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

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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 Smad 1, 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 (Bandeiraea) 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 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 α; TGF.

BASIC–ALIMENTARY TRACT

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 bone morphogenetic protein receptor type 1A 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

Abbreviations used in this paper: BMP, bone morphogenetic protein; ERK, extracellular signal-regulated kinase; GSII, Griffonia (Bandeiraea) simplicifolia lectin II; IF, intrinsic factor; PCNA, proliferating cell nuclear antigen; p-Smad, phospho-Smad; QRT-PCR, quantitative real-time polymerase chain reaction; SPEM, spasmolytic polypeptide expressing metaplasia; TFF, trefoil factor; TG, transgenic.

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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 (Bandeiraea) simplicifolia lectin 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 1*A*).[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 reports[20] (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 methods[21] (see Supplementary Materials and Methods).

Gastrin Radioimmunoassay

Plasma gastrin was measured using radioimmunoassay according to previously described methods[25] (see Supplementary Materials and Methods).

Statistical Analysis

Data are expressed as means ± 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 1*B*, that 2 of these lines, Noggin 3 (Nog.3) and Noggin 4 (Nog.4), exhibited a greater then 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 1*C*, the anti-phospho-Smad (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 1*C*, 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