (9, 10). Therefore, the accuracy of combination of IGFBP-2 with other biomarkers in the diagnosis of early CRC deserves further investigation.

The role of IGFBP-2 in the CRC carcinogenesis remained controversial. Overexpression of IGFBP-2 was observed in invasive ovarian carcinoma, breast cancer, and CRC (20, 30, 31). However, a recent animal model in IGFBP-2 transgenic mice showed that IGFBP-2 overexpression before the onset of CRC inhibited the growth of colorectal adenoma (32). One epidemiological study showed that higher serum IGFBP-2 levels before the oc-

currence of cancer were associated with reduced risk of colon cancer (33), but another nested case-control study failed to find the association (34). Although the predictive value of IGFBP-2 on CRC risk remains controversial, the marked elevation of IGFBP-2 at the time of cancer diagnosis and the result from the present study indicated that IGFBP-2 has the potential to become a diagnostic biomarker for CRC. In clinical practice, because subjects with higher plasma IGFBP-2 levels are more likely to have CRC, they should undergo colonoscopic screening. However, we should keep in mind that higher IGFBP-2 levels are also present in several other cancers, such as ovarian cancer, prostate cancer, and advanced solid tumor (6, 12, 28, 29).

Several studies also demonstrated the crucial role of IGFBP-2 on the progression of cancer. Dunlap *et al.* reported that IGFBP-2 was required for the development and progression of glioma (19). Expression of IGFBP-2 in tumor was correlated with tumor grade and stage in malignancies of colon, ovary, prostate, and childhood acute myelogenous leukemia (8, 12, 21, 28). Low or absent expression of IGFBP-2 in patients with glioblastoma multiforme was associated with better survival (35). Our study lends further support that higher plasma IGFBP-2 levels at the time of cancer diagnosis was independently associated with a worse overall survival in CRC. From these studies, it seems that IGFBP-2 may exert direct effect on cellular function and play an independent role in carcinogenesis. This viewpoint is supported by the *in vitro* studies showing that the administration of OGX-225, an antisense oligonucleotide of IGFBP-2, in breast cancer cells lead to decreased IGFBP-2 expression and attenuated the aggressive phenotype (30). In a transgenic mouse model, immunization with IGFBP-2 peptides and transfer of IGFBP-2-competent T cells also showed antitumor effect of breast cancer (36). The result from the present study provided epidemiological evidence that IGFBP-2 might be a potential therapeutic target for CRC therapy.

The IGF-II bioactivity in the cellular microenvironment was assumed to play an important role in determining the cellular fate. It is also of interest whether serum levels of IGF-II have any influence in certain contexts. However, previous studies on the issue of whether serum levels of IGF-II levels are elevated or reduced in cancer patients compared with healthy subjects remains controversial in various cancers and even in the same malignancy. Elevated serum IGF-II levels were reported in cervical cancer, non-small-cell lung cancer, and CRC (6, 8, 37, 38). In contrast, reduced serum IGF-II levels were reported in ovarian cancer, childhood acute lymphoblastic leukemia, and breast cancer (9, 10, 22, 39). Our result showed that plasma IGF-II levels are lower in patients with stage I disease compared with normal controls and patients with more advanced disease. However, the plasma IGF-II levels were not significantly different between controls and patients with more advanced CRC. There were some possible explanations for the contradictory result for the role of IGF-II as compared with previous studies. First, most of the previous studies used serum rather than plasma in the measurement of IGF-II levels. Although biomarkers usually showed similar predictive values using either plasma or serum, some studies showed that plasma and serum levels of matrix metalloproteinase-9 (MMP-9) exhibited different predictive values in the prediction of gastric cancer progression and survival (40). Therefore, further studies are warranted to compare the diagnostic values of serum and plasma IGF-II levels in CRC. Second, IGF-II must activate receptor in cells to exert its effect, which might be influenced by its binding to IGFBP-2. Therefore, one of the possible explanations was that the level of IGF-II in blood cannot reflect the effect in local environment. For example, IGFBP-2 could modulate the plasma IGF-II levels and consequently lead to variable results. This viewpoint was supported by our observation that plasma IGF-II levels correlated negatively with IGFBP-2 levels.

Overexpression of IGF-II in tumor tissues has been reported to be associated with more advanced disease and survival in several cancers (14–17). In contrast, recent studies showed that higher circulating levels of IGF-II were associated with better overall survival in patients with metastatic CRC and advanced non-small-cell lung cancer (38, 41). Our results showed that higher plasma IGF-II levels were associated with better overall survival in CRC patients. However, the presence of LOI of IGF-II, which was associated with overexpression of IGF-II in tumor tissues, was associated with a worse overall survival in patients with metastatic CRC. Our results indicated that overexpression of IGF-II in tumor tissue might not correlate with circulating IGF-II levels. Previous study showed that the exogenous IGFBP-2 could be degraded by colon cancer extracts but not by normal colon tissue extracts (42). Therefore, the bioavailability of IGF ligands might be higher in tumor tissues. Recently, Kaneda and colleagues (43) have found that mice with LOI of IGF-II also have enhanced sensitivity to IGF-II signaling, not simply increased IGF-II levels, indicating that LOI itself may have an independent effect on carcinogenesis. In the present study, we showed that the presence of LOI of IGF-II was associated with a worse overall survival in patients with stage IV disease. Miyamoto *et al.* (44) also reported that neutralization of IGF-I and IGF-II suppressed the growth of liver metastasis of CRC and prolonged survival in mice. These results suggested that IGF-II pathway blockade may be beneficial for patients with advanced CRC. Therefore, it would be interesting to investigate whether patients with

LOI of IGF-II would benefit more from the IGF-II neutralization therapy in the future.

There were some limitations in our study. First, whether IGFBP-2 is protective or pro-metastatic in CRC cannot be concluded from the present study. Further mechanistic studies are warranted to address this issue. However, our data suggested that plasma IGFBP-2 levels seemed to directly correlate with tumor burden and were indicative of tumor load. Therefore, it can still serve as a diagnostic and prognostic marker of CRC, although its actual role in colorectal carcinogenesis remains inconclusive. Second, we did not examine the IGF-II expression levels. Our study showed that plasma IGF-II levels were not different according to the LOI status of IGF-II and were not associated with survival in CRC patients. However, previous studies showed that the IGF-II expression levels in cancer tissues correlated with LOI of IGF-II and survival in CRC patients (17, 45). The results indicated that the IGF-II expression levels in cancer tissues may be a more reliable biomarker in the prediction of prognosis than the plasma IGF-II levels in CRC patients. Third, we did not measure insulin level in this study. A recent study showed that IGFBP-2 plays an important role in cross talk of the insulin-IGF system and is linked with insulin resistance (46). Future studies to examine the plasma insulin levels and their associations with plasma IGF-II and IGFBP-2 levels in CRC patients are warranted.

In conclusion, IGFBP-2 is a potential diagnostic and prognostic serological biomarker for CRC. Plasma IGF-II levels and LOI of IGF-II are predictive of survival in CRC patients. The result from this study provided epidemiological evidence that IGFBP-2 is a potential biomarker in the CRC screening and that IGF-II and IGFBP-2 are potential therapeutic targets for CRC.

Acknowledgments

Address all correspondence and requests for reprints to: Ming-Shiang Wu, M.D., Ph.D., Department of Internal Medicine, National Taiwan University Hospital, National Taiwan University, College of Medicine, No. 7, Chung-Shan S. Road, Taipei 100, Taiwan. E-mail: mingshiang@ntu.edu.tw.

Disclosure Summary: J.-M.L. received research grants from the National Taiwan University Hospital (Grant NTUH 99-S1300) for the present study. All the other authors do not have any conflict of interest to be declared.

References

1. **Sung JJ, Lau JY, Young GP, Sano Y, Chiu HM, Byeon JS, Yeoh KG, Goh KL, Sollano J, Rerknimitr R, Matsuda T, Wu KC, Ng S, Leung SY, Makharia G, Chong VH, Ho KY, Brooks D, Lieberman DA, Chan FK; Asia Pacific Working Group on Colorectal Cancer** 2008 Asia Pacific consensus recommendations for colorectal cancer screening. Gut 57:1166–1176
2. **Levin B, Lieberman DA, McFarland B, Andrews KS, Brooks D, Bond J, Dash C, Giardiello FM, Glick S, Johnson D, Johnson CD, Levin TR, Pickhardt PJ, Rex DK, Smith RA, Thorson A, Winawer SJ; American Cancer Society Colorectal Cancer Advisory Group; US Multi-Society Task Force; American College of Radiology Colon Cancer Committee** 2008 Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. Gastroenterology 134:1570–1595
3. **Liou JM, Lin JT, Huang SP, Chiu HM, Wang HP, Lee YC, Lin JW, Shun CT, Liang JT, Wu MS** 2007 Screening for colorectal cancer in average-risk Chinese population using a mixed strategy with sigmoidoscopy and colonoscopy. Dis Colon Rectum 50:630–640
4. **Hassan AB, Howell JA** 2000 Insulin-like growth factor II supply modifies growth of intestinal adenoma in $Apc^{Min/+}$ mice. Cancer Res 60:1070–1076
5. **Renehan AG, Painter JE, O'Halloran D, Atkin WS, Potten CS, O'Dwyer ST, Shalet SM** 2000 Circulating insulin-like growth factor II and colorectal adenomas. J Clin Endocrinol Metab 85:3402–3408
6. **el Atiq F, Garrouste F, Remacle-Bonnet M, Sastre B, Pommier G** 1994 Alterations in serum levels of insulin-like growth factors and insulin-like growth-factor-binding proteins in patients with colorectal cancer. Int J Cancer 57:491–497
7. **Manousos O, Souglakos J, Bosetti C, Tzonou A, Chatzidakis V, Trichopoulos D, Adami HO, Mantzoros C** 1999 IGF-I and IGF-II in relation to colorectal cancer. Int J Cancer 83:15–17
8. **Renehan AG, Jones J, Potten CS, Shalet SM, O'Dwyer ST** 2000 Elevated serum insulin-like growth factor (IGF)-II and IGF binding protein-2 in patients with colorectal cancer. Br J Cancer 83:1344–1350
9. **Visintin I, Feng Z, Longton G, Ward DC, Alvero AB, Lai Y, Tenthorey J, Leiser A, Flores-Saaib R, Yu H, Azori M, Rutherford T, Schwartz PE, Mor G** 2008 Diagnostic markers for early detection of ovarian cancer. Clin Cancer Res 14:1065–1072
10. **Mor G, Visintin I, Lai Y, Zhao H, Schwartz P, Rutherford T, Yue L, Bray-Ward P, Ward DC** 2005 Serum protein markers for early detection of ovarian cancer. Proc Natl Acad Sci USA 102:7677–7682
11. **Miraki-Moud F, Jenkins PJ, Fairclough PD, Jordan S, Bustin SA, Jones AM, Lowe DG, Monson JP, Grossman AB, Besser GM, Camacho-Hübner C** 2001 Increased levels of insulin-like growth factor binding protein-2 in sera and tumours from patients with colonic neoplasia with and without acromegaly. Clin Endocrinol (Oxf) 54:499–508
12. **Baron-Hay S, Boyle F, Ferrier A, Scott C** 2004 Elevated serum insulin-like growth factor binding protein-2 as a prognostic marker in patients with ovarian cancer. Clin Cancer Res 10:1796–1806
13. **Corcoran RB, Bachar Raveh T, Barakat MT, Lee EY, Scott MP** 2008 Insulin-like growth factor 2 is required for progression to advanced medulloblastoma in patched1 heterozygous mice. Cancer Res 68:8788–8795
14. **Liao Y, Abel U, Grobholz R, Hermani A, Trojan L, Angel P, Mayer D** 2005 Up-regulation of insulin-like growth factor axis components in human primary prostate cancer correlates with tumor grade. Hum Pathol 36:1186–1196
15. **Lu L, Katsaros D, Wiley A, Rigault de la Longrais IA, Risch HA, Puopolo M, Yu H** 2006 The relationship of insulin-like growth factor-II, insulin-like growth factor binding protein-3, and estrogen receptor-α expression to disease progression in epithelial ovarian cancer. Clin Cancer Res 12:1208–1214
16. **Braconi C, Bracci R, Bearzi I, Bianchi F, Sabato S, Mandolesi A, Belvederesi L, Cascinu S, Valeri N, Cellerino R** 2008 Insulin-like growth factor (IGF) 1 and 2 help to predict disease outcome in GIST patients. Ann Oncol 19:1293–1298
17. **Peters G, Gongoll S, Langner C, Mengel M, Piso P, Klempnauer J, Rüschoff J, Kreipe H, von Wasielewski R** 2003 IGF-1R, IGF-1 and

IGF-2 expression as potential prognostic and predictive markers in colorectal-cancer. Virchows Arch 443:139–145
18. **Zhao R, Berho M, Nogueras J, Sands D, Weiss E, Wexner S, Giardiello FM, Cruz-Correa M** 2005 Positive correlation of insulin-like growth factor-II with proliferating cell index in patients with colorectal neoplasia. Cancer Epidemiol Biomarkers Prev 14:1819–1822
19. **Dunlap SM, Celestino J, Wang H, Jiang R, Holland EC, Fuller GN, Zhang W** 2007 Insulin-like growth factor binding protein 2 promotes glioma development and progression. Proc Natl Acad Sci USA 104:11736–11741
20. **Lee EJ, Mircean C, Shmulevich I, Wang H, Liu J, Niemistö A, Kavanagh JJ, Lee JH, Zhang W** 2005 Insulin-like growth factor binding protein 2 promotes ovarian cancer cell invasion. Mol Cancer 4:7
21. **Dawczynski K, Steinbach D, Wittig S, Pfaffendorf N, Kauf E, Zintl F** 2008 Expression of components of the IGF axis in childhood acute myelogenous leukemia. Pediatr Blood Cancer 50:24–28
22. **Vorwerk P, Mohnike K, Wex H, Röhl FW, Zimmermann M, Blum WF, Mittler U** 2005 Insulin-like growth factor binding protein-2 at diagnosis of childhood acute lymphoblastic leukemia and the prediction of relapse risk. J Clin Endocrinol Metab 90:3022–3027
23. **Woodson K, Flood A, Green L, Tangrea JA, Hanson J, Cash B, Schatzkin A, Schoenfeld P** 2004 Loss of insulin-like growth factor-II imprinting and the presence of screen-detected colorectal adenomas in women. J Natl Cancer Inst 96:407–410
24. **Tang SH, Yang DH, Huang W, Zhou HK, Lu XH, Ye G** 2006 Hypomethylated P4 promoter induces expression of the insulin-like growth factor-II gene in hepatocellular carcinoma in a Chinese population. Clin Cancer Res 12:4171–4177
25. **Randhawa GS, Cui H, Barletta JA, Strichman-Almashanu LZ, Talpaz M, Kantarjian H, Deisseroth AB, Champlin RC, Feinberg AP** 1998 Loss of imprinting in disease progression in chronic myelogenous leukemia. Blood 91:3144–3147
26. **Liou JM, Wu MS, Lin JT, Wang HP, Huang SP, Chiu HM, Lee YC, Lin YB, Shun CT, Liang JT** 2007 Loss of imprinting of insulin-like growth factor II is associated with increased risk of proximal colon cancer. Eur J Cancer 43:1276–1282
27. **Cui H, Horon IL, Ohlsson R, Hamilton SR, Feinberg AP** 1998 Loss of imprinting in normal tissue of colorectal cancer patients with microsatellite instability. Nat Med 4:1276–1280
28. **Kanety H, Madjar Y, Dagan Y, Levi J, Papa MZ, Pariente C, Goldwasser B, Karasik A** 1993 Serum insulin-like growth factor-binding protein-2 (IGFBP-2) is increased and IGFBP-3 is decreased in patients with prostate cancer: correlation with serum prostate-specific antigen. J Clin Endocrinol Metab 77:229–233
29. **Eiseman JL, Guo J, Ramanathan RK, Belani CP, Solit DB, Scher HI, Ivy SP, Zuhowski EG, Egorin MJ** 2007 Evaluation of plasma insulin-like growth factor binding protein 2 and Her-2 extracellular domain as biomarkers for 17-allylamino-17-demethoxygeldanamycin treatment of adult patients with advanced solid tumors. Clin Cancer Res 13:2121–2127
30. **So AI, Levitt RJ, Eigl B, Fazli L, Muramaki M, Leung S, Cheang MC, Nielsen TO, Gleave M, Pollak M** 2008 Insulin-like growth factor binding protein-2 is a novel therapeutic target associated with breast cancer. Clin Cancer Res 14:6944–6954
31. **Hoeflich A, Reisinger R, Lahm H, Kiess W, Blum WF, Kolb HJ, Weber MM, Wolf E** 2001 Insulin-like growth factor-binding protein 2 in tumorigenesis: protector or promoter? Cancer Res 61:8601–8610
32. **Diehl D, Hessel E, Oesterle D, Renner-Müller I, Elmlinger M, Langhammer M, Göttlicher M, Wolf E, Lahm H, Hoeflich A** 2009 IGFBP-2 overexpression reduces the appearance of dysplastic aberrant crypt foci and inhibits growth of adenomas in chemically induced colorectal carcinogenesis. Int J Cancer 124:2220–2225
33. **Kaaks R, Toniolo P, Akhmedkhanov A, Lukanova A, Biessy C, Dechaud H, Rinaldi S, Zeleniuch-Jacquotte A, Shore RE, Riboli E** 2000 Serum C-peptide, insulin-like growth factor (IGF)-I, IGF-binding proteins, and colorectal cancer risk in women. J Natl Cancer Inst 92:1592–1600
34. **Jenab M, Riboli E, Cleveland RJ, Norat T, Rinaldi S, Nieters A, Biessy C, Tjønneland A, Olsen A, Overvad K, Grønbaek H, Clavel-Chapelon F, Boutron-Ruault MC, Linseisen J, Boeing H, Pischon T, Trichopoulos D, Oikonomou E, Trichopoulou A, Panico S, Vineis P, Berrino F, Tumino R, Masala G, Peters PH, van Gils CH, Bueno-de-Mesquita HB, Ocké MC, Lund E, Mendez MA, Tormo MJ, Barricarte A, Martínez-García C, Dorronsoro M, Quirós JR, Hallmans G, Palmqvist R, Berglund G, Manjer J, Key T, Allen NE, Bingham S, Khaw KT, Cust A, Kaaks R** 2007 Serum C-peptide, IGFBP-1 and IGFBP-2 and risk of colon and rectal cancers in the European Prospective Investigation into Cancer and Nutrition. Int J Cancer 121:368–376
35. **McDonald KL, O'Sullivan MG, Parkinson JF, Shaw JM, Payne CA, Brewer JM, Young L, Reader DJ, Wheeler HT, Cook RJ, Biggs MT, Little NS, Teo C, Stone G, Robinson BG** 2007 IQGAP1 and IGFBP2: valuable biomarkers for determining prognosis in glioma patients. J Neuropathol Exp Neurol 66:405–417
36. **Park KH, Gad E, Goodell V, Dang Y, Wild T, Higgins D, Fintak P, Childs J, Dela Rosa C, Disis ML** 2008 Insulin-like growth factor-binding protein-2 is a target for the immunomodulation of breast cancer. Cancer Res 68:8400–8409
37. **Mathur SP, Mathur RS, Underwood PB, Kohler MF, Creasman WT** 2003 Circulating levels of insulin-like growth factor-II and IGF-binding protein 3 in cervical cancer. Gynecol Oncol 91:486–493
38. **Han JY, Choi BG, Choi JY, Lee SY, Ju SY** 2006 The prognostic significance of pretreatment plasma levels of insulin-like growth factor (IGF)-1, IGF-2, and IGF binding protein-3 in patients with advanced non-small cell lung cancer. Lung Cancer 54:227–234
39. **Singer CF, Mogg M, Koestler W, Pacher M, Marton E, Kubista E, Schreiber M** 2004 Insulin-like growth factor (IGF)-I and IGF-II serum concentrations in patients with benign and malignant breast lesions: free IGF-II is correlated with breast cancer size. Clin Cancer Res 10:4003–4009
40. **Wu CY, Wu MS, Chiang EP, Chen YJ, Chen CJ, Chi NH, Shih YT, Chen GH, Lin JT** 2007 Plasma matrix metalloproteinase-9 level is better than serum matrix metalloproteinase-9 level to predict gastric cancer evolution. Clin Cancer Res 13:2054–2060
41. **Fuchs CS, Goldberg RM, Sargent DJ, Meyerhardt JA, Wolpin BM, Green EM, Pitot HC, Pollak M** 2008 Plasma insulin-like growth factors, insulin-like binding protein-3, and outcome in metastatic colorectal cancer: results from intergroup trial N9741. Clin Cancer Res 14:8263–8269
42. **Michell NP, Langman MJ, Eggo MC** 1997 Insulin-like growth factors and their binding proteins in human colonocytes: preferential degradation of insulin-like growth factor binding protein 2 in colonic cancers. Br J Cancer 76:60–66
43. **Kaneda A, Wang CJ, Cheong R, Timp W, Onyango P, Wen B, Iacobuzio-Donahue CA, Ohlsson R, Andraos R, Pearson MA, Sharov AA, Longo DL, Ko MS, Levchenko A, Feinberg AP** 2007 Enhanced sensitivity to IGF-II signaling links loss of imprinting of IGF-II to increased cell proliferation and tumor risk. Proc Natl Acad Sci USA 104:20926–20931
44. **Miyamoto S, Nakamura M, Shitara K, Nakamura K, Ohki Y, Ishii G, Goya M, Kodama K, Sangai T, Maeda H, Shi-Chuang Z, Chiba T, Ochiai A** 2005 Blockade of paracrine supply of insulin-like growth factors using neutralizing antibodies suppresses the liver metastasis of human colorectal cancers. Clin Cancer Res 11:3494–3502
45. **Cui H** 2007 Loss of imprinting of IGF2 as an epigenetic marker for the risk of human cancer. Dis Markers 23:105–112
46. **Arafat AM, Weickert MO, Frystyk J, Spranger J, Schöfl C, Möhlig M, Pfeiffer AF** 2009 The role of insulin-like growth factor (IGF) binding protein-2 in the insulin-mediated decrease in IGF-I bioactivity. J Clin Endocrinol Metab 94:5093–5101