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Evidence of Selection for Clones Having Genetic Inactivation of the Activin A Type II Receptor (*ACVR2*) Gene in Gastrointestinal Cancers[1]

Paula M. Hempen, Lin Zhang, Ravi K. Bansal, Christine A. Iacobuzio-Donahue, Kathleen M. Murphy, Anirban Maitra, Bert Vogelstein, Robert H. Whitehead, Sanford D. Markowitz, James K. V. Willson, Charles J. Yeo, Ralph H. Hruban, and Scott E. Kern[2]

Departments of Oncology [P. M. H., L. Z., R. K. B., B. V., R. H. H., S. E. K.], Pathology [C. A. I-D., K. M. M., A. M., R. H. H., S. E. K.], and Surgery [C. J. Y.], The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland 21231; Vanderbilt-Ingram Cancer Center, Vanderbilt University, Nashville, Tennessee 37232 [R. H. W.]; Ireland Cancer Center, University Hospitals of Cleveland and Case Western Reserve University, Cleveland, Ohio 44106 [S. D. M., J. K. V. W.]; and Howard Hughes Medical Institutes, Chevy Chase, Maryland 20815 [B. V., S. D. M.]

ABSTRACT

The activin signaling pathway parallels the transforming growth factor (TGF)-β pathway. Both use extracellular ligands and cell surface receptors that are structurally and functionally related, as well as the same intracellular mediators (SMADs 2–4) to transmit these signals. Members of both pathways have been characterized previously as tumor suppressor genes on the demonstration of inactivating mutations in human neoplasms, *e.g.*, genetic inactivation of the activin type I receptor was reported recently in pancreatic cancer. Here, we present evidence of selection for mutations of the activin A type II receptor (*ACVR2*) gene during human gastrointestinal carcinogenesis. Two 8-bp polyadenine tracts of the *ACVR2* gene are targets for inactivating frameshift mutations in gastrointestinal neoplasms having microsatellite instability (MSI). These mutations are similar to those of the 10-bp polyadenine tract within the TGF-β type II receptor (*TGFBR2*), a well-characterized target of frameshift mutations in the same neoplasms. We identified biallelic mutations of *ACVR2* in 25 of 28 MSI colorectal and pancreatic cancers. In addition, a mutation in the *ACVR2* gene combined with loss of the wild-type allele was found in a non-MSI pancreatic cancer. This evidence is compatible with a high degree of selection for inactivation of the *ACVR2* gene in tumorigenesis, supporting *ACVR2* as a candidate tumor suppressor gene in gastrointestinal cancers.

INTRODUCTION

The activin signaling pathway shares many features with the TGF[3]-β signaling pathway (reviewed in Ref. 1). Activin and TGF-β are homologous and structurally similar, as are their respective heterodimeric receptors. Activin and TGF-β directly bind their respective type II receptors, activin A receptor type II (Acvr2) or activin A receptor type IIB (Acvr2b) and TGF-β receptor type II (Tgfbr2; Refs. 2 and 3). All of the type II receptors have a constitutively active serine/threonine kinase and share a degree of sequence similarity at the amino acid level. Acvr2 and Acvr2b proteins share 69% identity at the amino acid level (4). Acvr2 and Tgfbr2 are 37% identical (5), and their serine/threonine kinase domains have an identity of 46% (5).

In both pathways, the type II receptors form an active complex with the bound ligand and corresponding type I receptors: (*a*) activin A receptor type I (Acvr1/Alk2); (*b*) activin A receptor type IB (Acvr1b/Alk4); and (*c*) TGF-β receptor type I (Tgfbr1/Alk5; Refs. 2 and 3). The type I receptors phosphorylate SMADs, resulting in their activation (reviewed in Ref. 6). Both the activin and TGF-β pathways are mediated by the r-SMADs 2 and 3 (7–9). The activated r-SMADs associate with the co-SMAD, SMAD4 (9–11). Smad proteins accumulate within the nucleus (12, 13) and have sequence-specific DNA binding capabilities that enable transcriptional activation (14–16).

The genes encoding the members of these signaling pathways have been found to have inactivating mutations in several tumor types, including pancreatic and colorectal cancers. This suggests a role of each of these genes as tumor suppressors. Homozygous deletions of the *TGFBR1* (ALK5) gene have been discovered in pancreatic and biliary cancers (17) and a lymphoma (18). Mutations of the *TGFBR2* gene have been discovered in the mononucleotide tract in microsatellite unstable tumors (19). Homozygous deletions and mutations have been found in non-MSI pancreatic and nongastrointestinal cancers (17, 20, 21). Mutations in the *MADH2* (*SMAD2*) gene occur in colorectal and lung cancers (7, 22, 23). Mutations in the *MADH4* (*SMAD4/DPC4*) gene are seen in pancreatic, colorectal, and other cancers (24–27). Genetic inactivation of the *ACVR1B* (*ALK4*) gene has been described recently in pancreatic cancers (28).

The *TGFBR2* gene has a 10-bp polyadenine [$(A)_{10}$] tract, which is a hot spot for mutation in neoplasms that have MSI (19). The common finding of biallelic polyadenine tract mutations in these tumors, combined with the finding of mutations in coding regions outside the polyadenine tract in MSI and non-MSI tumors (20, 29, 30), has supported the mutational evidence for *TGFBR2* as a tumor suppressor gene. The *ACVR2* gene has two 8-bp polyadenine [$(A)_8$] tracts, one located in exon 3 of the gene and another located in exon 10. We found mutations in the exon 10 $(A)_8$ tract of the *ACVR2* gene and therefore initiated the present study. Independently, another group reported that one of these polyadenine tracts in the *ACVR2* gene experiences mutation in 58% of colorectal MSI neoplasms (31) and in 44% of gastric MSI neoplasms (32). In both reports, only a minority of the neoplasms had evidence for homozygous alteration (31, 32). The issue of selective pressures that may favor the inactivation of *ACVR2* in these tumors having not yet been addressed, we gather the evidence for such selection in this work.

MATERIALS AND METHODS

Xenografts and Cell Lines Used in this Study. Pancreatic and peripancreatic cancer xenografts have been described previously (33, 34), as have the MSI colorectal carcinoma xenografts and cell lines (35).

Preparation of Genomic DNA from Primary Tumor Specimens. DNA was purified from histologically microdissected fresh frozen and archival paraffin tissues harvested from surgically resected primary tumors that corresponded to the xenografts used in this study. In general, the neoplastic cellularity of tumors studied was ≥65%.

Identification of LOH on 2q. Previous work (34) demonstrated a low frequency of LOH on 2q in pancreatic tumors by use of dinucleotide markers. To identify additional cases with LOH of 2q, data from an ongoing separate study were surveyed.[4] In this study, genomic DNA from our pancreatic xenografts and pancreatic cell lines was subjected to automated genotyping

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[2] To whom requests for reprints should be addressed, at Cancer Research Building 451, 1650 Orleans Street, Baltimore, MD 21231. E-mail: sk@jhmi.edu.

[3] The abbreviations used are: TGF, transforming growth factor; MSI, microsatellite instability; r-SMAD, receptor-associated SMAD; LOH, loss of heterozygosity; SMT, short mononucleotide tract; nt, nucleotide; RT-PCR, reverse transcription-PCR.

[4] C. A. Iacobuzio-Donahue *et al.*, unpublished data.

analysis by the NIH Center for Inherited Disease Research. On the basis of these data, 7 of 96 pancreatic xenografts were selected because of regional LOH according to one of two criteria. Seven cases (PX91, PX283, PX53, PX105, PX139, PX221, and PX280) were selected that had LOH in at least one marker flanking *ACVR2* (D2S1399 and D2S1353). PX53 was demonstrated previously to have regional LOH, providing a confirmation of the Center for Inherited Disease Research data (34). All exons of the *ACVR2* gene were sequenced in these seven cases.

Sequencing of ACVR2 in Tumors Having 2q LOH. PCR-amplified products were studied by automated sequencing. All samples that were found to have a mutation in the *ACVR2* gene were sequenced in the reverse direction for initial confirmation of the mutation. Verification of the mutation was accomplished by sequencing of a second PCR product derived independently from the original template.

Allelic Separation by Cloning. In the case of multiple mutations of *ACVR2*, allelic separation by cloning was performed using genomic DNA (unless otherwise indicated) with the TA cloning PCR 2.1 Kit (Invitrogen Life Technologies, Inc., Carlsbad, CA).

RT-PCR. RT-PCR was performed on samples where multiple mutations were located several exons apart from one another, for the purpose of allelic separation and cloning or to determine whether a mutated form of an allele was transcribed.

Mutational Studies of *TGFBR2* and *BAX* Genes. The 10 nt polyadenine tract in exon 3 of the *TGFBR2* gene and the 8 nt polyguanine tract in exon 3 of the *BAX* gene were surveyed by sequencing of genomic DNA of the 24 MSI colorectal tumors as reported (35) and were also sequenced in the four pancreatic MSI cases. For *TGFBR2*, cases heterozygous for frameshift mutations were further examined for additional mutations. In the case of multiple mutations, allelic status by cloning was performed in cases where a cDNA sample was available by ligating PCR products into EcoRV-digested pZEO plasmid (Invitrogen).

Statistical Analysis. A statistical test based on the binomial distribution was applied using the expected frequencies of biallelic mutations predicted by a mathematical model (36) and as described in "Results" and "Discussion." Each tumor having a mutation was considered as an individual Bernoulli trial, where each observation is independent and all tumors have the same assumed chance of a "success." For this purpose, a "success" is defined as a tumor having a biallelic mutation, and a "failure" is considered a tumor with a simple heterozygous mutation. The binomial sum reported (Table 2) represents the sum of the probabilities for observed biallelic mutation counts equal to or more extreme than observed. The binomial sum can be equated to a single-tailed estimate of the expected distribution under the null hypothesis.

ACVR2 Sequences in Familial Pancreatic Cancer. To determine whether germ-line mutations in the *ACVR2* gene were associated with familial pancreatic cancer, all exons were sequenced in 29 affected individuals from the National Familial Pancreas Tumor Registry, in which three or more first-degree relatives had pancreatic cancer (37–40). Information on the National Familial Pancreas Tumor Registry is available on the Internet.[5]

RESULTS AND DISCUSSION

ACVR2 in MSI Pancreatic Cancer Xenografts. Three pancreatic cancer xenografts known to exhibit MSI, PX26, PX196, and PX287 were studied (17). All three had frameshift mutations in the $(A)_{10}$ tract in exon 3 of the *TGFBR2* gene (17). A xenograft of a duodenal carcinoma known to have MSI, PX335, was also studied. Exons 3 and 10 of the *ACVR2* gene were PCR amplified from these xenografts and sequenced to examine the $(A)_8$ tracts. In these four cases, no mutations in the exon 3 $(A)_8$ tract were found. In three of four cases (PX26, PX196, and PX287), the *ACVR2* gene was biallelically inactivated by a 1-bp contraction in the exon 10 $(A)_8$ tract (Fig. 1*A*). The mutations in these three xenografts were also detected in DNA of their corresponding primary tumors and were somatic (Fig. 1*A*).

In the duodenal cancer xenograft, PX335, a 2-bp contraction was found in the exon 10 $(A)_8$ tract of the *ACVR2* gene; however, the remaining allele appeared to be wild type on sequencing of the entire coding region. This mutation was somatic and confirmed to be present in the corresponding primary tumor DNA.

ACVR2 in MSI Colorectal Cancer Cases. The status of the *ACVR2* gene was examined in colorectal cancers: 17 cell lines and 7 xenografts known to have MSI (35). Of these 24 cases, 22 had a biallelic inactivation of the *ACVR2* gene. A majority of the cases that were biallelically inactivated (17 of 22) had a 1-bp contraction of the $(A)_8$ tract in exon 10 (see Table 1 for summary). Paired normal DNAs were available for the VACO457 cell line and colorectal cancer xenografts CX2, CX10, and MX17; no mutations were found.

Two of the 22 colorectal cancers were biallelically inactivated by dissimilar mutations of the $(A)_8$ tract in exon 10 (Table 1). The RKO cell line had a 2-bp contraction in one allele and a 1-bp contraction in the other allele of the *ACVR2* gene. A colorectal cancer xenograft, CX7, had a 1-bp contraction and a 1-bp expansion of this $(A)_8$ tract on the two alleles of the *ACVR2* gene. In the paired normal DNA for this cancer, neither mutation was found.

Two of the 22 colorectal cancers were biallelically inactivated because of mutations in both the exons 3 and 10 $(A)_8$ tracts of the *ACVR2* gene. The VACO444 cell line was found to have a 1-bp contraction in both $(A)_8$ tracts of the *ACVR2* gene. Using allelic separation of cDNA by cloning, these mutations were demonstrated to exist on separate alleles.

The VACO481 cell line had a 1-bp expansion of the exon 3 $(A)_8$ tract and a 1-bp contraction at the exon 10 tract (Fig. 1*B*). These mutations were demonstrated to exist on separate alleles. Sequencing of paired normal DNA demonstrated their somatic nature (Fig. 1*B*).

One of the 24 MSI colorectal cancers had a biallelic inactivation represented by a frameshift mutation and a missense mutation in exon 10 (Fig. 1*C*). LIM1215, a colorectal cancer cell line, had a 1-bp contraction of the exon 10 $(A)_8$ tract and a C to A missense mutation just upstream of this tract at nt 1268 (GI:10862696, numbering from the translational start site), resulting in an amino acid change from proline to glutamine at amino acid 423 of the Acvr2 protein. The missense and frameshift mutations were demonstrated to exist on separate alleles (Fig. 1*C*). RT-PCR and sequencing of the *ACVR2* gene were also used to study LIM1215; this demonstrated that both mutant alleles were transcribed (data not shown). Two of the 24 MSI cases, the colorectal xenografts CX12 and MX23, had only wild-type *ACVR2* sequences.

A Non-MSI Pancreatic Carcinoma with an ACVR 2 Mutation. Genomic sequencing of all exons of the *ACVR2* gene was performed on all seven pancreatic carcinomas that had LOH flanking the *ACVR2* gene. In PX280, a deletion of a thymidine at nt 1363 was identified, resulting in a frameshift starting at codon 455 (Fig. 1*D*). No second allele was present. The mutation was not present in the paired normal DNA (Fig. 1*D*). The mutation was confirmed in an independent PCR product from this xenograft and in genomic DNA isolated from the corresponding primary tumor.

Mutational Status of TGFBR2 in MSI Gastrointestinal Cancers. A survey of the $(A)_{10}$ tract of the *TGFBR2* gene was performed on genomic DNA from the same 28 MSI gastrointestinal cancers described above. Twenty-one of 28 tumors were biallelically inactivated by frameshift mutations in this tract. These included the pancreatic cancers PX26 and PX287 and the duodenal cancer PX335. Biallelic frameshift mutations were also found in the colorectal cancers SW48, LOVO, LS180, VACO5, VACO6, HCT116, C, LIM1215, LIM2412, VACO444, VACO 457, CX2, CX7, CX10, CX12, CX29, MX17, and MX23. Carcinomas heterozygous at this $(A)_{10}$ tract included the pancreatic cancer xenograft PX196 and the colorectal cancer cell lines DLD-1, RKO, VACC1430, Co53, VACO481, and KM12.

[5] Internet address: http://www.path.jhu.edu/pancreas_nfptr/.

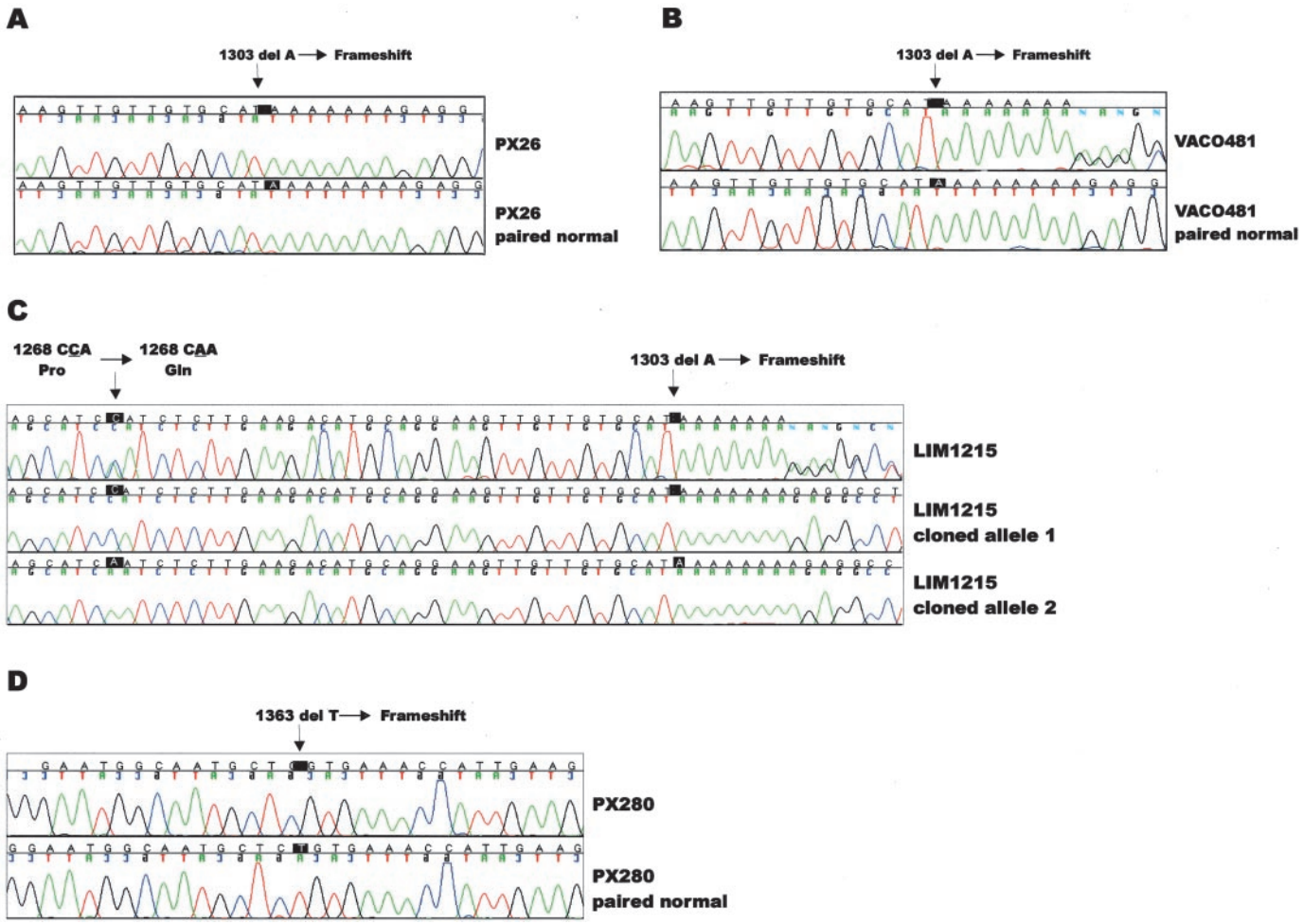

Fig. 1. *ACVR2* gene mutations in gastrointestinal cancers. *A*, a biallelic frameshift mutation resulting from deletion of an "A" in the exon 10 $(A)_8$ tract of the *ACVR2* gene in the MSI cancer PX26 (*top*) but not in the paired normal DNA (*bottom*). *B*, an exon 10 $(A)_8$ frameshift mutation present in a single allele of the *ACVR2* gene in VACO481, an MSI tumor (*top*), but not in its paired normal DNA (*bottom*). Deletion of an "A" at nt 1303 moves the sequence of one allele out of frame with the sequence of the other allele, seen as overlapping sequences after the $(A)_8$ tract. *C*, a combination of missense and frameshift mutations in the exon 10 of the *ACVR2* gene in the MSI cell line LIM1215 (*top*). One cloned allele of LIM1215 contains only the frameshift mutation caused by the deletion of an "A" at nt 1303 (*middle*). A second cloned allele of LIM1215 contains only the CCA to CAA missense mutation at nt 1268 (*bottom*). In *D*, a frameshift mutation with LOH at the *ACVR2* locus in a the non-MSI cancer PX280 is evidenced by the deletion of a "T" at nt 1363, with no wild-type allele present (*top*). Its paired normal DNA is wild type (*bottom*). Chromatograms are of direct sequencing of tumor DNA except as noted.

As reported previously, some of these heterozygous mutant tumors had additional missense mutations in the *TGFBR2* gene (19, 29). The cell lines Co53 and DLD-1 had a T-to-C change at nt 1690, resulting in a leucine-to-proline change at amino acid 452. This mutation had been reported previously for DLD-1 but not Co53 (19, 29). Allelic cloning of a cDNA sample from DLD-1 showed that the mutations existed on separate alleles; however, no cDNA sample was available to study the allelic pattern of the Co53 mutations.

The cell line VACO481 was found to have an expansion of a 6-bp GT repeat to an 8-bp GT repeat, resulting in a frameshift at amino acid 533. In KM12, a T-to-C change at nt 1696 resulted in a leucine-to-proline change at amino acid 454 in the protein. Both cell lines are known to be compound heterozygote mutants in the *TGFBR2* gene (19, 29), and VACO481 is null for expression of the Tgfbr2 protein (19). However, a cDNA sample was not available to determine the allelic pattern of the KM12 mutations.

The mutations identified in this study are summarized in Table 1.

Comparison of Mutational Data in *ACVR2* and *TGFBR2* Genes. A mathematical model has been proposed to evaluate whether a gene containing a polynucleotide tract is under selective pressures that favor inactivation in MSI tumors (36). This model is based on a predicted relationship between the frequency of biallelic and heterozygous mutations for any gene containing a polynucleotide repeat region. Assigning the spontaneous mutation rate per allele as "p," we expect under the null hypothesis (the mutations in the alleles not being subject to selective biases) that the observed biallelic mutant frequency in tumors would equal the square of the spontaneous mutation frequency per allele, or p^2. The heterozygote frequency is estimated by $2p - 2p^2$ in diploid tumors. The equation $1 - (2p - 2p^2) - p^2$ therefore estimates the proportion of tumors homozygous for the

Table 1 *ACVR2 mutations in gastrointestinal cancers*

Sample	Status of each allele	Exons	Predicted change
Multiple cancers[a]	1303 del A/1303 del A	10/10	Frameshift/frameshift
CX7[b]	1303 del A/1303 ins A	10/10	Frameshift/frameshift
LIM1215[b]	1268 CCA to 1268 CAA/ 1303 del A	10/10	P 423 Q/Frameshift
PX335	1303 del A/wild-type	10/none	Frameshift/none
RKO[b]	1303 del A/1303 del AA	10/10	Frameshift/frameshift
VACO444[b]	278 del A/1303 del A	3/10	Frameshift/frameshift
VACO481[b]	278 ins A/1303 del A	3/10	Frameshift/frameshift
PX280 (non-MSI)	1363 del T/deletion (LOH)	11	455 Frameshift

[a] Cell lines and xenografts containing this biallelic mutation include the following: C, CX2, CX10, CX29, DLD-1, HCT116, KM12, LIM2412, LOVO, LS180, Co53, MX17, PX26, PX196, PX287, SW48, VACC1430, VACO5, VACO6, and VACO457.

[b] Biallelic mutants (compound mutant heterozygotes).

Table 2 *Expected versus observed mutation frequencies in microsatellite unstable tumors*[a]

Locus or gene	SMT	Obs. hom. wt. freq.	Obs. het. mut. freq.	Obs. biallelic mut. freq.	Exp. het. mut. freq.[b]	Exp. biallelic mut. freq.[b]	Binomal sum[c]
Examples		10%			46%	44%	
		25%			50%	25%	
		50%			42%	8%	
		75%			22%	2%	
		90%			10%	0.2%	
Published data							
SMT 14	$(G)_8$	54%	38%	8%	39%	7%	>0.1
SMT 4	$(A)_8$	71%	25%	4%	27%	3%	>0.1
SMT 28	$(G)_8$	71%	25%	4%	27%	3%	>0.1
SMT 10	$(A)_8$	75%	25%	0%	23%	2%	>0.1
SMT 1	$(G)_8$	75%	25%	0%	23%	2%	>0.1
SMT 25	$(G)_8$	79%	17%	4%	20%	1%	>0.1
SMT 19	$(A)_8$	79%	21%	0%	20%	1%	>0.1
SMT 18	$(G)_8$	88%	13%	0%	12%	0.4%	>0.1
SMT 26	$(A)_8$	88%	13%	0%	12%	0.4%	>0.1
SMT 2	$(A)_8$	92%	8%	0%	8%	0.2%	>0.1
SMT 3, 7, 17, 21, 22, 27	$(A)_8$	96%	4%	0%	4%	0.04%	N/A
SMT 24	$(A)_8$	96%	0%	4%	4%	0.04%	N/A
SMT 5, 8, 9, 11, 12, 13, 20, 23	$(A)_8$	100%	0%	0%	0%	0%	N/A
BAX							
SMT mutations	$(G)_8$	43%	29%	29%	45%	12%	0.009
Current study							
TGFBR2							
All mutations		0%[d]	7%[e]	93%[e]	29%	68%	0.004
SMT mutations	$(A)_{10}$	0%[d]	21%	79%	29%	68%	0.24
ACVR2							
All mutations		7%	4%	89%	39%	54%	0.00001
SMT mutations	$(A)_8$ x 2	7%	7%	86%	39%	54%	0.0001

[a] Obs., observed; hom., homozygous; wt., wild type; freq., frequency; mut., mutation; het., heterozygous; SMT, short mononucleotide tracts.
[b] Expected values are derived from the mathematical model on the assumption of the given hypothetical or observed wild-type frequencies.
[c] The binomial sum is not reported for expected biallelic mutation frequencies of 0.
[d] 3% was imputed for calculation of expected frequencies, as described in the text. The binomial sum will be lower with higher imputed values and *vice versa.*
[e] Assumption of biallelic mutations in Co53 and KM12.

wild-type allele, where the mutational accumulation is the product of unselected chance events (A related formula first reported by G. H. Hardy is used in another setting to describe the equilibrium of allele association in a natural population; Ref. 41).

Solving for the spontaneous mutation rate per allele ("p") produces a set of wild-type, heterozygote mutant, and biallelic mutant proportions that are expected under the null hypothesis. In cases of selection for clones having genetic inactivation during tumorigenesis, the observed frequency of biallelic mutant tumors would be higher than the predicted proportions and that of the heterozygous mutant tumors, lower. As suggested (36), the observed frequency of homozygous wild-type tumors can be used to solve for "p." The equation for the estimated wild-type homozygous tumors can then be solved in a quadratic equation of the form $ax^2 + bx + c = 0$, where $0 < x < 1$.

Data reflecting the mutational status of *ACVR2* and *TGFBR2* were assembled for the 28 MSI tumors studied. The observed data were considered in two different ways. The first was to only consider frameshift mutations, a conservative means by which to handle the data, restricted to mutations that are obviously "obliterative." In the second treatment of the data, missense mutations are included and presumed to inactivate gene function. In the few cases where the allelic mutational status was not studied (Co53 and KM12 for the *TGFBR2* gene), the mutational frequencies were calculated, assuming that the mutations found in each tumor occurred on separate alleles. This analysis is summarized in Table 2, along with examples of expected biallelic and heterozygous mutation frequencies based on given homozygous wild-type frequencies. A binomial sum was calculated based on the expected frequency of biallelic mutations for each locus analyzed.

A previous study (35) had examined SMTs in noncoding DNA of the 24 MSI colorectal tumors, identifying considerable locus-specific variation in tract lengths. The mathematical model accommodates such variation and was used to analyze the data for 25 of the 29 SMTs from the previous study (Table 2). Four of the 29 SMT markers known to have germ-line polymorphisms were excluded (35, 42). As expected, there was no evidence of selection for inactivation of these SMTs; the binomial sums in each case were >0.1, within expected variation. In contrast, the mutational frequencies of the poly$(G)_8$ tract of the *BAX* gene, a well-characterized candidate tumor suppressor gene (43), had evidence of selection for biallelic inactivation (binomial sum of 0.00915; Table 2).

In the case of *ACVR2*, 2 of 28 carcinomas were homozygous wild type, an observed frequency of 7% (Table 2). In both treatments of the *ACVR2* data, the data indicate selection against wild-type *ACVR2* alleles, with a significant increase of biallelic mutant carcinomas accomplished at the expense of a decrease of the expected heterozygous carcinomas (binomial sums of 0.00013 and 0.000013, respectively).

Although previous studies reported considerable variability in the pattern of longer, and presumably more unstable, mononucleotide runs (29), our current work (Table 2) and a recent study (35) indicate a high concordance of the observed data and the mathematical model for unselected 8-bp mononucleotide tracts.

In the case of the *TGFBR2* gene, containing a 10-bp mononucleotide tract, no wild-type genotypes were observed among 28 tumors (Table 2). The true wild-type genotype frequency for *TGFBR2* inactivation in tumors is known not to be 0 (29), and so a wild-type frequency value >0 should probably be imputed for the mathematical model (Table 2). In the treatment of the *TGFBR2* gene with all mutations, 93% biallelic mutant tumors were observed (26 of 28, assuming biallelic mutations in Co53 and KM12), and 7% heterozygous mutant tumors (2 of 28) were observed (binomial sum of 0.004).

Familial Pancreatic Cancer and ACVR2. No germ-line mutations of the *ACVR2* gene were found in samples of 29 affected individuals.

Selection Against Wild-type ACVR2 in Cancer. Results of the mathematical model suggest that biallelic inactivation is over-represented in gastrointestinal MSI carcinomas, even when the high rate of

mutation is taken into account. Interestingly, previous studies indicated that poly(A) tracts < 10 bp are rarely mutated in MSI tumors (29, 31, 32, 35, 42, 44). The $(A)_8$ tract in exon 10 of the *ACVR2* gene, despite its small size, appears to have prevalence of mutation at least comparable with the slightly larger and well-characterized $(A)_{10}$ tract located in exon 3 of the *TGFBR2* gene (31, 32).

The frequency of biallelic frameshift mutations in the exon 10 $(A)_8$ tract of the *ACVR2* gene reported in this study is significantly higher (86 *versus* 58 and 44%) than reported in two previous studies of a single mononucleotide tract of the gene (31, 32). The increased observed frequency of biallelic frameshift mutations seen here is attributable to the use of enriched neoplastic samples, such as xenografts and cell lines (previous studies used only primary tumors, which can harbor a significant proportion of non-neoplastic cells), and to the determination of the entire gene sequence.

The biallelic inactivation of the *ACVR2* gene was also seen in one of seven non-MSI carcinomas having LOH. Although the low frequency of LOH of chromosomal arm 2q in pancreatic cancer limited the tumors available for study, *ACVR2* mutation is not merely a property of the MSI phenotype. Similarly, mutations in both MSI and non-MSI tumors are reported for the *TGFBR2* gene (17, 19, 20, 29, 30, 45–47).

The *ACVR2* gene therefore may be a tumor suppressor gene in gastrointestinal cancers and other tumors. *ACVR2* is part of an extended family of extracellular signaling receptors and mediators of the TGF-β superfamily (48, 49), which has numerous tumor suppressors as members (*TGFBR2*, *TGFBR1*, *ACVR1B*, *MADH2/SMAD2*, and *MADH4*/*SMAD4/DPC4*). It is possible that the heterozygous mutations found in MSI carcinomas may cause expression of a truncated dominant-negative type II receptor. Transgenic mice expressing a truncated form of the Acvr2 protein suffered impaired pancreatic differentiation (50). This functional evidence, together with the results of this and the previous study (31), emphasizes the importance of activin signaling through the activin type II receptor for appropriate cellular growth and differentiation.

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