Research Article

Cancer Epidemiology, Biomarkers & Prevention

Reduced Insulin-like Growth Factor I Receptor and Altered Insulin Receptor Isoform mRNAs in Normal Mucosa Predict Colorectal Adenoma Risk

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Abstract

Background: Hyperinsulinemia resulting from obesity and insulin resistance is associated with increased risk of many cancers, but the biology underlying this risk is unclear. We hypothesized that increased mRNA levels of the insulin-like growth factor I receptor (IGFIR) versus the insulin receptor (IR) or elevated ratio of IR-A:IR-B isoforms in normal rectal mucosa would predict adenoma risk, particularly in individuals with high body mass index (BMI) or plasma insulin.

Methods: Biopsies from normal rectal mucosa were obtained from consenting patients undergoing routine colonoscopy at University of North Carolina Hospitals (Chapel Hill, NC). Subjects with colorectal adenomas were classified as cases ($n = 100$) and were matched to adenoma-free controls ($n = 98$) based on age, sex, and BMI. *IGFIR* and *IR* mRNA levels were assessed by qRT-PCR, and IR-A:IR-B mRNA ratios by standard PCR. Plasma insulin and crypt apoptosis were measured by ELISA and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL), respectively. Logistic regression models examined relationships between receptor mRNAs, BMI, plasma insulin, and adenoma risk.

Results: Unexpectedly, cases were significantly more likely to have lower *IGFIR* mRNA levels than controls. No overall differences in total *IR* mRNA or IR-A:IR-B ratios were observed between cases and controls. Interestingly, in patients with high plasma insulin, increased IR-A:IR-B ratio was associated with increased likelihood of having adenomas.

Conclusions: Our work shows novel findings that reduced *IGFIR* mRNA and, during high plasma insulin, increased IR-A:IR-B ratios in normal rectal mucosa are associated with colorectal adenoma risk.

Impact: Our work provides evidence supporting a link between IGFIR and IR isoform expression levels and colorectal adenoma risk. *Cancer Epidemiol Biomarkers Prev; 23(10); 2093–100. ©2014 AACR.*

Introduction

Despite increased colonoscopy-based screening and improved treatment strategies (1), colorectal cancer remains the second leading cause of cancer-related deaths in the United States (2). Obesity, insulin resistance, and type II diabetes are considered risk factors for colorectal cancer (3–5). Previous work by our group has linked elevated plasma insulin and low apoptosis in normal rectal mucosa to increased adenoma risk (6–8). Elevated plasma insulin (hyperinsulinemia) can increase the levels of free insulin-like growth factor I (IGFI) in the circulation by inhibiting the production of IGF-binding protein 1 (IGFBP1; refs. 9, 10). In recent years, there has been increasing interest in targeting the insulin/IGF pathway for cancer treatment, as a large body of evidence links insulin/IGFI-mediated activation of insulin receptor (IR) or IGFI receptor (IGFIR) to cancer of multiple organs (11–16). Furthermore, a number of studies have shown that IGFIR confers resistance to radiation therapy and chemotherapy (17, 18), and clinical evidence links IGFIR overexpression to colorectal tumor formation and progression (19, 20). Although IGFIR inhibitors showed a potential to reduce tumor growth (21, 22), recent reports suggested that IR may permit tumors to resist IGFIR inhibition, which led to the development of dual IGFIR/IR inhibitors (23–26).

Considerable evidence has highlighted the potential significance of different IR isoforms in growth and cancer (14, 27, 28). The *IR* gene yields two distinct IR isoforms due to alternate pre-mRNA splicing. IR-B is encoded by an mRNA that includes exon 11 and is the primary mediator

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of the metabolic actions of insulin (12, 29). IR-A is encoded by an mRNA that lacks exon 11, plays a role in fetal growth, and may mediate proliferative or antiapoptotic actions of insulin or the IGFs (27, 28). Evidence that IR-A may be the predominant IR isoform in tumors or tumor cells (12, 14, 27, 28, 30), including colon tumors (31, 32), has increased attention on this isoform as a possible mediator of cancer development or growth. However, the finding that IR-A knockdown increased viability of a colon cancer cell line via enhanced IGFIR activation (32) indicates that when IGFIR signaling is intact, IR may limit IGFIR signaling. Thus, the roles of IGFIR versus IR in promoting colorectal tumorigenesis are not defined.

Little attention has been given to *IGFIR* and *IR* mRNA expression patterns during preneoplastic stages of malignancy, including whether *IGFIR* or *IR* levels in normal colorectal tissue differ between patients with adenomas and patients without adenomas. We hypothesized that elevated mRNA levels of *IGFIR* versus *IR* or elevated IR-A:IR-B ratio in normal mucosa are associated with increased colorectal adenoma risk, elevated plasma insulin, and overweight/obese body mass index (BMI). To address this hypothesis, biopsies from normal rectal mucosa were obtained from adenoma or adenoma-free patients undergoing routine colonoscopy. Levels of mRNAs encoding *IGFIR*, *IR*, and IR isoforms were quantified and the relationship between their expression, adenoma status, BMI, and plasma insulin was evaluated.

Materials and Methods

Participants

Participants were randomly selected from eligible subjects enrolled in the Diet and Health Study V (DHS V) who provided written informed consent and underwent routine colonoscopy at the University of North Carolina Hospitals (UNC; Chapel Hill, NC). The DHS V cohort has been described in previous studies (33–36). For the present study, a subset of 100 cases and 98 controls was selected so that the two groups were matched on the basis of age, gender, and BMI. Patients were excluded from the study if they had cancer, colitis, 100 or more polyps (polyposis), prior resection of the colon, or history of colorectal adenomas. Colonoscopy was performed by certified gastroenterologists and all polyps were removed for pathologic examination and were not available for research purposes. Adenomas were confirmed and defined according to standard pathologic criteria. Subjects with one or more adenomas were classified as "cases" and those without adenomas as "controls." The study was approved by the School of Medicine Institutional Review Board at UNC.

Data collection

Methods for data collection were previously described (33–36). Briefly, participants fasted overnight and body weight, height, and waist and hip circumference were measured at the time of colonoscopy. Within 3 months after colonoscopy, patients were interviewed by telephone to provide information about their lifestyle, diet, and demographics. BMI between 18.5 kg/m^2 and 24.9 kg/m^2 (lean) was defined as "normal" and BMI equal to or higher than 25 kg/m^2 (overweight/obese) was defined as "Ovt/Ob."

Biologic specimens and laboratory assays

Before the endoscopic procedure, normal mucosal pinch biopsies were obtained 8 to 12 cm from the anal verge using standard disposable, fenestrated forceps. Sampling site was the same in all patients. Two biopsies were pooled for RNA extraction and immediately flash frozen in liquid nitrogen and later transferred to −80°C. Another biopsy was fixed in 10% buffered formalin for histology and evaluation of apoptosis. Blood was collected via an intravenous catheter before administration of medication. Plasma was separated and insulin levels assayed for 95 controls and 79 cases by ELISA (Diagnostic Systems Laboratory) as previously described (8). Plasma insulin levels below or above the median were defined as "low" or "high," respectively.

Assays for apoptosis

Formalin fixed rectal biopsies were embedded in paraffin. Apoptosis was scored by terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) using ApopTag Peroxidase In Situ Apoptosis Detection Kit (Millipore). This technology detects apoptotic cells by incorporating digoxigenin-conjugated nucleotides to the 3′OH termini of DNA fragments utilizing terminal deoxynucleotidyl transferase (TdT). Briefly, samples were deparaffinized in 100%, 95%, and 70% ethanol, digested in proteinase K, and blocked in 2% hydrogen peroxide. TdT reaction was performed for 1.5 hours at 37°C. Anti-digoxigenin conjugate was applied to the slides for 30 minutes, followed by a 3, 3′-diaminobenzidine reaction for 1 minute. Samples were counterstained with hematoxylin and dehydrated with 95% and 100% ethanol and xylene. Slides were coverslipped using Eukitt mounting medium (Sigma-Aldrich) and visualized with a bright-field microscope. Open crypts with good orientation were selected for scoring. The mean number of TdT-labeled apoptotic cells per crypt was calculated for each patient sample by investigators blinded to adenoma status. Because of the low number of samples available for apoptosis scoring (21 controls and 68 cases), it was only possible to compare apoptosis in cases versus controls without further stratification.

RNA extraction, reverse transcription (RT), and PCR

RNA was extracted from biopsies using RNeasy Kit (Qiaen) and reverse transcribed with High Capacity cDNA Reverse Transcription Kit, including RNase inhibitor (Applied Biosystems) according to the manufacturer's protocol. qRT-PCR used the 7500 Real-Time PCR System (Applied Biosystems) to quantify *IGFIR* and *IR* mRNA levels. Hydroxymethylbilane synthase (HMBS), which we have found to be invariant across

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rectal biopsy mRNAs, was used as the housekeeping gene for normalization. The following TaqMan primer/probes (Applied Biosystems) were used: Hs00951562_m1 (*IGFIR*), Hs00961550_m1 (*IR*), and Hs00609297_m1 (*HMBS*). Pooled cDNA from colorectal cancer cell lines (Caco-2, SW480, Colo205) was run in all assays as a positive, internal control to account for inter-run variability. Samples were run in duplicate and water was run as a negative control. Reaction cycles consisted of initial denaturation at 95°C for 5 minutes, 45 cycles of 95°C denaturation for 15 seconds, and 60°C annealing for 45 seconds. Data were analyzed using the Applied Biosystems 7500 software v2.0.1 and expression levels were calculated using the standard curve method. These values were then normalized to *HMBS* and to the internal control.

IR isoforms A and B were assessed by traditional, semiquantitative PCR using 150 ng of cDNA template. Forward primer 5′-GAATGCTGCTCCTGTCCAAA -3′ and reverse primer 5′-TCGTGGGCACGCTGGTCGAG -3′ (Integrated DNA Technologies) were designed to flank exon 11, resulting in 250 bp (IR-B) and 214 bp (IR-A) amplified fragments. PCR protocol was modified from Brierley and colleagues (32) and consisted of initial denaturation at 92°C for 5 minutes followed by 40 cycles of 92°C denaturation for 30 seconds, 60°C annealing for 30 seconds, and 72°C extension step for 30 seconds. Water and the internal control cDNA mentioned above were included in every assay. PCR products were run and visualized in a 2.5% agarose gel and band intensities were measured using ImageJ software (NIH). Ratios of IR-A to IR-B were calculated for each patient sample and normalized to the internal control. All PCR assays were performed by an investigator blinded to case–control and BMI status, and samples were randomly organized by another investigator so that all the groups were represented in each assay run.

Statistical analysis

Mean and SEs were computed for continuous variables. Differences in continuous or categorical variables between adenoma cases and adenoma-free controls were compared by Student *t* test or χ^2 test, respectively. BMI was divided into "normal" and "Ovt/Ob" (overweight/obese) and plasma insulin levels into "low" (below the median) and "high" (above the median) subgroups as described above. For each receptor mRNA, the levels in controls were used to generate quartiles, and the lowest quartile was considered as reference. Logistic regression models were used to compute ORs and 95% confidence intervals (CI) to examine the association between mRNA quartiles (predictors) and adenoma status (response). We also calculated *P* values for interactions between mRNA variables and BMI/insulin subgroups in a model testing for an association with case status. The relationship between plasma insulin and receptor mRNA levels was evaluated by Spearman correlation coefficient. *P* values less than 0.05 were considered statistically significant. All analyses were performed using SAS Version 9.3 (SAS Institute).

Results

Patient samples in this study were selected so that cases and controls were matched on age, sex, and BMI. Subject characteristics are summarized in Table 1. Race was not associated with control or case status, and no differences

Table 1. Descriptive characteristics of study participants

Variable	Control	Case	*P*[a]
Age (mean; SE)	55.4 (0.7)	55.5 (0.7)	0.92
Race (*n*; %)			
White	76 (92)	79 (84)	0.17
Black	7 (8)	15 (16)	
Sex (*n*; %)			
Female	41 (48)	43 (45)	0.66
Male	44 (52)	53 (55)	
BMI (*n*; %)			
Normal	45 (48)	49 (49)	0.98
Overweight	29 (31)	29 (29)	
Obese	20 (21)	21 (21)	
Physical activity in MET-minutes per week (mean; SE)	2,981 (341)	2,485 (263)	0.25
Apoptosis (mean; SE)	1.42 (0.12)	1.08 (0.06)	0.008[b]
Plasma insulin (mean; SE)	7.1 (0.8)	10.8 (1.7)	0.055
Calories (mean; SE)	2,101 (88)	1,949 (79)	0.20
Waist/hip ratio (mean; SE)	0.908 (0.01)	0.915 (0.01)	0.54

Abbreviation: MET, metabolic equivalent of task.

[a]χ^2 for age, race, and gender and Student *t* test for remaining variables.

[b]Significant at $P < 0.05$.

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in waist/hip ratio (WHR), reported calorie intake or reported physical activity were observed between cases and controls. Consistent with previous studies from our group (6, 7), adenoma cases showed lower apoptosis ($P = 0.008$) and a trend toward increased plasma insulin ($P = 0.055$) relative to adenoma-free controls.

IGFIR and *IR* mRNA levels in normal rectal mucosa were quantified by qRT-PCR. IR-A and IR-B mRNAs were assessed by standard PCR, where amplification of both isoforms by identical primers allowed us to calculate the ratio of IR-A:IR-B amplicon in each patient sample. We first compared mean mRNA levels between controls and cases overall and after stratifying for BMI and plasma insulin (Table 2). Overall, cases had significantly lower *IGFIR* mRNA levels ($P = 0.0003$) than controls. This reduction in *IGFIR* mRNA was statistically significant in both normal ($P = 0.02$) and Ovt/Ob ($P = 0.01$) BMI subgroups and in subjects in the lower half of plasma insulin ($P = 0.007$). Because cases had slightly higher plasma insulin levels than controls, and elevated insulin can downregulate IGFIR as a consequence of increased free IGFI in the circulation (37, 38), we asked whether the lower *IGFIR* mRNA observed in cases could be associated with higher plasma insulin. Therefore, we examined *IGFIR* mRNA levels in controls versus cases after adjusting for plasma insulin. This analysis showed that even after controlling for insulin, cases still had lower *IGFIR* than controls ($P = 0.005$). Total *IR* mRNA levels did not differ between cases and controls in any subgroup categorized for BMI or plasma insulin. IR-A:IR-B ratio was 1.96 ± 0.04 in controls and 1.96 ± 0.03 in cases, demonstrating approximately 2-fold higher IR-A mRNA expression in human rectum compared with IR-B, but no significant difference in cases and controls as a whole or when stratified for BMI (Table 2). Interestingly, among patients with high plasma insulin, adenoma cases had small but significant increases in IR-A:IR-B ratios relative to controls ($P = 0.006$), which qualitatively reflected reduced IR-B mRNA (Fig. 1).

To further evaluate the potential relationship between mRNA levels and colorectal adenoma risk, we studied the association between quartiles of *IGFIR*, *IR*, and IR-A:IR-B mRNA expression and the odds of being a case, with the lowest quartile set as the reference (Table 3). Subjects in the highest two quartiles for *IGFIR* mRNA were significantly less likely to be cases. There were no significant associations between *IR* mRNA, IR-A:IR-B ratio, and case status. To explore the association between receptor mRNA expression and adenoma risk in each subgroup, we used a logistic regression model to test for interactions between mRNA levels and BMI or plasma insulin status (Table 4). We found no interactions between BMI or plasma insulin and either *IGFIR* or *IR* mRNA levels. We did, however, observe a significant interaction between plasma insulin and IR-A:IR-B ratio ($P = 0.005$). With increasing IR-A:IR-B mRNA ratios, patients with high plasma insulin were more likely to have adenomas than were patients with low plasma insulin (Supplementary Fig. S1).

We next compared mRNA expression between subgroups in controls and cases separately. We found that in the control group, subjects with high plasma insulin had reduced mean *IGFIR*, *IR*, and IR-A:IR-B mRNA levels ($P = 0.048$, $P = 0.02$, $P = 0.01$, respectively) relative to subjects with low plasma insulin. This association was not found in cases. Qualitative evaluation of the IR isoforms suggested that the reduced IR-A:IR-B ratio observed in

Table 2. Mean expression of *IGF1R*, *IR*, and IR-A:IR-B mRNAs in controls versus cases overall and grouped by BMI and plasma insulin status

Variable	Subgroup		*n*	Control mean (SE)	*n*	Case mean (SE)	*P*[a]
IGFIR	Everyone		98	1.04 (0.03)	100	0.90 (0.02)	0.0003[b]
	BMI	Normal	45	1.06 (0.05)	49	0.91 (0.04)	0.02[b]
		Ovt/Ob	49	1.00 (0.04)	50	0.88 (0.03)	0.01[b]
	Insulin	Low	48	1.09 (0.05)	36	0.93 (0.03)	0.007[b]
		High	47	0.97 (0.04)	43	0.88 (0.03)	0.09
IR	Everyone		98	1.17 (0.03)	100	1.17 (0.06)	0.94
	BMI	Normal	45	1.14 (0.05)	49	1.18 (0.11)	0.78
		Ovt/Ob	49	1.17 (0.04)	50	1.15 (0.04)	0.82
	Insulin	Low	48	1.24 (0.05)	36	1.12 (0.05)	0.10
		High	47	1.08 (0.04)	43	1.12 (0.04)	0.51
IR-A:IR-B	Everyone		98	1.96 (0.04)	100	1.96 (0.03)	0.94
	BMI	Normal	45	2.01 (0.07)	49	1.96 (0.05)	0.52
		Ovt/Ob	49	1.91 (0.04)	50	1.95 (0.03)	0.48
	Insulin	Low	48	2.07 (0.07)	36	1.95 (0.05)	0.18
		High	47	1.85 (0.04)	43	2.01 (0.04)	0.006[b]

[a]Student *t* test.
[b]Significant at $P < 0.05$.

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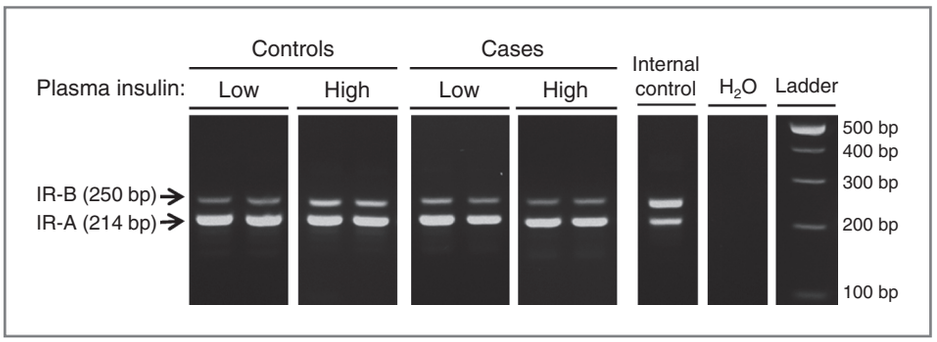

Figure 1. Representative gels showing IR-A and IR-B mRNAs in cases and controls with low and high plasma insulin. Controls with high insulin have decreased IR-A:IR-B ratios compared with controls with low insulin, potentially due to increased IR-B and maintained IR-A. Among patients with high insulin, cases have higher IR-A:IR-B ratios than controls, and this seems to result from decreased IR-B and unaltered IR-A. Samples were run in groups of 19 per gel, and representative images were obtained from different originals or multiple fields from the same image.

controls with high insulin reflected higher IR-B (Fig. 1). To further examine the possible effect of elevated insulin on gene expression, we calculated the correlation coefficients between plasma insulin and *IGFIR*, *IR*, and IR-A:IR-B mRNA levels (Table 5). We indeed found significant negative correlations between plasma insulin and all three mRNA variables in controls, whereas in cases, this relationship was significant only for *IGFIR* mRNA. In fact, in cases there was a nonsignificant trend for a positive correlation between IR-A:IR-B ratio and plasma insulin ($P = 0.06$).

Discussion

This case–control study provides novel evidence that, compared with adenoma-free controls, rectal biopsies from grossly normal mucosa of patients with adenomas are likely to have (i) significantly lower levels of *IGFIR* mRNA, (ii) unaltered *IR* mRNA, and (iii) higher ratios of IR-A:IR-B isoforms in those individuals with elevated plasma insulin. Consistent with our previous findings in three different patient groups (6–8), the presence of adenomas was associated with reduced apoptosis in normal appearing rectal mucosa and increased plasma insulin, although the latter was borderline significant in this smaller study population.

Table 3. ORs and 95% CIs for the association between colorectal adenomas and *IGFIR*, *IR*, and IR-A:IR-B mRNA expression

Variable	*n* (control/case)	OR[a] (95% CI)	*P*
IGFIR			
Q1	25/46	1.0 (Reference)	—
Q2	25/23	0.5 (0.2–1.1)	0.07
Q3	25/19	0.4 (0.2–0.9)	0.02[b]
Q4	23/12	0.3 (0.1–0.7)	0.004[b]
IR			
Q1	25/26	1.0 (Reference)	—
Q2	24/28	1.1 (0.5–2.4)	0.77
Q3	25/30	1.2 (0.5–2.5)	0.71
Q4	24/16	0.6 (0.3–1.5)	0.30
IR-A:IR-B			
Q1	25/29	1.0 (Reference)	—
Q2	24/20	0.7 (0.3–1.6)	0.42
Q3	25/23	0.8 (0.4–1.7)	0.56
Q4	24/28	1.0 (0.5–2.2)	0.99

[a]Odds of being a case.
[b]Significant at $P < 0.05$.

Identifying molecular biomarkers that predict early precancerous lesions could significantly improve our understanding of factors that promote colorectal cancer risk, which could eventually contribute to better colorectal cancer prevention or screening. This study aimed to establish whether elevated mRNA expression of *IGFIR*, *IR*, or relative expression of isoforms IR-A and IR-B in normal rectal mucosa predicts adenomas and whether this is influenced by BMI or plasma insulin levels. IGFIR signaling can be activated during elevated insulin and has been linked to reduced apoptosis and cancer progression in a number of organs, including the intestine (11, 13, 39). Thus, we hypothesized that patients with adenomas would have upregulated *IGFIR* mRNA expression in their normal rectal mucosa, particularly in those with high plasma insulin. Unexpectedly, we found that cases had significantly lower *IGFIR* mRNA levels than controls, and the odds of having colorectal adenomas diminished with increasing *IGFIR* mRNA expression. We considered whether elevated insulin could be linked to the reduced *IGFIR* mRNA in cases, because elevated insulin is known to downregulate IGFBP1, resulting in higher levels of free circulating IGFI (9, 10) that can downregulate IGFIR (38). However, the association between decreased *IGFIR* mRNA and presence of adenomas persisted even after adjusting for plasma insulin, suggesting that the reduced *IGFIR* mRNA observed in cases was not merely a result of elevated plasma insulin in this group. We next tested for interactions between mRNA levels and BMI or plasma insulin that may impact case status. We found a significant interaction between IR-A:IR-B ratio and plasma insulin,

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Table 4. Association between colorectal adenomas and *IGFIR*, *IR*, and IR-A:IR-B mRNA expression influenced by BMI and plasma insulin

	$P_{interaction}$	
Variable	BMI (normal *vs.* Ovt/Ob)	Insulin (low *vs.* high)
IGFIR	0.78	0.47
IR	0.74	0.11
IR-A:IR-B	0.34	0.005[a]

[a]Significant at $P < 0.05$.

where increased IR-A:IR-B ratio was associated with increased colorectal adenoma risk in patients with high plasma insulin compared with those with low plasma insulin. This suggests that circulating insulin levels may play an important role in influencing tumor risk associated with high IR-A:IR-B expression, and that more attention should be given to the impact of hyperinsulinemia on relative tissue expression of these IR isoforms.

Insulin has long been known to downregulate its own receptor by negative feedback to properly regulate glucose uptake in a number of tissues (40–44), and some evidence suggests that hyperinsulinemia and insulin resistance can impact isoform expression (45–48). Insulin can also downregulate IGFIR, potentially by increasing levels of free IGFI in plasma (16, 37, 38). Downregulation of *IGFIR* transcript in situations of high insulin has been described in skeletal muscle of diabetic db/db mice, where reduced *IgfIr* mRNA relative to normoglycemic littermates was associated with increased *IgfIr* promoter methylation (49). These numerous lines of evidence for negative feedback effects of elevated insulin are supported by the present study showing that in adenoma-free controls, levels of *IGFIR* and *IR* mRNAs, and IR-A:IR-B ratios each negatively and significantly correlated with plasma insulin. Qualitative analysis of IR isoforms suggested that reduced IR-A:IR-B ratio in controls with high plasma insulin seemed to be primarily due to increased IR-B. Patients with adenoma differed from controls in which only *IGFIR* mRNA levels significantly and negatively correlated with insulin, and for IR-A:IR-B mRNA ratios, there was actually a trend for a positive correlation with insulin. This suggests a difference in the relationship between plasma insulin and *IR* mRNA levels or IR-A:IR-B mRNA ratios in cases versus controls that may be relevant to mechanisms underlying adenoma risk.

Table 5. Correlation between plasma insulin and *IGFIR*, *IR*, and IR-A:IR-B mRNA expression

	Plasma insulin			
	Controls ($n = 95$)		Cases ($n = 79$)	
Variable	r^a	P	r^a	P
IGFIR	−0.21	0.045[b]	−0.30	0.01[b]
IR	−0.26	0.01[b]	−0.14	0.21
IR-A:IR-B	−0.31	0.002[b]	0.21	0.06

[a]Spearman correlation coefficient.
[b]Significant at $P < 0.05$.

IR isoforms in humans have been studied primarily in breast and prostate cancers and it is well established that IR-A exerts proliferative actions and is overexpressed in tumor tissue (14, 27, 28, 50). However, little is known about the relative expression of IR isoforms in normal gastrointestinal organs, including the colorectum. Our findings that mean levels of IR-A mRNA are about 2-fold higher than IR-B mRNA in the human rectal mucosa are relevant to normal and aberrant growth of colon epithelium. A predominance of IR-A might contribute to the relatively low levels of spontaneous colonocyte apoptosis (51) and increased susceptibility to insulin-mediated reductions in apoptosis. Our recent publication demonstrated a switch from predominance of IR-A in proliferative intestinal stem or progenitor cells to IR-B predominance in differentiated enterocytes (31). Furthermore, IR-B expression was reduced in mouse precancerous adenomas versus normal colon and was dramatically reduced in aggressive, poorly differentiated human colorectal cancer cell lines versus differentiated colorectal cancer cells (31). Consistent with this finding, other studies have recently shown that the relative mRNA levels of IR-A versus IR-B are elevated in both tumor and grossly normal adjacent tissue of human breast and prostate, compared with purely benign tissue (52, 53). However, whether IR isoform expression is altered in normal colorectum in the presence or absence of premalignant lesions had not to our knowledge been investigated previously. Our study suggests that among patients in the upper half of plasma insulin, those with adenomas had higher mean IR-A:IR-B ratio in their normal rectal mucosa compared with controls, which seemed to result from decreased IR-B and maintained IR-A as observed by qualitative examination. These data were supported by logistic regression analyses, which showed that increasing IR-A:IR-B ratios predicted adenomas in patients with elevated plasma insulin. A limitation of these findings is that they resulted from a subgroup comparison, in a relatively small number of patients. However, they do suggest that the relationship between plasma insulin and relative IR-A:IR-B expression in normal tissues should be further explored, as they may be relevant to improved understanding of the roles of hyperinsulinemia and impact of IR isoforms on colorectal tumorigenesis.

A limitation of this study is that alterations in receptor mRNA levels do not necessarily reflect changes in protein expression and phosphorylation, as increased activation

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of IGFIR and IR has been reported in cancer (54, 55). This is particularly difficult to address for IR-A due to the lack of available antibodies to permit IHC or Western immunoblot analysis for this isoform. We chose to analyze RNA because sufficient RNA for evaluation of receptor levels is readily obtained from biopsies but we recognize the limitation with regard to predicting protein expression or activation. Another limitation of our study is the lack of access to actual adenomas as these are considered clinical specimens and were not available to us for research. Recent findings from our group using preclinical adenoma models provided evidence for increased IR-A:IR-B ratios in colon adenomas relative to normal colon mucosa in mice (31), but whether this is altered in humans and in the context of elevated plasma insulin needs further investigation. An additional limitation is that the differences in mean receptor mRNA expression across patient groups in this study are relatively small. Despite these limitations, the potential significance of our observations is highlighted by the growing interest in the role of the insulin/IGF pathway in cancer and IR/IGFIR inhibitors as potential therapies (22, 24, 26). To date, IR and IR isoforms have been understudied in the gastrointestinal tract, and our work suggests that further studies focusing on these receptors and relative IR-A and IR-B expression are needed to better understand their roles in initiation and pathophysiology of colorectal precancerous lesions. Therefore, our previous and current work indicates that additional attention to the relative expression levels and biologic roles of IR-A and IR-B is warranted.

Overall, this is to our knowledge the first study to show that the presence of colorectal adenomas is associated with decreased *IGFIR* mRNA and, during elevated plasma insulin, increased IR-A:IR-B mRNA ratio in normal rectal mucosa. Particularly, our data raise the important possibility that high IR-A:IR-B mRNA ratio may contribute to colorectal adenoma initiation during elevated plasma insulin. In addition, reduced *IGFIR* expression and increased relative expression of IR-A:IR-B in normal mucosa should be further investigated as potential predictive biomarkers of premalignant colorectal lesions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: M.A. Santoro, R.S. Sandler, T.O. Keku, P.K. Lund
Development of methodology: S.F. Andres, P.K. Lund
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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M.A. Santoro, J.A. Galanko, T.O. Keku, P.K. Lund
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