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Bone Morphogenetic Protein Signaling Regulates Gastric Epithelial Cell Development and Proliferation in Mice

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Abstract

BACKGROUND & AIMS—We investigated the role of bone morphogenetic protein (BMP) signaling in the regulation of gastric epithelial cell growth and differentiation by generating transgenic mice that express the BMP inhibitor noggin in the stomach.

METHODS—The promoter of the mouse *H+/K+-ATPase β-subunit* gene, which is specifically expressed in parietal cells, was used to regulate expression of noggin in the gastric epithelium of mice. The transgenic mice were analyzed for noggin expression, tissue morphology, cellular composition of the gastric mucosa, gastric acid content, and plasma levels of gastrin. Tissues were analyzed by immunohistochemical, quantitative real-time polymerase chain reaction, immunoblot, microtitration, and radioimmunoassay analyses.

RESULTS—In the stomachs of the transgenic mice, phosphorylation of Smad1, 5, and 8 decreased, indicating inhibition of BMP signaling. Mucosa were of increased height, with dilated glands, cystic structures, reduced numbers of parietal cells, and increased numbers of cells that coexpressed intrinsic factor, trefoil factor 2, and griffonia simplicifolia lectin II, compared with wild-type mice. In the transgenic mice, levels of the *H+/K+-ATPase α-subunit* protein and messenger RNA were reduced, whereas those of intrinsic factor increased. The transgenic mice

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were hypochloridric and had an increased number of Ki67- and proliferating cell nuclear antigen-positive cells; increased levels of plasma gastrin; increased expression of transforming growth factor- α , amphiregulin, and gastrin; and activation of extracellular signal-regulated kinase 2.

CONCLUSIONS—Inhibiting BMP signaling in the stomachs of mice by expression of noggin causes loss of parietal cells, development of transitional cells that express markers of mucus neck and zymogenic lineages, and activation of proliferation. BMPs are therefore important regulators of gastric epithelial cell homeostasis.

Keywords

Trefoil Factor 2; TFF2; Transforming Growth Factor; TGF; Griffonia Simplicifolialectin II; GSII; Smads; Chief Cells; Mucus Neck Cells

The bone morphogenetic proteins (BMPs) have been shown to regulate a broad array of biologic actions during both embryonic and postnatal vertebrate development.¹⁻¹⁵ BMP-2, BMP-4, and BMP-7 are among the most studied members of the BMP family of regulatory factors. The actions of the BMPs can be specifically blocked, in vivo, by inhibitory proteins that are expressed in tissues to modulate the level of activation of BMP signaling. Of these, noggin, a secreted polypeptide present in several mammalian tissues, has been shown to bind to and inhibit the actions of BMP-2, BMP-4, and, to a lesser degree, BMP-7.^{3,5,9,16,17}

One of the best-characterized signaling pathways that mediate the intracellular actions of the BMPs is that leading to the activation of the regulatory proteins Smad 1, 5, and 8.^{18,19} In particular, binding of the BMPs to the BMP type I receptor leads to the dimerization of BMPR-I with the BMP type II receptor, a molecule that has serine/threonine kinase activity. This event triggers the phosphorylation of both BMP type I receptor and of Smad 1, 5, and 8, allowing the association of Smad 1, 5, and 8 with Smad 4 in a heterodimeric complex that translocates to the nucleus where it activates gene transcription.^{18,19}

Studies have shown that either transgenic expression of noggin in the mouse intestinal epithelium or conditional inactivation of BMPR1A leads to the formation of new crypts and to an increased number of proliferative cells in normally differentiated areas of the villi.^{9,10} Similar abnormalities have been detected in patients with Juvenile Polyposis Syndrome, a disease characterized by the formation of hamartomatous polyps throughout the gastrointestinal tract and by increased colon cancer risk.^{7,8,11} Genetic analysis of these subjects has revealed that these abnormalities are due to the presence of germ-line mutations in genes involved in BMP signal transduction such as BMPR1A and Smad 4.^{7,8,11} The importance of BMP signaling in gastrointestinal carcinogenesis has been further substantiated by the observations that BMP-2 and BMP-7 inhibit the growth of gastric and colonic cancer cells and that epigenetic silencing of the BMP-2 gene through methylation can be detected in gastric cancers.¹²⁻¹⁴ Reports have also indicated that conditional inactivation of BMPR1A leads to increased cellular proliferation and to the development of cancers at the level of gastric epithelial transition zones.¹⁵

In support of a possible role of the BMPs in the regulation of gastric epithelial cell maturation and differentiation, studies from our laboratory have shown that incubation of cultured parietal cells with BMP-4 leads to stimulation of H⁺/K⁺-ATP-ase α -subunit gene expression and to enhancement of secretagogue-stimulated gastric acid production.²⁰ Moreover, our laboratory has demonstrated that BMP-4 can block extracellular signal-regulated kinase (ERK)-dependent inhibition of H⁺/K⁺-ATPase gene expression, suggesting that activation of BMP signaling might regulate the induction and maintenance of a differentiated parietal cell phenotype.²⁰

Accordingly, because the mechanisms that regulate the growth, differentiation, and maturation of the cells of the gastric epithelium have been poorly defined, we sought to test the hypothesis that induction of BMP signaling in the stomach is an important mechanism for the inhibition of cell proliferation and for the induction of programs of cell activation that leads to cellular maturation and differentiation. Toward this goal, we generated transgenic (TG) mice that express the BMP inhibitor noggin in the gastric epithelium. Our studies demonstrate that inhibition of BMP signaling in vivo leads to enhanced gastric epithelial cell proliferation, reduced parietal cell numbers, and expansion of transitional cells coexpressing intrinsic factor (IF), trefoil factor 2 (TFF2), and griffonia simplicifolialectin II (GSII).

Material and Methods

Generation of Noggin Transgenics

The H/K-noggin transgene construct contains the mouse noggin complementary DNA driven by the mouse H⁺,K⁺-ATPase β -subunit promoter (−1035 to +24) followed by human growth hormone sequences, which provided introns and a poly A⁺ site (Figure 1A).²¹ Construction of the transgene was performed by Intrexon (Blacksburg, VA) (for additional details see Supplementary Materials and Methods).

Quantitative Real-time Polymerase Chain Reaction Analysis

Quantitative real-time polymerase chain reaction (QRT-PCR) was performed using primer sequences and protocols that were obtained from either previously published reports^{21,22} or commercially available sources (SA Biosciences Corp, Frederick, MD) (see Supplementary Materials and Methods).

Western Blots

Western blots were performed according to previously published reports²⁰ (see Supplementary Materials and Methods).

Histochemical Analysis

These studies were carried out according to previously reported methods with minor modifications^{20,21,23,24} (see Supplementary Materials and Methods).

Basal Gastric Acid Content

Gastric acid content was measured according to previously described methods²¹ (see Supplementary Materials and Methods).

Gastrin Radioimmunoassay

Plasma gastrin was measured using radioimmunoassay according to previously described methods²⁵ (see Supplementary Materials and Methods).

Statistical Analysis

Data are expressed as means $\pm$ standard error. Statistical analysis was performed using Student *t* test. *P* values < .05 were considered to be significant.

Results

To define the role of BMP signaling in gastric epithelial homeostasis, we generated TG mice that express noggin in the gastric epithelium. Seven TG-lines were established. Analysis at 12 weeks after birth of noggin expression by QRT-PCR demonstrated, as shown in Figure

1B, that 2 of these lines, Nog.3 and Nog.4, exhibited a greater than 100-fold increase in noggin messenger RNA abundance. Similar results were obtained in QRT-PCR assays using primers that measured transgene noggin messenger RNA (data not shown). For our studies, we used mice from line Nog.4. We confirmed that expression of noggin leads to inhibition of BMP signaling. First, we examined the localization of cells receiving BMP-mediated signals in the gastric mucosa of non-TG mice. As shown in the representative images depicted in Figure 1C, the anti-p-Smad1-5-8 antibody positively stained the nuclei of cells predominantly located in the isthmus and neck of the glands, sections known to contain mucus cells, parietal cells, endocrine cells, and rare gastric progenitor cells. As depicted in Figure 1C, some of the positively stained cells had the morphologic appearance of parietal cells. Immunohistochemical staining of the mucosa of the TG mice with the anti-p-Smad1-5-8 antibody demonstrated, when compared with age-matched, non-TG littermates, a marked decrease in the number of p-Smad1-5-8-positive nuclei. Western blot analysis of phosphorylated forms of Smad1, 5, and 8 in both non-TG and TG mice, confirmed, as shown in Figure 1D and E, a decreased level of expression of p-Smad 1, 5, and 8 in the TG mice.

We investigated the morphologic consequences of inhibition of BMP signaling in the gastric epithelium of 4-, 12-, and 24-week-old TG mice. As indicated in Figure 2B, it was possible to detect morphologic abnormalities of the fundic mucosa, mainly characterized by a decrease in the number of cells that had the morphologic appearance of parietal cells, as early as 4 weeks after birth. These changes were, however, more pronounced in the 12-week-old TG mice, whose mucosa exhibited increased height, dilated glands, and an altered epithelium characterized by extensive pale staining cells and by a prominent decrease in the number of cells that had the morphologic appearance of parietal cells (Figure 2D and E). Moreover, the epithelium of the 12-week-old TG mice contained cystic structures filled with amorphous material (Figure 2E). Similar changes were detected in the gastric mucosa of the 24-week-old TG mice (data not shown). In view of these observations, we opted to conduct a detailed phenotypic analysis in 12-week-old mice.

Counting of cells that had the morphologic appearance of parietal cells in H&E-stained sections from the gastric mucosa of both 12-week-old TG- and non-TG mice demonstrated the presence of a decreased number of parietal cells in the TG mice (Figure 3A). Similar results were detected in both 4- and 24-week-old animals (data not shown). Analysis of parietal cells by immunostaining for H^+, K^+ -ATPase α -subunit confirmed the presence of a decreased numbers of parietal cells in the TG mice (Figure 3B). In addition, the parietal cells of the TG mice exhibited an irregular and disorganized distribution. To further investigate the role of BMP signaling in parietal cell biology, we performed Western blots with anti- H^+/K^+ -ATPase α -subunit antibodies. Analysis of gastric mucosal lysates demonstrated decreased expression of the H^+/K^+ -ATPase α -subunit protein (Figure 3C). Similar results were observed in QRT-PCR assays with specific H^+/K^+ -ATPase α -subunit gene primers that demonstrated a significant decrease in the expression of the H^+/K^+ -ATPase α -subunit gene in 4-, 12-, and 24-week-old animals (data not shown). To assess the functional significance of these findings, we measured the basal acid content and showed that the TG mice exhibited a statistically significant decrease in acid production (Figure 3D). Thus, inhibition of BMP signaling affects parietal cell number, H^+/K^+ -ATPase expression, and, ultimately, gastric acid secretion.

Some pathologic states of the stomach are characterized by loss of parietal cells and by expansion of an aberrant mucus cell type, which can be visualized with Alcian blue,²¹ a stain that detects mucins normally present in more distal areas of the gut. Accordingly, we stained sections of the gastric mucosa with periodic acid Schiff/Alcian blue, to detect the presence of neutral (pink) and acidic (blue) mucins, respectively. The mucosa of the TG

mice, but not that of TG-negative littermates (Figure 4A and B), exhibited extensive regions in the middle of the glands that stained positive with both periodic acid Schiff and Alcian blue. The aberrant mucus cells were also stained with the lectin GSII (Figure 4D), which in the normal fundus is a marker of mucus neck cells^{21,26} (Figure 4C).

In the mouse stomach, IF is a well-established marker of zymogenic cell differentiation. Loss of parietal cells is commonly associated with a decrease in the number of chief cells.^{21,27-30} We therefore investigated whether inhibition of BMP signaling led to significant perturbations in zymogenic cell differentiation. Accordingly, we stained sections of the fundic mucosa of both TG- and non-TG mice with anti-IF antibodies. As shown on Figure 5A, whereas the non-TG mice demonstrated the presence of positively stained cells only at the base of the glands, the TG mice displayed a significant increase in the number of IF-stained cells, which could be detected in both the base and the neck of the glands. To validate the observation of increased IF-expressing cells, we performed Western blots with anti-IF antibodies and QRT-PCR using specific IF gene primers. The QRT-PCR analysis, depicted in Figure 5B and the Western blots, shown in Figure 5C, demonstrate increased expression of both the IF protein and transcript in the TG mice. Thus, inhibition of BMP signaling achieved by transgenic expression of noggin, elicits expansion of IF-expressing cells.

Studies have shown that mucus neck cells differentiate from preneck cells and that the mucus neck cells subsequently transdifferentiate into fully differentiated zymogenic cells as they migrate toward the base of the fundic gland.³¹ Accordingly, we investigated whether inhibition of BMP signaling could lead to the development of transitional cells expressing both mucus neck and zymogenic cell markers. Sections of the fundic mucosa of both TG- and non-TG mice were costained with Alexa 488-conjugated GSII and anti-IF antibodies. As shown on the overlay images, the mucosa of the TG mice exhibited a marked increase in the number of double-stained cells (Figure 6B). TFF2 is a peptide growth factor that, in the normal stomach, is predominantly expressed in deep gland cells of the antrum and in the mucus neck cells of the fundic glands.^{21,23,24,26,32,33} Moreover, aberrant expression of TFF2 at the base of the fundic glands has been associated with the occurrence of spasmodic polypeptide expressing metaplasia (SPEM), an event linked to the development of both dysplasia and neoplasia in *Helicobacter felis*-infected mice.³² Staining of fundic mucosal sections with both anti-IF and anti-TFF2 antibodies demonstrated costaining (Figure 6C and D) in cells through the lower half of the fundic glands. Thus, inhibition of BMP signaling leads to the expansion of populations of transitional cells expressing markers of both mucus neck and zymogenic lineages. To further investigate the role of BMP signaling in the regulation of mucus neck cell differentiation, we measured, using QRT-PCR, the expression of MUC-6 and TFF2, 2 well-established mucus neck cell markers. Consistent with the increased histologic stains of mucus neck cell markers, the expression of both MUC-6 and TFF2 was markedly increased in the TG mice when compared with age matched non-TG littermates (Figure 6E and F), suggesting that BMP signaling exerts regulatory actions on the expression of markers of the mucus neck cell/zymogenic cell lineage.

We showed that expression of noggin leads to hypochlorhydria, an event known to cause hypergastrinemia. Thus, we examined whether the noggin TG mice exhibited changes in both gastrin expression and release. As shown in supplementary Figure 1A, depicting gastrin plasma levels measured in both TG- and non-TG mice by radioimmunoassay, the TG mice demonstrated a greater than 2-fold increase in plasma gastrin. Similarly, the TG mice displayed a significant increase in antral gastrin gene expression as indicated by the QRT-PCR data depicted in Supplementary Figure 1B. In a series of immunohistochemical studies conducted in the TG mice, we also confirmed that, in these animals, gastrin is exclusively

expressed in the antrum and not in the fundus (data not shown), confirming the notion that inhibition of BMP signaling in the fundus does not cause antralization of the fundic mucosa.

We examined whether the epithelium of the TG mice is hyperproliferative. Accordingly, we stained sections of the fundic mucosa with antibodies directed against proliferating cell nuclear antigen (PCNA) and Ki67, well-established markers of cell proliferation. As shown in Figure 7A, the TG mice exhibited a marked increase in the number of PCNA-positive nuclei. Similar results were seen when the sections were stained with anti-Ki67 antibodies. Moreover, as shown in Figure 7A, some of the positively stained cells were located in deeper regions of the glands, in areas that do not normally contain mitotic cells. In some of the TG animals, we also noted robust staining in the nuclei of cells lining the lumen of cysts, suggesting that these structures can be lined with proliferating cells. To obtain a more accurate assessment of the hyperproliferative state of the fundic epithelium of the TG mice, we counted the number of positively stained nuclei in the fundic glands of 3 separate sections derived from at least 4 different TG and non-TG mice. The TG mice exhibited a significant increase in the number of both PCNA- and Ki67-positive nuclei when compared with that of age-matched TG-negative littermates (Figure 7B). Because expression of noggin and induction of cell proliferation could lead to the development of apoptosis, we measured gastric epithelial cell apoptosis in the TG mice. Staining of sections of the gastric mucosa of both TG mice and of non-TG, age-matched littermates, with anticaspase-3 antibodies,³⁴ failed to reveal the presence of any significant amount of cellular apoptosis. Similar results were observed in Western blots with antibodies directed against cleaved PARP (Asp214), a target of cleaved caspase-3, which specifically marks apoptotic cells.³⁵ Review of H&E-stained sections of the stomach of the TG mice also failed to identify signs of significant gastric epithelial cell necrosis.

Induction of ERK activation and increased expression of epidermal growth factor (EGF) receptor (EGFR) ligands such as TGF- α and amphiregulin have been linked to stimulation of gastric epithelial cell proliferation.³⁶⁻³⁹ Accordingly, we investigated whether inhibition of BMP signaling leads to perturbations in the level of activation of these signaling mechanisms. Analysis of TGF- α and amphiregulin gene expression by QRT-PCR demonstrated increased level of expression of these growth factors in the fundic mucosa of the TG mice when compared with that of non-TG, age-matched littermates (Figure 7C). Similarly, as shown on the Western blots with anti-phospho-ERK2 antibodies, the TG mice displayed a marked increase in ERK2 activation, whereas no differences were noted in the level of expression of the nonphosphorylated form of the kinase (Figure 7D). Accordingly, inhibition of BMP signaling *in vivo* leads to increased expression of EGFR ligands and to induction of ERK activation, suggesting that these events might be responsible, at least in part, for the hyperproliferative activity of the gastric epithelium of the noggin TG mice.

Discussion

In this paper, we reported a series of novel observations that underscore the importance of the BMPs in the regulation of gastric epithelial cell proliferation and differentiation. In particular, we present evidence that inhibition of BMP signaling leads to the expression of a novel gastric phenotype characterized by increased epithelial cell proliferation, decreased parietal cell number, and by expansion of cells that express markers of both mucus neck and zymogenic cell differentiation.

Several studies have shown that the parietal cells play an important role in the process of differentiation and development of other cell lineages in the gastric mucosa. Loss of mature parietal cells leads to a block in the differentiation program of the zymogenic lineages and to the occurrence of different types of mucosal cell remodeling.^{21,27-30} A possible explanation

for the occurrence of these events might reside in the observation that the parietal cells are a major site for the production of growth factors and morphogens in the gastric epithelium.^{4,40,41} Indeed, the parietal cells have been shown to produce EGF receptor ligands and Sonic hedgehog, a peptide that plays an important role in the regulation of gastric epithelial cell maturation and differentiation.^{4,42,43} Interestingly, in contrast to other models of parietal cell loss, the noggin TG mice exhibit increased number, rather than loss, of cells expressing IF, a marker of zymogenic cells. It is conceivable that this phenotype might be the result of the type of experimental approach used for our studies. Whereas, in the other models, the parietal cells were completely ablated by the use of either bacterial toxins or pharmacologic compounds, our mice were engineered to express noggin, an agent that does not kill the parietal cells, but rather blocks a physiologic signal that could be involved in the regulation of their differentiation and maturation. Indeed, it is possible that the primary event caused by inhibition of BMP signaling is the occurrence of perturbations in the normal development and differentiation of the parietal cells. The previously reported observation that BMP-4 induces H/K-ATPase α -subunit gene expression in parietal cells would support a possible role of the BMPs in the regulation of parietal cell maturation.²⁰ Because parietal cells are known to direct zymogenic cell differentiation, the presence of abnormalities in parietal cell census and function might then, in turn, lead to secondary epithelial cell alterations. Although we did not observe any differences in the expression of Sonic hedgehog (data not shown), it is possible that other parietal cell-specific factors might have been affected by inhibition of the BMP signal transduction pathway. Alternatively, BMP signaling might directly regulate the census and lineage determination of cells belonging to the zymogenic lineage. Thus, inhibition of BMP signaling appears to cause severe abnormalities in the normal mechanisms that regulate the census and, possibly, the maturation and differentiation of cells belonging to both the parietal and the mucus neck/zymogenic lineages (Supplementary Figure 2).

One interesting finding of our study is the observation that inhibition of BMP signaling leads to the development of an increased number of TFF2-expressing cells in the fundic mucosa, an event that has been associated with SPEM, a type of metaplasia that appears to be linked to the development of intestinal type gastric adenocarcinomas.³² It appears that loss of parietal cells and alterations in the complex milieu of growth factors and morphogens of the gastric epithelium, such as those that might occur during chronic inflammatory states of the stomach, could be linked to the appearance of SPEM.³² One interesting theory is that SPEM could evolve from transdifferentiation of chief cells.³² Accordingly, it is possible that inhibition of BMP signaling might lead to profound changes in the normal mechanisms that regulate zymogenic cell differentiation leading to the aberrant expression of TFF2 at the base of the fundic glands.

In our study, we have also shown that expression of noggin in the epithelium of the corpus leads to induction of ERK activation and to stimulation of EGFR ligands gene expression, events that are likely to contribute to the hyperproliferative state of the fundic epithelium of the noggin TG mice. Accordingly, activation of BMP signaling appears to play an important role in the regulation of several mitogenic mechanisms of the gastric mucosa. These observations are in agreement with previous reports conducted both in vivo and in vitro that have indicated that BMP-4 inhibits EGF-stimulated ERK activation in isolated parietal cells and that prolonged overexpression of growth factors in the stomach alters the normal architecture of the gastric mucosa leading to increased ERK activation, loss of parietal cells, and foveolar hyperplasia.^{37,38}

Another finding of our report is that inhibition of BMP signaling leads to hypergastrinemia, an event that might represent a consequence of decreased parietal cell number and hypochloridria. Because gastrin has been shown to stimulate gastric epithelial cell growth

and to induce ERK activation,^{41,44,45} the hypergastrinemic state of our mice could represent one of the mechanisms responsible for the increased proliferative activity of the epithelium of the noggin TG mice. Future studies with gastrin knock out mice will be necessary to define better the complex interplay that exists between BMP signaling and gastrin in the stomach.

In contrast to our findings, a recent report has indicated that transgenic expression of noggin in the gastric epithelium by means of the Keratin 19 promoter fails to cause a significant gastric phenotype.⁴⁶ However, crossing of K19-Nog mice with genetically engineered animals that express an elevated level of PGE2s in the stomach appears to lead to the development of gastric hamartomas, suggesting that inhibition of BMP signaling by itself might not be sufficient to alter gastric epithelial homeostasis. It is possible that the differences in phenotypic outcome observed between our mice and the K19-Nog mice might be due to a higher level of expression of noggin achieved by our transgenic vector.

Alternatively, the ability of our mice to express an abnormal gastric phenotype could be due to differences in the site of noggin expression in the gastric epithelium. Indeed, whereas the K19 promoter directs the expression of noggin in surface epithelial cells, the H⁺/K⁺-ATPase β -subunit promoter, which is a parietal specific promoter, drives the expression of noggin in a microenvironment that contains not only fully differentiated cells, but also uncommitted gastric progenitors, cells that are likely to be particularly sensitive to the physiologic actions of the BMPs.

In conclusion, our study demonstrates that BMP signaling plays a crucial role in the regulation of gastric physiology because inhibition of this signaling mechanism leads to profound abnormalities in the census, maturation, and differentiation of several gastric epithelial cell lineages. These findings shed new insight into the complex signal transduction pathways that mediate the actions of growth factors in the stomach, providing new clues for a better understanding of the mechanisms that regulate gastric epithelial cell growth and differentiation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used in this paper

BMP	bone morphogenetic protein
ERK	extracellular signal-regulated kinase
GSII	griffonia simplicifolialectin II
IF	intrinsic factor
PCNA	proliferating cell nuclear antigen
QRT-PCR	quantitative real-time polymerase chain reaction
SPEM	spasmolytic polypeptide expressing metaplasia
TG	transgenic
TFF	trefoil factor

Figure 1.

Generation of noggin TG mice. (A) Diagram of the H/K-Noggin transgene. Noggin was placed under the control of the mouse H⁺,K⁺-ATPase β-subunit promoter. A mutated human growth hormone (*mut hGH*) cassette provided intron and polyadenylation sequences. (B) Transgene messenger RNA (mRNA) levels in 12-week-old mice from TG-lines Nog.3 and Nog.4 were compared with background levels in non-TG mice using QRT-PCR and displayed as fold increase over the non-TG negative controls. Values are shown as means ± standard error, n = 4. (C) Gastric paraffin sections from 12-week-old non-TG and TG mice were stained with the anti-p-Smad1-5-8 antibody and a biotin-conjugated secondary antibody. Scale bars, 50 μm. Arrows point to p-Smad1-5-8 positive cells. The *magnified window* depicts rare p-Smad1-5-8 positive nuclei in epithelial cells of the TG mice. Similar results were observed in 3 other 12-week-old non-TG and TG mice. (D) Expression of p-Smad1, 5, and 8 and GAPDH in gastric extracts obtained from 12-week-old non-TG and TG mice from line Nog.4 was studied by Western blots. (E) Bar graphs represent results obtained from densitometric analysis of the blots. P-Smad 1, 5, and 8 expression levels were normalized to GAPDH expression. Data are expressed as means ± standard error, n = 4. *P < .05.

Figure 2. Development of morphologic abnormalities in the gastric epithelium of noggin TG mice. Representative H&E-stained gastric paraffin sections of the corpus of 4-week-old non-TG (A) and TG mice (B) and of 12-week-old non-TG (C) and TG mice (D and E). *Scale bar*, 50 μ m. Similar histologic changes were observed in three 4-week-old and in seven 12-week-old TG mice from line Nog.4 and in four 12-week-old TG mice from line Nog.3.

Figure 3.

Diminished parietal cell number in noggin TG mice. (A) Number of cells per gland exhibiting the morphologic appearance of parietal cells in H&E-stained sections from 12-week-old TG and non-TG mice. Values are shown as means ± standard error, n = 3. **P* < .01. The sections from 12-week-old mice were stained with the anti-H⁺,K⁺-ATPase α-subunit antibody and a FITC-conjugated secondary antibody (B). Scale bar, 50 μm. Similar results were observed in 7 other 12-week-old TG mice. H⁺,K⁺-ATPase α-subunit protein expression in non-TG and TG mice was measured by Western blots (C). Basal gastric acid content in non-TG and TG mice was determined by automated titration (C). Acid content values are shown as means ± standard error, n = 3. **P* < .05.

Figure 4.

Expression of Alcian blue-positive mucus and expansion of mucous neck cells in noggin TG mice. Gastric paraffin sections from non-TG (A and C) and TG mice (B and D) were stained with periodic acid Schiff (PAS)/Alcian blue (*top panels*) and Alexa 488-GS II (*bottom panels*). Scale bar, 50 μ m. Similar results were observed in 3 other 12-week-old TG mice.

Figure 5.

Expression of noggin in TG mice leads to increased IF-expressing cells. Gastric paraffin sections from non-TG and TG mice were stained with the IF antibody and a biotin-conjugated secondary antibody (A). Scale bar, 50 μ m. Similar results were observed in 7 other 12-week old TG mice. IF messenger RNA (mRNA) abundance in non-TG mice was compared with that detected in TG mice using QRT-PCR and displayed as fold increase over the non-TG negative controls (B). Values are shown as means $\pm$ standard error, $n = 8$. $*P < .05$. IF protein levels in non-TG and TG mice were measured by Western blots with anti-IF antibodies (C).

Figure 6.

Alterations in gastric epithelial cell differentiation in *noggin* TG mice. Gastric paraffin sections from non-TG and TG mice were stained with anti-IF primary antibodies and Cy-3 conjugated secondary antibodies (*red*) together with Alexa 488-conjugated GS II (*green*) (A and B), anti-TFF2 primary antibodies, and Cy-3-conjugated secondary antibodies (*red*) together with anti-IF primary antibodies and FITC-conjugated secondary antibodies (*green*) (C and D). Scale bar, 50 μ m. Arrows point to cells expressing both markers. Similar results were observed in 2 other TG mice. QRT-PCR analysis of MUC-6 (E) and TFF2 (F) mRNA abundance in fundic RNA samples. Values are shown as fold change (means $\pm$ standard error, $n = 4$ for MUC-6 and $n = 8$ for TFF2) compared with non-TG levels. $*P < .05$.

Figure 7.

Increased cell proliferation and expression of mitogenic signaling pathways in noggin TG mice. (A) Gastric paraffin sections from non-TG and TG mice were stained with anti-proliferating cell nuclear antigen (PCNA) (*top panels*) and anti-Ki67 antibodies (*bottom panels*). Scale bar, 50 μ m. (B) Graph bars represent the number of Ki67 and PCNA-positive nuclei detected in both TG- and non-TG littermates. Values are shown as means $\pm$ standard error, $n = 4$ for PCNA and $n = 3$ for Ki67. $*P < .05$. (C) TGF- α and amphiregulin mRNA signals in non-TG mice were compared with those detected in TG mice using QRT-PCR and displayed as fold increase over the non-TG negative controls. Values are shown as means $\pm$ standard error, $n = 4$. $*P < .05$. (D) Phosphorylation and activation of ERK2 in non-TG and TG mice was studied by Western blots using an anti-phospho-ERK2 antibody. Total ERK2 levels were monitored by Western blots with an antibody recognizing ERK2 independent of its phosphorylation state.