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Microsatellite instability in the insulin-like growth factor II receptor gene in gastrointestinal tumours

Sir — Microsatellites are oligonucleotide repeat sequences present throughout the human genome. The appearance of mutations within these regions, consisting of additions or deletions of repeat units, are known as the replication/repair error-positive (RER+) phenotype or microsatellite instability (MI). MI has been demonstrated in multiple primary human tumour types. However, most MI has thus far been described in noncoding DNA, within introns or intergenic regions of the genome.

Recently, the first target of MI within a coding region was identified in the transforming growth factor-beta type II receptor (*TGFβ1RII*) gene in colon cancer cells[1]. Deletions or insertions of one or two bases were reported in a poly-deoxyadenine tract within the upstream portion of the cDNA. These mutations generated nonsense codons downstream and were predicted to yield truncated, nonfunctional proteins. Microsatellite mutation within the coding region of *TGFβ1RII* is present in RER+ primary gastric and ulcerative colitis-associated cancers, in addition to sporadic colorectal cancers[2–4]. Thus, there is strong evidence supporting the importance of *TGFβ1* and its receptors in gastrointestinal tumorigenesis.

Reasoning that other coding portions of cancer-related genes might constitute targets of MI in tumours, we searched the GenBank and European Molecular Biology Library (EMBL) databases for genes containing coding region microsatellites. One gene encoded the insulin-like growth factor II receptor (*IGFIIR*) and contained several microsatellites within its coding sequence. This gene has several mechanistic and clinical links to human cancer. Most importantly, IGFIIR binds the latent complex of *TGFβ1*, and this binding is essential for *TGFβ1* activation[5–9]. It is the active form of *TGFβ1* that exerts growth suppressive effects. Moreover, loss of heterozygosity (LOH) at the *IGFIIR* locus on chromosome 6q26–27 is common in hepatocellular[10] and breast[11] cancers. Similarly, a splice donor mutation resulting in intron inclusion within the *IGFIIR* mRNA was described in a hepatic tumour[12], and missense mutations occurred in breast cancers demonstrating LOH at 6q26–27 (ref. 11). IGFIIR is also essential for the degradation of IGFII, itself a potent growth stimulant[13,14]. The ligand IGFII binds to both the insulin-like growth factor I receptor (IGFIR) and IGFIIR, but with higher affinity to IGFIR. Binding of IGFII by IGFIR promotes cellular proliferation and suppresses apoptosis[15–17]. In contrast, binding of IGFII to IGFIIR results in internalization and subsequent degradation of the ligand, making it unavailable to activate IGFIR. Thus IGFIIR, by antagonizing the growth stimulatory effect of IGFII, effectively operates as a growth suppressor gene. Furthermore, recent studies in human breast cancer cells support the role of IGFIIR as a tumour suppressor[18].

Taken together, the above-cited work suggested that MI occurring within the *IGFIIR* gene could contribute to carcinogenesis. We therefore screened a panel of both RER+ and RER– tumours for mutations within *IGFIIR* coding region microsatellites.

A total of 92 RER+ tumours were analysed, of which 12 (13%) demonstrated *IGFIIR* mutation. Analysed by specific tissue type, these included 7 of 29 RER+ gastric adenocarcinomas (24%); 1/8 RER+ HNPCC tumours (12.5%); 3/35 RER+ sporadic colorectal cancers (9%); 1/8 RER+ ulcerative colitis-associated colorectal cancers (12.5%); 0/9 RER+ ulcerative colitis-associated dysplasias; 0/2 RER+ pancreatic tumours; and 0/1 RER+ esophageal tumours (examples, Fig. 1). In addition, 52 RER– tissues were assayed, none of which contained mutations in *IGFIIR*. Interestingly, 6/11 mutations for which histologic grade was known occurred in poorly differentiated tumours, while 3/11 occurred in moderately differentiated lesions and only 1/11 occurred in a well-differentiated cancer.

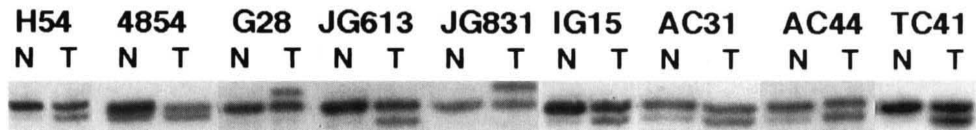

Fig. 1 Mutation of *IGFIIR*. H54 (N and T), normal and tumour DNAs from a patient with ulcerative colitis-associated colorectal cancer; 4854, G28, JG613, JG831 and IG15 (N and T), DNAs from patients with gastric cancer; AC31and AC44 (N and T), DNAs from patients with sporadic colorectal adenocarcinoma; TC41 (N and T), DNAs from a patient with HNPCC. An abnormally migrating band located just above or below the wild-type band is visible in each of the tumour (T) lanes. Mutations were demonstrated using primer set *R4*, which amplified nucleotides 4030-4140 and contained an 8-deoxyguanine repeat (5'-GCAGGTCTCCTGACTCAGAA-3' upstream, 5'-GAAGAAGATGGCT-GTGGAGC-3' downstream), except for sample 4854T, which contained a mutation demonstrated using primer set R5, amplifying nucleotides 6141- intron 40 (ref. 12) and including a 5-CT repeat (5'-GAAACACAAAACCTACGACC-3' upstream, 5'-AGAACCCAAAAGAGCCAA CC-3' downstream). PCR conditions consisted of 35 cycles at 94 °C x 1 min, 58 °C x 1 min and 72 °C x 2 min. PCR products were denatured in 95% formamide, electrophoresed on 6% denaturing polyacrylamide gels and visualized by autoradiography. Mutation was defined as the presence of bands in tumour DNA that were not visualized in corresponding normal DNAs. All matching normal and tumour tissues were obtained at the time of surgical resection or endoscopic biopsy. Tissues were obtained fresh, dissected grossly free of normal surrounding tissues, and immediately frozen in liquid nitrogen. None of the specimens evaluated were derived from xenografts or cell lines. The human tumours included 30 gastric cancers (29 RER+, 1 RER–), 35 sporadic colorectal adenocarcinomas (35 RER+, 0 RER–), 8 hereditary non-polyposis coli (HNPCC) colorectal cancers, 10 pancreatic carcinomas (2 RER+, 8 RER–), 1 esophageal carcinoma (RER+), 46 ulcerative colitis-associated cancers (8 RER+, 38 RER–) and 14 ulcerative colitis-associated dysplasias (9 RER+, 5 RER–).

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Table 1 Tumours with mutation in an *IGFIIR* microsatellite (R4 or R5) or *TGFβ1RII*

Specimen	Tissue Type	Tissue Grade	*IGFIIR* (R4)	*IGFIIR* (R5)	*TGFβ1RII*	# of unstable loci
4854	gastric	WD	N	P	N	2
G28	gastric	MD	P	N	N	3
IG15	gastric	unk	P	N	N	1
JG831	gastric	PD	P	N	N	3
JG613	gastric	PD	P	N	N	5
JG933Tp1	gastric	PD	P	N	N	3
JG933Tp2	gastric	PD	P	N	N	3
AC1	sporadic colon	MD	N	N	P	2
AC3	sporadic colon	MD	N	N	P	3
AC4	sporadic colon	MD	N	N	P	1
AC5	sporadic colon	MD	N	N	P	3
AC7	sporadic colon	MD	N	N	P	3
AC8	sporadic colon	MD	N	N	P	3
AC13	sporadic colon	MD	N	N	P	2
AC18	sporadic colon	MD	N	N	P	2
AC23	sporadic colon	PD	N	N	P	4
AC25	sporadic colon	PD	N	N	P	2
AC28	sporadic colon	MD	N	N	P	4
AC29	sporadic colon	PD	P	N	N	4
AC31	sporadic colon	MD	P	N	N	4
AC32	sporadic colon	MD	N	N	P	4
AC35	sporadic colon	WD	N	N	P	1
AC43	sporadic colon	MD	N	N	P	2
AC44	sporadic colon	PD	P	N	P	2
AC56	sporadic colon	MD	N	N	P	1
AC59	sporadic colon	MD	N	N	P	1
TC41	HNPCC	unk	P	N	P	2
H42	UCAN	WD	N	N	P	0
715D	UCAN-DALM	unk	N	N	P	1
750D	UCAN-DALM	unk	N	N	P	5
H54	UCAN	MD	P	N	P	2

Top row: primer set used for each coding microsatellite of *IGFIIR*, poly (A)[+] site of *TGFβ1RII* or number of unstable loci. HNPCC: hereditary nonpolyposis colon cancer; UCAN: ulcerative colitis-associated neoplasia; DALM: dysplasia-associated lesion or mass; N: negative for mutation; P: positive for mutation; PD: poorly differentiated; MD: moderately differentiated; WD: well-differentiated; unk: unknown. Markers used for determining RER phenotype were as follows: DNAs from 12 of the gastric cancers, 1 esophageal cancer and all ulcerative colitis-associated neoplasias were amplified at microsatellite loci *D2S123*, *D2S147*, *D2S119*, *D10S197* and *D11S904*. Among the remaining gastric tumours, 5 were tested at loci *D5S346*, *D17S804* and *p53* intron 1, while 13 were tested at loci *D1S116*, *D6S86*, *D10S197*, *D2S136*, *D17S261*, *MXS2* and *TP53*. Sporadic colorectal cancers were tested for MI at loci *MYCL-tet*, *AT3-tri*, *D2S123* and *F13B*. Pancreatic tumours were tested at either of two sets of loci: (i) *VWF*, *FABP2*, *D2S123*, *TNFβ* and *GCK* or (ii) *D14S52*, *D2S123*, *TNFβ*, *GCK* and *D11S937*. Lesions were classified as RER+ if they manifested instability at one or more of the microsatellite loci tested. Case H42 was RER–.

Of particular interest was the anticorrespondence of *IGFIIR* and *TGFβ1RII* mutation in our tumours (Table 1). Of 31 lesions with mutations in *IGFIIR* or *TGFβ1RII*, 28 (90%) contained mutation of one or the other, but not both, of these genes. Only 3 lesions (10%) were mutated in both *IGFIIR* and *TGFβ1RII*.

All but one of the mutations occurred within an 8 poly-deoxyguanine (8G) tract spanning nucleotides 4089 to 4096 of the *IGFIIR* coding sequence (locus R4, Fig. 1). The single exception was a mutation in 1 gastric adenocarcinoma at an interrupted poly (CT) tract spanning nucleotides 6169 to 6180 (locus R5, Fig. 1). These mutations all comprised one- or two-basepair (bp) deletions or insertions within the microsatellite region, causing frameshifts and premature stop codons downstream. Finally, as an additional control, an 8G microsatellite within the histone gene was amplified in eight of the twelve tumours with *IGFIIR* mutations; none of these eight lesions contained mutations in the histone 8G locus. This finding supports the concept of nonrandom targeting of *IGFIIR* by MI, that is, that *IGFIIR* mutation confers some specific growth advantage on cells and is not simply a passenger or bystander event. Moreover, targeting of *IGFIIR* by MI is also supported by the fact that 0/52 RER– lesions possessed *IGFIIR* mutations, while 12/92 (13%) of RER+ lesions contained them. Finally, the majority of our *IGFIIR* mutations occurred in poorly differentiated tumours. Poorly differentiated tumours may more closely resemble early fetal tissue, which has been shown to be highly responsive to IGFII overexpression caused by inactivation of *IGFIIR*[13].

The mechanisms by which MI contributes to cancer are not yet precisely known, but it has been assumed that MI must alter gene structure or function to have a carcinogenic effect. Until now, the only gene known to undergo MI within its coding region was *TGFβ1RII*. Teleologically, it makes sense for *TGFβ1RII* to be targeted by MI, since it is the receptor for *TGFβ1*, an important growth-inhibitory signal for epithelial cells. We have now shown that another gene, *IGFIIR*, is targeted by MI. Teleologically, this finding also makes sense: IGFIIR activates the *TGFβ1* complex from its latent to its active form, and this activation is required for normal *TGFβ1* function. The current study suggests that the *IGFIIR* and *TGFβ1RII* genes indeed comprise serial points in the same tumorigenesis pathway, since mutation of either, but not both, occurred in 90% of our tumours. Furthermore, since 31 of 92 tumours (34%) possessed abnormalities in *TGFβ1RII*,

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IGFIIR, or both genes, our findings suggest that active *TGFβ* function was compromised in at least one-third of our tumours. Therefore, targeting of *IGFIIR* by MI may represent another step at which disruption of the *TGFβ1* growth control pathway occurs during tumorigenesis.

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Rhonda F. Souza[1]
Rebecca Appel[1]
Jing Yin[1]
Suna Wang[1]
Kara N. Smolinski[1]
John M. Abraham[1]
Tong-Tong Zou[1]
Ying-Qiang Shi[1]
Junyi Lei[1]
John Cottrell[2]
Karina Cymes[6]
Kelli Biden[3]
Lisa Simms[3]
Barbara Leggett[3]
Patrick M. Lynch[4]
Marsha Frazier[4]
Steven M. Powell[5]
Noam Harpaz[6]
Hirohashi Sugimura[7]
Joanne Young[3]
Stephen J. Meltzer[1,9]

Departments of [1]Medicine (GI Division), and [2]Pathology, University of Maryland School of Medicine and Baltimore VA Hospital, Baltimore, Maryland 21201, USA
[3]Glaxo Conjoint Gastroenterology Laboratory, Royal Brisbane Hospital, Herston, Q4029 Australia
[4]Department of Gastrointestinal Medical Oncology and Digestive Diseases, The University of Texas M.D. Anderson Cancer Center, Houston, Texas 77030, USA
[5]Department of Medicine, Indiana University School of Medicine, Indianapolis, Indiana 46202, USA.
[6]Mt. Sinai Medical Center, New York, New York 10029, USA
[7]First Department of Pathology, Hamamatsu University School of Medicine, Hamamatsu 431-31, Japan

S.P. current address: Department of Medicine, GI Division, University of Virginia, PO Box 1031, Charlottesville, Virginia 22906, USA

Correspondence should be addressed to S.J.M.

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