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Advances in Brief

Sequence Alterations of Insulin-like Growth Factor Binding Protein 3 in Neoplastic and Normal Gastrointestinal Tissues[1]

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Abstract

Insulin-like growth factor binding protein 3 (IGFBP-3) is an important regulator of normal and malignant cell growth. It modulates the mitogenic effects of insulin-like growth factors (IGFs) by inhibiting growth through mechanisms both dependent on and independent of IGF binding. IGF-I and IGF-II levels are regulated by binding to the IGF-II receptor, which is inactivated by mutation in human gastrointestinal (GI) tumors. We have previously demonstrated elevated IGF-II ligand expression in IGF-II receptor-mutant GI tumors, implicating the IGF signaling system in GI tumorigenesis. Therefore, to investigate the potential involvement of IGFBP-3 in human GI carcinogenesis, direct DNA sequencing of exons 1–4 and intron-exon boundaries of the *IGFBP-3* gene was performed in 10 colorectal cancers, 10 gastric cancers, and 10 esophageal cancers. Four distinct sequence alterations were identified: (*a*) in one gastric and one esophageal tumor, an A to C transversion occurred at nucleotide 5795 (CAC→CCC), leading to a His→Pro substitution at codon 179; (*b*) a second esophageal tumor had a C to T transition at nucleotide 8291 (ACC→ATC), leading to a Thr→Ile substitution at codon 277 of IGFBP-3; (*c*) one alteration comprised a G to C transversion in exon 1 at nucleotide 2132 (GGG→GCG), leading to a Gly→Ala substitution at codon 32 in two gastric cancers, seven esophageal cancers, and nine colon cancers; and (*d*) a C to G transversion located 17 nucleotides from the 3′ splice site in intron 1 was observed in three colon cancers and four esophageal cancers. All of these DNA sequence alterations were present in matched normal DNA from the same subjects, which suggests that some or all of them may represent polymorphisms. However, we cannot exclude the possibility that the germ-line nonconservative amino acid substitutions predicted to occur as a result of these alterations result in subtle changes to IGFBP-3 protein function and a predisposition to developing GI malignancy.

Introduction

The aberrant expression and regulation of IGFs[3] and their receptors have been implicated in the deregulation of cell growth, with resultant transformation and tumorigenesis (1–3). IGFBP-3 can regulate the biological activity of IGFs, which are produced at high levels by many tumors and neoplastic cells (4–6). At least seven IGFBPs interact with IGFs and thereby exert effects on the IGF signaling system (2, 7–10). IGFBP-3 is the most abundant IGFBP in adult serum. It binds IGFs with high affinity, prolonging their half-lives and modulating their proliferative and anabolic effects on target cells (2). Recent evidence suggests a role for IGFBP-3 in the regulation of cancer cell growth. This regulation may occur by various mechanisms that are both dependent on, and independent of, the effect of IGFBP-3 on the IGF signaling system (2, 11). In addition, IGFBP-3 mediates the potent growth inhibitory action of TGF-β1 as well as the induction of apoptosis by the tumor suppressor gene *p53* (12–15). We previously described (16) alterations in the IGF-IIR in GI tumors, whereby mutations of microsatellite tracts within the coding region of IGF-IIR functionally inactivate this gene. The IGF-IIR normally has two growth-suppressive functions: (*a*) it binds and stimulates the plasmin-mediated cleavage and activation of the latent TGF-β1 complex; and (*b*) it mediates the internalization and degradation of the IGF-II ligand, a mitogen. Therefore, mutation of the IGF-IIR is associated with growth stimulation due to (*a*) diminished levels of the active form of the epithelial cell growth suppressor TGF-β1; and (*b*) decreased internalization and degradation of the IGF-II ligand. Of relevance to the first of these functions is the finding that IGFBP-3 also inhibits binding of TGF-β1 to the type V TGF-β1 receptor and that IGFBP-3 and TGF-β1 share this same receptor (17). Taken together, the above findings suggest that IGFBP-3 may have TGF-β-like growth-suppressive activity through action at the dual BP-3/type V TGF-β receptor and point to a possible role for IGFBP-3 as a tumor suppressor in the GI tract. Therefore, to determine whether intragenic *IGFBP-3* alterations occurred in GI malignancies, direct DNA sequencing of exons 1–4 and intron-exon boundaries in the *IGFBP-3* gene was performed in 10 colorectal cancers, 10 gastric cancers, and 10 esophageal cancers.

Materials and Methods

Tissue Samples. Matching normal and tumor tissues were obtained at the time of surgical resection or endoscopic biopsy. All of the tissues were obtained fresh, grossly dissected free of normal surrounding tissue, and immediately frozen in liquid nitrogen.

Exon-specific PCR Amplification and Sequencing of Genomic DNA. Exon-specific PCR amplification was carried out using genomic DNA from 10 colorectal cancers, 10 gastric cancers, and 10 esophageal cancers as well as their matching normal control tissues. The following primer sets (based on GenBank accession number M35878) were used: (*a*) for exon 1, bp3-ipf (sense), TTCCTGCCTGGATTCCACAGCTT and bp3-cr4 (antisense), GCATCTACACCGAGCGCTGT as well as bp3-cf3 (sense), TGCTGCCTGACGTGCGCACT and bp3-ipr (antisense), CCGCTTTCTTCTCACTGGAGAT; (*b*) for exon 2, bp3–2sf (sense), ACCTCACTTGGATTGCCAAC and bp3–2pr (antisense), TACCCAGGCTTGGCAGGTCTT; (*c*) for exon 3, bp3–3sf (sense), TTGGTAGTTGTGCAGCATCG and bp3–3pr (antisense), GAAGAAAACACACTGAGGACC; and (*d*) for exon 4, bp3–4pf (sense), TCTCAGCATAGCAGAGTCAC and bp3–4pr (antisense), CTCCTGAGTACTCACCCTT.

PCR amplification for exon 1 consisted of 1 cycle at 95°C for 5 min; 34 cycles at 94°C for 1 min, 46°C for 0.5 min, and 72°C for 0.75 min; and an end extension step (72°C for 20 min). PCR amplification for exons 2–4 consisted of 35 cycles at 94°C for 30 s, 50°C for 30 s, and 72°C for 45 s after an initial

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[3] The abbreviations used are: IGF, insulin-like growth factor; BP, binding protein; TGF, transforming growth factor; IGF-IIR, IGF-II receptor; GI, gastrointestinal.

denaturation step (95°C for 2 min). PCR products were directly sequenced using the same primers as those used for PCR amplification.

Results and Discussion

To investigate whether IGFBP-3 was inactivated by mutation in primary human GI cancers, exon-specific sequencing of genomic DNA was performed using PCR primers based on intron-exon boundary sequences (GenBank Accession number M35878). These genomic DNA-based PCRs were successfully carried out in 10 colorectal cancers, 10 primary gastric cancers, and 10 esophageal cancers.

Sequence alterations differing from the publicly available DNA sequence of *IGFBP-3* (GenBank accession number M35878) were identified in two gastric cancers, seven esophageal cancers, and nine colorectal cancers (Table 1). In samples G21T and JE5T (Fig. 1), an A to C transversion occurred at nucleotide 5795 (CAC→CCC), leading to a His→Pro substitution at codon 179; and sample E225T (Fig. 2) had a C to T transition at nucleotide 8291 (ACC→ATC), leading to a Thr→Ile substitution at codon 277 of *IGFBP-3*. One G to C transversion in exon 1 occurred at nucleotide 2132 (GGG→GCG), leading to Gly→Ala substitution at codon 32 in two gastric cancers, seven esophageal cancers, and nine colon cancers. These alterations were heterozygous in tumor tissues. In fact, three specimens (G21T, JE5T, and E225T) contained dual mutations; G21T and JE5T had mutations in exons 1 and 2, and E225T manifested them in exons 1 and 4. Additional studies showed that these sequence alterations were present in matched normal samples. However, these three alterations are predicted to change amino acids in a nonconservative fashion, suggesting the possibility that they may also possess functional significance. In addition, a C to G transversion in intron 1 (17 nucleotides from the 3′ splice site) was found in three colon cancers and four esophageal cancers, as well as in matched normal samples (Fig. 3); this noncoding substitution is not part of a splice site and probably does represent a polymorphism. Although no purely somatic muta-

Table 1 *Summary of IGFBP3 sequence alterations in colorectal, gastric, and esophageal cancers and normals*

Tissue	Samples	Exon/ Intron	Codon	Nt position[a]	Sequence changes	Predicted effect
Gastric	G21T	Exon 1	32	2132	GGG→GCG	Gly→Ala
		Exon 2	179	5795	CAC→CCC	His→Pro
	G23T	Exon 1	32	2132	GGG→GCG	Gly→Ala
Esophageal	JE5T	Exon 1	32	2132	GGG→GCG	Gly→Ala
		Exon 2	179	5795	CAC→CCC	His→Pro
	E105T	Exon 1	32	2132	GGG→GCG	Gly→Ala
	E185T	Exon 1	32	2132	GGG→GCG	Gly→Ala
	E225T	Exon 1	32	2132	GGG→GCG	Gly→Ala
		Exon 4	277	8291	ACC→ATC	Thr→Ile
	E294T	Exon 1	32	2132	GGG→GCG	Gly→Ala
	E366T	Exon 1	32	2132	GGG→GCG	Gly→Ala
	E369T	Exon 1	32	2132	GGG→GCG	Gly→Ala
	E105T	Intron 1		5709	C→G	
	E225T	Intron 1		5709	C→G	polymorphism
	E294T	Intron 1		5709	C→G	
	E369T	Intron 1		5709	C→G	
Colon	C605T	Exon 1	32	2132	GGG→GCG	Gly→Ala
	C607T	Exon 1	32	2132	GGG→GCG	Gly→Ala
	C611T	Exon 1	32	2132	GGG→GCG	Gly→Ala
	C624T	Exon 1	32	2132	GGG→GCG	Gly→Ala
	C637T	Exon 1	32	2132	GGG→GCG	Gly→Ala
	C10591T	Exon 1	32	2132	GGG→GCG	Gly→Ala
	C10636T	Exon 1	32	2132	GGG→GCG	Gly→Ala
	C10818T	Exon 1	32	2132	GGG→GCG	Gly→Ala
	C11346T	Exon 1	32	2132	GGG→GCG	Gly→Ala
	C637T	Intron 1		5709	C→G	
	C607T	Intron 1		5709	C→G	polymorphism
	C611T	Intron 1		5709	C→G	

[a] Nt position, nucleotide number mutated (as per GenBank accession number M35878).

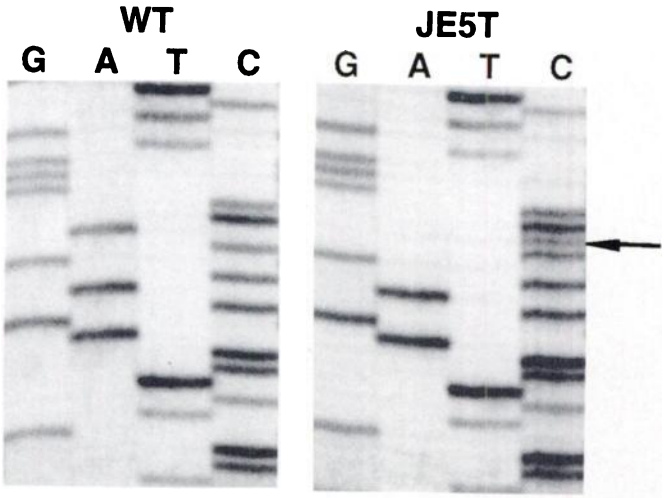

Fig. 1. DNA sequencing of cloned PCR product from *IGFBP-3* exon 2 in esophageal cancer JE5T shows an A to C transversion (*arrow*) at nucleotide 5795 (codon 179), leading to a His→Pro substitution. This substitution was also seen in normal tissue from the same patient.

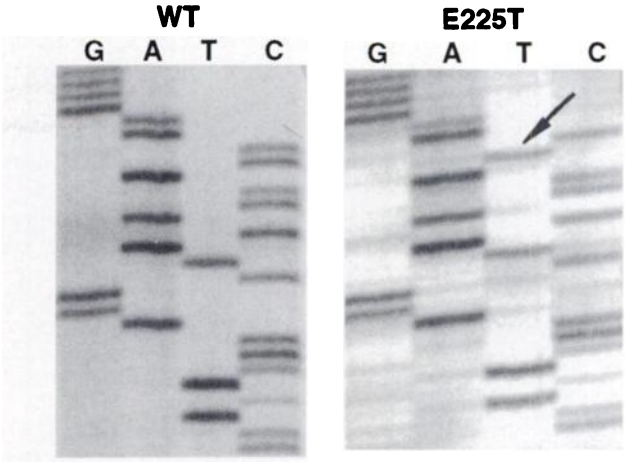

Fig. 2. DNA sequencing (cloned PCR product) of *IGFBP-3* exon 4 in esophageal cancer E225T shows a C to T transition (*arrow*) at nucleotide 8291, leading to a Thr→Ile substitution at codon 277. This substitution was also seen in normal tissue from the same patient.

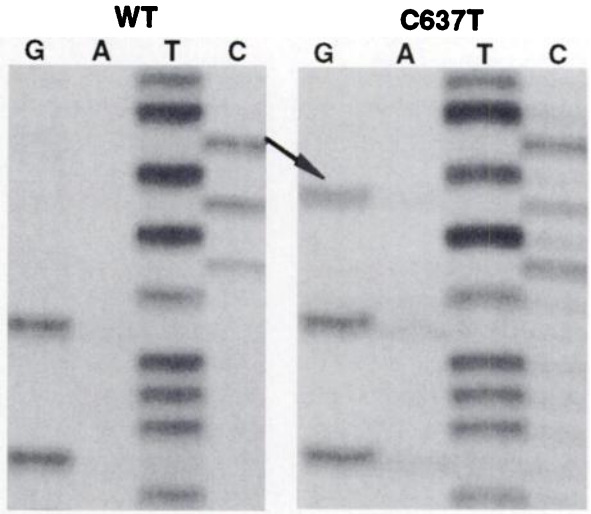

Fig. 3. A C to G transversion (*arrow*) in intron 1 (17 nucleotides from the 3′ splice site) was found in three colon cancers and four esophageal cancers and in matched normal tissues.

tions of *IGFBP-3* were found in this study, we cannot exclude the possibility that the germ-line nonconservative amino acid substitutions predicted to occur within IGFBP-3 have a definite, albeit subtle, effect on IGFBP-3 protein function.

In conclusion, three discrete missense *IGFBP-3* gene alterations were observed in a significant proportion of GI tumors and matching normal tissues, raising the possibility that mutations in this gene predispose to the development of GI malignancies in at least a subset of cases. However, some or all of the sequence alterations that we observed may represent polymorphisms.

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