Insulin-like Growth Factor II and Colorectal Cancer Risk in Women

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Abstract

Recently, a number of prospective studies showed evidence that the growth hormone/insulin-like growth factor I (IGF-I) axis may be important in the development of colorectal cancer. However, only a few studies have reported on the possible relationship of colorectal cancer risk with circulating levels of IGF-II, which are not growth hormone dependent and which do not vary with alterations in energy balance. In a case-control study of 102 cases and 200 matched controls nested within a cohort of 14,275 women in New York, we examined the relationship between colorectal cancer risk and prediagnostic serum levels of IGF-II. Conditional logistic regression analysis showed an odds ratio (OR) for colorectal cancer of 2.02 (95% confidence interval (CI): 0.83–4.93), comparing the upper to lower quintile of IGF-II. This association was slightly attenuated after excluding IGF-II measurements in serum samples taken within 1 year before case diagnosis (OR of 1.81; 95% CI: 0.71–4.64) and moderately attenuated after excluding IGF-II measurements in serum samples taken within 2 years before case diagnosis (OR of 1.47; 95% CI: 0.56–3.91). Adjustment for IGF-1, IGF binding protein (BP)-1, IGFBP-3, smoking, or body mass index did not substantially alter the association, whereas adjustment for IGFBP-2 moderately attenuated the relationship. Our results confirm those of three recent case-control studies, and collectively these results suggest a possible increase in colorectal cancer risk among subjects with comparatively elevated serum IGF-II. Mechanisms that might cause the increase in IGF-II levels are unknown but may include loss of parental imprinting of the IGF-II gene.

Introduction

Recently, a number of prospective epidemiological studies have shown an increase in colorectal cancer risk among men and women with comparatively elevated plasma or serum levels of IGF[2]-I, measured either as absolute concentrations or relative to levels of IGFBP-3 (1–3). These findings suggested that the growth hormone/IGF-I axis may be important in the development of colorectal carcinoma. However, few studies have reported on the relationship between circulating levels of IGF-II and colorectal carcinoma. IGF-II, a 67 amino acid polypeptide with a paternally imprinted gene, is the member of a family of insulin-like peptides also including insulin and IGF-I. In contrast to IGF-I, which is known to be regulated by growth hormone and nutritional status, the regulators of IGF-II are not known. Furthermore, although circulating levels of IGF-II are higher in adults than circulating levels of IGF-I, IGF-II is believed to be especially crucial during fetal life and perinatal growth and development, whereas IGF-I reaches peak levels during puberty.

Recently, Renehan *et al.* (4) reported findings of a case-control study suggesting that IGF-II could play a role in the pathogenesis of colorectal cancer. We report on the relationship between serum levels of IGF-II and colorectal cancer in a case-control study nested within the New York University Women's Health Study cohort.

Materials and Methods

A case-control study, including 102 cases and 200 matched controls, was conducted within the New York University Women's Health Study, a prospective cohort study that was designed primarily to address the role of endogenous hormones and diet in the etiology of cancer in women. From March 1985 through June 1991, 14,275 women ages 35–65 years who had not used hormonal medication or been pregnant in the previous 6 months, were enrolled at a mammography-screening center in New York City. One-hundred two colorectal cancer cases were identified through January 31, 1998. A total of 200 matched control subjects was selected randomly from all cohort members alive and free of cancer at the time of diagnosis of each index case. Matching criteria were age, date and menopausal status at enrollment into the cohort, number of blood samples given over time, and time of the day at which the first sample had been given. All subjects had given written informed consent at entry into the cohort, and the study was approved by the Institutional Board of New York University School of Medicine.

Serum samples were collected during each participant's initial visit and in a subset of participants (46 cases and their 88 matched controls) at a subsequent visit. Using these serum samples, levels of IGF-II, total IGF-I, IGFBP-1, IGFBP-2, and

Received 4/13/01; revised 4/23/02; accepted 5/13/02.
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[2] The abbreviations used are: IGF, insulin-like growth factor; IGFBP, IGF binding protein; IGF-R, IGF receptor type; OR, odds ratio; CI, confidence interval; P_{trend}, *P* for trend; BMI, body mass index.

IGFBP-3 were measured. When duplicate serum samples were available, measurements for each protein were averaged.

Samples pertaining to matched study subjects were always analyzed on the same day with the same immunoassay kit. To control for the quality of each type of peptide measurement, analytical batches systematically included three standard serum samples and an unidentified aliquot of a pooled serum. The analyses were performed in two laboratories, one at the Hôpital de l'Antiquaille and the other at the International Agency for Research on Cancer, both located in Lyon, France. Laboratory personnel were blinded as to whether serum samples were from cases or controls. Levels of IGF-I, IGF-II, IGFBP-1, IGFBP-2, and IGFBP-3 were measured by double-antibody immunoradiometric assay. Reagents were from the following companies: for IGF-I and IGFBP-3, Immunotech (Marseille, France); and for IGF-II, IGFBP-1, and IGFBP-2, DSL-Diagnostic System Laboratory (Webster, TX). The IGF-I and IGF-II measurements included an acid-ethanol extraction step to release IGF-I and IGF-II from their binding proteins. The mean intrabatch coefficients of variation in this study were 5.6, 13, 2.7, 17.1, and 2.6%, respectively, for IGF-I, IGF-II, IGFBP-1, IGFBP-2, and IGFBP-3. The reproducibility of the various peptide measurements between serum samples collected at the first and second visits was evaluated by calculating Spearman correlation coefficients. Finally, conditional logistic regression models were used to calculate ORs for colorectal cancer for quintile levels of IGF-II. Quintile cutpoints were determined on distributions of case and control subjects combined. Smoking status (current, former, never), body mass index [BMI = height in meters/(weight in kilograms)^2], IGFBP-1, IGFBP-2, IGFBP-3, and IGF-I were considered as covariates. Likelihood ratio tests were used to assess linear trends in ORs over the quintiles, scoring the five levels quantitatively as 1, 2, 3, 4, and 5. All statistical tests used were two-sided.

Results

Among participants with serum samples collected at two time points, Spearman rank correlations were moderate to high for measurements of levels of IGF-I ($r = 0.87$), IGF-II ($r = 0.81$), IGFBP-2 ($r = 0.73$), and IGFBP-3 ($r = 0.87$) between the first and second visits, whereas correlation coefficients for IGFBP-1 ($r = 0.63$) were somewhat lower.

Characteristics of the case-control study population have been reported previously (3). Briefly, the time between the initial blood donation and cancer diagnosis ranged from 0.2 to 9.4 years, with an average of 4.8 years. Of the 102 cases of colorectal cancer, 96 (94%) were diagnosed >1 year and 84 (82%) >2 years after the initial collection of blood. At baseline, colorectal cancer cases were of the same height, had a higher BMI, and were more likely to smoke in comparison to control participants. Mean values of serum IGF-II levels adjusted for age were 933.0 ng/ml (95% CI: 894.6–971.3) in the case population and 896.0 ng/ml (95% CI: 868.6–923.4) in the control population. Among the controls, after adjusting for age at recruitment, the partial Spearman rank correlations between IGF-II and height, BMI, IGFBP-1, IGFBP-2, IGFBP-3, and IGF-I were 0.07, 0.11, −0.28, −0.13, 0.71, and 0.31, respectively. Finally, mean serum IGF-II levels adjusted for age were 915.4 mg/ml in current smokers, 865.4 ng/ml in former smokers, and 918.7 ng/ml in the never smokers.

Logistic regression analyses showed an OR for colorectal cancer of 2.02 [95% CI: 0.83–4.93] comparing the upper quintile to the lower quintile ($P_{trend} = 0.07$). Adjustment for IGFBP-3, which was strongly associated with IGF-II ($r = 0.71$), did not substantially alter this relationship and neither did adjustments for smoking, BMI, IGF-I, or simultaneously adjusting for IGFBP-3 and IGF-I (Table 1). However, adjustment for IGFBP-1, which was inversely associated with IGF-II ($r = -0.28$), somewhat attenuated this relationship [OR = 1.83 (95% CI: 0.73–4.60); $P_{trend} = 0.13$], and adjustment for IGFBP-2, also inversely associated with IGF-II ($r = -0.13$), moderately attenuated this relationship [OR = 1.50 (95% CI: 0.56–3.99); $P_{trend} = 0.24$]. Restricting the study population to the 75 cases of colon cancer and their 147 matched controls resulted in ORs very comparable with those reported for the 102 colorectal cancer cases and their 200 matched controls when comparisons were made between the upper and lower quintile (Table 2).

When IGF-II measurements taken within 1 year before case diagnosis were excluded, restricting the study population to 96 colorectal cancer cases and 188 controls, the OR comparing the upper quintile to the lower quintile, using the quintile cutpoints defined before the exclusion, was somewhat lower [OR = 1.81 (95% CI: 0.71–4.64); $P_{trend} = 0.15$]. Additional adjustment in this restricted population, with the exception of adjustment for IGFBP-2, did not alter this association (Table 1). Finally, when measurements taken within 2 years before case diagnosis were excluded, restricting the study population to 84 colorectal cancer cases and 164 controls, the OR comparing the upper quintile to the lower quintile was moderately attenuated [OR = 1.47 (95% CI: 0.56–3.91); $P_{trend} = 0.22$]. In this restricted population, adjusting for IGFBP-3 partially attenuated the remaining association between IGF-II and colorectal cancer [OR = 1.26 (95% CI: 0.28–5.70); $P_{trend} = 0.62$], whereas adjusting for IGFBP-2 entirely eliminated this association [OR = 1.00 (95% CI: 0.33–2.96); $P_{trend} = 0.62$].

Discussion

In summary, our results suggest that elevated circulating levels of IGF-II might be associated with the development of colorectal cancer. However, this association was of borderline statistical significance, was somewhat attenuated after restricting the analysis to serum samples obtained at least 1 year before the diagnosis of colorectal cancer, and further attenuated after restricting the analysis to serum samples obtained at least 2 years before the diagnosis of colorectal cancer. Additionally, in each analysis, adjustment for IGFBP-2 moderately attenuated the relationship between IGF-II and colorectal cancer.

In addition to studying the relationship between IGF-II and the combined end point colorectal cancer, we were able to study the relationship limited to the colon cancer cases and their matched controls. Overall, relationships between IGF-II and colon cancer were similar to relationships between IGF-II and colorectal cancer.

IGF-II, as a member of the family of insulin-like peptides also including insulin and IGF-I, is part of an extremely complex system that involves endocrine, paracrine, and autocrine interactions between the IGFs, at least six identified IGFBPs, and cellular receptors (5–7). It is not entirely known how circulating IGF-II levels and the IGFBPs may be quantitatively related to IGF-II bioactivity in tissues. The IGFBPs are known to serve as IGF transport proteins in the plasma, prolong IGF half-life, provide a means of tissue and cell type localization, and directly modulate interactions between IGFs and their receptors (5, 6). Approximately 75% of circulating IGF-II is found in a M_r 150,000 ternary complex composed of IGF-II, IGFBP-3, and an acid-labile subunit. Of the remaining five IGFBPs, only circulating concentrations of IGFBP-1 and IGFBP-2 are believed sufficient to play a

Table 1 ORs of cancer of the colorectum for quintiles of serum IGF-II[a]

	Quintile level					P_{trend}
	1	2	3	4	5	
Study population: 102 cases and 200 controls						
OR in the basic model	1.00	1.32	0.87	1.82	2.02	
(95% CI)		(0.57–3.09)	(0.37–2.00)	(0.84–3.93)	(0.83–4.93)	0.07
No. cases/controls	17/43	20/40	16/45	25/35	24/37	
Mean exposure (ng/ml)	643	805	898	1000	1192	
OR adjusted for smoking	1.00	1.46	0.85	1.67	2.26	0.08
(95% CI)		(0.61–3.48)	(0.36–2.02)	(0.76–3.67)	(0.90–5.69)	
OR adjusted for BMI	1.00	1.77	0.93	1.95	2.19	0.11
(95% CI)		(0.70–4.43)	(0.38–2.28)	(0.85–4.49)	(0.84–5.67)	
OR adjusted for IGFBP-1	1.00	1.21	0.82	1.66	1.83	0.13
(95% CI)		(0.51–2.90)	(0.35–1.91)	(0.75–3.69)	(0.73–4.60)	
OR adjusted for IGFBP-2	1.00	1.12	0.75	1.62	1.50	0.24
(95% CI)		(0.46–2.76)	(0.31–1.79)	(0.74–3.57)	(0.56–3.99)	
OR adjusted for IGFBP-3	1.00	1.33	0.88	1.85	2.07	0.25
(95% CI)		(0.55–3.20)	(0.34–2.23)	(0.68–5.02)	(0.54–7.88)	
OR adjusted for IGF-I	1.00	1.30	0.85	1.78	1.95	0.12
(95% CI)		(0.55–3.09)	(0.36–2.01)	(0.79–4.01)	(0.74–5.16)	
OR adjusted for IGF-I and IGFBP-3	1.00	1.33	0.88	1.88	2.13	0.25
(95% CI)		(0.55–3.19)	(0.35–2.25)	(0.69–5.16)	(0.55–8.27)	
Study population after excluding serum samples taken within 1 year of cancer diagnosis: 96 cases and 188 controls						
OR in the basic model	1.00	1.25	0.81	1.65	1.81	0.15
(95% CI)		(0.52–2.99)	(0.35–1.88)	(0.75–3.64)	(0.71–4.64)	
No. cases/controls	17/40	19/37	16/45	23/33	21/33	
Mean exposure	640	805	898	1001	1191	
OR adjusted for IGFBP-1	1.00	1.17	0.78	1.55	1.67	0.22
(95% CI)		(0.48–2.85)	(0.34–1.82)	(0.69–3.49)	(0.63–4.40)	
OR adjusted for IGFBP-2	1.00	1.14	0.71	1.45	1.39	0.38
(95% CI)		(0.46–2.86)	(0.29–1.70)	(0.65–3.26)	(0.50–3.90)	
OR adjusted for IGFBP-3	1.00	1.25	0.81	1.64	1.80	0.38
(95% CI)		(0.50–3.09)	(0.31–2.09)	(0.59–4.61)	(0.45–7.26)	

[a] Conditional logistic regression analyses were matched for age, menopausal status, day of menstrual cycle (for premenopausal women) and time of last food consumption.

role in the bioavailability of circulating IGF-II. IGFBP-1, which has equal affinity for IGF-I and IGF-II, is believed to modulate IGF action through inhibition and/or potentiation, whereas IGFBP-2, which has greater affinity for IGF-II than IGF-I, is believed to modulate IGF action solely through inhibition.

We observed some attenuation of the IGF-II colorectal cancer relationship after adjustment for circulating IGFBP-2 level. This weak confounding effect was explained mathematically by a minor inverse correlation between IGF-II and IGFBP-2 (-0.13), plus a lower level of IGFBP-2 among cases than among controls (485.2 *versus* 573.8. ng/ml). The relative decrease in IGFBP-2 level among the cases may be the result of higher insulin levels as described previously (3). However, it is unclear whether the weakly negative association between IGF-II and IGFBP-2 in our data reflects a general finding.

Compared with IGF-I, the affinity of IGF-II for the receptors that bind IGFs is very low for the insulin receptor, moderate for the IGF-R1, and very high for the IGF-R2. Recent reports on the potential involvement of the IGF axis in cancer development, independently of the insulin hypothesis, have focused on the action of IGF-I through the IGF-R1. The IGF-R1 is known to stimulate DNA and RNA synthesis and affect cell proliferation, differentiation, and survival. The biological action of IGF-II is also thought to occur through the IGF-R1, and IGF-II, like IGF-I, is thought to favor tumor development by stimulating cell proliferation and by inhibiting apoptosis through the IGF-I receptor (8). Furthermore, although the IGF-R2 was long believed to be involved mainly in the degradation of IGF-II (9), it recently has also been studied as a potential mediator of the biological effects of IGF-II (10, 11), and additional mechanisms for the involvement of IGF-II in the development of colorectal cancer could thus also include the IGF-R2.

Currently, loss of maternal imprinting (only the paternal gene remains expressed) and other genetic factors (*e.g.*, chromosomal rearrangements) affecting the expression of the *IGF-2* gene are the only reasonably well-documented determinants of variation in *IGF-2* mRNA expression and protein secretion. In a study of 27 individuals with colorectal tumors heterozygous at either of two polymorphisms for the *IGF-2* gene, loss of imprinting for the *IGF-2* gene occurred in 12 individuals; additionally, among these 12 individuals, a loss of imprinting was identified in other examined tissues, including normal colorectal tissue (10 of 10 examined) and blood samples (4 of 4 examined; Ref. 12). This suggests that in some instances the loss of imprinting of the *IGF-2* gene may occur simultaneously in different, and possibly even all, body tissues. In addition, a loss of imprinting of the *IGF-2* gene was identified in 2 of 15

Table 2 ORs of cancer of the colon for quintiles of serum IGF-II[a]

	Quintile level					P_{trend}
	1	2	3	4	5	
Study population: 75 cases and 147 controls						
OR in the basic model	1.00	1.24	0.73	1.25	2.02	0.24
(95% CI)		(0.47–3.31)	(0.29–1.84)	(0.55–2.99)	(0.73–5.61)	
No. cases/controls	14/30	15/29	11/34	16/28	19/26	
Mean exposure (ng/ml)	668	818	902	997	1189	
OR adjusted for IGFBP-1	1.00	1.18	0.70	1.17	1.92	0.30
(95% CI)		(0.44–3.18)	(0.27–1.79)	(0.48–2.88)	(0.68–5.41)	
OR adjusted for IGFBP-2	1.00	1.21	0.62	1.19	1.51	0.53
(95% CI)		(0.43–3.39)	(0.23–1.64)	(0.49–2.88)	(0.51–4.47)	
OR adjusted for IGFBP-3	1.00	1.20	0.69	1.15	1.77	0.67
(95% CI)		(0.43–3.35)	(0.24–1.99)	(0.37–3.57)	(0.37–8.44)	

[a] Conditional logistic regression analyses were matched for age, menopausal status, day of menstrual cycle (for premenopausal women) and time of last food consumption.

blood samples and 2 of 16 colorectal tissue samples taken from the general population. This implies that loss of imprinting may be more frequent in those with a diagnosed colorectal tumor or possibly a risk factor for the development of colorectal cancer. Disorders of imprinting of the *IGF-2* gene have also been implicated in the development of other tumors such as Wilms' tumor (13). Furthermore, individuals with Beckwith-Wiedemann Syndrome, a genetic disorder of excessive prenatal growth thought to be caused by chromosomal rearrangements resulting in increased levels of IGF-II, are believed to have an increased risk of developing embryonic tumors of childhood containing cell types known to express IGF-II during normal fetal development (*e.g.*, rhabdomyosarcomas, hepatoblastomas, and Wilms' tumors; Refs. 14, 15).

In addition to the hypothesized involvement of IGF-II in the initial development of colorectal tumors, it has been proposed that IGF-II may act in an autocrine or paracrine manner to stimulate colorectal tumor growth. Colorectal cancer cell lines have been shown to express mRNA for both IGF-II and IGF-R2 and to secrete both proteins (16, 17). Comparisons between normal and colorectal tumor tissue indicate that in ~30–40% of colorectal tumors IGF-II mRNA is overexpressed (18–20). Recently, a clinical study conducted by Kawamoto *et al.* (21) provided evidence that the overexpression of *IGF-2* mRNA and protein within colorectal tumors was correlated with tumor progression, tumor proliferation, and patient survival, whereas a second study conducted by the same group indicated a possible paracrine mechanism involving IGF-II in colorectal cancer metastasis to the liver (22). Furthermore, a recent study of 345 volunteers attending a sigmoidoscopy trial found individuals with adenomas ($n = 52$), known precursors of colorectal cancer, had elevated mean serum levels of IGF-II and IGFBP-2 that dropped after removal of the adenoma ($n = 31$; Ref. 23).

Results of several (4, 24, 25) but not all (1) previous epidemiological studies have suggested a possible direct relationship between circulating IGF-I and colorectal cancer risk. In a study of 23 colorectal carcinoma cases and multiple control groups, a 2-fold increase in serum levels of IGF-II was reported in cases when compared with controls (24). A study including 41 cases and 50 controls reported an OR of 2.7 (95% CI: 0.7–10.5) for colorectal cancer comparing the upper two-thirds of the IGF-II distribution to the lower one-third after controlling for age, gender, BMI, height, education level, IGF-I, IGFBP-3, and date of blood sampling (25). Likewise, Renehan *et al.* (4) reported that mean IGF-II scores were elevated in Dukes A ($n = 12$) and Dukes B ($n = 25$) colorectal cancer cases when compared with controls ($n = 57$), although not in Dukes C ($n = 13$) or in advanced colorectal cancer ($n = 42$). However, one prospective cohort study showed no case-control differences in age- and IGFBP-3-adjusted IGF-II levels (case subjects, 623 ng/ml; control subjects, 622 ng/ml; $P = 0.82$; Ref. 1).

Results of the current study are similar in direction and magnitude to results of previous case-control studies. However, because our study was a case-control study nested within a prospective cohort with serum samples collected before development of disease, we were able to specify the temporal relationship studied. In addition to this advantage, our study incorporated measurements on a second blood sample for almost half of the study subjects. This allowed us to establish the remarkable stability of IGF-II levels over time and diminished the possibility that degradation of the samples lead to bias toward the null.

The objective of our study was to determine whether elevated circulating levels of IGF-II were predictive of a diagnosis of colorectal cancer after excluding the possibility that IGF-II levels were elevated because of the presence of colorectal cancer. Unfortunately, a lack of statistical power, especially after exclusion of serum samples obtained within 1 or 2 years of cancer diagnosis, limited our ability to document significant elevations in risk. In addition, throughout the analyses there was a consistent departure from a monotonic relationship with the lowest relative risk observed in the third quintile, and although we believe this result was most likely because of chance, it made interpretation of the results more difficult. Finally, very few studies have addressed relationships between IGF-II and IGFBP-2 in the circulation or possible physiological mechanisms through which the two peptides might be correlated. In each analysis in our study, adjustment for IGFBP-2 attenuated the associations between IGF-II and colorectal cancer; however, because of the complex nature of the IGF system, it is hard to determine whether it is appropriate to control for IGFBP-2 when looking at the association between IGF-II and colorectal cancer. Consequently, it is difficult to determine whether our results reflect a true causal relationship between elevated plasma IGF-II concentrations and increased colorectal cancer risk, or if this association is an artifact driven by increased levels of IGF-II in people with undiagnosed cancerous or precancerous lesions.

In summary, circulating IGF-II is being studied both as a

marker of colorectal adenomas and colorectal cancer and as a risk factor for the development and progression of colorectal cancer. It is important for future studies of IGF-II in relation to colorectal cancer to consider all proposed mechanisms involving IGF-II.

Acknowledgments

We thank Francine Claustrat, Béatrice Vozar, and David Achaintre for technical assistance with laboratory assays, Carine Biessy for technical assistance with the analysis, and Jennie Dehedin for secretarial assistance.

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Cancer Epidemiol Biomarkers Prev 2002;11:901-905.