BMPs are necessary for stomach gland formation in the chicken embryo: a study using virally induced BMP-2 and Noggin expression

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SUMMARY

Epithelial-mesenchymal interactions are necessary for the normal development of various digestive organs. In chicken proventriculus (glandular stomach), morphogenesis and differentiation of the epithelium depend upon the inductive signals coming from underlying mesenchyme. However, the nature of such signals is still unclear despite extensive analyses carried out using experimental tissue recombinations. In this study we have examined the possible involvement of bone morphogenetic proteins (BMPs) in the formation of stomach glands in the chicken embryo. Analysis of the expression patterns of BMP-2, -4 and -7 showed that these BMPs were present in the proventricular mesenchyme prior to the initiation of the proventricular gland formation. BMP-2 expression, in particular, was restricted to the proventriculus among anterior digestive organs. Virus-mediated BMP-2 overexpression resulted in an increase in the number of glands formed. Moreover, ectopic expression of Noggin, which antagonizes the effect of BMPs, in the proventricular mesenchyme or epithelium, led to the complete inhibition of gland formation, indicating that BMP signals are necessary for the proventricular gland formation. These findings suggest that BMPs are of prime importance as mesenchymal signals for inducing proventricular glands.

INTRODUCTION

The digestive organs comprise the endodermal epithelium and mesenchyme derived from the splanchnic mesoderm. For morphogenesis and cytodifferentiation of these organs, epithelial-mesenchymal interactions are essential (Le Douarin, 1975; Wessels, 1977; Haffen et al., 1987; Yasugi and Mizuno, 1990; Yasugi, 1993). We have been studying the mechanisms of differentiation of the proventricular epithelial cells using experimental and molecular approaches. The proventriculus is a glandular stomach situated anterior to the gizzard (muscular stomach) and is characterized by the development of compound glands (Romanoff, 1960). Morphogenesis of the proventricular glands starts around day 6.5 of incubation. Groups of epithelial cells invaginate into the underlying mesenchyme to form the gland structures. These cells then express the embryonic chicken pepsinogen (ECPg) gene, a differentiation marker of the glandular epithelial cells, from day 9 of incubation (Hayashi et al., 1988). In contrast, non-invaginating epithelial cells express the chicken spasmolytic polypeptide (cSP) gene, encoding a mucus-related protein, and differentiate into luminal cells (Tabata and Yasugi, 1998). Expressions of ECPg and cSP are almost completely exclusive (Tabata and Yasugi, 1998). Previous studies have reported that the formation of the glands depends upon induction from the underlying mesenchyme and competence of the epithelium to this inductive signal. The epithelia of the esophagus, proventriculus and gizzard can form glands under the influence of the proventricular mesenchyme, while intestinal epithelium never responds to the same influence (Takiguchi et al., 1986; Urase et al., 1996), indicating that the competence is restricted to epithelia of anterior digestive organs (Yasugi and Mizuno, 1990). Results of tissue recombination experiments using lung mesenchyme suggest that molecules identical or similar to the proventricular mesenchymal factors also exist in lung mesenchyme (Urase et al., 1996). Conversely the mesenchymal cells of the gizzard, situated posterior to the proventriculus, inhibit the differentiation of associated epithelium into glands (Urase and Yasugi, 1993). These results suggest the existence in the mesenchyme of proventriculus or gizzard of specific factors involved in the differentiation of the epithelium. We have further confirmed that proventricular mesenchymal factors are soluble (Koike and Yasugi, 1999), although the exact molecular nature of the mesenchymal factors is still unknown.
Bone morphogenetic proteins (BMPs) are expressed during mouse development in epithelia and/or mesenchymes at many sites of epithelial-mesenchymal interactions, such as digestive organs, lung, tooth and hair, implying that BMP signals serve as important mediators of various morphogenetic events (Hogan, 1996; Bitgood and McMahon, 1995). Recent studies have demonstrated that BMPs also function in organogenesis. In the developing chicken feather bud, BMP-2 is expressed in the anterior side of the placodes and the results of ectopic expression of BMP-2 suggest that BMP-2/4 are inhibitors of the development of feather bud patterning (Jung et al., 1998; Noramly and Morgan, 1998). In development of the mouse lung, BMP-4 expression is restricted to the tips of distal bud and adjacent mesenchyme (Bellusci et al., 1996). Overexpression of BMP-4 in the epithelia of distal buds results in development of a smaller lung due to an inhibition of epithelial proliferation (Bellusci et al., 1996). In developing kidney, BMP-7 is expressed initially in the ureteric bud and expression is later observed in the metanephric mesenchyme and the early tubules (Luo et al., 1995; Dudley et al., 1995). BMP-7 mutant mice demonstrate failure of kidney development (Luo et al., 1995; Dudley et al., 1995).

Noggin inhibits the activities of BMP and is involved in the patternings of the mesoderm and the somite (Smith et al., 1993; Zimmerman et al., 1996; Capdevila and Johnson, 1998). The inhibition of BMP activity by Noggin is attained by direct binding to BMP with higher affinity to BMP-2 and -4 than BMP-7 in vitro (Zimmerman et al., 1996). Noramly and Morgan (1998) used Noggin as an antagonist for BMP activity to test the role of BMP-2/4 in feather bud patterning.

Roberts et al. (1995, 1998) studied the expression patterns of BMPs in the developing chicken gut. BMP-4 was expressed throughout the mesenchyme of the gut, except in the proventriculus and gizzard. BMP-2, -5 and -6 were not expressed in the stomach, while BMP-7 was expressed in the dorsal stomach. Ectopic expression of Sonic hedgehog (Shh), normally expressed throughout the digestive epithelium from early stages of development (Roberts et al., 1995; Narita et al., 1998), in the midgut and hindgut can induce BMP-4 expression, and misexpression of BMP-4 in the presumptive stomach region results in a smaller stomach phenotype, suggesting that BMP-4 regulates the proliferation of cells in the mesodermal layer (Roberts et al., 1998).

In this paper we have investigated the role of BMP signals in the induction of the proventricular glands in chicken embryogenesis. First, the detailed expression patterns of BMP-2, -4 and -7 were studied in the digestive organs using in situ hybridization of histological sections. As BMP-2 was found only in the proventricular mesenchyme, the effect of overexpression of BMP-2 was tested using tissue-restricted gene expression by retrovirus (Fekete and Cepko, 1993). We also expressed Noggin in the proventricular mesenchyme to antagonize BMPs. These experiments unequivocally demonstrate that BMP signals are important mesenchymal mediators in the formation of the proventricular glands.

MATERIALS AND METHODS

Virus

Replication-competent avian retroviruses (belonging to subgroup A) were constructed by ligating pDS derivatives with pREP(A) and transfecting them into chicken embryonic fibroblasts (Murakami et al., 1997). BMP-2 virus was prepared using pDS carrying the chicken BMP-2 gene as described previously (Yokouchi et al., 1996). Noggin virus, encoding the Xenopus Noggin gene (a gift from Dr Y. Sasai) was constructed from a derivative of pDS, pIR-2, that carries the IRES sequence just before the exogenous gene to ensure high-level expression (Murakami et al., 1997; Kameda et al., 1999). Control vector lacking an exogenous gene was prepared from pDS.

Chicken strain

In order to introduce exogenous genes using the avian subgroup A retrovirus into either epithelial cells or mesenchymal cells selectively, we used two kinds of chicken embryos: one originated from the C/O strain (WL-M/O), which is fully susceptible to any subgroup of avian retrovirus, and the other originated from the C/ABE (GSN/1, GSN/2) strain, which is insensitive to infection with subgroup A, B or E avian retroviruses. These strains were established, maintained and supplied by the Nippon Institute for Biological Science (Kobuchizawa, Japan).

Tissue recombination culture and virus infection

Tissue recombination culture was performed as described by Takiguchi et al. (1988) and Koike and Yasugi (1999). Proventriculi and gizzards were obtained from embryos incubated for around 6 days. To separate the epithelial fragments from mesenchymal fragments, the proventriculi and gizzards were treated with 0.03% type I collagenase (Cooper Biochemical) in Tyrode’s solution at 37°C for 40 minutes and 90 minutes, respectively. Two proventricular fragments or one gizzard mesenchymal fragment were recombined with one proventricular or gizzard epithelial fragment on a Nuclepore filter (Corning, 110409). They were then settled on a stainless steel grid placed in a well of one 24-well culture plate (Falcon, 3047), with medium was replaced with fresh medium every second day. The explants were then cultivated for 6 days. The culture medium was replaced with fresh medium every second day.

Plasmid construction

A 1.1kb SalI-BglII fragment carrying the env gene was excised from pDS and subcloned into the SalI and BamHI site of pBluescript SK (+) to generate pBS-DS1. The antisense riboprobe of env was synthesized by the transcription with T3 RNA polymerase after linearization of pBS-DS1 by SalI.

In situ hybridization

Digoxigenin-labeled RNA probes for in situ hybridization were prepared from cDNA clones of chicken BMP-2, -4, -7, BRK-1, -2, -3 (Kawakami et al., 1996), cSP (Tabata and Yasugi, 1998), ECPg (Hayashi et al., 1988), Xenopus Noggin (Sasai and De Robertis, 1996) and env. Embryos and explants was fixed with 4% paraformaldehyde, embedded into OTC compound (Miles, 4583) and 14 μm sections cut in a cryostat. In situ hybridization was performed as previously described (Ishii et al., 1997).

RESULTS

BMP expression in proventricular mesenchyme at early stages of gland formation

To elucidate the possible involvement of BMPs in proventricular gland formation, we analyzed the detailed expression patterns of BMPs (BMP-2, -4 and -7) and BMP
Stomach gland formation by BMP receptors (BRK-1, -2 and -3) in the anterior digestive organs (the esophagus, proventriculus and gizzard). Furthermore we analyzed these expression patterns in developing lung, because a previous study (Urase et al., 1996) suggested that the same molecule inducing proventricular glands exists in the lung mesenchyme.

**BMP-2** transcripts were first detected weakly in the proventricular mesenchyme of 5-day embryo (Fig. 1D) and found until day 6.5 of incubation (Fig. 1B), but not at day 7 of incubation when some parts of the epithelium invaginated into the mesenchyme (Fig. 1E). **BMP-2** expression was also found in the developing lung mesenchyme (D). E, epithelium; Es, esophagus; GE, glandular epithelium; Gz, gizzard; M, mesenchyme; LE, luminal epithelium; Lg, lung; Pv, proventriculus. Bars, 200 μm (A,B,C,E),(D).

**BMP-4** expression was widely detected in the anterior digestive organs. **BMP-4** expression in esophagus, proventriculus and gizzard was first detected in mesenchyme of 6-, 4- and 5-day embryos, respectively (Fig. 2A-C). In 7-day embryos **BMP-4** expression was observed in the entire mesenchyme of the esophagus and proventriculus, except in the region just beneath the epithelium (Fig. 2D,E). In gizzard, it was restricted to the zone that lies some distance away from the epithelium, not overlapping with the smooth muscle layer (Fig. 2F). In developing lung, **BMP-4** expression was detected at a high level in the entire mesenchyme from day 4 of incubation (data not shown).

**BMP-7** was expressed broadly in the anterior digestive organs. In 4-day embryos, high-level **BMP-7** expression was observed in the mesenchyme near the boundary of the proventriculus and gizzard. In esophagus, **BMP-7** expression was first found in the mesenchyme of 5-day embryos (data not shown). In the proventriculus of 6-day embryos, **BMP-7** expression was detected in the mesenchyme beneath the epithelium (Fig. 3A). In 7-day embryos it was restricted in the mesenchyme under the luminal epithelium, whereas it was not found in the mesenchyme beneath the invaginated epithelium (Fig. 3B). However, by 9 days of development **BMP-7** was expressed in the mesenchyme beneath both the luminal and the glandular epithelia (Fig. 3C). In gizzard, **BMP-7** expression was found in the anterior half of the mesenchyme until day 7. In 9-day embryos it was distributed irregularly in the entire gizzard mesenchyme (data not shown). In developing lung, **BMP-7** expression was not detected (data not shown).

Expression of **BRK-1** and **BRK-3** was not detected in the anterior digestive organs and lung. In the proventriculus **BRK-2** was first expressed on day 6 unevenly in the epithelium of the posterior half of the organ (Fig. 4A). In 7-day embryos expression persisted only in the non-invaginating epithelium (Fig. 4B). In 9-day embryos expression was observed in some parts of the luminal and glandular epithelia, but was higher in the former (Fig. 4C). In the gizzard, a low level of **BRK-2** was found in the entire organ of 4-day embryos (data not shown).

![Fig. 1. BMP-2 expression in anterior digestive organs (D), esophagus (A), proventriculus (B,E) and gizzard (C), of day 5 (D), day 6 (A,B,C) and day 7 (E) of incubation. BMP-2 expression was restricted to the proventricular mesenchyme (B,D). It was not detected in the esophagus (A,D) and gizzard (C,D). The expression in the proventriculus was transient and was not detected at 7 days of incubation (E). BMP-2 expression was also found in the developing lung mesenchyme (D). E, epithelium; Es, esophagus; GE, glandular epithelium; Gz, gizzard; M, mesenchyme; LE, luminal epithelium; Lg, lung; Pv, proventriculus. Bars, 200 μm (A,B,C,E),(D).](image)

![Fig. 2. BMP-4 expression in esophagus (A,D), proventriculus (B,E) and gizzard (C,F) of day 5 (A,B,C) and day 7 (D,E,F) of incubation. The expression was detected as a broad band in the mesenchyme at a short distance from the epithelium. **BMP-4** expression was also found in lung mesenchyme (Lg) (A,B). Abbreviations as in Fig. 1. Bar, 200 μm.](image)
In 9-day embryos its expression was found in the entire gizzard epithelium whereas in the mesenchyme it was restricted to the smooth muscle layer (Fig. 4D). In esophagus and lung, BRK-2 expression was not detected (data not shown).

Effect of overexpression of BMP-2 in proventricular mesenchyme

BMP-2 expression was restricted to the mesenchyme of the proventriculus among the anterior digestive organs and was also expressed in the lung mesenchyme at a high level. A previous study suggested that the molecule responsible for the induction of the proventricular glands is also expressed in the lung mesenchyme (Urase et al., 1996). Thus the expression patterns indicate that BMP-2 is a candidate for the mesenchymal factor that induced proventricular glands. To test for a role of BMP-2 in development of the proventriculus, we performed tissue recombination experiments with forced expression of BMP-2 using a retrovirus vector. In this experiment, we used gizzard epithelium that has not been exposed to induction from the proventricular mesenchyme, but can differentiate into the $ECP_g$-positive glandular epithelium under such induction. The gizzard epithelium was prepared from the C/AB strain of embryos that cannot be infected with a virus. It is evident that forced BMP-2 expression was restricted within the mesenchymal tissue of explants treated with the BMP-2 virus (BMP-2 explants) (Fig. 5C,D,G,H). In control explants endogenous BMP-2 expression was not detected since it ceases on day 7 of incubation in the normal course of development (Fig. 5G).

In control explants a few glands developed under the influence of the proventricular mesenchyme and cSP expression was found in non-invaginating epithelial cells (Fig. 5E,F). In contrast, BMP-2 explants showed differentiation of many glands expressing $ECP_g$ (5 out of 10 explants). Epithelial cells that did not differentiate into $ECP_g$-positive glandular cells expressed the cSP gene (Fig. 5A,B). These results demonstrate that BMP-2 overexpression enhanced the differentiation towards proventricular gland cells.

BMP-4 expression together with BMP-2 expression was observed in the proventricular mesenchyme. A previous study demonstrated that the effect of BMP-4 is identical to that of BMP-2 in tooth development (Vainio et al., 1993). To address whether BMP-4 is involved in gland formation, we performed tissue recombination experiments with forced expression of BMP-4 in the proventricular mesenchyme. Differentiation of the glands in BMP-4 explants was the same as in control explants (Fig. 5E,I,J) ($n=16$).

Effect of ectopic BMP-2 expression in the gizzard mesenchyme

We tested whether gland formation is induced in the associated gizzard epithelium if BMP-2 is ectopically expressed in the gizzard mesenchyme, where it is not
expressed normally (Fig. 1C,D). Control explants, infected with control virus (Fig. 6H), expressed cSP in the entire epithelium (Fig. 6F) and ECPg expression was not detected (Fig. 6E). All epithelial cells thus differentiated into luminal cells. Ectopic BMP-2 expression in the gizzard mesenchyme (Fig. 6B) led to the same result as the control explants (4 out of 4 explants); BMP-2 expression in the gizzard mesenchyme could not induce the ECPg-positive glandular cells (Fig. 6A). All epithelial cells differentiated into cSP-positive luminal cells (Fig. 6B).

Effect of ectopic Noggin expression in the mesenchyme or epithelium
To test whether BMP signals are necessary in the formation of the proventricular glands, we expressed Noggin at a high level using a retrovirus vector in order to antagonize the activities of BMP-2, -4 and -7 proteins in the proventricular mesenchyme. We performed tissue recombination experiments using proventricular epithelium and the mesenchyme of 5.25-day embryos, when epithelial cells may not yet be determined to differentiate into glandular cells. Noggin virus infected only the mesenchyme (Fig. 7C,D). In control explants infected with control vector, the epithelium formed many glandular structures and epithelial cells expressed either ECPg or cSP (Fig. 7E-H), depending on whether they were in glands or on the luminal surface. In contrast, neither the gland formation nor ECPg expression was observed in Noggin explants (10 out of 10 explants) and cSP expression was detected in all epithelial cells (Fig. 7A,B). This result demonstrates that BMPs are indispensable for gland formation in the proventriculus.

We further investigated whether BMPs act directly on epithelial cells in inducing gland formation by misexpression of Noggin in epithelial cells and shutting off the transmission of mesenchymal BMP signals to epithelial cells. Control explants infected with control virus showed many ECPg-positive glands and also cSP-expressing luminal cells (Fig. 8E-H). When Noggin virus infected epithelial cells (Fig. 8C,D), the entire epithelium of Noggin explants (6 out of 6 explants) differentiated into the luminal epithelium expressing cSP; the gland structures and ECPg expression were not observed (Fig. 8A,B). This result suggests that BMPs may serve as mesenchymal factors that exert their influence on inducing proventricular glands directly on epithelial cells.

DISCUSSION
Expression patterns of BMPs in digestive organs
Study of the precise expression patterns of genes, both temporal and spatial, is a prerequisite for the conjecture of function of the genes considered. We examined the expression of BMP genes (BMP-2, -4 and -7) in the anterior digestive organs (esophagus, proventriculus and gizzard) in the chicken embryo to elucidate the possible involvement of these genes in the differentiation of proventricular epithelium. BMP-2 expression was detected only transiently in the proventricular mesenchyme from day 5 to 6.5 of incubation, when the proventricular epithelium shows initial signs of gland formation (Fig. 1). BMP-4 expression was observed as a broad band in the mesenchyme.

Fig. 5. In situ hybridization of ECPg (A,E,I), cSP (B,F), BMP-2 (C,G), env (D,H) and BMP-4 (J) in the explants composed of gizzard epithelium and proventricular mesenchyme. The mesenchyme was derived from the C/O strain and was infected with BMP-2 virus (A-D), control virus (E-H) or BMP-4 virus (I,J), whereas the epithelium was derived from the C/AB strain. BMP-2 explants formed many more ECPg-positive glands (5 out of 10 explants) than the controls. On the other hand, there was no difference between BMP-4 explants and controls with regard to gland formation. Expression of env is not detectable in tissue fragments derived from C/AB strain embryos, indicating that the virus cannot infect cells derived from C/AB strain embryos. The black stripe under the explant is the Nuclepore filter used in the culture. Bar, 300 μm.
at a short distance from the epithelium in the proventriculus and gizzard (Fig. 2), while BMP-7 expression in proventriculus was found in mesenchymal cells beneath the epithelium (Fig. 3). Roberts et al. (1995, 1998) reported somewhat different patterns of BMP expression in the gut; they did not detect BMP-2 and -4 in the proventriculus and gizzard. This discrepancy of expression patterns of BMPs in the proventricular mesenchyme may be attributable to the methods of detection; Roberts et al. (1995, 1998) utilized whole-mount in situ hybridization while we used in situ hybridization on sections.

The fact that BMP-2 is expressed in the proventricular mesenchyme just when the epithelium begins to form glands is indicative of the participation of this molecule in the morphogenetic movement of the epithelium. Moreover, we remarked that BMP-2 transcripts also exist in lung mesenchyme. We have hypothesized that the mesenchymes of proventriculus and lung share the same molecule(s), which can evoke gland formation and ECPg expression in the associated gizzard and esophageal epithelia (Urase et al., 1996; Koike and Yasugi, 1999). These results directed us to assume that BMP-2 is an important mesenchymal factor for the induction of gland formation of proventricular epithelium.

**Role of BMPs in development of proventriculus**

Noggin can block the action of BMPs by binding directly to BMP-2 or BMP-4 with a high affinity (Zimmerman et al., 1996). Ectopic Noggin expression to prevent BMP activity in the proventricular mesenchyme led to the inhibition of gland formation and ECPg expression and all epithelial cells of the explants differentiated into the luminal cells expressing cSP (Fig. 7). These findings suggest that BMPs are necessary for the proventricular gland formation. Moreover, overexpression of BMP-2 in the proventricular mesenchyme resulted in increased gland formation and ECPg expression (Fig. 5), indicating that BMP-2 is involved in this process. In tooth development, BMP-2 and BMP-4 showed identical effects in experiments with BMP-soaked beads, suggesting that these BMPs are functionally redundant (Vainio et al., 1993). However, in our present experiment, no overt effect of BMP-4 overexpression was detected in the gland formation (Fig. 2A). Taken together, these data suggest that a BMP-2 signal, but not a BMP-4 signal, is important for inducing proventricular glands.

Ectopic BMP-2 expression in the gizzard mesenchyme did not induce gland formation and ECPg expression in associated gizzard epithelium (Fig. 6). A previous study suggests that gizzard mesenchymal cells secrete inhibitory factor(s) that suppress the differentiation of glands (Urase et
al., 1996). It is reasonable to assume that inhibitory factors in gizzard mesenchyme are inhibitors of BMP-2. Several molecules such as Noggin, Chordin, Cerberus or Follistatin are known to directly bind BMPs and counteract their actions (Zimmerman et al., 1996; Piccolo et al., 1996, 1999; Iemura et al., 1998). In our preliminary analysis, Noggin expression was detected weakly as a band in the mesenchyme and Follistatin was expressed in a part of the mesenchyme (data not shown), implying that Noggin and Follistatin are not responsible for preventing virus-mediated BMP-2 action. Examination of the precise expression patterns of other molecules is necessary to confirm our hypothesis.

Alternatively, it is possible that other molecules together with the BMP-2 signal are necessary for gland formation. Since the virus vector in an infected cell should yield a great quantity of the protein of an inserted gene, which would be expected to be sufficient to overcome the activity of any presumptive endogenous inhibitor in the gizzard mesenchyme, we favor the hypothesis that the gizzard lacks an essential cofactor(s).

A possible mode of BMP action as a mesenchymal factor inducing glands

It is important to establish whether BMPs can act directly on the epithelial cells, or induce secondary signal molecules in the mesenchyme while then affecting the epithelium. To approach the problem, we examined genes encoding receptor molecule of BMPs. We detected transcripts of BRK-2, encoding a BMP type I receptor, in the proventricular epithelium, but not in the mesenchyme (Fig. 4A). Although mRNA of BMP type II receptors was not detected, the Activin type II receptor, which can transduce BMP signals together with a BMP type I receptor, is expressed in the digestive epithelium of chicken embryos (Ohuchi et al., 1992; Yamashita et al., 1995). Furthermore, ectopic Noggin expression in the epithelium led to the complete inhibition of the gland formation (Fig. 8). These findings strongly suggest that BMPs are mesenchymal factors directly inducing glands. However, Noggin has a long-range activity to prevent BMP action (Dosch et al., 1997). The suppression of the gland formation by ectopic Noggin expression may therefore result from the inhibition of induction of another signaling molecule by BMP in the mesenchymal cells. To determine whether BMP is a direct mesenchymal factor inducing glands, it will be necessary to carry out an experiment using a dominant-negative type BMP receptor.

Epithelial-mesenchymal interactions in development of the proventriculus

In the development of the chicken digestive organs we have studied mainly the properties of mesenchymal factors affecting epithelial differentiation (Yasugi, 1993). Recently, it has been reported that Shh, whose expression is found in the digestive epithelium from esophagus to colon (Roberts et al., 1995; Narita et al., 1998), is a morphogenetic signal from the epithelium to mesenchyme. A. Sukegawa, T. N., T. K., K. S., T. Nohno, H. Iba, S. Y. and K. Fukuda (unpublished results) have shown that Shh plays a role in patterning the stratification of the mesenchyme into connective tissue and smooth muscle layers. In addition, Shh misexpression in the mesenchyme cultivated without the epithelium led to the proliferation of mesenchymal cells, suggesting that a Shh signal from the epithelium is involved in increasing the thickness of the connective tissues in the gut. Moreover, Shh-secreting cells can induce BMP-4 expression in surrounding mesenchymal cells (A. Sukegawa, T. N., T. K., K. S., T. Nohno, H. Iba, S. Y. and K. Fukuda, unpublished results). Thus the signal from the epithelium affects various steps of differentiation of mesenchymal cells and may also be associated with the production of a mesenchymal factor important for the differentiation of epithelium. BMP-2 is a candidate for such a factor.

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REFERENCES

Bellusci, S., Henderson, R., Winnier, G., Oikawa, T. and Hogan, B. L. M.


