TGFBR1 AND CANCER SUSCEPTIBILITY

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ABSTRACT

Transforming growth factor beta (TGF-β) is a potent inhibitor of cell growth. *TGFBR1**6A is a polymorphism consisting of a 9-base pair in-frame deletion within exon 1 of the type I TGF-β receptor (*TGFBR1*), which results in a receptor with decreased TGF-β signaling capability. The discovery of an association between *TGFBR1**6A and cancer susceptibility led to the hypothesis that hypomorphic variants of the TGF-β signaling pathway may predispose to the development of cancer. This hypothesis was tested *in vivo* with the development of a mouse model of *Tgfbr1* haploinsufficiency. $Tgfbr1^{+/-}$ mice developed twice as many intestinal tumors as $Tgfbr1^{+/+}$. *Tgfbr1* haploinsufficiency was also associated with early onset adenocarcinoma and increased tumor cell proliferation. A case control study identified two haplotypes associated with constitutively decreased *TGFBR1* and substantially increased colorectal cancer risk indicating that *TGFBR1* may act as a potent modifier of cancer risk.

INTRODUCTION

During the past two decades, the transforming growth factor beta (TGF-β) signaling pathway has emerged as a central mediator of cancer progression because of its capability to regulate cell growth, differentiation, and migration. There is growing evidence that the TGF-β signaling pathway and various members of the TGF-β superfamily, which include bone morphogenic proteins, inhibins, and activins, are frequently mutated in cancer (1–3). We identified the first mutation of the type I TGF-β receptor (*TGFBR1*), a 9-base pair in-

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frame deletion within *TGFBR1* exon 1, which results in the deletion of three alanines from a 9 alanine repeat segment (4). This mutation was initially named *TβR-I(6A)* (4), which has since been updated to *TGFBR1**6A in keeping with the official HUGO gene nomenclature of the type I TGF-β receptor, *TGFBR1* (5). Despite not being a single nucleotide polymorphism (SNP), as it consists of a 9-nucleotide deletion, *TGFBR1**6A was later assigned a unique SNP number, rs11466445, which has been used in several recent reports (6–8). Hence, *TβR-I(6A)*, *TGFBR1**6A, and rs11466445 refer to the same truncated variant of *TGFBR1*.

Functional studies have revealed that the mature TGFBR1*6A receptor transduces TGF-β signals less effectively than its wild type counterpart, TGFBR1 (9,10). Having observed a higher than expected number of *TGFBR1**6A homozygotes among tumor and nontumor DNA from patients with a diagnosis of cancer, we hypothesized that *TGFBR1**6A may act as a tumor susceptibility allele (10). To test this hypothesis, we genotyped *TGFBR1* exon 1 in a case control study of 851 patients with a diagnosis of cancer and 735 healthy volunteers and found that there was a significantly higher proportion of *TGFBR1**6A heterozygotes and homozygotes among patients with a diagnosis of cancer compared to healthy individuals (10).

The findings of an increased frequency of *TGFBR1**6A carriers among patients with a diagnosis of cancer, together with the discovery that TGFBR1*6A has impaired TGF-β signaling, led us to more broadly hypothesize that decreased TGFBR1 signaling may predispose to the development of cancer (11). During the past decade, we have further tested this hypothesis with the use of novel mouse models (12,13), and current findings suggest that constitutively decreased TGFBR1 signaling is a potent modifier of cancer susceptibility and progression (12–17).

TGF-β Signaling

The TGF-β ligand exists in three different isoforms: TGFB1, TGFB2, and TGFB3. The TGF-β isoforms are secreted as latent homodimeric polypeptides. Each isoform is encoded by a distinct gene and is expressed in a tissue-specific fashion. The amino acid sequences of the three isoforms are 70% to 80% homologous. TGFB1 is expressed in endothelial, hematopoietic, and connective tissue cells; TGFB2 in epithelial and neuronal cells; and TGFB3 primarily in mesenchymal cells (18). Upon activation, TGF-β binds first to a type II receptor (TGFBR2). The TGF-β ligand/TGFBR2 complex then binds to TGFBR1

and triggers TGFBR1 phosphorylation resulting in downstream signaling activation. The activated ligand/receptor complex is a pentameric unit consisting of one ligand (TGFB1, TGFB2, or TGFB3), two TGFBR1 receptors, and two TGFBR2 receptors (19). The activated ligand/receptor complex initiates both SMAD signaling and SMAD-independent downstream signaling.

The activated complex initiates SMAD signaling by phosphorylation of the carboxy-terminal serine residue of the SMAD intracellular messenger proteins, SMAD2 and SMAD3 (20,21). This phosphorylation results in oligomerization of SMAD2 and SMAD3 with SMAD4, a necessary step for nuclear translocation (22). Once in the nucleus, the SMAD complex acts as transcriptional activator or repressor of several genes. Non-SMAD signaling consists in the activation of several other pathways including the MAPK, RHOA, and PI3K-AKT pathways by the same ligand/receptor complex (23).

It has been generally accepted that the SMAD signaling pathway controls the proliferation of epithelial cells and the non-SMAD signaling pathway induces cell migration and invasion. The downstream signaling events associated with TGF-β–mediated growth inhibition involve various pathways including downregulation of cyclin-dependent kinase 4 (CDK4) and v-myc avian myelocytomatosis viral oncogene homolog (MYC), as well as upregulation of cyclin-dependent kinase inhibitor 1A (CDKN1A), formerly known as p21, and cyclin-dependent kinase inhibitor 2B (CDKN2B), formerly known as p15 (24–26). Additionally, the SMAD-dependent pathway results in programmed cell death through activation of Kruppel-like factor 10 (KLF10), death-associated protein kinase 1 (DAPK1), and BCL2-like 11 (BCL2L11) (27). The downstream signaling events associated with migration and invasion involve inhibition of inhibitor of DNA binding 1 (ID1), which results in decreased expression of cadherin 1 (CDH1) and zona occludens 1 (TJP1), two factors involved in the maintenance of the epithelial phenotype (28).

Alterations of the TGF-β Signaling Pathway in Cancer

TGF-β effectively inhibits the growth of both epithelial and hematopoietic cell lines. In normal cells, TGF-β acts as a tumor suppressor by inhibiting cell growth, promoting cellular differentiation, and/or inducing apoptosis in a context-dependent manner. However, during the stepwise transition from premalignant to malignant cells, TGF-β growth inhibitory properties are progressively lost by virtually all transformed cells due to the functional loss of receptors and intracel-

lular messengers of the TGF-β pathway (29,30). The functional role of the TGF-β pathway in malignant diseases such as colorectal cancer is further exemplified by the fact that 4 of the 69 most frequently mutated genes are constitutive elements of the TGF-β signaling pathway: *TGFBR2*, *SMAD2*, *SMAD3*, and *SMAD4* (31). Loss of TGF-β responsiveness can occur through loss-of-function mutations, loss of expression, or promoter methylation of genes. Several studies suggest that inactivation of the TGF-β signaling pathway is predominantly due to promoter methylation of TGF-β superfamily components (32-34). Importantly, loss of TGF-β signaling appears to be associated with advanced disease and poor prognosis in some malignancies such as colorectal cancer (35, 36), whereas in others such as head and neck tumors, decreased levels of phosphorylated SMAD2 or SMAD3 (pSMAD2/pSMAD3) are associated with a better prognosis (37).

Concomitantly with loss of TGF-β responsiveness, cancer cells secrete larger amounts of TGF-β ligand than their normal counterparts. Increased TGF-β ligand secretion is particularly prominent in the most advanced stages of tumor progression. TGFB1 is the TGF-β isoform most commonly overexpressed in several common tumor types including breast cancer (38), pancreatic cancer (39), and lung cancer (40). In each of these diseases, high levels of TGFB1 are associated with advanced stage and/or shorter survival (38–40). Increased TGF-β ligand levels result in evasion of immune surveillance as TGF-β is one of the most potent naturally occurring inhibitor of lymphocytes. We have recently comprehensively reviewed the duality of TGF-β functions between tumor prevention and carcinogenesis (41).

TGFBR1 and Cancer Susceptibility

Hypothesis and Proof of Concept. Unrestricted cell growth due to a lack of growth inhibitory activity is probably the most important of the possible consequences resulting from a defect in TGF-β function. This hypothesis was originally confirmed by the discovery that *Tgfb1*$^{+/-}$ and *Tgfbr2*$^{+/-}$ haploinsufficient mice, both of which contain genetic alterations that result in decreased TGF-β signaling, have an increased susceptibility to develop cancer relative to their wild type counterparts (42,43). Whether these conceptual findings in mice are relevant in humans were unknown until the discovery of *TGFBR1**6A, a common variant with decreased TGFBR1-mediated TGF-β signaling capabilities, which was also found to be associated with risk for cancer (9,10). This led us to hypothesize that constitutively decreased TGFBR1 signaling may be causatively involved in the development of cancer (11).

To test the hypothesis that constitutively decreased TGFBR1 may contribute to the development of cancer, we developed a novel mouse model of *Tgfbr1* haploinsufficiency, in which part of *Tgfbr1* exon 1 as well as 1.1 kb mouse genomic sequence upstream of *Tgfbr1* exon 1 were removed (12). *Tgfbr1* haploinsufficient mice were viable and fertile and were normal in their morphology and behavior. Tgfbr1 expression levels in tissues were decreased by approximately 50%, resulting in decreased TGF-β signaling by approximately 22% to 25% (12). Because the gastrointestinal tract is a common site of cancer in humans with constitutively altered signaling due to mutations within the type 1A bone morphogenic protein receptor gene (*BMPR1A*) (44), we assessed the impact of *Tgfbr1* haploinsufficiency on the development of $Apc^{Min/+}$-mediated intestinal tumors. Mice were sacrificed at 12 weeks and examined for intestinal tumors. We did not observe any tumors in the small and large bowels of $Tgfbr1^{+/+}$ and $Tgfbr1^{+/-}$ mice in wild type *Apc* background. However, $Apc^{Min/+}$;$Tgfbr1^{+/+}$ mice developed an average of 5.4 $\pm$ 1.7 tumors (mean $\pm$ SEM), whereas the number of tumors observed in $Apc^{Min/+}$;$Tgfbr1^{+/-}$ mice was almost three times higher: 14.5 $\pm$ 1.1 tumors, a highly significant difference of 9.8 tumors (95% confidence interval [CI], 4.8–13.4; $P = .0004$) (12).

To determine the reproducibility of our early findings obtained in a mixed 129SvIm $\times$ C57BL/6 background in 2006, we repeated these experiments with $Tgfbr1^{+/-}$ mice, which were fully backcrossed into the C57BL/6 background. There was an average of 30.2 $\pm$ 0.9 tumors in $Apc^{Min/+}$;$Tgfbr1^{+/+}$ mice and 61.4 $\pm$ 3.4 tumors in $Apc^{Min/+}$;$Tgfbr1^{+/-}$ mice (mean $\pm$ SEM). Again, the difference in the number of tumors between the two groups was highly significant: 31.2 tumors (95% CI, 25.3–37.2; $P = 4.8 \times 10^{-5}$). Importantly, the number of colonic tumors alone was significantly higher among $Apc^{Min/+}$;$Tgfbr1^{+/-}$ mice (4.9 $\pm$ 0.3) than among $Apc^{Min/+}$;$Tgfbr1^{+/+}$ mice (3.0 $\pm$ 0.4; $P = .0005$) (12).

To characterize the *in vivo* consequences of constitutively decreased TGF-β signaling, we performed pSmad2 immunostaining of intestinal tissue and tumor sections. Although pSmad2 staining was homogeneous throughout the intestinal mucosa of $Apc^{Min/+}$;$Tgfbr1^{+/+}$ mice, we observed reduced pSmad2 staining in the crypts of $Apc^{Min/+}$;$Tgfbr1^{+/-}$ mice. We also performed pSmad3 immunostaining of the same tissues and observed homogeneous pSmad3 staining in the crypts of $Apc^{Min/+}$;$Tgfbr1^{+/+}$ mice whereas pSmad3 staining was markedly reduced in the crypts of $Apc^{Min/+}$;$Tgfbr1^{+/-}$ mice, providing strong evidence that *Tgfbr1* haploinsufficiency results in decreased phosphorylation of both receptor Smads within the intestinal epithe-

lial crypts, thus resulting in overall decreased Smad-mediated TGF-β signaling *in vivo*. To determine whether the differential expression of Smads within the intestinal crypts modifies cellular proliferation *in vivo*, we assessed the levels of proliferating cell nuclear antigen (PCNA) in the normal intestinal epithelium of $Apc^{Min/+}$;$Tgfbr1^{+/+}$ and $Apc^{Min/+}$;$Tgfbr1^{+/-}$ mice. PCNA staining was significantly more intense in the intestinal crypts of $Apc^{Min/+}$;$Tgfbr1^{+/-}$ mice than in their wild type counterpart, thus confirming *in vivo* the observed *in vitro* increased cellular proliferation of $Tgfbr1^{+/-}$ upon exposure to TGF-β.

To determine whether *Tgfbr1* haploinsufficiency has an impact on tumor proliferation *in vivo*, we assessed the levels of PCNA in tumors of $Apc^{Min/+}$; $Tgfbr1^{+/+}$ and $Apc^{Min/+}$;$Tgfbr1^{+/-}$ mice. PCNA staining was significantly more intense in $Apc^{Min/+}$; $Tgfbr1^{+/-}$ tumors than in their wild type counterpart. This provided the first *in vivo* evidence that decreased but not abrogated Tgfbr1-mediated signaling confers a selective growth advantage to tumor cells.

Several $Apc^{Min/+}$;$Tgfbr1^{+/-}$ mice, both in the mixed background and in the pure C57BL/6 background, exhibited large colonic tumors with a maximal diameter greater than 7 mm. Histologic analysis of these tumors revealed the presence of adenocarcinoma. In contrast, the largest tumors in the $Apc^{Min/+}$;$Tgfbr1^{+/+}$ mice in either the mixed 129SvIm/C57BL/6 or the pure C57BL/6 backgrounds were 3 mm in size and none of them harbored carcinoma. Among all mice examined at 12 weeks, the proportion of $Apc^{Min/+}$;$Tgfbr1^{+/-}$ mice with colonic tumors greater than 7 mm (35.3%) harboring carcinoma was significantly higher than that of $Apc^{Min/+}$;$Tgfbr1^{+/+}$ mice (0%; $P = .018$).

Current Evidence From Case Control Studies. In the 16 years since identification of *TGFBR1**6A, numerous case control studies and five meta-analyses have been published (5,15–17,45). We first identified and reported an association with colorectal cancer in 1999 (10). An association with breast cancer was first reported by Baxter et al in 2002 (46). The first meta-analysis of seven case-control studies was published in 2003. It found an association between *TGFBR1**6A and cancer risk, odds ratio (OR) 1.26; 95% CI 1.07–1.49. Cancer risk for *TGFBR1**6A homozygotes (OR, 2.53; 95% CI, 1.39–4.61) was higher than that of *TGFBR1**6A heterozygotes (OR, 1.26; 95% CI, 1.04–1.51) (5). Importantly, it unveiled an association with ovarian cancer and hematologic malignancies in addition to breast cancer (5). An association with colorectal cancer risk was only found among *TGFBR1**6A carriers from the United States (OR, 1.38; 95% CI, 1.02–1.86) but not among European *TGFBR1**6A carriers. A subsequent meta-analysis of 12 case-control studies confirmed the broad association between

*TGFBR1**6A and cancer risk (OR, 1.24; 95% CI, 1.10–1.40), as well as the higher risk associated with *TGFBR1**6A homozygosity (OR, 1.70; 95% CI, 1.11–2.59) than with *TGFBR1**6A heterozygosity (OR, 1.70; 95% CI, 1.11 to 2.59) (45).

Subsequent analyses included more than twice as many case-control studies. In 2010, Liao et al performed a meta-analysis of 32 case-control studies including 13,662 cases and 14,147 controls (15). Cancer risk was associated with *TGFBR1**6A in all genetic models analyzed. Analysis by cancer types revealed significant association with breast cancer (OR, 1.16; 95% CI, 1.01–1.34) and ovarian cancer (OR, 1.24; 95% CI, 1.00–1.54). No significant association between *TGFBR1**6A and risk for colorectal cancer, bladder cancer, prostate cancer, and lung cancer were found. However, in 2012 a meta-analysis of nine case-control studies including 6765 patients with colorectal cancer and 8496 unrelated controls was performed by Zhang et al and reported a significant association between *TGFBR1**6A heterozygosity and colorectal cancer risk (OR, 1.12; 95% CI, 1.02–1.23). In contrast, *TGFBR1**6A homozygosity was not associated with risk for colorectal cancer (OR, 1.13; 95% CI, 0.80–1.58). The most recent and comprehensive meta-analysis published to date included 35 case-control studies and confirmed an association between *TGFBR1**6A and cancer risk in all genetic models studied (dominant OR, 1.11; 95% CI, 1.04–1.18; recessive OR, 1.36; 95% CI 1.11–1.66; additive OR, 1.13; 95% CI 1.05–1.20) (17). In conclusion, there is strong evidence that *TGFBR1**6A is associated with cancer risk.

Following our original discovery that constitutively decreased Tgfbr1 signaling is a potent modifier of intestinal tumor development in mice (12), we hypothesized that a similar phenotype might exist in humans. We chose patients with microsatellite instability (MSI) −negative colorectal cancer to exclude most patients with somatically acquired *TGFBR2* mutations, a common finding in MSI-positive colorectal cancer (47). Using a method based on allelic specific expression, we genotyped 242 patients with MSI-negative colorectal cancer and 195 geographically matched healthy individuals for three SNPs located in *TGFBR1* 3' untranslated region. Of these individuals, 138 patients with a diagnosis of colorectal cancer and 109 healthy individuals were heterozygous for these SNPs. Significant differences in *TGFBR1* allelic expression, for example, one allele's expression level being at least 1.5-fold than the other one, were observed in cases and controls. Interestingly, this phenotype was observed in 29 of 138 (21%) informative patients with a diagnosis of colorectal cancer but only in 3 of 105 (3%) healthy individuals (14).

We then determined whether the observed ratios falling outside 1.5 were due to increased or decreased expression of one allele. Using hybrid clones monoallelic for chromosome 9 created from two individuals with allelic ratios higher than 1.5, reverse transcriptase polymerase chain reaction (RT-PCR) experiments were performed to assess TGFBR1 expression. Each of the four hybrid clones contained either the maternal or paternal copy of chromosome 9, plus the mouse genome (14). TGFBR1 expression levels for each of the four mono allelic hybrid clones were compared with the corresponding value for a mouse reference gene. One allele showed reduced TGFBR1 expression in both patients. These experiments provided strong support for the novel notion of lowered TGFBR1 expression in one allele as the cause for differential allelic TGFBR1 expression. To assess the impact of this phenotype on TGF-β signaling, we exposed lymphoblastoid cell lines from four patients exhibiting this phenotype and compared them with lymphoblastoid cell lines from four patients that did not exhibit the same phenotype. We observed significantly lower pSMAD2 and pSMAD3 levels in lymphoblastoid cell lines from patients exhibiting the phenotype. Hence, constitutively decreased TGFBR1 expression on one allele was associated with decreased TGF-β signaling.

In a separate study of 118 consecutive patients with biopsy-proven adenocarcinoma of the colon or the rectum, 74 (62.7%) of them being heterozygous for informative *TGFBR1* SNPs, 11 (9.3%) patients had evidence of constitutively decreased TGFBR1 allelic expression (48). We selected 18 tag SNPs in addition to *TGFBR1**6A and genotyped the 19 variants in all colorectal cancer cases. *TGFBR1**6A, rs7034462, and rs11568785 were associated with decreased TGFBR1 expression.

Current Evidence From Functional Studies. Using mink lung epithelial cell lines devoid of TGFBR1, we established stably transfected TGFBR1 and TGFBR1*6A cell lines. Upon exposure to various concentrations of TGF-β1 ranging from 0.5–300 pM, we found that TGFBR1*6A cell lines transduced TGF-β growth inhibitory signals less effectively than TGFBR1 cell lines (10). A separate group of investigators conducted experiments using the same mink lung epithelial cell lines, which were transiently transfected with either TGFBR1 or TGFBR1*6A. These experiments also revealed that TGFBR1*6A was less effective than TGFBR1 with respect to TGF-β signaling (9). TGF-β binding assays did not reveal any difference in TGF-β binding between TGFBR1 and TGFBR1*6A (10). Furthermore, the half-life of TGFBR1 and TGFBR1*6A proteins in the stably transfected cell lines, which was assessed by pulse-chased metabolic label-

ing experiments, did not show any difference in metabolic stability between TGFBR1 and TGFBR1*6A (10).

Although it had been proposed that the TGFBR1 signal sequence cleavage site was located within the TGFBR1 polyalanine tract, for example, where the *TGFBR1**6A 9 bp deletion is located (49), computerized prediction using the SignalP program (50) suggested that the signal sequences of TGFBR1 and TGFBR1*6A were likely to be cleaved by the signal peptidase enzyme between Ala33 and Leu34 for TGFBR1 and between Ala30 and Leu31 for TGFBR1*6A. Indeed, this site had all the hallmarks of a classical signal sequence cleavage site with Ala at positions -1 and -3 and Pro at position -5 relative to the cleavage site (51). In theory, the deletion of 3 alanines in TGFBR1*6A should not affect the cleavage of *6A and *9A signal sequences. On the other hand, differences in TGF-β–mediated growth inhibition (9,10) and epidemiological evidence that *TGFBR1**6A acts as a tumor susceptibility allele suggested that the deletion of three alanines might have significant functional consequences. To resolve this quandary, we determined the amino terminus of the mature TGFBR1*6A and TGFBR1 receptors. We found that the signal sequence of TGFBR1 and TGFBR1*6A are cleaved at the same site resulting in identical mature receptors (52).

Interestingly, we discovered that *TGFBR1**6A was somatically acquired in a small fraction (1.8 to 2.5%) of primary colorectal and head and neck cancers as well as in a large fraction (30%) of colorectal cancer metastases (52). We showed that *TGFBR1**6A somatic acquisition was not associated with a mutated phenotype, loss of heterozygosity, or MSI. These findings prompted us to investigate whether *TGFBR1**6A might confer a selective growth advantage to cancer cells. We transfected DLD-1 colorectal cancer cells and MCF-7 breast cancer cells with *TGFBR1**6A and found that TGFBR1*6A was capable of switching growth inhibitory signal into growth stimulatory signals in both cell lines (52).

Subsequently, we sought to determine whether *TGFBR1**6A may act as an oncogene. We assessed its effects on NIH-3T3 cells as well as its effects on migration and invasion (53). *TGFBR1**6A did not have any effect on H-Ras–induced transformation whereas *TGFBR1* enhanced transformation. Hence, the previously identified TGFBR1*6A hypomorphic properties with respect to TGF-β signaling result in decreased oncogenesis when compared to TGFBR1. These experiments provide strong evidence that *TGFBR1**6A is not an oncogene.

To broadly explore possible roles for TGFBR1*6A in tumor development and progression, we studied its ability to modify cell migra-

tion and invasion of MCF-7 cells. Both migration and invasion of *TGFBR1**6A MCF-7 cells were significantly higher than that of *TGFBR1* MCF-7 cells (53). To investigate the molecular mechanisms accounting for the differences in migration and invasion of *TGFBR1**6A and *TGFBR1* cells, we assessed and compared their respective gene expression profile. We identified two genes involved in cell migration that were downregulated in *TGFBR1**6A cells compared with *TGFBR1* cells, Rho GTPase activating protein 5 (*ARHGAP5*), and fibronectin 1 (*FN1*). ARHGAP5 and FN1 expression was downregulated in MCF-7 cells stably transfected with a kinase-inactivated TGFBR1*6A construct, which provides strong evidence that downregulation of these two genes is independent of TGF-β signaling. Functional assays showed that TGFBR1*6A-mediated decreased ARHGAP5 expression is associated with higher RhoA activation, a crucial mediator of cell migration. We also found that EPH receptor B2 (EPHB2 formerly named ERK) activation was higher in cells that harbor the TGFBR1*6A allele. In summary, these findings indicate that *TGFBR1**6A is not an oncogene but enhances MCF-7 cell migration and invasion through RhoA and EPHB2 pathway activation and downregulates two crucial mediators of this phenotype. These results provide the first evidence that *TGFBR1**6A may contribute to cancer progression in a TGF-β signaling independent manner.

CONCLUSIONS AND FUTURE DIRECTIONS

The results from five successive meta-analyses conducted and published between 2003 and 2012 consistently show an association between *TGFBR1**6A and risk for cancer. The latest and most comprehensive results that included 32 case-control studies with 19,767 cases and 18,516 controls shows an association between *TGFBR1**6A and cancer risk in all genetic models used (dominant OR = 1.11, 95% CI = 1.04,1.18; recessive: OR = 1.36, 95% CI = 1.11,1.66; additive: OR = 1.13, 95% CI = 1.05,1.20) (17). Several mutually nonexclusive mechanisms of action have been identified that account for *TGFBR1**6A effects on cancer development and progression. First, TGFBR1*6A transduces TGF-β less effectively than TGFBR1, which allows for increased cell proliferation both *in vitro* (9, 10), as well as *in vivo* at sites of rapid cell proliferation such as in the intestinal crypts (12). Second, TGFBR1*6A switches TGF-β growth inhibitory signals into growth stimulatory signals in breast and colorectal cancer cells (52). Third, TGFBR1*6A enhances tumor cell migration and invasion through activation of RhoA and EPHB2 (53). TGFBR1*6A may there-

fore emerge as a therapeutic target for chemoprevention for treatment of established breast and colorectal tumors.

Constitutively decreased TGFBR1 signaling is emerging as a potent modifier of colorectal cancer risk. However, specific SNPs and/or genotypes have not yet been characterized and validated that would allow for identification of at-risk individuals. Additional studies are needed to further characterize biomarkers of TGFBR1 haploinsufficiency as well as the exact magnitude of cancer risk associated with phenotype.

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