Correction

MEDICAL SCIENCES

The authors note that Fig. 6 appeared incorrectly. The corrected figure and its legend appear below.

Fig. 6. GH induces EMT, suppresses apoptosis, and increases motility. Western blot analysis of PTEN and EMT factors in (A) hNCC and (B) HCT116 cells treated with indicated doses of GH for 24 h. (C) Western blot analysis of cleaved caspase 3 in cells treated with GH (500 ng/mL) for 24 h. Experiments were performed at least three times, and representative results shown. (D) Migration of hNCC and HCT116 cells treated with GH (500 ng/mL) and harvested 48 h after plating. (E) Migration of HCT116 cocultured for 48 h with hCF infected with lentivirus or lentiGH. In D and E, for quantification, the number of migrated cells per 1,000 cells in five randomly chosen fields in each duplicate transwell were counted and means calculated. Results are presented as mean ± SEM of three independent experiments; *P < 0.05. (F) Number of colonies and arbitrary colony size formed in soft agar by HCT116 cells cocultured with hCF infected with lentivirus or lentiGH, and tested for anchorage independent growth. Colony size was determined using ImageJ software. Results are presented as mean ± SEM of duplicates from two independent experiments; **P < 0.01 vs. control. In D–F, the differences between groups were analyzed using two-tailed unpaired Student t test.

www.pnas.org/cgi/doi/10.1073/pnas.1612785113
Growth hormone is permissive for neoplastic colon growth

Vera Chesnokova,a Svetlana Zonis,b Cuizi Zhou,a Maria Victoria Recouvreur,a Anat Ben-Shlomo,a Takako Araki,b Robert Barrettb,c Michael Workman,c,d Kolja Wawrowsky,a Vladimir A. Ljubimov,a Magdalena Uhart,a and Shlomo Melmed*a,b1

aPituitary Center, Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA 90048; bDepartment of Medicine, Board of Governors Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA 90048; and cDepartment of Medicine, F. Widjaja Foundation Inflammatory Bowel and Immunobiology Research Institute, Cedars-Sinai Medical Center, Los Angeles, CA 90048

Growth hormone (GH) excess in acromegaly is associated with increased precancerous colon polyps and soft tissue adenomas, whereas short-stature humans harboring an inactivating GH receptor mutation do not develop cancer. We show that locally expressed colon GH is abundant in conditions predisposing to colon cancer and in colon adenocarcinoma-associated stromal fibroblasts. Administration of a GH receptor (GHR) blocker in acromegaly patients induced colon p53 and adenomatous polyposis coli (APC), reversing progrowth GH signals. p53 was also induced in skin fibroblasts derived from short-statured humans with mutant GHR. GH-deficient prophet of pituitary-specific positive transcription factor 1 (Prop1)−/− mice exhibited induced colon p53 levels, and cross-breeding them with Apcmin+/− mice that normally develop intestinal and colon tumors resulted in GH-deficient double mutants with markedly decreased tumor number and size. We also demonstrate that GH suppresses p53 and reduces apoptosis in human colon cell lines as well as in induced human pluripotent stem cell-derived intestinal organoids, and confirm in vivo that GH suppresses colon mucosal p53/p21. GH excess leads to decreased colon cell phosphatase and tensin homolog deleted on chromosome 10 (PTEN), increased cell survival with down-regulated APC, nuclear β-catenin accumulation, and increased epithelial–mesenchymal transition factors and colon cell motility. We propose that GH is a molecular component of the “field change” milieu permissive for neoplastic colon growth.


Edited by David W. Russell, University of Texas Southwestern Medical Center, Dallas, TX, and approved May 2, 2016 (received for review January 12, 2016)

The pituitary gland secretes growth hormone (GH), which acts as an endocrine regulator by signaling through membrane-associated GH receptors (GHR) to elicit direct peripheral actions as well as to induce insulin growth factor (IGF1) production (1–4). Most growth-promoting endocrine actions of GH are mediated by IGF1; however, GH also acts independently of IGF1 to regulate muscle, bone, and adipose tissue functions (5–7).

Local GH (structurally identical to pituitary GH) is expressed in nonpituitary tissues, including the colon, prostate, and breast (8–10), where it similarly binds the GHR to signal in a paracrine/autocrine fashion (11, 12). Intracranial GH also acts within cells in an intracrine fashion, directly targeting intracellular GHR to regulate nuclear genes (9, 11, 12).

GH deficiency appears to confer protection against development of malignancies. Abrogating GH signaling by inducing GH deficiency as seen in Ames [prophet of pituitary-specific positive transcription factor 1 (Prop1)−/−] mice, or by disrupting GHR, as in GHR−/− dwarf mutant mice, is protective of cancer development (13, 14). Indeed, 20% of follow-up have shown that individuals who harbor inactivating GHR mutations do not develop cancer, whereas unaffected relatives develop cancer at rates similar to those in the general population (15). These clinical observations are buttressed by animal studies showing that GHR inhibition suppresses colon carcinoma xenograft growth in nude mice (16) and reduces susceptibility to induced colon cancer in GH-deficient rats (17).

In contrast, transgenic mice expressing universally high circulating and tissue GH exhibit an increased incidence of soft tissue tumors (18–21), and mice overexpressing bovine GH exhibit preneoplastic liver lesions. The latter are believed to be a consequence of the direct effect of GH on the liver rather than mediated by IGF1, as transgenic mice overexpressing IGF1 do not exhibit similar liver pathology (22, 23). Furthermore, acromegaly patients with excess systemic GH elaborated by a GH secreting pituitary tumor have increased prevalence of colon polyps (24–26) as well as increased colon length with prominent mucosal folds and overgrowth (27), and also exhibit fourfold increased rates of colon adenocarcinoma (28–31).

Colorectal cancer results from inactivating mutations of tumor-suppressor genes, such as adenomatous polyposis coli (APC), p53, deleted in colorectal cancer (DCC), deleted in pancreatic cancer locus 4 (DPC4), and Kristen rat sarcoma viral oncogene homolog (K-ras), as well as DNA damage-repair abnormalities and chromosomal instability. Many of these genomic events target the transition from normal mucosa to small adenomas, then to large adenomas, and ultimately to carcinomas (32–34). Furthermore, the surrounding milieu for colon tumor development includes the extracellular matrix, cancer-associated fibroblasts (CAFs), vascular endothelial and smooth muscle cells, and immune responses (35). For example, ulcerative colitis (UC) is associated with increased rates of colon adenocarcinoma, and


The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Data deposition: Information on the pegvisomant clinical trial protocol is available at https://clinicaltrials.gov/ct2/show/NCT01261000.

1To whom correspondence should be addressed. Email: melmed@csmc.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1600561113/-/DCSupplemental.
mucosal mapping indicates that chronically inflamed colonic mucosa undergoes a “field change” of cancer-associated molecular alterations before histological evidence of dysplasia (36). Multiple factors sustain colon proliferative signaling and enable resistance to cell death and evasion of growth suppressors. CAF-derived growth factors act through MAPK and PI3K mammalian target of rapamycin (mTOR) to mediate cell proliferation, survival, cytoskeletal rearrangement, and invasion (37–39), enabling normal mucosa to undergo premalignant changes within a pro-growth milieu. Thus, colon polyp recurrence is common in UC, presumably because of an underlying field effect (36).

Here, we present evidence supporting a novel mechanism whereby GH mediates the colon microenvironment by suppressing p53. This mechanism appears to underlie the linkage between GH and colon cell proliferative control. As GH appears to potentiate colon tissue growth (27), we treated acromegaly patients with the GHR inhibitor pegvisomant and observed induced p53 and APC expression in colon tissue biopsy specimens. Furthermore, using skin fibroblasts derived from short-stature human subjects harboring an inactivating GHR mutation (40) and insensitive to GH (41, 42), we show high p53 levels, as well as enhanced p53/p21 induction in response to DNA damage. Consistent with these clinical findings, we show that Prop1−/− mutant mice devoid of GH exhibit strong colon p53 expression. Because APC-deficient Apcmin/+ mice develop multiple intestinal tumors (43), we generated doubly mutant Apcmin/+Prop1−/− mice, and observed markedly diminished tumor incidence. We elucidate mechanisms for these observations and show that GH acts to attenuate p53 stabilization, as well as suppress APC, phosphatase and tensin homolog deleted on chromosome 10 (PTEN), and apoptosis, while inducing cell motility. In intestinal organoids generated from induced human pluripotent stem cells (iPSCs), GH treatment suppressed p53, APC, and E-cadherin expression. Finally, in vivo, high GH resulted in murine colon tissue p53 and p21 suppression. These findings represent a novel protective effect of GH deficiency against tumor growth are mediated by p53 and that GH excess enables a protumorigenic cell phenotype by inhibiting tumor suppressors.

Results
Deficient GH Signaling Is Protective for Colon Neoplasia Growth.
Disrupted human GH signaling is associated with elevated p53 levels. We first tested mechanisms underlying the protective effects of GH signaling deficiency. We examined primary skin fibroblasts derived from short-stature GHRmut Laron syndrome subjects harboring an inactivating GHR mutation, and which are defective in GH signaling (44). When actively proliferating asynchronous cells were pulsed with BrdU, incorporation was 40% lower in mutated than in WT fibroblasts (Fig. S1A), indicating decreased proliferation in cells lacking a functional GHR. Higher baseline p53 expression was observed in mutated compared with normal sibling age-, sex-, and passage-matched fibroblasts (Fig. 1A). p21, a cell cycle inhibitor, was also increased, and was associated with decreased Ki67 and proliferating cell nuclear antigen (PCNA) proliferation markers (Fig. 1A). Observed changes in p53 and p21 proteins reflect increased levels of both p53 and p21 mRNAs in mutant cells (Fig. S1B).

To further confirm that deficient GH signaling results in p53 accumulation, we transfected GHRmut human fibroblasts with increasing amounts of pcDNA3.1hGHR plasmid using electroporation. Reinstatement of WT GHR in these cells led to dose-dependent suppression of baseline p53 abundance (Fig. 1B). When GHR expression was attenuated by transfecting WT human skin fibroblasts with specific GHR siRNA, p53 levels were also induced (Fig. 1C). Decreased GHR levels were concordant with down-regulation of the proliferation markers Ki67 and PCNA (Fig. S1C), similar to that observed in GHR mutated cells (Fig. 1A). Moreover, transfection of WT fibroblasts with siGHR RNAi decreased BrdU incorporation by 37% (Fig. S1D). These results
show that disrupted human GH signaling induces p53 abundance and decreases normal skin fibroblast proliferation.

Acromegaly patients with high levels of circulating GH exhibit increased incidence of colon adenomas (45). Pegvisomant, a GHR antagonist, prevents GH binding by disrupting internal rotation and activation of downstream GH signaling (46–48). To examine whether clinical inhibition of GH signaling alters colon tissue p53 expression, seven acromegaly subjects were prospectively enrolled to undergo colon mucosal biopsy before and after 8 wk of pegvisomant treatment (Table S1). The efficacy of pegvisomant treatment was confirmed by observed decreased serum IGF1 levels in all subjects (Table S1). GHR blockade resulted in induced colon tissue p53 expression in all seven subjects, which was accompanied by APC induction in five of seven subject specimens (Fig. 1D). p21, a proximal marker of proliferation, was induced in these same subjects concordantly with p53 induction. The remaining two subjects (#3 and #6) showed no change or decrease in APC as well as reduced p21 expression.

Murine GH deficiency increases colon p53 in vivo. Because GH signaling deficiency appears to protect against tumor formation, we tested colon tissue derived from Prop1<sup>−/−</sup> mice devoid of pituitary GH (49) (Fig. S2A). We observed markedly induced colon p53 and p21 levels, and cleaved caspase 3, a marker of apoptosis, was also induced, whereas p56 abundance, an end-point marker of mTORC1 activity, was attenuated (Fig. 1E). Basal colon GH mRNA levels, already low in WT mice (32 ± 0.8 Ct), were almost undetectable in colon tissue derived from Prop1<sup>−/−</sup> mice (36.2 ± 0.9 Ct) (Fig. S2B).

Mice lacking GHR (GHR<sup>−/−</sup>) also exhibited high colon p53 expression (Fig. 1F), supporting the hypothesis that GH signaling deficiency is associated with p53 induction. Similar to our observations in GHR-mutated and WT fibroblasts where GHR was suppressed (Fig. 1A and Fig. S1C), GHR<sup>−/−</sup> mice exhibit markedly decreased colon Ki67, indicative of decreased proliferation in colon tissue devoid of GHR (Fig. 1F).

Murine GH deficiency attenuates intestinal tumor development. We next tested whether enhanced colon p53 abundance could account for protection of GH-deficient mice from tumor formation. Homozygous APC<sup>min+/-</sup> mice are not viable, but APC<sup>min+/-</sup> Prop1<sup>−/−</sup> mice develop multiple small intestine tumors by 9 mo with 100% penetrance (43). These mice also develop colon tumors when kept on a high-fat diet (50). As Prop1<sup>−/−</sup> mice are not fertile, Prop1<sup>−/−</sup> mice were cross-bred with APC<sup>min+/-</sup>Prop1<sup>−/−</sup> mice, and tumor development examined in doubly mutant APC<sup>min+/-</sup>Prop1<sup>−/−</sup> animals, all maintained on high-fat diets.

All 30 male and all 22 female APC<sup>min+/-</sup>Prop1<sup>−/−</sup> mice, as well as 10 of 16 male and 1 of 8 female APC<sup>min+/-</sup>Prop1<sup>−/−</sup> mice, developed small intestine tumors by 9 mo. Both tumor size and number were strikingly decreased in doubly mutant animals (Fig. 2A and B). By this age, 67% (20 of 30) of APC<sup>min+/-</sup>Prop1<sup>−/−</sup> males also developed colon adenomas (one to two tumors each), whereas a single colon tumor was observed in 12.5% (2 of 16) of double mutant male mice (Fig. 2C). Colon tumors were not observed in female mice of either genotype. Thus, GH deficiency constrains both the size and number of intestinal tumors in these compound transgenic male mice.

We then analyzed colon tissue, carefully dissecting non-tumorous colons of 7-mo-old APC<sup>min+/-</sup> and APC<sup>min+/-</sup>Prop1<sup>−/−</sup> male mice. Colon p53 was induced in doubly mutant compared with APC<sup>min+/-</sup> mice (Fig. 2D). Immunofluorescence analysis indicated that APC<sup>min+/-</sup> mice exhibit high GH expression in colon tumor stromal cells, as well as in epithelial tumor cells (Fig. 2E). In contrast, GH was not detected in tumor-free APC<sup>min+/-</sup>Prop1<sup>−/−</sup> colon tissue (Fig. 2E).

To directly study effects of GH deficiency on colon cells, we used HCT116 colon cancer cells harboring WT p53 and primary nontumorous human colon cells (hNCC). These cells are stable for up to 10–12 population doublings; only cells from passage 4...
were used. We previously detected immunoreactive GH in both cell lines (51). Nontransformed hNCC were stably infected with lentivirus expressing shGH RNA, whereas HCT116 cells were transfected with siGH RNAi. In both cell lines, GH suppression resulted in p53 up-regulation, with increased levels of p21, a transcriptional target for p53 (52), and S6 phosphorylation was suppressed in malignant HCT116 cells but not in non-tumorous colon cells (Fig. 2F), confirming our in vivo observations (Fig. 1E).

**GH Is a Component of the Field Change Enhancing Progression of Colon Epithelial Cell Neoplasia.** In contrast to GH signaling deficiency, GH excess is associated with increased incidence of colon tumors. We therefore hypothesized that GH participates in the field change enabling neoplastic colon growth, and tested mechanisms underlying colon GH action.

**GH expression in human colon tissue.** Immunofluorescent staining and confocal microscopy of human colon tissue arrays show that normal colon GH expression is low. Because patients with UC exhibit higher rates of colon adenocarcinomas (36), we tested GH expression in inflamed human colon specimens. Although GH is undetectable in stromal cells in normal colon tissue, GH expression was enhanced in 11 of 20 (55%) colon specimens derived from patients with UC (Fig. 3A). In these specimens, GH colocalizes with IgA, a plasma cell marker (Fig. 3B). Similar stromal expression patterns were also observed in benign colon adenomas (Fig. 3C). In human colon adenocarcinoma specimens, GH, although not expressed within epithelial tumor cells, was expressed in fibroblasts surrounding malignant colon carcinomas and colocalized with α-SMA (smooth muscle actin), a marker for CAFs (Fig. 3D and E). Eight of 32 (25%) adenocarcinoma specimens and 12 of 32 (38%) colon adenocarcinoma metastases analyzed exhibited high (>11% positive cells) fibroblast GH expression. GH immunopositivity was not detected in normal colon mucosa fibroblasts. We found that the GHR is expressed in both colon epithelial and stromal cells, and that receptor expression was markedly induced in 25 of 70 (35%) analyzed epithelial colon adenocarcinoma cells compared with normal mucosa (Fig. 3F).

**GH suppresses p53 expression.** We tested endocrine effects of GH by treating colon cells with increasing doses of GH for 24 h. Although p53 mRNA levels were not altered (Fig. 4A), we observed dose-dependent p53 protein attenuation in both hNCC and HCT116 cell lines (Fig. 4B). Accordingly, p21 was also dose-dependently reduced (Fig. 4B). Consistent with previous findings (2), GH treatment of hNCC resulted in early increase in STAT5 phosphorylation (for up to 3 h), indicative of GHR activation, and phospho STAT5 levels were normalized 24 h later (Fig. S3A).

To further confirm that the GH effects on p53 occur through GHR activation, we pretreated hNCC with 20 μg/mL pegvisomant for 1 h, treated cells with 500 ng/mL GH, and harvested them 24 h later. Suppressive effects of GH on p53 and p21 were abolished and both proteins were up-regulated when GHR was blocked with pegvisomant (Fig. S3B), indicating that GH effects on p53 are GHR-mediated.

We did not observe IGF1 changes in the two cell lines after GH treatment (Fig. S4A). Treatment with increasing doses of IGF1 did not suppress p53 protein expression (Fig. S4B). Moreover, IGF1 suppression with lentivirus expressing shIGF-1 RNA did not affect GH-induced decreases in p53 (Fig. S4C). The results suggest that GH action on colon cell p53 may be IGF1-independent.

**Mechanisms for p53 suppression by GH.** p53 half-life is short and, in unstressed cells, the protein is maintained at low levels by ubiquitination and subsequent degradation (53). We explored mechanisms for GH-mediated p53 suppression by treating hNCC with MG-132, a proteasome inhibitor that blocks degradation of ubiquitin-conjugated proteins, in the presence of GH. In cells not treated with MG-132, GH suppressed p53, as expected, whereas p53 levels accumulated in transfectants treated with MG-132 despite the presence of GH (Fig. 4C). Because GH enhances p53 binding to ubiquitin, we immunoprecipitated protein lysates derived from hNCC treated with IgG or antiubiquitin antibodies, and

![Fig. 3.](image-url) GH expression in human colon tissue. Human colon tissue arrays comprising specimens from normal colon tissue, ulcerative colitis, colon adenocarcinoma, and colon adenocarcinoma metastasis. Representative images are shown. (A) GH (green) induced in colon of patients with ulcerative colitis vs. normal adjacent tissue (NAT). (Scale bar, 100 μm.) (B) GH (green) coexpressed in stromal cells with IgA (red), a marker for plasma cells. (Scale bar, 25 μm.) (C) GH (brown) expressed in benign colon adenoma stromal tissue. (Scale bar, 100 μm.) (D) GH (green) and α-SMA (red) expressed in CAFs in (D) adenocarcinoma and in (E) metastasis. (Scale bars, 100 μm.) (F) GHR (brown) in normal adjacent tissue (NAT) and in colon adenocarcinoma. (Scale bar, 100 μm.)
immunoblotting showed that GH enhanced p53 degradation by increasing ubiquitin binding (Fig. 4D).

Ubiquitin-dependent p53 degradation is primarily mediated by ubiquitin E3 ligase MDM2 (53). GH treatment did not alter either total or pMDM colon cell levels (Fig. S3B), however, GH enhanced expression of Pirh2, another p53-specific E3 ligase, likely responsible for accelerated p53 ubiquitination. (Fig. 4E). Indeed, GH treatment of hNCC for 6 h increased p53 binding to Pirh2 (Fig. 4F). When Pirh2 was suppressed in hNCC stably expressing shPirh2, high GH was unable to fully suppress p53 protein (Fig. 4G). GH-induced Pirh2 up-regulation was abolished in cells pretreated with pegvisomant (Fig. S3C), further supporting a strong association between GHR activation and p53 degradation.

Tripartite motif-containing 29 (TRIM29), another E3 ligase (54), binds p53, resulting in nuclear sequestration, rendering p53 susceptible to ubiquitination (55). Because GH treatment enhanced TRIM29 levels in hNCC (Fig. 4E), we treated hNCC cells stably expressing TRIM29 shRNA with GH for 6 h. p53 was not decreased by GH in the presence of suppressed TRIM29 (Fig. 4H), indicating that p53 degradation in response to GH treatment is mediated, at least in part, by Pirh2 and TRIM29.

**GH inhibition of p53 results in APC suppression.** APC, acting as a tumor suppressor, is protective for intestinal tumor development, and APC mutations result in familial adenomatous polyposis in humans and mice (56). We tested GH effects on APC in hNCC and observed dose-dependent attenuation of APC protein levels (Fig. 5A).

The APC promoter is activated by caudal-related homeobox transcription factor (CDX2) (57), which is suppressed by phosphorylated extracellular signal-regulated kinase (ERK1/2) (58). Although pERK was dose-dependently induced by GH, CDX2 levels were decreased, likely accounting for the observed suppression of APC in GH-treated cells (Fig. 5A).

We assessed whether GH suppression of APC is p53-dependent by generating hNCC stably infected with lentivirus expressing p53 shRNA. With no GH treatment, these cells exhibited induced ERK phosphorylation, followed by decreased CDX2 and decreased APC expression (Fig. 5B), similar to that observed after treating WT hNCC with GH (Fig. 5A). Furthermore, p53-deficient cells did not respond to GH and expressed very low APC levels, whereas, in control cells stably expressing scramble shRNA, GH significantly attenuated APC levels (Fig. 5C). The results suggest that p53 suppression may be required for GH-dependent APC down-regulation in nontumorous colon cells.

**GH suppression of APC induces nuclear β-catenin.** When APC is suppressed, β-catenin undergoes nuclear translocation, expediting progresion actions (59). We therefore examined whether GH-mediated APC suppression regulates β-catenin localization. Because HCT116 cells express a mutated β-catenin isomor that cannot be phosphorylated and is not degraded (60), we used only hNCC. When treated with GH, both p53 and APC were suppressed in the cytoplasmic fraction, whereas p53 was suppressed in both cytoplasmic and nuclear fractions. β-Catenin abundance was also decreased in cytoplasmic fractions and up-regulated in nuclear fractions (Fig. 5D). Cytoplasmic β-catenin is abundant, and quantification showed cytoplasmic expression decreased ~20%, whereas intranuclear β-catenin expression was increased by 67% (Fig. 5S).

We next isolated cytoplasmic and nuclear protein fractions from hNCC stably expressing shp53. In these cells, GH did not increase nuclear β-catenin, whereas in control hNCC stably expressing scramble shRNA, nuclear β-catenin was increased (Fig. S6), consistent with markedly attenuated APC (Fig. 5D).
Testing β-catenin transcriptional activity after treatment of hNCC with GH showed a modest but consistent increase in canonical Wnt pathway activity as measured by assays of β-catenin-mediated transcription through T-cell factor/lymphoid enhancer binding factor (TCF/LEF) binding sites (Fig. 5E). β-Catenin targets, including matrix metalloproteinase 2 (MMP2) and MMP9 mRNA, as well as c-myc protein levels, were up-regulated after GH exposure (Fig. 5F and G), possibly as a result of β-catenin nuclear translocation.

**GH suppresses PTEN and inhibits apoptosis.** In both HCT116 and hNCC, we observed that PTEN, a transcriptional target of p53, was down-regulated in response to GH, likely as a result of p53 suppression (Fig. 6A and B). PTEN is a critical mediator of mitochondria-dependent apoptosis (61). Consistent with this function, we observed that, in both hNCC and HCT116 cells, GH treatment for 24 h suppressed cleaved caspase 3 expression, thereby decreasing apoptosis (Fig. 6C).

**GH enhances cell motility.** We further explored consequences of GH excess on markers of epithelial–mesenchymal transition (EMT) (62). The GH-induced EMT transcription factor Snail1, which activates mesenchymal markers, was induced in HCT116 cells, whereas in hNCC, Twist2 levels were also dose-dependently up-regulated (Fig. 6A and B). Induction of these transcription factors reflects EMT activation in response to GH treatment, permitting a permissive tumor microenvironment toward a mesenchymal phenotype promoting cell motility and invasion. Accordingly, consistent with Western blotting results, we observed enhanced migration of hNCC and HCT116 cells (50% and 40%, respectively) 24 h after GH treatment (Fig. 6D and Fig. S7).

Because GH expression was detected in human colon adenocarcinoma specimen stromal cells, we cocultured HCT116 epithelial tumor cells with human colon fibroblasts (hCF) infected with lentivirus expressing human GH. Using a GH-specific ELISA, we confirmed that GH-expressing fibroblasts secreted GH in culture (338 ng/mL), whereas in cultured fibroblasts expressing empty vector, GH was undetectable. In the presence of GH-secreting fibroblasts, HCT116 migration was enhanced twofold (Fig. 6E).

When anchorage-independent growth was assessed, we found that nontransformed hNCC did not form colonies in soft agar, but HCT116 cells cultured in the presence of GH-expressing fibroblasts formed fourfold more colonies, and colony size was 4.3 times larger than in cells cultured with control fibroblasts expressing empty vector (Fig. 6F). Thus, high GH levels appear to support protumorigenic cell properties, as reflected by decreased apoptosis, enhanced EMT transcription factors, and increased cell motility and colony formation.

**GH effects in colon mucosa of 3D primary human colon organoids.** We tested GH actions in induced pluripotent 3D intestinal organoid cultures that recapitulate cellular heterogeneity of human colon mucosa. Human fibroblast cell lines derived from healthy individuals were reprogrammed to generate iPSCs using a non-integrating reprogramming system (63). Cells were induced to support protumorigenic cell properties, as reflected by decreased apoptosis, enhanced EMT transcription factors, and increased cell motility and colony formation.

**Fig. 5.** GH suppresses APC and increases β-catenin nuclear translocation. Western blot analysis of ERK/APC pathway in (A) hNCC treated with indicated doses of GH for 24 h and (B) hNCC stably expressing scramble (Scr) shRNA or pShp3 shRNA. Experiments were repeated twice and representative blots shown. (C) Western blot analysis of hNCC stably infected with lentivirus expressing shp53 RNA or scramble (Scr) shRNA and untreated (C) or treated with GH (500 ng/mL). (D) Western blot analysis of cytoplasmic and nuclear fractions of hNCC untreated (C) or treated with GH (500 ng/mL) for 24 h. Experiments were each performed twice and representative results are shown. (E) Assay of canonical Wnt pathway signaling through activation of a TCF/LEF luciferase reporter construct (TOPFlash) or a control reporter (FOPFlash). hNCC were nucleofected with reporter constructs and treated with GH (500 ng/mL) for 24 h. Fold-induction was calculated as normalized relative light units of TOPFlash divided by those of FOPFlash. Results are mean ± SEM of triplicate measurements; *P < 0.05. The experiment was repeated four times with similar results and representative results shown. (F) Real-time PCR assessment of β-catenin target genes MMP2 and MMP9 expression in hNCC treated with GH (500 ng/mL) for 24 h. Normalized PCR results are expressed as fold-change vs. control taken as 1. Results are depicted as mean ± SEM of triplicates measurements. Two independent experiments were performed, and representative results depicted; *P < 0.05. (G) Western blot analysis of c-myc in hNCC treated with GH (500 ng/mL) for 24 h. In E and G, the differences between groups were analyzed using two-tailed unpaired Student t test.
and confirming our in vitro observations that effects of GH in the colon may be independent of IGF1 in this experimental model.

**Discussion**

We demonstrate novel mechanisms underlying the relationship between GH and specific tissue growth (21, 45, 66, 67). Our results suggest that the known protective effect of GH signaling deficiency on neoplastic tissue growth is mediated, at least partially, by regulating p53 expression. In contrast, GH may act as a tumor promoter in colon tissue by suppressing p53, PTEN, and APC.

In skin fibroblasts derived from GHRmut−/− Laron and from normal subjects, baseline p53 were higher in GHRmut−/− cells. Reconstituting the WT GHR in mutant fibroblasts resulted in dose-dependent p53 attenuation, whereas suppressing GH signaling in WT human fibroblasts led to p53 accumulation. Comparable results were obtained in mice with GH signaling deficiency. Colon tissue derived from Prop1−/− dwarf mice exhibited markedly increased p53 and p21 levels associated with restrained cell proliferation. These specific observed changes are likely responsible for the striking decrease in intestinal tumor number and size, as well as in the rate of tumor formation in these mice. Colon tumors observed in doubly mutant APCmut−/−/Prop1−/− animals that express high colon p53 levels.

We cannot exclude that tumor protective effects observed in Prop1−/− mice may also be attributed to other hormone deficiencies, as these animals may also lack thyroid stimulating hormone and prolactin, and may have low gonadotropin levels (68). However, GHR−/− mice exhibit increased colon p53 expression as well, and direct in vitro GH suppression in hNCC and HCT116 colon cells also leads to p53 induction. Importantly, blocking of colon cell GHR by pegvisomant in vitro, and co-culturing of HCT116 cells with GH, leading to decreased CDX2 and, subsequently, APC expression may therefore underlie the progrowth potential of GH.

**Low APC expression effects of GH signaling deficiency**

GHR-induced p53 suppression leads to increased ERK1/2 dephosphorylation (74). Based on our observations, it appears that GH-induced p53 suppression leads to increased ERK phosphorylation and decreased APC expression. It is not clear whether APC expression is p53-dependent (75, 76). Our results showing decreased APC in cells where p53 is suppressed suggest that, in colon cells, APC is at least partially dependent on intact p53.

**Low APC expression effects of GH signaling deficiency**

Low APC expression effects of GH signaling deficiency and increased Wnt signaling through activation of protooncogenic genes (59, 77). In our experiments, in nontumorous colon cells, GH induced β-catenin nuclear translocation with subsequent activation of β-catenin–mediated transcriptional activity, p53 and APC suppression may therefore underlie the progrowth potential of GH. Furthermore, by suppressing both p53 and PTEN, GH abrogated apoptosis thereby favoring cell survival (78). We also show GH-induced cell migration and anchorage-independent growth with expression of transcription factors Snail1 and Twist 2, early steps in EMT activation (62).

Because GH is induced in CAFs surrounding colon adenocarcinoma, and GHR is abundantly expressed in colon cancer cells, our results suggest that CAF-derived GH may enhance cell transformation as evidenced by increased anchorage-independent growth of HCT116 cells cocultured with GH-expressing fibroblasts. These results are consistent with observations by others that intracellular
GH promotes breast cancer cell transformation (79, 80) and induces an invasive phenotype by triggering EMT (81).

GH treatment of intestinal organoids closely recapitulating normal human intestinal and colon mucosa resulted in suppression of p53 and APC. Moreover, GH treatment led to down-regulation of E-cadherin, which maintains cell adhesion (82), whereas N-cadherin, indicative of a mesenchymal phenotype (62), was induced, underscoring the role of colon GH in EMT.

Five of nine mice responded to high circulating GH concentrations with suppressed colon p53, and seven of nine mice responded with p21 suppression, validating our in vitro results. The response of colon tissue to the circulating GH was not universal, which may reflect individual sensitivity to high GH.

Several studies show that GH acts independently of IGF1 in multiple tissues (7, 22, 83), and in our studies IGF1 suppression does not alter suppressive effects of GH on p53. Effects of IGF1 on p53 appear to be tissue specific as IGF1 induces p53/21 in human skin fibroblasts and primary liver cells (84–86), while reducing p53 in rat myocytes (87). However, we cannot exclude that our observed in vivo GH effects may be mediated, at least in part, by IGF1, as high circulating GH increased IGF1 blood levels and liver expression. Circulating IGF1 may induce murine colon cancer growth (88), whereas in humans, the colon cancer risk for high IGF1 is modest compared with that seen in acromegaly (89). GH overexpression in transgenic mice is associated with increased abundance of tumor suppressor proteins and protective effects of GH signaling d...

**Methods**

**Human Subjects.** Seven acromegaly patients in whom pegvisomant treatment was indicated were recruited for the clinical trial (https://clinicaltrials.gov/ct2/show/NCT01261000) and underwent screening evaluation for inclusion criteria (see Table S1 and SI Methods for inclusion and exclusion criteria).

Before initiating pegvisomant treatment, patients underwent colonoscopy, during which pretreatment colon mucosal biopsies were obtained. Patients then received a loading dose of 40 mg pegvisomant by subcutaneous injection, followed by a daily subcutaneous pegvisomant injection of 20 mg/d for 8 wk.

Following 8 wk of pegvisomant treatment, patients underwent sigmoidoscopy, during which posttreatment colon mucosal biopsies were obtained. Patients were evaluated for signs and symptoms of acromegaly at this time and IGF1 levels were again measured. Patients were monitored for potential side effects for the duration of the protocol, and continued to receive daily subcutaneous pegvisomant treatment for 6 mo after the study.

**Human colon tissue arrays, which include pathology diagnosis, were purchased from US Biomax.**

**Mice.** Prop1min−/− (Oca2 Prop1ββ) and APCmin−/− breeding pairs were purchased from Jackson Laboratory. Both Prop1−/− and APCmin−/− mice were backcrossed to the parental strains at least four times. WT and Prop1−/− mice were obtained by breeding Prop1+− females and males. APCmin−/− Prop1−/− mice were obtained by breeding APCmin−/− males to Prop1−/− females. APCmin−/− mice usually do not develop colon tumors unless they are fed with a high-fat diet. Therefore, mice were placed on a high-fat diet starting from age 2 mo.
Cells. Human skin fibroblasts derived from a 21-y-old short-stature male carrying a GHR mutation and from his 23-y-old normal height male sibling (Donotor: Δ22) were generous gifts from Ron Rosenfeld (Oregon Health Science University, Portland, OR). Human fibroblasts were cultured in αMEM medium (Invitrogen) supplemented with 10% (vol/vol) FBS.

Human colon carcinoma HCT116 cells were obtained from American Type Culture Collection (ATCC) and cultured in McCoy’s 5A medium (Invitrogen) and 10% (vol/vol) FBS. Primary hNCC were purchased and cultured in plates pretreated with Applied Cell Extracellular Matrix in PriGrow III Media (both from Applied Biological Materials) supplemented with 5% (vol/vol) FBS. Cells from passage ≤4 were used for experiments.

Normal hCF were purchased and cultured in full fibroblasts media (both from Cell Biologics). Cells from passage 3 were used for experiments. All cell lines tested negative for mycoplasma.

Constructions and Transfections. For constructions and transfections, see SI Methods.

Three-Dimensional Intestinal Organoïds. Healthy control fibroblasts were obtained from the Coriell Institute for Medical Research or derived from healthy human volunteer donors at Cedars-Sinai (IRB# 00027264). Three-dimensional intestinal organoids were generated from a control fibroblast “831” iPSC line generated using an episomal plasmid reprogramming system, which has been extensively characterized elsewhere (63). To induce differentiation, all iPSCs were cultured with a high dose of Activin A (100 ng/mL, R&D Systems) with increasing concentrations of FBS over time [0%, 0.2%, and 2% (vol/vol) on days 1, 2, and 3, respectively]. Wnt3A (25 ng/mL, R&D Systems) was also added on the first day of endoderm differentiation. To induce hindgut formation, cells were cultured in Advanced DMEM/F12 with 2% (vol/vol) FBS along with CHIR99021 (2 μM; Tocris), noggin, and EGF (both 100 ng/mL; all R&D Systems) and B27 (1×; Invitrogen). Organoids were passaged every 7–10 d thereafter.

In Vitro Treatments. Recombinant human GH (Bio Vision or R&D Systems) was reconstituted in culture medium containing 0.1% BSA. Cells were placed in culture medium free of serum containing 0.1% BSA, GH was added, and cells were harvested 24 h later. If treatment was extended to 48 h, GH was added daily. MG-132 (Sigma-Aldrich; 10 μM in DMSO) was added for 24 h. Control cells were treated with DMSO.

Protein Analysis. Cells were homogenized and lysed in RIPA buffer (Sigma-Aldrich) with 10 M protease inhibitors (Sigma-Aldrich). For Western blot analysis, proteins were separated by SDS-PAGE, electrophoblotted onto Trans-Blot Turbo Transfer Pack 0.2-μm PVDV membrane (Bio-Rad), and incubated overnight with antibodies, followed by corresponding secondary antibodies (Sigma-Aldrich). See SI Methods for antibody information.

For immunohistochemical analysis of human tissue, we used antibodies to GH followed by secondary antibodies conjugated with chicken anti–rabbit AlexaFluor 488 or donkey anti-goat Alexa Fluor568 (Invitrogen). Antigen retrieval was performed in 10 mM sodium citrate, and control reactions were devoid of primary antibodies or stained with blocking antibodies. Staining of GHR was performed using antibodies from LSBio (LS-C171952). Samples were imaged with a Leica TCSPP spectral confocal scanner (Leica Microsystems) in dual-emission mode to distinguish autofluorescence from specific staining.

NE-Per Nuclear and cytoplasmic Extraction Reagents (Thermo Scientific, #78833) were used to isolate cytoplasmic and nuclear fractions in hNCC.

Immunoprecipitation and real-time PCR, SA-galactosidase Activity, cell proliferation assay and motility assay were performed as previously described (S1, 95). For details, see SI Methods.

TOPFlash Reporter Assay. hNCC were nucleofected with 1 μg DNA of the standardized pair of Super16xTOPFlash and Super16xTOPFlash plasmids (#12456, #12457 Addgene), each with a Renilla control luciferase plasmid applied for signal normalization. Luciferase assays were performed using a Dual-Luciferase Reporter Assay Kit (Promega), and fold-induction was calculated as normalized relative light units of TOPFlash divided by those of FOPFlash.

Mouse Xenograft Model. HCT116 cells stably infected with lenti-murine GH (5 × 10⁵ cells in 0.05 mL PBS) were mixed (1:1) with Matrigel (Corning) and injected subcutaneously into the right flank of nine athymic nude female mice (Jackson Laboratory) to establish a model of excess systemic GH. Control mice were injected with HCT116 cells infected with lentiv. All nine mice developed xenograft tumors and were killed 5 wk after injection. Blood, liver, and colon tissues were collected and analyzed.

Hormone Measurements. Plasma GH was measured using mouse/rat GH ELISA from Millipore and plasma IGF1 was measured using mouse/rat IGF1 ELISA from ALPCO Diagnostics according to the manufacturer’s instructions.

Statistics. Differences between groups were analyzed using two-tailed unpaired Student t test. Differences in average tumor counts and size between groups by genotype were tested by way of ANOVA followed by Tukey test to adjust for multiple comparisons. All testing was done two-sided. Probability of P < 0.05 was considered significant.

Study Approval. The pegvisomant clinical trial protocol (https://clinicaltrials.gov/ct2/show/NCT01261000) was approved by the Cedars-Sinai Institutional Review Board and informed consent for use of biopsy tissue or paraffin slides for research was obtained from each patient before study procedure. Experiments performed on mice were approved by the Cedars-Sinai Institutional Animal Care and Use Committee.

ACKNOWLEDGMENTS. The authors thank Dr. M. Pimentel for performing colon biopsies and Ms. Shira Berman for assistance in preparing the manuscript. This work was supported by NIH Grants DK103198 and DK007770; the Doris Dorsey Molecular Endocrinology Laboratory; and investigator-initiated research Grant WS921563 from Pfizer (to S.M.).


Supporting Information

Chesnokova et al. 10.1073/pnas.1600561113

SI Methods

Human Subjects Inclusion and Exclusion Criteria. Inclusion criteria included: (i) diagnosis of acromegaly established on the basis of symptoms and signs at presentation, evidence of a pituitary adenoma on magnetic resonance imaging, elevated serum concentrations of IGF1 (>1.3× upper limit of normal), and inadequate GH suppression (>0.4 ng/mL) following an oral glucose load; (ii) patients were candidates to receive pegvisomant therapy following pituitary adenoma surgery, or were intolerant of other medical treatments or had not undergone previous therapy; (iii) normal liver function tests before treatment; (iv) dynamic testing of the pituitary axis and, if applicable, appropriate hormone replacement.

Exclusion criteria included: (i) long-acting somatostatin analog within 12 wk before enrollment; (ii) macroadenoma with visual field defects as a result of chiasmatic compression; (iii) clinically significant hepatic abnormalities and/or aspartate aminotransferase and/or alanine aminotransferase >3× the upper limit of normal during the screening period; (iv) known hypersensitivity to any of the test materials or related compounds; (v) history of, or known current, problems with alcohol or drug abuse; (vi) any mental condition rendering the patient unable to understand the nature, scope, and possible consequences of the study, and/or evidence of an uncooperative attitude.

Constructs and Transfections. Lentiviral particles expressing human shTRIM29 RNA, shp53 RNA, shPirh2 RNA, shGH RNA, shIGF1-RNA, or nontargeted shRNA control (GFP Control Lentiviral Particles) are all from Santa Cruz Biotechnology. Cells were selected in 8 μg/mL puromycin.

Lentiviral particle expressing murine (EF1-luc2-GH-Ubic) or human GH (EF1-GH1-IR-GFP) and the respective control lentiviral particles were generated at the Cedars-Sinai Virus Core facility. Specific human siGH1 RNAi and shGH RNAi were purchased from Santa Cruz Biotechnology.

pCDNA3.1hGHR was cloned in the laboratory. The hGHR (1,917 bp) was PCR-amplified from mRNA derived from LNCap cells, and resulting amplified fragments were cloned into BamHI/XhoI sites in pcDNA3.1 vector and sequenced. Indicated amounts of pcDNA3.1hGHR supplemented with empty vector pcDNA3.1 DNA for a total amount of 1 μg were nucleofected into 5 × 10^5 primary human GHRmut fibroblasts. Control samples were nucleofected with 1 μg pcDNA3.1. Ten μg purified RNA by the SuperScript II First-Strand cDNA synthesis system (Thermo Fisher Scientific). Quantitative PCR was performed in 20-μL reactions using IQ SYBR Green Master Mix in Bio-Rad IQ5 instrument (Bio-Rad). Specific validated primers for human p53, MMP2, and MMP9, as well as murine GH were purchased from SuperArray (Qiagen). Triplicate PCR reactions yielded threshold cycle (Ct) averages, with coefficient of variance of <0.05%, and ΔCt values (ΔCt = Ct of target gene minus Ct of housekeeping gene) determined. All experiments included template-free (water) and reverse-transcriptase minus controls to prevent contamination. Relative mRNA quantities in experimental samples were determined, normalized to housekeeping genes, as indicated, and expressed in arbitrary units as fold-difference from control.

SA-β-Galactosidase Activity. Senescence-associated (SA)-β-galactosidase enzymatic activity was assayed in vitro by β-galactosidase staining (Senescence Cell Staining Kit, Sigma-Aldrich). Briefly, 10,000 cells were plated in 12-well plates, treated with nutlin for the indicated times, incubated overnight, washed with PBS (pH 6.0), fixed, and stained with 5-bromo-4-choro-3-indolyl-b-D-galactopyranoside (X-Gal) overnight at 37 °C. Only senescent cells stain at pH 6.0.

Cell Proliferation. Asynchronized human skin fibroblasts were pulsed with BrdU (BrdU Labeling and Detection Kit, Roche) diluted 1:1,000 in medium with 10% (vol/vol) PBS for 1 h at 37 °C. Cells were washed in PBS, trypsinized, and fixed in glycine/70% ethanol fixative at pH 2.0 for 1 h at −20 °C. Fixed cells were washed with PBS, pelleted, and immunostained with BrdU mouse monoclonal antibody with the nucleases for 30 min at 37 °C, followed by goat anti-mouse Alexa488 secondary antibody (Invitrogen) for 1 h at room temperature. Cells then were washed, resuspended in 1 mL PBS, and analyzed by FACScan (Becton Dickinson).

Real-Time PCR. Total RNA was isolated from cells with TRIzol (Invitrogen) followed by RNAeasy mini Kit (Qiagen). After DNase I treatment (TURBO DNA free, Ambion), cDNA was synthesized from 3 μg purified RNA by the SuperScript II First-Strand cDNA synthesis system (Thermo Fisher Scientific). Quantitative PCR was performed in 20-μL reactions using IQ SYBR Green Master Mix in Bio-Rad IQ5 instrument (Bio-Rad). Specific validated primers for human p53, MMP2, and MMP9, as well as murine GH were purchased from SuperArray (Qiagen). Triplicate PCR reactions yielded threshold cycle (Ct) averages, with coefficient of variance of <0.05%, and ΔCt values (ΔCt = Ct of target gene minus Ct of housekeeping gene) determined. All experiments included template-free (water) and reverse-transcriptase minus controls to prevent contamination. Relative mRNA quantities in experimental samples were determined, normalized to housekeeping genes, as indicated, and expressed in arbitrary units as fold-difference from control.
In Vitro Motility Assays. For in vitro motility assays, 50,000 hNCC or HCT116 cells were seeded in BD Biocoat Growth Factor Reduced Matrigel Invasion Chambers (BD Biosciences) in 0.5 mL of serum free medium supplemented with 0.1% BSA. For GH treatment, 500 ng/mL GH was added to the medium. After 24 h, nonmigrated cells on the upper surface of the membrane were removed with a cotton swab. Cells that migrated through membrane pores to the lower part of membranes were fixed with 70% (vol/vol) ethanol, washed, and stained with Crystal violet. Each experiment was performed three times in duplicate. The number of migrating cells was counted in five random fields of each transwell.

For coculturing experiments, human colon fibroblasts stably infected with either lentivirus or lentihuman GH were plated on the bottom of 24-well plate (40,000 cells per well) in 1 mL of full fibroblast media (Cell Biologics) in duplicate. After 48 h, transwells with 50,000 HCT116 cells in 0.5 mL of McCoy media supplemented with 0.1% BSA were inserted. After 24 h, the HCT116 cells were fixed, and migrated cells were counted in six random fields. Culture media was collected for GH measurement. The experiment was repeated twice.

For soft agarose assay, 200,000 human colon fibroblasts infected with either lenti-human GH or lentivector were plated in six-well plates; the next day, media was removed and replaced by 0.6% agarose in full fibroblast media. After 48 h, HCT116 cells (5,000 per well) were placed on the top in 0.3% agarose in serum free McCoy media supplemented with 0.1% BSA. After 10 d, colony number was counted and colony size measured using ImageJ software. The experiment was conducted in duplicate and repeated twice.

**Fig. S1.** GH deficiency results in decreased proliferation and increased senescence. (A) Percent BrdU incorporation in skin fibroblasts derived from normal human (Cont) and from GHRmut subjects. The graph depicts mean ± SEM of measurements obtained from three independent experiments; *P < 0.05. (B) Real-time PCR of p53 and p21 mRNA levels in skin fibroblasts derived from normal human (Cont) and from GHRmut subjects. Normalized PCR results are expressed as fold-change vs. control taken as 1. Results are shown as mean ± SEM of triplicate measurements. (C) Western blot analysis of normal human skin fibroblasts transfected with 10 pM scramble (Scr) or hGHRsiRNA and harvested 48 h later. Experiments were repeated twice. (D) Percent BrdU incorporation in normal human skin fibroblasts transfected with scramble (Scr) or siGHR RNA for 48 h. Graph depicts mean ± SEM of triplicate measurements. *P < 0.05. In A, B, and D, differences between groups were analyzed using two-tailed unpaired Student t test.

**Fig. S2.** GH in pituitary and colon of Prop1−/− mice. (A) Western blot analysis of p53 in pituitary of Prop1−/− mice. (B) Real-time PCR of GH mRNA levels in colon tissue derived from WT and Prop1−/− mice. Normalized PCR results are expressed as fold-change vs. control taken as 100%. n = 7 mice per group. Results are shown as mean ± SEM of triplicate measurements.

**Fig. S3.** GH in pituitary and colon of Prop1−/− mice. (A) Percent BrdU incorporation in skin fibroblasts derived from normal human (Cont) and from GHRmut subjects. The graph depicts mean ± SEM of measurements obtained from three independent experiments; *P < 0.05. (B) Real-time PCR of p53 and p21 mRNA levels in skin fibroblasts derived from normal human (Cont) and from GHRmut subjects. Normalized PCR results are expressed as fold-change vs. control taken as 1. Results are shown as mean ± SEM of triplicate measurements. (C) Western blot analysis of normal human skin fibroblasts transfected with 10 pM scramble (Scr) or hGHRsiRNA and harvested 48 h later. Experiments were repeated twice. (D) Percent BrdU incorporation in normal human skin fibroblasts transfected with scramble (Scr) or siGHR RNA for 48 h. Graph depicts mean ± SEM of triplicate measurements. *P < 0.05. In A, B, and D, differences between groups were analyzed using two-tailed unpaired Student t test.
Fig. S3. Effect of GH on p53/p21 is MDM2 independent and is mediated by GHR. Western blot analysis: (A) of total and pSTAT5 in hNCC cells treated with 500 ng/mL GH and harvested at indicated time points, (B) of total and phospho MDM2 in hNCC treated with indicated doses of GH for 24 h, (C) of p53, p21 and Pirh2 in hNCC pretreated with 20 μg/mL pegvisomant for 1 h and then treated with 500 ng/mL GH for 24 h.

Fig. S4. GH effects on colon cells is not mediated by IGF1. Western blot analysis of (A) IGF1 in colon hNCC and HCT116 cells treated with indicated doses of GH, (B) p53 in hNCC treated with indicated doses of IGF1 for 24 h, and (C) hNCC cells stably infected with lentivirus expressing shIGF-1 RNA or scramble (Scr) shRNA and treated with 100 ng/mL GH for 24 h. Experiments were repeated twice and representative blots are shown.

Fig. S5. GH induces nuclear β-catenin accumulation. Intensities of protein bands from Western blot analysis of cytoplasmic and nuclear fractions of hNCC untreated (control) or treated with GH (500 ng/mL) for 24 h quantified, normalized to β-actin, and depicted as percent from control, taken as 100%. The experiment was performed twice and mean results are shown.
Fig. S6. GH induced nuclear β-catenin accumulation depends on p53. Western blot analysis of β-catenin in cytoplasmic and nuclear fractions of hNCC stably infected with lentivirus expressing shp53 RNA or scrambled shRNA (shScr) and untreated (C) or treated with GH (500 ng/mL). Experiments were repeated twice with similar results and representative blots are shown.

Fig. S7. GH enhances cell motility. Migration of hNCC and HCT116 cells treated with GH (500 ng/mL) and harvested 48 h after plating. Representative images are shown. (Magnification, 10×.)

Fig. S8. Three-dimensional intestinal organoids. (A) iPSC intestinal organoids are polarized and form crypt-like structures. (B) Organoids immunostained for phalloidin (red), a marker for actin fibers. (Magnification, 4×.)
Fig. S9. Effects of high circulating GH in mice bearing mGH-secreting tumor xenografts. Athymic nude mice were injected subcutaneously with 500,000 HCT116 cells stably infected with lentimGH or lentilV and killed 5 wk after injection. \( n = 9 \) mice/treatment group. (A) Western blot analysis of p53 and p21 in representative colon tissues not shown in Fig. 7. (B) Serum GH and IGF1. *\( P < 0.05 \), **\( P < 0.01 \). (C) Western blot analysis of IGF1 and p53 in representative liver and colon (from Fig. 7) tissues.

Table S1. Acromegaly subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age (y)</th>
<th>Gender</th>
<th>Pegvisomant study protocol (20 mg/d)</th>
<th>Colonic mucosa biopsy pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pretreatment IGF1</td>
<td>8 wk Posttreatment IGF1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[ng/mL (normal range)]</td>
<td>[ng/mL (normal range)]</td>
</tr>
<tr>
<td>1</td>
<td>67</td>
<td>F</td>
<td>447 (47–264)</td>
<td>151 (47–264)</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>F</td>
<td>598 (121–423)</td>
<td>162 (83–456)</td>
</tr>
<tr>
<td>3</td>
<td>79</td>
<td>F</td>
<td>361 (34–245)</td>
<td>83 (34–245)</td>
</tr>
<tr>
<td>4</td>
<td>31</td>
<td>F</td>
<td>636 (53–331)</td>
<td>448 (53–331)</td>
</tr>
<tr>
<td>5</td>
<td>39</td>
<td>M</td>
<td>435 (53–331)</td>
<td>66 (53–331)</td>
</tr>
<tr>
<td>6</td>
<td>54</td>
<td>M</td>
<td>749 (50–317)</td>
<td>256 (50–317)</td>
</tr>
<tr>
<td>7</td>
<td>62</td>
<td>F</td>
<td>386 (41–279)</td>
<td>132 (41–279)</td>
</tr>
</tbody>
</table>

F, Female; M, Male; — no significant histopathological changes.