Gain of allelic gene expression for IGF-II occurs frequently in Barrett’s esophagus

Linda A. Feagins, Nathan Susnow, Hui Ying Zhang, Stephanie Pearson, Charles Owen, William F. Schmalstieg, Lance S. Terada, Stuart J. Spechler, Ruben D. Ramirez, and Rhonda F. Souza. Gain of allelic gene expression for IGF-II occurs frequently in Barrett’s esophagus. Am J Physiol Gastrointest Liver Physiol 290: G871–G875, 2006. First published December 8, 2005; doi:10.1152/ajpgi.00383.2005.—The IGF-II gene normally exhibits genomic imprinting, a DNA modification that allows the expression of only one of the two inherited alleles. With loss of imprinting, there is a gain of allelic gene expression (GOAGE) due to IGF-II being expressed by both alleles. GOAGE for IGF-II has been demonstrated in a number of malignancies and in normal epithelia surrounding malignancies, but not in epithelia without associated neoplasia. We hypothesized that nonneoplastic Barrett’s epithelium might have GOAGE for IGF-II that could facilitate its progression to neoplasia. Endoscopic biopsies were obtained from metaplastic esophageal, normal gastric, and normal duodenal epithelia from 43 patients with Barrett’s esophagus. Genomic DNA were analyzed using PCR followed by A↓pol restriction enzyme digestion or allele-specific PCR to identify an I↓f↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I↓I→
first reported in childhood tumors such as Wilms’ tumors but has since been found in adult tumors, including hepatocellular, gastric, colonic, and esophageal squamous cell carcinomas (3, 5, 13, 14, 28). GOAGE of IGF-II has also been found in adenomatous polyps in the colon, an observation suggesting a role for the growth factor early in the process of neoplastic progression (5, 27). We have studied GOAGE for IGF-II in the specialized intestinal metaplasia of patients with Barrett’s esophagus.

MATERIALS AND METHODS

Patients and tissue samples. Patients with biopsy-verified Barrett’s esophagus who were scheduled for endoscopic surveillance at the Dallas Veterans Affairs Medical Center were invited to participate in the study. A total of 43 patients with Barrett’s esophagus were enrolled, including 31 patients with long-segment Barrett’s esophagus (≥3 cm of esophageal specialized intestinal metaplasia), 11 patients with short-segment Barrett’s esophagus (<3 cm of esophageal specialized intestinal metaplasia), and one patient whose length of Barrett’s esophagus was not determined. Surveillance biopsy specimens from 39 patients exhibited no histological evidence of dysplasia or cancer, 2 patients had low-grade dysplasia, and 2 patients had high-grade dysplasia or cancer. Written informed consent to participate in the study was obtained from all patients. Biopsies of Barrett’s esophagus, gastric body, and duodenal epithelia were obtained using a jumbo biopsy forceps (Olympus FB-50K-1), immediately snap-frozen, and subsequently stored at −80°C. For RNA extraction, samples were placed in RNA later solution (Ambion, Austin, TX) and stored at 4°C. The study was approved by the Institutional Review Board at the Dallas Veterans Affairs Medical Center.

DNA and RNA isolation. In biopsy samples from the first 25 patients, DNA were extracted using phenol chloroform and RNA were extracted using TRizol reagent as previously described (21, 23). Technically simpler extraction procedures were used for the biopsy samples from the remaining 18 patients [DNA were extracted using the DNeasy mini kit protocol (Qiagen, Valencia, CA) and RNA were obtained using the RNeasy mini kit (Qiagen) according to the manufacturer’s instructions]. The DNA and RNA were quantitated using spectrophotometry. Total RNA were treated with RNase-free DNase (Ambion) in accordance with the manufacturer’s instructions to remove any contaminating genomic DNA (gDNA). cDNA were synthesized using equal amounts of DNA-free total RNA and 100 ng of random primer using SuperScript II RT (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol. As a control, the reaction was performed on DNA-free total RNA in the absence of RT.

Identification of IGF-II genomic polymorphism. Allelic heterozygosity in IGF-II was determined by performing PCR amplification followed by restriction enzyme digestion or by performing allele-specific PCR amplification using the Apal restriction enzyme site in exon 9 of the IGF-II gene on gDNA. For biopsy samples from 22 patients, PCR was performed in 50 μl of reaction mixture containing 10 mM Tris⋅HCl (pH 8.3), 50 mM KCl, 4 mM MgCl₂, 1 mM 2-deoxyxynucleotide 5’-triphosphate (dNTP), and 500 nM primers (sense primer, 5’-GGACGTTGAAGCTTTAGGG-3’; antisense primer, 5’-GGGTTGCTTGATTTATGCA-3’) (20). These primers were designed to amplify a segment of IGF-II containing an Apal polymorphism. Aliquots (1 μl) of DNA were added to the reaction mixture. Initial denaturation was performed at 95°C for 10 min, followed by 37 cycles of denaturation at 95°C for 30 s, annealing at 62°C for 30 s, and extension at 72°C for 30 s, with a final extension at 72°C for 10 min. AmpliTaq Gold (1.25 U; Applied Biosystems, Foster City, CA) was used in the “hot start” PCR. Amplified DNA were electrophoresed on agarose gels. IGF-II DNA PCR products were digested overnight using Apal restriction enzyme (Roche Molecular Biochemicals, Indianapolis, IN). After digestion, PCR products were again electrophoresed on 6% polyacrylamide gels and visualized using ethidium bromide staining. Informative samples were identified by the presence of three distinct bands after gel electrophoresis (20).

For the remaining DNA from 12 patients, the technique of allele-specific PCR was performed as previously described (26). Briefly, we used allele-specific 20-mer sense primers designed to anneal with the 3’-end to the intact Apal site at nt 820 (HSIGF-II/7; GenBank accession no. X07868) primer Y4 (5’-CAGCAAAGAGAAAGAAGAGAG-3’) or to the corresponding Apal polymorphism primer N2 (5’-CAGCAAGAGAAAGAAGAAGT-3’) (26). Primer specificity for either allele was determined on the basis of the last nucleotide at the 3’ position of the primers, which corresponds to the polymorphism (bold), and allele specificity was improved by the introduction of a nt mismatch at position 1 at the 3’-end of the primer (underlined) (26). Both alleles were amplified using the same antisense primer B (5’-GGGTGCTGCGCAATTACATTTC-3’). PCR was performed in a total volume of 50 μl for 37 cycles consisting of denaturation at 95°C for 1 min, annealing at 53°C for 30 s, and extension at 72°C for 30 s. Each reaction contained 10 ng of genomic DNA, 2.5 μM primer (12.5 pmol total), 1.7 U of AmpliTaq Gold (Roche Applied Biosystems, Indianapolis, IN), and standard amounts of MgCl₂, PCR buffer, and dNTP. PCR products were then electrophoresed on 2% agarose gels and stained using ethidium bromide. Samples informative for Apal polymorphism were identified using a PCR product resulting from primers Y4 and B (allele containing the polymorphism designated allele A) and primers N2 and B (allele not containing the polymorphism designated allele B) corresponding to each of the two IGF-II alleles (26). In a subset of patients, we performed the Apal PCR followed by the Apal digestion method in addition to using allele-specific PCR and found that these techniques yielded identical results. The remainder of the study was therefore performed using the far more efficient method of allele-specific PCR.

Allele-specific gene expression using exon connection PCR. After identifying the informative samples as described above, corresponding cDNA were then amplified to assess for monoallelic or biallelic mRNA expression of IGF-II using a nested exon connection RT-PCR as previously described (26). The nested exon connection strategy was used to minimize the interference of small amounts of remaining gDNA in the cDNA preparation in the PCR.

In the first PCR, a CDNA-specific primer pair that spanned the boundaries between exons 8 and 9 of IGF-II was used as described previously (26). The sense primer Z (5’-CCTCCCGGATCCCTGTC- GGAGAC-3’) was a sequence in exon 8 of IGF-II, was used along with an exon 9-specific antisense primer E (5’-GGATGGGAGAATGATGTAAG-3’) to amplify a 1,101-bp cDNA fragment of the IGF-II gene (26). Each 50 μl of PCR product contained 1 or 5 μl of cDNA (corresponding to 25 or 125 ng of total RNA), 2.5 μM concentrations of each primer (12.5 pmol total), 1.7 U of AmpliTaq Gold (Roche Applied Biosystems), and standard amounts of MgCl₂, PCR buffer, and dNTP. PCR was begun with 5-min predenaturation at 95°C, followed by 37 cycles at 95°C for 1 min, 55°C for 1 min, and 72°C for 2 min. In the nested secondary PCR, a sense primer A (5’-CCTGGACCTTTGAGTCAATGG-3’) and the antisense primer B described above were used to amplify a 293-bp fragment of exon 9 of the IGF-II gene, which included the Apal site (26). The 50-μl reaction mixture contained 1 μl of the PCR exon connection PCR product, 2.5 μM concentrations of each primer (12.5 pmol total), 1.7 U of AmpliTaq Gold (Roche Applied Biosystems), and standard amounts of MgCl₂, PCR buffer, and dNTP. PCR was begun with a 5-min predenaturation cycle at 95°C, followed by 35 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 30 s. After being amplified, PCR products were electrophoresed on 2% agarose gels and stained with ethidium bromide.

RT-PCR products (30 μl) resulting from the nested primers were then digested with 20 U of Apal restriction enzyme (Roche Applied
Biosystems) in 1× ApaI buffer (Roche Applied Biosystems) with a total reaction mixture of 40 μL. After overnight digestion at 30°C, the resulting reaction mixture was then electrophoresed on 2% agarose gels and stained with ethidium bromide. ApaI digestion of the amplification product from the allele containing an intact ApaI site results in two products: a small 63-bp product (not observed on the gel) and a 230-bp product that can be observed after gel electrophoresis (26). Because the allele without the ApaI site is not digested by the ApaI restriction enzyme, only a single 293-bp product is observed after gel electrophoresis (26). Normally, IGF-II is expressed from only one of the two alleles (8). The finding of both 293- and 230-bp products after gel electrophoresis (26). Normally, IGF-II is expressed from only one of the two alleles (8). Gain of allelic gene expression of IGF-II in Barrett’s esophagus was demonstrated by the finding of biallelic gene expression (Fig. 4). Of those five samples, four were from patients with long-segment Barrett’s esophagus and one sample exhibited low-grade dysplasia.

Gain of allelic gene expression in matched normal mucosal samples. cDNA from matching normal duodenal and gastric biopsies from six of the nine patients with Barrett’s esophagus demonstrating GOAGE of IGF-II were analyzed to determine whether GOAGE of IGF-II was present in normal tissues. (Analysis could not be performed on 3 informative patients with Barrett’s esophagus because of insufficient tissue sample.) Of the nine Barrett’s esophagus samples, five (56%) demonstrated GOAGE of IGF-II as determined by the finding of biallelic gene expression (Fig. 4). Of those five samples, four were from patients with long-segment Barrett’s esophagus and one sample exhibited low-grade dysplasia.

Identification of the IGF-II genomic polymorphism in Barrett’s esophagus. Genomic DNA from 43 Barrett’s esophagus biopsy samples were analyzed for the ApaI polymorphism in the IGF-II gene, which allows the detection of two IGF-II alleles. Two PCR-based methods were used to identify the IGF-II alleles. In one method, PCR amplification was performed using primers that flanked the ApaI site, followed by ApaI digestion, and identified 8 (32%) of 25 samples as informative for IGF-II (Fig. 1). In the other method, we used the technically easier approach of allele-specific PCR and identified 5 (28%) of 18 samples as informative for IGF-II (Fig. 2). We found no significant difference in the percentage of patients informative for IGF-II determined on the basis of these two methods (P < 0.05). We then compared the results of PCR and ApaI restriction enzyme digestion with those of allele-specific PCR in a subset of samples and found that both methods produced identical results (Fig. 3). Overall, 13 (30%) of 43 Barrett’s esophagus samples were heterozygous and thus informative for IGF-II. Of the 31 patients with long-segment Barrett’s esophagus, 11 (35%) were informative for IGF-II, whereas in the 11 patients with short-segment Barrett’s esophagus, only 2 (18%) were informative.

Gain of allelic gene expression of IGF-II in Barrett’s esophagus. Monoallelic or biallelic expression of IGF-II was determined in cDNA from nine informative patients with Barrett’s esophagus using exon connection PCR designed to avoid amplification of contaminating gDNA. (Analysis could not be performed on four patients with Barrett’s esophagus because the amount of remaining sample was insufficient.) Of the nine Barrett’s esophagus samples, five (56%) demonstrated GOAGE of IGF-II as determined by the finding of biallelic gene expression (Fig. 4). Of those five samples, four were from patients with long-segment Barrett’s esophagus and one sample exhibited low-grade dysplasia. (Analysis could not be performed on 3 informative patients with Barrett’s esophagus because of insufficient tissue sam-
GOAGE of IGF-II in Barrett’s Esophagus

In all four patients containing GOAGE in the Barrett’s esophagus tissues, GOAGE for IGF-II was also found in the matched normal duodenal and gastric tissue that was available for analysis (Fig. 4). In Barrett’s tissues that did not demonstrate GOAGE, no GOAGE was found in the matched normal duodenal and gastric tissues.

DISCUSSION

We found a gain in allelic gene expression for IGF-II in five (56%) of nine informative samples of Barrett’s specialized intestinal metaplasia. Genomic imprinting is a reversible epigenetic process involving differential DNA methylation between parental alleles (i.e., one allele is methylated and thereby silenced, whereas the other is not). This leads to differential expression of the maternal and paternal genes in somatic cells during development and in adult life. It has been established that the IGF-II gene is normally imprinted so that only the paternal allele is expressed (7). However, when this differential methylation is lost (i.e., when there is LOI), there is a gain in gene expression as a result of IGF-II being expressed by both alleles. GOAGE for the IGF-II gene has been documented in a number of human tumors, including squamous cell carcinoma of the esophagus (3, 14). Expression of IGF-I-R, the receptor through which IGF-II exerts its mitogenic effects, has been detected in metaplastic Barrett’s esophagus. IGF-I-R expression has been shown to increase as the degree of dysplasia in Barrett’s epithelium increases, a phenomenon suggesting a role for the IGF pathway in the neoplastic progression of Barrett’s esophagus (10). To our knowledge, our present report is the first to demonstrate GOAGE of IGF-II in metastatic Barrett’s esophagus.

In most other studies of IGF-II GOAGE, genomic polymorphism identification and IGF-II allelic expression have been assessed on the basis of PCR or RT-PCR in combination with Apal restriction enzyme digestion. However, these methods can be flawed for various reasons, including partial digestion of the PCR products, the presence of digestion-resistant heteroduplexes in the PCR products, and contamination by gDNA (16, 18). These flaws might be responsible for the wide variations in the incidence of IGF-II GOAGE reported in human tumors (29). To overcome the problems introduced by Apal digestion, the technique of allele-specific PCR was developed in which the sequences of the 3’-oligonucleotide ends are absolutely complementary to the DNA template: one primer to the intact Apal restriction enzyme site and the other to the Apal site containing the polymorphism (29). This technique has been shown to be superior to conventional Apal digestion for investigating IGF-II imprinting (29). In our study, we used both the conventional Apal restriction enzyme digestion method and the allegedly superior allele-specific PCR method to identify the Apal genomic polymorphism in DNA from our patients with Barrett’s esophagus. We found no significant difference in the percentage of patients informative for IGF-II determined using these two methods, and in a subset of patients, we found that both methods produced identical results. Overall, we found a frequency of informative cases of 30% among our patients with Barrett’s esophagus, which is slightly less than the previously reported frequency range of 34–57% in other tissues (26).

Allele-specific PCR does not entirely eliminate the potential error of amplifying gDNA that are contaminating the cDNA preparations. To minimize this error, we treated our total RNA with DNase and followed this with reverse transcription to generate cDNA that were then amplified using exon connection RT-PCR. In this method, cDNA were first PCR amplified using primers that span exons 8 and 9 (the exon connection) of the IGF-II gene, followed by PCR using primers within exons 8 and 9 alone (26). By using primers that span exon 8 and 9 in the first of these two PCRs, the amplification of gDNA, which retains intervening intronic sequences, is markedly reduced. Using this technique, we assessed biallelic gene expression of IGF-II in cDNA from metaplastic Barrett’s, normal gastric, and normal duodenal epithelia from nine informative patients with Barrett’s esophagus. We found GOAGE of IGF-II in five (56%) of nine samples of Barrett’s specialized intestinal metaplasia. To our knowledge, this study is the first to demonstrate IGF-II GOAGE in a metastatic epithelium. The frequency of GOAGE of IGF-II has been reported to range from 25 to 100% in a variety of human tumors (9, 27). In the past few years, however, it has become clear that GOAGE is not limited solely to neoplastic tissue. Indeed, biallelic gene expression of IGF-II has been found in 12–17% of samples of colonic tissue from individuals without colonic neoplasia (6, 27). Furthermore, GOAGE of IGF-II has been found in peripheral blood lymphocytes from patients with a personal or familial history of colorectal neoplasia (4). This finding of biallelic IGF-II ex-

Fig. 3. Identification of the Apal polymorphism in IGF-II using allele-specific PCR in gDNA from metaplastic Barrett’s esophagus previously analyzed using the Apal restriction enzyme digestion method. Representative samples 29B and 45B demonstrate both allele A and allele B, indicating the presence of the Apal polymorphism and thus are informative for IGF-II. The same results were found on the basis of analysis using the Apal restriction enzyme digestion method shown in Fig. 1.

Fig. 4. Identification of gain of allelic gene expression (GOAGE) of IGF-II using exon connection PCR in cDNA from informative samples of metaplastic Barrett’s esophagus and normal gastric and duodenal samples. Representative Barrett’s samples 45B, 46B, 53B, and 54B demonstrate biallelic expression of IGF-II. Samples 29B and 51B retain monoallelic expression of IGF-II. Like the corresponding Barrett’s tissues, the normal gastric (G) and duodenal (D) samples from patient 51 demonstrated monoallelic IGF-II expression, whereas biallelic expression of IGF-II is detected in the duodenal and gastric samples from patients 53 and 54, respectively.
pression in apparently normal blood lymphocytes suggests that IGF-II GOAGE may be part of a systemic genetic process. The fact that GOAGE in lymphocytes did not correlate with environmental factors such as smoking, alcohol consumption, use of nonsteroidal anti-inflammatory agents, or nutrient ingestion suggests that IGF-II GOAGE may not be acquired in adulthood, but rather can occur early in life (4).

We found GOAGE for IGF-II in five (56%) of nine informative cases of Barrett’s specialized intestinal metaplasia. Our study is the first to demonstrate GOAGE for IGF-II in metaplastic Barrett’s epithelium. Although the nonneoplastic colonic epithelium surrounding colonic neoplasm has been shown to exhibit GOAGE for IGF-II, the investigators did not search for GOAGE in extracolonic tissues (6, 27). In our patients who had GOAGE for IGF-II in Barrett’s epithelium, we also found IGF-II GOAGE in biopsy specimens from the stomach and the duodenum. In contrast, the normal tissues from patients whose metaplastic epithelium retained monoallelic expression of IGF-II did not demonstrate GOAGE. These findings further support the hypothesis that GOAGE is a systemic genetic feature but do not provide insight into whether this feature is an inherited characteristic or an epigenetic alteration that occurs during early development (12).

In conclusion, we have shown that GOAGE for IGF-II occurs frequently in the metaplastic mucosa of patients with long- and short-segment Barrett’s esophagus. We have also shown that GOAGE for IGF-II occurs in normal gastric and duodenal epithelia from Barrett’s patients in whom the metaplastic epithelium demonstrates GOAGE, but not in those without GOAGE in Barrett’s esophagus. To our knowledge, this report is the first to describe GOAGE of IGF-II occurring in the metaplastic epithelium of patients with Barrett’s esophagus.

GRANTS

This work was supported by the American College of Gastroenterology Clinical Research Award (to L. A. Feagins); the Office of Medical Research, Department of Veterans Affairs, Dallas, TX (to R. F. Souza, R. D. Ramirez, and L. S. Terada); the Harris Methodist Health Foundation, Dr. Clark R. Gregg Clinical Research Award (to L. A. Feagins); the Office of Medical Research, National Heart, Lung, and Blood Institute Grants HL-61897 and HL-67256 (to L. S. Terada); and National Heart, Lung, and Blood Institute Grants AstraZeneca (to S. J. Spechler); National Institute of Diabetes and Digestive and Endocrine Diseases Grants HL-61897 and HL-67256 (to L. S. Terada); and National Heart, Lung, and Blood Institute Grants HL-61897 and HL-67256 (to L. S. Terada).

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