Loss of Stromal IMP1 Promotes a Tumorigenic Microenvironment in the Colon

Kathryn E. Hamilton1,2, Priya Chatterji1,2, Emma T. Lundsmith1,2, Sarah F. Andres1,2, Veronique Giroux1,2, Philip D. Hicks1,2, Felicite K. Noubissi3,4,5, Vladimir S. Spiegelman3,4, and Anil K. Rustgi1,2,6,7

Abstract

The colon tumor microenvironment is becoming increasingly recognized as a complex but central player in the development of many cancers. Previously, we identified an oncogenic role for the mRNA-binding protein IMP1 (IGF2BP1) in the epithelium during colon tumorigenesis. In the current study, we reveal the contribution of stromal IMP1 in the context of colitis-associated colon tumorigenesis. Interestingly, stromal deletion of Imp1 in the azoxymethane/dextran sodium sulfate (AOM/DSS) model of colitis-associated cancer resulted in increased tumor numbers of larger size and more advanced histologic grade than controls. In addition, Imp1 hypomorphic mice demonstrated an increase in hepatocyte growth factor (HGF), which has not been associated with regulation via IMP1. Genetic knockdown of Imp1 in human primary fibroblasts confirmed an increase in HGF with Imp1 loss, demonstrating a specific, cell-autonomous role for Imp1 loss to increase HGF expression. Taken together, these data demonstrate a novel tumor-suppressive role for IMP1 in colon stromal cells and underscore an exquisite, context-specific function for mRNA-binding proteins, such as IMP1, in disease states.

Implications: The tumorous suppressive role of stromal IMP1 and its ability to modulate protumorigenic factors suggest that IMP1 status is important for the initiation and growth of epithelial tumors. Mol Cancer Res; 13(11); 1478–86. ©2015 AACR.

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Introduction

The colon tumor microenvironment is becoming recognized as an increasingly important component in the development of colorectal cancer. Chronic inflammation, particularly in cases arising from colitis (colitis-associated colorectal cancer, CAC), is a major contributor to the protumorigenic microenvironment (1–3). In addition to inflammatory pathways, growth factors, such as EGF, FGF, hepatocyte growth factor (HGF) produced by cancer-associated fibroblasts (CAF), may potentiate tumor growth (reviewed in ref. 4, 5). For example, HGF from stromal myofibroblasts has been shown to facilitate Wnt signaling and "stemness" in colon cancer cells (6). Understanding mechanisms regulating these pathways is of utmost importance, as mounting evidence in patients suggests that high expression of certain growth factors in the stroma may confer chemoresistance to traditional therapeutics (7–9).

Posttranscriptional regulation of tissue homeostasis via mRNA-binding proteins, such as Igf2 mRNA binding protein 1 (IMP1), has been implicated in critical processes, including development, stem cell dynamics, and cancer. While it has been appreciated that mRNA-binding proteins, including IMP1, Lin28b, and Musashi, are increased in epithelial tumors, it is not yet known if these factors play a role in the tumor microenvironment (10–13). Imp1 hypomorphic mice exhibit dwarfism, decreased organ size, morphologically defective intestines, and death near birth for a large portion of mice (14, 15). Recent studies demonstrated a specific role for IMP1 to promote expansion of fetal neural stem cells via posttranscriptional enhancement of self-renewal gene products including Hmga2 (16). Because of the severe phenotype of Imp1 hypomorphic mice, studying postnatal roles of Imp1 in these animals is not feasible.

Imp1 expression is low or absent in most adult tissues, yet is increased during tumorigenesis, likely due to its induction by and subsequent stabilization of oncogenic gene products including those within Wnt/b-catenin and cMyc pathways (12, 17–20). We and others have observed low IMP1 expression in the adult intestine and colon, suggesting a potential, functional homeostatic role in the adult gut (11, 12). Despite the severe intestinal phenotype observed in Imp1-hypomorphic mice (14), intestine–epithelium–specific conditional knockout mice (VillinCre; Imp1Loxp/Loxp or Imp1Δ476) revealed a minimal gross phenotype...
during homeostasis, whereas crossing Imp\(_1^{\text{loxP/loxP}}\) onto the Apc\(^{\text{Min+}}\) background of intestinal tumors exhibited a dramatic reduction in tumor lesions (unpublished data; ref. 11). We therefore reasoned that nonepithelial Imp1 might be important for normal homeostasis of the adult intestine and colon. In support of this hypothesis, a recent study revealed that Imp1 expression is upregulated in colonic stromal cells within days following focal wounding (21).

In the present study, we generated and characterized mice with mesenchyme-specific loss of Imp1 (Dermo1Cre;Imp1\(^{\text{loxP/loxP}}\), or Imp1\(^{\text{loxP/loxP}}\)) during homeostasis and a mouse model of colitis-associated colorectal tumorigenesis. We report that stromal Imp1 is dispensable for adult tissue homeostasis and mice exhibit no changes in epithelial cell proliferation or differentiation. Induction of colitis-associated cancer, however, revealed a significant increase in tumor burden in Imp1\(^{\text{loxP/loxP}}\) mice accompanied by upregulation of known protumorigenic factors previously unassociated with Imp1, including HGF. Taken together, our data suggest a novel role for mesenchymal Imp1 to modulate stromal factors in the context of colitis-associated cancer that directly influence the initiation and growth of epithelial tumors in the colon.

**Materials and Methods**

**Animals**

Previously generated Imp1\(^{\text{floxed}}\) mice (11) were maintained on a C57Bl/6 background and crossed with Dermo1Cre animals (Jackson Laboratories), which were maintained as heterozygous by crosses with CD1 mice. The resulting Dermo1Cre; Imp1\(^{\text{floxed}}\) mice, referred to as Imp1\(^{\text{loxp/loxp}}\), were thus mixed strain. Control mice consisted of age-matched Dermo1Cre; Imp1\(^{\text{floxed}}\), Imp1\(^{\text{fl+/fl}}\), and Imp1\(^{\text{fl/+}}\) mice, all of which are functionally wild type and were thus used to reduce overall animal numbers. Rosa-CAG-LSL-TdTomato mice (Jackson Laboratories) were used to confirm recombination in Dermo1Cre mice. All mice were cared for in accordance with University Laboratory Animal Resources requirements and under an IACUC-approved protocol. Mice were housed in specific pathogen-free conditions and fed standard, irradiated chow, and water ad libitum.

**Azoxymethane/dextran sodium sulfate tumor model**

Adult (8–12 weeks) male and female control and Imp1\(^{\text{loxP/loxP}}\) mice were given a single intraperitoneal injection of azoxymethane (AOM; Sigma) at a dose of 10 mg/kg, followed by three cycles of 2.5% dextran sodium sulfate (DSS; Abbemethix) for 5 days in the drinking water ad libitum with 1 week of normal water in between each cycle. Mice were sacrificed 10 weeks after the AOM injection. A total of ≥9 mice per genotype were tested across 2 independent experiments. Upon sacrifice, gross colon lesions were evaluated in a blinded fashion (Kathryn E. Hamilton) using a Nikon SMZ645 dissecting stereomicroscope. Colonos were then processed further for histology and gene expression analysis. Pathologic scoring was performed blinded by expert veterinary pathologist Dr. Elizabeth Buza of the University of Pennsylvania School of Veterinary Medicine’s Comparative Pathology Core using previously published scoring methods (22). For tumor stroma scoring, the presence of lymphocytes, plasma, and neutrophils was noted, as well as necrosis and crypt abscesses on a scale of 0 (not present) to 5 (marked).

**Immunohistochemistry**

Mouse colons were Swiss-rolled and fixed in 10% formalin followed by paraffin embedding. Hematoxylin and eosin (H&E), alcin blue, Sirus red, and Masson’s trichrome staining were performed using standard procedures. Immunofluorescence (IF) and/or immunohistochemistry (IHC) were performed using heat antigen-retrieval and staining with the following antibodies: Ki67 (1:250 dilution; Abcam; 16667), α-SMA (1:2,000 dilution; Sigma; A5228), IMP1 (1:100 dilution; Cell Signaling; 2852S), β-catenin (1:100 dilution; Sigma; C2206). For IHC, biotin-conjugated secondary antibodies were as follows: anti-mouse IgG (1:250 dilution; Vector Laboratories; BA-9200) and anti-rabbit IgG (1:250 dilution; Vector Laboratories; BA-1000). For IF, anti-mouse Cy3-conjugated (Jackson Immunoresearch) was used at 1:1,000.

**Epithelial and mesenchymal isolations and antibody array**

A one-centimeter piece of nontumor tissue from mid-distal colon was excised from AOM/DSS-treated animals and subjected to epithelial dissociation using EDTA and DTT (23). The epithelium was then mechanically dissociated from the tissue via shaking, and remaining mesenchyme washed twice with PBS. Epithelial and mesenchymal fractions were then lysed in Triton lysis buffer (1% Triton X-100, 50 mmol/L Tris-HCl, pH 7.5, 100 mmol/L NaCl, 5 mmol/L EDTA, 40 mmol/L β-glycerophosphate, 5% glycerol, 50 mmol/L NaF) plus protease and phosphatase inhibitors (Pierce and Sigma, respectively). Total protein was quantified using Pierce BCA assay and tested for purity via Western blot for E-cadherin (epithelial cells, 1:1,000 dilution; BD Biosciences; 610182) and α-SMA (mesenchyme/fibroblasts, 1:2,000 dilution; Sigma; A5228). Protein samples were assayed using the RayBiotech, Inc. Mouse QuantiLib Array 4000, which includes 200 targets.

**Immunoblotting**

Cells were lysed in Triton lysis buffer and total protein quantified using Pierce BCA assay (Thermo Scientific; 23225). Protein lysates were resolved in reducing conditions on 4% to 12% gradient gels for electrophoresis and detected with ECL Prime Western Blotting Detection Reagent (Amersham; RPN2232) according to the manufacturer’s instructions. Primary antibodies were as follows: Imp1 (1:400 dilution; Cell Signaling; 2852S), mouse HGF (1:1,000 dilution; Abcam; 83760), human HGF (1:1,000 dilution; Santa Cruz; H-145), and GAPDH (1:20,000 dilution; Chemicon; MAB374). Secondary antibodies from GE Healthcare ECL anti-rabbit IgG, horseradish peroxidase (HRP)-linked and ECL anti-mouse IgG, HRP-linked were used at 1:1,000 to 1:10,000 dilutions.

**Gene expression analysis**

Total RNA was isolated from cell lines using the GeneJet RNA purification Kit (Thermo Scientific) and reverse transcribed using the Applied Biosystems/Life Technologies Taqman Reverse Transcriptase Reagents. qRT-PCR was performed using Taqman Fast Universal Master Mix (Applied Biosystems) using the following primers/probe sets: human HGF (Hs00300159) and housekeeping gene Hmbs (Hs00609297).

**Cell lines**

Human fetal esophageal fibroblast lines (FF 2008 and FF 3303) were generated by the Rustgi laboratory as described previously (24). All cells were cultured in DMEM with 10% FBS plus penicillin/streptomycin and maintained at 37°C with 5% CO\(_2\).
Cell proliferation assays

Fetal esophageal fibroblast cell proliferation was evaluated by staining with Trypan blue and enumeration via the Countess Automated Cell Counter (Life Technologies). Manual cell counting via hemocytometer was also used to confirm results obtained via automated cell counting. Cell counts were performed in duplicate across 4 independent experiments.

Statistical analyses

For AOM/DSS-treated animals, statistical significance of comparisons between control and experimental mice was determined by applying the Student t test, with \( P < 0.05 \) as statistically significant. For all other analyses, unless noted otherwise, data from at least three experiments were presented as mean \( \pm \) SE and analyzed by applying the Student t test, with \( P < 0.05 \) as statistically significant.

Results

Stromal Imp1 is dispensable for normal intestinal development and homeostasis

Imp1-hypomorphic mice exhibit severe growth defects in the intestine. Intriguingly, intestine-epithelium-specific conditional knockout mice (Imp1<sup>ΔIEC</sup>) are phenotypically normal during homeostasis (unpublished data). We therefore generated mice with mesenchyme-specific loss of Imp1 (Imp1<sup>ΔMes</sup>) by crossing Imp1<sup>-fl</sup>oxed mice (11) with Dermo1Cre mice (25, 26), causing Imp1 disruption in mesoderm-derived tissues, including fibroblasts, macrophages, and endothelial cells, starting at E9.5. We confirmed Cre-recombination in the mesenchymal compartment of the intestine and colon by crossing Dermo1Cre mice with Rosa<sup>LSL-tdTomato</sup> mice and evaluating tdTomato expression grossly and histologically (Supplementary Fig. S1A and S1B) as well as IMP1 expression (Supplementary Fig. S1C). Imp1<sup>ΔMes</sup> mice were born at comparable ratios to wild-type littermates and exhibited no differences in gross phenotype into adulthood. Initial histologic examination of adult Imp1<sup>ΔMes</sup> mice revealed normal crypt architecture of the colon (Fig. 1A), with no gross abnormalities. Furthermore, we found no difference in proliferation (Ki67) in Imp1<sup>ΔMes</sup> mice (Fig. 1B). Evaluation of goblet cells (alcan blue) also revealed comparable numbers of goblet cells between control and Imp1<sup>ΔMes</sup> mice (Fig. 1C), suggesting that lineage specification of the epithelium was unaffected. Analysis of proliferation and cell lineages in the small intestine were also unaffected (data not shown).

As Imp1 deletion was targeted to the subepithelial compartment, we specifically evaluated stromal cells and extracellular matrix components in these mice. There were no changes in \( \alpha \)-SMA-positive fibroblasts, which appeared in the same location and frequency in Imp1<sup>ΔMes</sup> and control mice (Fig. 1D). Trichrome staining for collagen and muscle fibers similarly revealed no significant difference between groups (Fig. 1E), nor did Sirius red staining for collagen (Fig. 1F). This is intriguing, because Imp1-hypomorphic mice exhibited a decrease in genes encoding

Figure 1. Stromal Imp1 is dispensable for colon homeostasis. A, representative H&E staining from control and Imp1<sup>ΔMes</sup> mice demonstrates no morphologic differences in colon crypts or mesenchymal layers. B, Ki67 staining is comparable between control and Imp1<sup>ΔMes</sup> mice. C, staining for goblet cell lineage via Alcian blue showed no significant differences between control and Imp1<sup>ΔMes</sup> mice. To evaluate stromal components, staining for \( \alpha \)-SMA (D), Masson's trichrome (E), and Sirius red (F) was performed; however, no differences were observed between control and Imp1<sup>ΔMes</sup> mice.
extracellular matrix components, including multiple procollagens (types I, II, IV, V, VIa1, VIa2, VIv3, VIv11), galectin-1, lumican, and tenascin-C (14). Taken together, these data indicate that Imp1 expression is dispensable in the mesenchymal compartment for homeostasis in the adult colon.

Mice lacking stromal Imp1 exhibit enhanced tumor burden in the colon

We have established previously a direct, oncogenic role of epithelial IMP1 in colorectal cancer xenografts and ApcΔMes/+ tumorigenesis (11). In addition, studies of IMP1 have demonstrated specific roles for IMP1 in cytoskeletal organization and cell movement (27–30). Most recently, studies have reported that IMP1 may regulate cell migration and polarization via regulation of MAPK4 and PTEN in osteosarcoma and ovarian tumor cell lines, and may promote wound repair in colonic mesenchymal stem cells via stabilization of Ptg2 mRNA (21, 31). We sought to determine the consequences of Imp1 loss in the mesenchyme in the setting of inflammation-associated colon tumorigenesis. Imp1ΔMes mice were treated with a single dose of AOM followed by three cycles of DSS to include colitis-associated tumors. The AOM/DSS model is an ideal approach to study the tumor microenvironment, as it combines a chemical carcinogen in the setting of inflammation to recapitulate tumor initiation and progression in a defined chronological (32). Furthermore, this model is characterized by activating mutations in epithelial pathways observed in humans, including Wnt/Apc/β-catenin and K-Ras (32, 33). We sacrificed mice at 10 weeks after AOM injection in order to evaluate the early establishment of adenomas and/or adenocarcinomas.

Intriguingly, loss of mesenchymal Imp1 promoted, rather than attenuated, tumor growth. Male Imp1ΔMes mice exhibited a significant increase in tumor number (14.4 ± 2.3 vs. 8.6 ± 1.6; Fig. 2A). Tumor size was also significantly increased in Imp1ΔMes mice (1.7 ± 0.09 vs. 1.3 ± 0.06), as well as tumor load (Fig. 2B–D). A nonsignificant trend was observed in female mice (data not shown), which is not surprising as female mice are less susceptible to DSS (34). Blinded, histologic scoring by an expert veterinary pathologist supported increases in gross tumor number in Imp1ΔMes mice. Furthermore, Imp1ΔMes mice had a higher incidence of both low-grade and high-grade adenomas and gastrointestinal neoplasia (GIN) compared with controls (Fig. 2E and F), whereas the majority of lesions found in control mice were characterized as hyperplasia (Fig. 2F). Histologic evidence of adenocarcinoma was observed in Imp1ΔMes mice but not controls; however, those lesions did not meet the full criteria for...
Together, these data suggest that adenocarcinoma based on published scoring systems (22).

Histologic examination of tumor stroma revealed higher inflammation and activated stroma scores in Imp1ΔMes mice compared with controls (Fig. 3A–C). Inflammatory infiltrates consisted of mostly lymphocytes and plasma cells. Inflammation and necrosis within tumors tended to be more severe in the high-grade adenomas versus low-grade adenomas versus hyperplasia, and inflammation within the neoplasms had more of a neutrophilic component. Overall stromal scores included the extent of inflammatory infiltrate, as well as evidence of necrosis, crypt abscesses, and crypt loss with replacement by fibrosis (Fig. 3D). Evidence of herniation was observed in Imp1ΔMes mice only. Sirius red scoring revealed a trend for enhanced fibrosis in Imp1ΔMes mice (scoring not shown), and tumor stromal fibrosis tended to be more prominent within the higher-grade adenomas. Collectively, we demonstrate that the increase in tumor burden in Imp1ΔMes mice is accompanied by enhanced inflammation and stromal components, suggesting that IMP1 in the colon mesenchyme may normally function to attenuate the effects of chronic inflammatory damage.

To elucidate mechanisms underlying enhanced tumorigenesis in Imp1ΔMes mice, we isolated mesenchyme from Imp1ΔMes and control mice treated with AOM/DSS and performed a cytokine/growth factor array of 200 targets. Tissues from the mid-distal colon, at least 1 centimeter from the closest gross lesion, were evaluated in order to ensure minimal contamination of sample with tumor epithelium (Fig. 4A). Stromal enrichment and epithelial depletion of isolated mesenchyme were confirmed via Western blotting for epithelial marker E-cadherin and activated fibroblast marker α-SMA (Fig. 4B). There was a global increase in several proinflammatory cytokines and chemokines, including IL6, II1β, and MCP1 in stroma from Imp1ΔMes mice (Fig. 4C), although not all were statistically significant. In addition, the BMP pathway inhibitor Gremlin 1, which has been implicated in colorectal cancer progression (35), was also upregulated in Imp1ΔMes mice. Interestingly, there was also a 60% increase in stromal HGF, a growth factor produced by fibroblasts with known roles in stimulation epithelial cell growth in the gut (36). We confirmed upregulation of HGF in stromal lysates of AOM/DSS-treated Imp1ΔMes mice (Fig. 4D and E). HGF has been implicated in the pathogenesis of colorectal cancer not only via direct stimulation of pathways downstream of its receptor, cMet, but also via synergy with the Wnt/β-catenin pathway in epithelial tumor cells (37). We therefore evaluated the presence of nuclear β-catenin in hyperplastic areas of control and Imp1ΔMes mice (Fig. 4F). Indeed, Imp1ΔMes mice exhibited enhanced nuclear β-catenin compared with controls, suggesting that the tumor microenvironment in Imp1ΔMes mice indeed contributes to the increase tumorigenesis in these mice.

Fibroblasts lacking Imp1 exhibit increased HGF expression and fibroblast proliferation in vitro

HGF has been implicated in the pathogenesis of colorectal cancer (38–40) and is a key paracrine signaling component relevant to several human cancers, including colorectal cancer. In order to determine if the increase in AOM/DSS tumorigenesis is due to direct signaling from Imp1-deficient fibroblasts, we evaluated the expression of HGF following Imp1 knockdown in human fibroblast cell lines and human colon primary fibroblasts. We first evaluated Imp1 knockdown in human fetal esophageal fibroblasts (FEF2008 and FEF 3303), as these cells produce low to intermediate levels of HGF and are highly amenable to transfection (24, 41). In addition, FEF cell lines recapitulate phenotypically the proliferative and invasive behavior characteristic of cancer-associated fibroblasts ‘CAFs,’ and were thus appropriate model for the current study (41). In both fibroblast cell lines...
evaluated, there was an increase in both pro-HGF and the active, α-HGF isoform with Imp1 knockdown (Fig. 5A). Interestingly, we do not observe a significant difference in Hgf mRNA with Imp1 knockdown (Fig. 5B). This is consistent with IMP1 regulating HGF at the level of posttranscription/translation. We next evaluated the proliferative capacity of Imp1 knockdown fibroblasts and found a 67% increase in proliferation via automated cell counting 72 hours after knockdown (Fig. 5C). The increase in HGF and proliferation with Imp1 knockdown was found in the absence of any differences in morphology of Imp1 knockdown fibroblasts and controls (Fig. 5D). Together, these data indicate that Imp1 knockdown may promote a “CAF-like” phenotype in fibroblasts.

**Discussion**

In the present study, we demonstrate dispensability of stromal Imp1 for homeostasis in the adult colon and that Imp1 loss renders mice more susceptible to a protumorigenic microenvironment and thus increases tumor burden in a mouse model of colitis-associated cancer. The lack of phenotype in Imp1ΔMes mice during homeostasis is perhaps not surprising, as expression of Imp1 in the mesenchyme of untreated adult mice is nearly undetectable; however, dramatic intestine defects in mice with global Imp1 deletion remain unexplained. It is possible that Cre-mediated excision of Imp1-flxed alleles using Derma1Cre (or VillinCre, unpublished) does not occur early enough during development to recapitulate any of the global hypomorphic phenotype. It is also possible that Imp1 must be lost in both epithelial and mesenchymal compartments simultaneously in order to elicit a phenotype at homeostasis, which will be a focus of future studies.

Our finding that Imp1ΔMes mice exhibit increased tumor burden was unexpected, as our previous studies in xenograft and genetic models identified an oncogenic role for epithelial Imp1 in the intestine (11). We now demonstrate a dichotomous role for Imp1 in the adult colon, whereby loss of Imp1 in the mesenchymal compartment promotes, rather than attenuates, inflammation-associated colon tumorigenesis. These data support a growing literature in which the discrete roles of Imp1 within specific contexts reveal either oncogenic or tumor-suppressive roles. For example, transgenic expression of Imp1 in mouse breast tissue promotes tumor growth, whereas other studies have shown that ectopic Imp1 expression can attenuate metastatic potential in breast cancer cell lines via modulation of cell motility (28, 42, 43). In colorectal cancer, IMP1 expression correlates with poor clinical outcome (12, 20); however, no studies have evaluated IMP1 expression in tumor stroma of colorectal cancer patients.

Our data demonstrating that Imp1 loss promotes an enhanced tumor microenvironment are the first to evaluate the nonepithelial roles of Imp1 in this context. While analyses of specific
inflammatory targets revealed few statistically significant upregulated targets, several proinflammatory cytokines exhibited a trend for increased expression with Imp1 loss. This, together with pathologic scoring of inflammation, supports the hypothesis that stromal Imp1 loss promotes an inflammatory environment in this context. Recent studies in a colon wound-healing model revealed that Imp1 expression is upregulated in the mesenchyme, where it binds to and promotes induction of Ptg2 mRNA during the initial phase of wound healing (21). This implicates Imp1 as a facilitator of the temporal orchestration of wound healing in the colon; however, it likely has a divergent role in chronic inflammation such as that found in the current study in AOM/DSS-treated mice. Interestingly, the tumor progression locus-2 (TPL2) kinase has also been shown to regulate Ptg2 during colon homeostasis, suggesting alternative or potentially overlapping pathways (44).

In AOM/DSS-treated Imp1+/− mice, the enhanced tumor microenvironment promotes tumorigenesis characterized by increased tumor load together with more advanced pathologic grading and increased nuclear β-catenin in epithelial cells. In addition to increased inflammation, Imp1+/− mice express also increased HGF levels, which are produced by stromal fibroblasts, and promote proliferation of epithelial cells (36). HGF is also thought to promote invasive growth of colorectal cancer cells via cooperation with β-catenin signaling, and has been shown to confer resistance to EGFR inhibitors by colon tumor-initiating cells (37, 45, 46). As such, we confirmed that Imp1 loss does indeed upregulate HGF in fibroblasts in vitro, implicating a novel, cell autonomous role for Imp1 in the modulation of HGF in fibroblasts. In addition, our data demonstrating that Imp1 loss increases fibroblast cell growth, while likely due to increased HGF, may also be attributed to other factors. Although the direct mechanisms for Imp1 modulation of Hgf are still under investigation, possible roles include (i) direct binding of Imp1 to Hgf mRNA, (ii) binding and modulation of negative regulators of HGF such as TPL2, which has been shown to have other pheno-

dic similarities to IMP1 (40), and (iii) modulation of mature HGF cleavage events, among other possibilities.

There are likely additional factors, including those identified in our antibody array and other cytokines (Fig. 4C), that contribute to the tumorigenic microenvironment in mice lacking stromal Imp1. For example, we demonstrate that GREM 1, an inhibitor of the BMP pathway, is upregulated with Imp1 loss. This is intriguing as GREM 1 has recently been implicated in the promotion and progression of colorectal cancer when aberrantly expressed in epithelial cells or via loss of cancer cell differentiation at invasive fronts, respectively (35, 47). The role of IMP1 in modulating the BMP pathway through suppression of GREM 1 will be a focus of future studies.

In addition to factors revealed in our antibody screen, previous studies revealed that IMP1 contributes to activation of the NF-κB pathway by stabilizing mRNA of βTrCP1 that results in increased levels of βTrCP1 protein and accelerated degradation of IkBs (17). Interestingly, βTrCP1 is minimally expressed in fibroblasts, whereas its homolog βTrCP2 (HOS) is a predominantly expressed gene in fibroblasts. Because IMP1 does not affect the expression of βTrCP2, the present data may suggest that knockout of Imp1 fails to inhibit NF-κB in fibroblasts, potentially due to the absence of βTrCP1. Additional cell-type-specific Cre-mice will be employed in future studies to target Imp1 specifically in fibroblasts or immune cells to evaluate the specific contribution of Imp1 loss on NF-κB and other proinflammatory pathways in the context of inflammation-associated colorectal cancer.

Recent genomic approaches have identified in patients a significant correlation between tumor stromal components and the most aggressive, therapy-resistant colorectal cancers (48, 49). Specifically, stromal markers and genes associated with CAFs correlate with higher disease recurrence, resistance to therapeutics, and enhancement of tumor-initiating cells. Our data that stromal Imp1 loss promotes colon tumorigenesis indicate that stromal...
**Impi** may normally play a tumor-suppressive role in the colon. As such, it would be interesting to evaluate IMP1 expression in the stroma of colorectal cancer patient samples in the future to determine its potential as a diagnostic or prognostic marker.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Authors’ Contributions**

Conception and design: K.E. Hamilton, A.K. Rustgi

Development of methodology: K.E. Hamilton, V.S. Spiegelman, A.K. Rustgi

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K.E. Hamilton, P. Chatterji, E.T. Lundsmith, P.D. Hicks

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K.E. Hamilton, P. Chatterji, E.T. Lundsmith, S.F. Andres, A.K. Rustgi

Writing, review, and/or revision of the manuscript: K.E. Hamilton, P. Chatterji, S.F. Andres, V. Giroux, V.S. Spiegelman, A.K. Rustgi

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): P. Chatterji, E.T. Lundsmith, P.D. Hicks, A.K. Rustgi

Study supervision: K.E. Hamilton, A.K. Rustgi

Other (generation of Imp1-floxed mice and review of the manuscript): F.K. Noubissi

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