Abnormal gastrointestinal development in PDGF-A and PDGFR-α deficient mice implicates a novel mesenchymal structure with putative instructive properties in villus morphogenesis

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SUMMARY

Development of the gastrointestinal (GI) tract depends on reciprocal epithelial-mesenchymal cell signaling. Here, we demonstrate a role for platelet-derived growth factor-A (PDGF-A) and its receptor, PDGFR-α, in this process. Mice lacking PDGF-A or PDGFR-α were found to develop an abnormal GI mucosal lining, including fewer and misshapen villi and loss of pericryptal mesenchyme. Onset of villus morphogenesis correlated with the formation of clusters of PDGFR-α positive cells, ‘villus clusters’, which remained located at the tip of the mesenchymal core of the growing villus. Lack of PDGF-A or PDGFR-α resulted in progressive depletion of PDGFR-α positive mesenchymal cells, the formation of fewer villus clusters, and premature expression of smooth muscle actin (SMA) in the villus mesenchyme. We found that the villus clusters were postmitotic, expressed BMP-2 and BMP-4, and that their formation correlated with downregulated DNA synthesis in adjacent intestinal epithelium. We propose a model in which villus morphogenesis is initiated as a result of aggregation of PDGFR-α positive cells into cell clusters that subsequently function as mesenchymal centers of signaling to the epithelium. The role of PDGF-A seems to be to secure renewal of PDGFR-α positive cells when they are consumed in the initial rounds of cluster formation.

Key words: Intestinal villus, Development, Platelet-derived growth factor, Bone morphogenetic protein, Epithelial-mesenchymal interaction

INTRODUCTION

The fully developed gastrointestinal (GI) tract displays a high degree of organization at both structural and cellular levels. Villus and crypt morphologies vary between different regions of the intestine, but are in any selected region regularly spaced and uniform in shape. Four component cell lineages are rapidly renewed in the intestinal epithelium through a geographically orchestrated sequence of proliferation, commitment and migration-associated differentiation. The connective tissue core of the villus is also highly organized, containing subepithelial smooth muscle cells, blood vessels and nerve fibers. Formation of the gastrointestinal (GI) tract starts during development by the association of the visceral endoderm with the splanchnic mesoderm. Intestinal organogenesis proceeds as a proximal-to-distal wave of morphogenetic events (Kaufman and Bard, 1999). First, the pseudostratified endoderm differentiates into a single-layered epithelium. Secondly, the epithelium folds to form intestinal villi and becomes compartmentalized into differentiating villus and proliferating inter villus epithelium. Basal crypts subsequently form by reshaping of the inter villus epithelium (reviewed in Gordon and Hermiston, 1994). In parallel, the GI tract mesenchyme differentiates into mucosal and submucosal connective tissue, and the outer muscle layers. It is thought that while the mesenchyme dictates the morphology and the cellular organization of the digestive mucosa, the epithelial cells induce the mesenchyme to develop the typical intestinal mesenchymal structures (Kedinger et al., 1981; Duluc et al., 1994; reviewed in Kedinger et al., 1998). Thus, development of the GI tract, like other epithelial organ systems, depends on reciprocal signaling between the epithelium and the mesenchyme (reviewed in Birchmeier and Birchmeier, 1993).

The mechanisms involved in the inductive events of gut development are poorly understood. However, expression patterns and the results of gene targeting have implicated a variety of signaling molecules and transcription factors in GI development. Growth and differentiation factors implicated in different aspects of GI development include members of the fibroblast growth factor (FGF) family (Colvin et al., 1999; Jung et al., 1999), epidermal growth factor (EGF) family (Luetteke et al., 1994; Miettinen et al., 1995), transforming growth factor-β (TGF-β) (Kaartinen et al., 1995), insulin-like growth factors (IGF)-1 and -2 (Liu et al., 1993), hepatocyte growth factor (HGF)/scatter factor (Sonnenberg et al., 1993; Schmidt et al., 1995; Uehara et al., 1995; Kermorgant et al., 1997), sonic and Indian hedgehog (Bitgood and McMahon, 1995; Apelqvist et al., 1997; Roberts et al., 1995), wnts and bone
morphogenetic proteins (BMPs) (Bitgood and McMahon, 1995; Simon and Gordon, 1995; Roberts et al., 1995). Knowledge of the GI cellular functions controlled by these factors is, however, limited (reviewed in Traber and Wu, 1995 and Montgomery et al., 1999).

In this paper we investigate the action of platelet derived growth factor-A (PDGF-A) and the PDGF-α-receptor (PDGFR-α) in the development of the GI tract by analysis of PDGF-A+/− and PDGFR-α+/− mice (Boström et al., 1996; Soriano, 1997). The platelet-derived growth factor (PDGF) family of ligands and receptors are known to have critical roles in a number of developmental processes. The three PDGF gene products, PDGF-A, PDGF-B (reviewed in Heldin, 1992) and PDGF-C (Li et al., 2000), signal through two related receptor tyrosine kinases, PDGFR-α and PDGFR-β. Gene knockout studies have already established roles for PDGF-A/-R-α signaling in the development of lung alveoli (Boström et al., 1996; Lindahl et al., 1997a), central nervous system oligodendrocytes (Calver et al., 1998; Fruttiger et al., 1999), hair follicles (Karlsson et al., 1999) and neural crest-derived mesenchyme (Soriano, 1997). PDGF-B and PDGFR-β signaling has proven critical in the development of the vascular wall (Levéen et al., 1994; Soriano, 1994; Lindahl et al., 1997b, 1998; Hellström et al., 1999). Here we show that PDGF-A and its receptor, PDGFR-α, are necessary for the correct structuring of the mucosal lining of the GI tract. Our data suggest that PDGF-A, acting by paracrine signaling through PDGFR-α, controls the multiplication and differentiation of a subset of the mucosal mesenchymal cells. This activity of PDGF-A is required for coordinated GI villus morphogenesis.

**MATERIALS AND METHODS**

PDGF-A+/− mice (Boström et al., 1996) and PDGFR-α+/− mice (Soriano, 1997) were bred as 129/C57BL6 hybrids. Heterozygotes were intercrossed to produce homozygous mutants. In C57BL6/129 hybrid genetic background, PDGF-A+/− mice display a range of survival restriction points, ranging between approximately 10 days post coitum (E10) and 6 weeks of postnatal age (P42). In this study, PDGF-A+/− mice surviving the E10 restriction point were subject to analysis of the GI tract.

Standard histological techniques were used. For immunohistochemistry (IHC), the following antibodies were used: anti-α-smooth muscle actin (SMA) (clone 1A4, Dako); biotin-conjugated anti-mouse IgM (Dako); anti-BrdU (#347580, Becton Dickinson); biotin-conjugated rabbit anti-mouse Ig (Dako). Non-radioactive in situ hybridization was performed as described by Boström et al. (1996) and combined with IHC as described by Karlsson et al. (1999). PDGF-A and PDGFR-α probes were generated as described previously (Boström et al., 1996). Bmp2 and Bmp4 probes were transcribed from 1.2 and 1.0 kb mouse cDNA fragments provided by Dr A. McMahon. For BrdU labeling, BrdU (Sigma; 100 mg/g body mass) was injected intraperitoneally to pregnant female mice or postnatal pups. Injected animals were killed 2 hours later, and tissues or embryos were fixed and processed for IHC.

The number of villi, crypts and villus clusters were counted on sections and related to the perimeter. The perimeter was measured using Easy Image Measurement software (Bergstrom Instruments) and expressed as arbitrary units. The results were statistically analyzed using the Student’s two-tailed t-test.

**RESULTS**

Abnormal intestinal villi in PDGF-A+/− mice

Intestinal villus formation starts around E14 and proceeds until postnatal day 28 (P28) (Simon and Gordon, 1995). Dissection of the PDGF-A+/− GI tract at different postnatal ages (P10 until P42) revealed an abnormal morphology of the mucosal lining. The abnormality was most prominent in the upper small intestine, but extended throughout the GI tract. In the small intestine, villi were lower in abundance, irregular in length, thickness and spacing, and were sometimes branched (Figs 1A, 2A-F). The reduction in villus number in PDGF-A+/− mutants was estimated to approximately 45% at P16-19 (Table 1). The PDGF-A+/− large intestine displayed abnormally shaped folds and a 30% reduction in the number of crypts at P16 (Table 1 and Figs 1B, 3A-F).

Histological analysis revealed tissue disorganization in the PDGF-A+/− small (Fig. 2) and large intestines (Fig. 3). A typical feature of the PDGF-A+/− villi in the small intestine was the presence of epithelial pleats (Fig. 2B,F, arrows). These pleats were not misplaced crypts as they lacked BrdU-labeled cells (Fig. 2F). In the PDGF-A+/− colon, folds contained fewer cuffs (Fig. 3). No individual epithelial subtypes were missing in PDGF-A+/− intestines and cellular morphologies appeared

![Abnormal intestinal mucosa in PDGF-A+/− mice.](image-url)
largely normal; however, some cell types were abnormally represented or distributed. In particular, the submucosal mesenchyme contained a reduced amount of cells, especially in older PDGF-A−/− pups (>P30) in which there was an almost complete lack of pericryptal fibroblasts in the small intestine, bringing the crypt epithelium in apposition to the smooth muscle cell wall (Fig. 2C,D, arrows). Also in the colon folds, the submucosal mesenchyme appeared more sparse (Fig. 3C,D, arrows). In particular, α-smooth muscle actin (SMA) labeled mesenchyme was thinner and showed abnormal distribution in PDGF-A−/− cuffs (Fig. 3E,F). In addition, goblet cells were reduced in number in the PDGF-A−/− small intestine (Fig. 2E,F) and abnormally distributed in the PDGF-A−/− colon cuffs (Fig. 3C,D).

**PDGF-A and PDGFR-α expression in the developing intestine**

Before the onset of villus formation PDGF-A and PDGFR-α were expressed in adjacent, alternating cell layers in the developing intestine. PDGF-A was uniformly expressed in the pseudostratified epithelium and in the developing intestinal wall smooth muscle layers (Fig. 4A), while PDGFR-α was expressed in the submucosal mesenchyme (strongest in cells just beneath the epithelium) and in the mesenchymal cells between the muscle layers (Fig. 4B).

At the onset of villus formation the pseudostratified epithelium becomes compartmentalized into differentiating villus and proliferating intervillus epithelium. This transition was accompanied with changed expression patterns for

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**Fig. 2.** Histological abnormalities of the upper small intestine in PDGF-A−/− mice. (A-D) Hematoxylin/Eosin staining at P25 revealed a lower abundance and disturbed morphology of villi in PDGF-A−/− (B) compared to wild-type (A) intestine, including epithelial pleats along villi (arrows in B,F). (C,D) The submucosal mesenchyme (indicated by arrows) was reduced in thickness in PDGF-A−/− (D) compared to wild-type (C) intestine. (E,F) Staining for goblet cells (blue) using Alcian Blue/Fast Red at P42 revealed a lower abundance of such cells in the PDGF-A−/− small intestine. (G,H) BrdU-labeling, detected with FITC (yellow) and counterstained with propidium iodide (red), at P10 showed that epithelial proliferation was associated with crypts and not with villus pleats.

**Fig. 3.** Histological abnormalities in the colon of PDGF-A mutant mice. (A,B) Hematoxylin/Eosin staining at P19 revealed an abnormal morphology of colonic mucosal folds in PDGF-A−/− (B) compared to wild-type (A) colon. (C,D) Alcian Blue/Fast Red staining of colon. Note the reduction of mesenchymal cells in the submucosa of the folds (arrows). Goblet cells (blue) were abnormally distributed in PDGF-A−/− colon. (E,F) α-smooth muscle actin (SMA) staining demonstrated reduced thickness of the SMA positive mesenchyme at the core of the folds, and a smaller number of epithelial crypts along each fold.
PDGF-A and PDGFR-α. PDGF-A expression became restricted to the intervillus epithelium (Fig. 4C), and later to the crypt epithelium (Fig. 4E,G). Villus formation correlated spatially and temporally with the formation of discrete strongly PDGFR-α positive clusters of mesenchymal cells (Figs 4D,F, 5). To our knowledge, this cell cluster has not been described before, and it will hereafter be referred to as the ‘villus cluster’. Villus clusters were seen underneath the earliest protrusions of epithelium (Fig. 4D arrowheads, Fig. 5C), and remained associated with the tip of mesenchymal core of the growing villus. During elongation of the villus, PDGFR-α positive cells became distributed along the villus epithelium (Fig. 4F). Accordingly, full-length villi in postnatal gut (P10-P30) lacked a villus cluster at the villus tip, while the discontinuous lining of the epithelium by PDGFR-α positive mesenchymal cells remained (Fig. 4F, arrow). A similar lining of the epithelial basement membrane by PDGFR-α positive cells was seen in the intervillus region (Fig. 4D,H arrows).

Patterns of PDGF-A and PDGFR-α expression similar in principal to the ones described for the upper small intestine were also seen in the developing ventricular antrum and in the developing colon (data not shown). In the colon, small clusters of PDGFR-α positive mesenchymal cells were located to the developing cuff regions, i.e. sites analogous to the villi of the small intestine.
Abnormal pattern of PDGFR-α expression in PDGF-A−/− intestine

PDGF-A−/− mice displayed altered expression of PDGFR-α during development of the GI tract. Before villus formation, the layer of PDGFR-α expressing cells underlying the early epithelium was discontinuous and less prominent (Fig. 5A,B). After onset of villus formation, the number of villus clusters formed per perimeter unit was reduced by 30% in E15.5 PDGF-A−/− embryos (Table 2 and Fig. 5E,F). The PDGF-A−/− intestine also displayed a reduction in the number of PDGFR-α positive villus clusters associated with the formation of new villi also at late embryonic and postnatal time-points (data not shown). There was also a significant loss of PDGFR-α positive cells subjacent to the intervillus epithelium at E15.5 (arrows in Fig. 5C,D).

Reduced proliferation of PDGFR-α positive cells in PDGF-A−/− intestine at E12.5

Proliferation studies using BrdU labeling showed that the layer of strongly PDGFR-α positive cells underlying the epithelium proliferated, whereas the clusters were postmitotic (see below). Quantification of the BrdU-labeling index in the total submucosa at E12.5 and E17.5 revealed no significant difference between PDGF-A mutant and wild type. We next determined the BrdU-labeling index in the innermost mesenchymal cell layer, where the strongly PDGFR-α positive cells are found. At E12.5, i.e. at a time point before the PDGFR-α positive cells are lost at this location in PDGF-A−/− intestine, we found that the BrdU-labeling index was reduced by 30% in PDGF-A−/− compared to PDGF-A+/+ intestine (Table 3). At E17.5, when the strongly PDGFR-α positive cell layer is depleted in the PDGF-A−/− intestine, BrdU labeling was indistinguishable between PDGF-A+/+ and PDGF-A−/− intestine. Determination of total cell numbers in the submucosal compartment of the intestine at E12.5 and E17.5 showed that there were approximately 30% less cells in the PDGF-A−/− submucosa (Table 3). TUNEL-labeling failed to reveal any difference between wild-type and PDGF-A−/− tissue, arguing against increased apoptosis in mutant submucosa (data not shown). Our data are compatible with the idea that PDGF-A promotes proliferation (but has no influence on survival) in a layer of strongly PDGFR-α positive cells located immediately beneath the intestinal epithelium.

Differentiation of villus smooth muscle cells

As the development of smooth muscle cells (SMC) in lung alveoli and in small blood vessels depends on PDGFs, we investigated the expression of the SMC marker α-smooth muscle actin (SMA) during embryonic and postnatal GI development. SMA staining at different postnatal ages demonstrated the presence of SMC in wild type as well as in PDGF-A−/− villi in the small intestine (not shown), hence PDGF-A is not required for villus SMC formation per se.

Differentiation of villus SMC normally occurs postnatally.

Table 2. Reduction in villus cluster formation in PDGF-A−/− mutants compared to wild type

<table>
<thead>
<tr>
<th>E15.5</th>
<th>Wild type</th>
<th>PDGF-A−/−</th>
<th>P-value</th>
<th>Reduction in PDGF-A−/− (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of villus clusters/10,000 perimeter units</td>
<td>39 (±10)</td>
<td>28 (±10)</td>
<td>&lt;7×10−5</td>
<td>30</td>
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Approximately 30 cross-sections of small intestine from four E15.5 embryos of each genotype were quantified. Values are means (s.d. in parentheses).
SMA staining was evident in the embryonic mesenchymal villus core in the PDGF-A<sup>−/−</sup> intestine at E18.5 when no or little SMA expression was seen in the wild-type mesenchymal villus core (Fig. 6A,B). This may suggest that premature differentiation of villus SMC occurs in the absence of PDGF-A.

The expression patterns for PDGFR-α and SMA in the mesenchymal villus core seemed spatially similar, indicating that the PDGFR-α positive cells might constitute villus SMC or their progenitors. However, double-labeling of P10 intestine showed that SMA and PDGFR-α are not expressed by the same cells (Fig. 6C). It also showed that the PDGFR-α positive cells are found closely associated with the epithelium, while the SMA-expressing cells are found more centrally in the villus mesenchyme, possibly in association with its blood vessels.

**Abnormal intestinal development in PDGFR-α<sup>−/−</sup> mice**

PDGFR-α<sup>−/−</sup> embryos usually die before E15, but in a C57Bl6/129sv hybrid genetic background, an appreciable number of mutants survive until E16.5 or later (Soriano, 1997). This permits analysis of the initial steps in GI villus formation. The intestines of two E16.5 PDGFR-α<sup>−/−</sup> embryos were compared with wild-type littermates. The mutants showed fewer and broader villi (Fig. 7A-D) and less mesenchymal cells in the submucosal compartment (Fig. 7C,D arrow). Thus, PDGFR-α<sup>−/−</sup> embryos display a villus phenotype similar to PDGF-A<sup>−/−</sup> embryos. Like the PDGF-A<sup>−/−</sup> embryos, PDGFR-α<sup>−/−</sup> villi showed premature expression of SMA in the villus mesenchyme (Fig. 7E,F arrow). Although PDGFR-α could not be used to stain the villus clusters in PDGFR-α<sup>−/−</sup> embryos, mesenchymal condensations associated with villus formation suggested normal induction of villus clusters, similar to the situation in PDGF-A<sup>−/−</sup> mice (Fig. 7C,D arrowheads).

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**Table 3. BrdU-labeling index in PDGF-A<sup>−/−</sup> cells at different locations in the intestine**

<table>
<thead>
<tr>
<th>Time</th>
<th>Wild type</th>
<th>PDGF-A&lt;sup&gt;−/−&lt;/sup&gt;</th>
<th>Significance of size difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>E12.5</td>
<td>Number of cells in the submucosa 537 (±132) 358 (±111)</td>
<td>358 (±111)</td>
<td>P&lt;0.003</td>
</tr>
<tr>
<td></td>
<td>BrdU-labeling index in the innermost cell layer 0.554 (±0.12) 0.367 (±0.19)</td>
<td>0.367 (±0.19)</td>
<td>P&lt;0.03</td>
</tr>
<tr>
<td></td>
<td>BrdU-labeling index in the total submucosa 0.467 (±0.068) 0.367 (±0.12)</td>
<td>0.367 (±0.12)</td>
<td>P=0.28</td>
</tr>
<tr>
<td>E17.5</td>
<td>Number of cells in the submucosa 86.6 (±26) 66.1 (±17)</td>
<td>66.1 (±17)</td>
<td>P&lt;0.005</td>
</tr>
<tr>
<td></td>
<td>BrdU-labeling index in the innermost cell layer 0.38 (±0.087) 0.31 (±0.09)</td>
<td>0.31 (±0.09)</td>
<td>P=0.60</td>
</tr>
<tr>
<td></td>
<td>BrdU-labeling index in the total submucosa 0.25 (±0.045) 0.25 (±0.06)</td>
<td>0.25 (±0.06)</td>
<td>P&lt;0.10</td>
</tr>
</tbody>
</table>

The number of BrdU-labeled and propidium iodide-labeled nuclei were counted in cross-sections of embryonic upper small intestine. Note that there is a significant difference in BrdU-labeling index of the cells just beneath the epithelium (i.e. where the strongly α-receptor positive cells are located) but not in the total submucosa. Values are means (s.d. in parentheses).

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Fig. 6. Alpha-smooth muscle actin (SMA) expression during gastrointestinal development. (A,B) SMA expression at E18.5 in wild-type (A) and PDGF-A<sup>−/−</sup> upper small intestine (B) revealed premature expression of SMA in the PDGF-A<sup>−/−</sup> intestine (arrow in B). (C) Combined PDGFR-α in situ hybridisation (blue) and SMA antibody staining (brown) at P10 showed that the two markers are expressed by different cells and that these cells have a slightly different position (C and data not shown). The PDGFR-α positive cells were located just beneath the villus epithelium and the SMA expressing cells were located at the centre of the villus core.
**Proliferative arrest and BMP expression in the PDGFR-α positive villus cluster of mesenchymal cells**

Cell proliferation in embryonic intestines was examined using BrdU-labeling. Before the onset of villus formation, a BrdU pulse uniformly labeled cell nuclei in the epithelium and in the mesenchyme (data not shown), in agreement with earlier studies (Calvert and Pothier, 1990). Following villus formation, epithelial proliferation became progressively restricted to the intervillus region, and later to the crypts (Fig. 8A,B and data not shown). In the mesenchyme, BrdU labeling was evident in the layer of PDGFR-α positive cells subjacent to the PDGF-A positive intervillus epithelium. The villus clusters, however, were invariably unlabeled by BrdU, arguing that these structures consisted of non-cycling or slowly cycling cells (Fig. 8B, cluster indicated by black hatched line). Since the zone of epithelial mitotic arrest correlates spatially and temporally with the presence of villus clusters (arrows and arrowheads in Fig. 8B), we hypothesize that the clusters may produce inhibitor(s) of epithelial cell proliferation. BMP-2 and BMP-4 have been reported to be expressed in intestinal mesenchyme, and are candidate inhibitors of epithelial cell growth in the intestine and elsewhere (Bitgood and McMahon, 1995; Roberts et al., 1995; Bellusci et al., 1996; Kaestner et al., 1997; Jung et al., 1998; Roberts et al., 1998; Pabst et al., 1999). Indeed, non-radioactive in situ hybridization localized the expression of both BMP-2 and BMP-4 mRNA to the villus cluster (Fig. 8C,D). PDGF-A−/− intestine displayed the formation of fewer villus clusters and, consequently, fewer sites of BMP expression (data not shown).

**DISCUSSION**

Development of the GI tract requires reciprocal epithelial-mesenchymal signaling. Several growth and differentiation factors have been implicated in these processes but the mechanisms by which GI epithelial-mesenchymal interactions proceed in vivo are still poorly understood. Here we show that PDGF-A is necessary for the normal development of the mucosal lining of the GI tract. In the absence of PDGF-A the structure of the GI tract is disorganized, presumably as a result of loss of a PDGF-A target cell population in the mesenchyme of the developing submucosa. The analysis also reveals some new aspects of villus formation. Our data suggest that PDGF-A/PDGFR-α signaling promotes proliferation and prevents premature differentiation of intestinal mesenchymal progenitor cells. We propose that these mesenchymal cells are recruited to a transient structure that we have tentatively named the ‘villus cluster’. The villus cluster appears to play a critical role during GI villus morphogenesis (see below).

**The villus cluster**

At E14, GI villus formation starts in the anterior portion of the small intestine and is first seen as a condensation of the mesenchyme. We show that the generation of the villus epithelial folds coincides with the formation of mesenchymal clusters that express PDGFR-α, BMP-2 and BMP-4. These clusters remain beneath the villus tip during its invagination into the gut lumen. The growing villus tip has previously been reported to be associated with condensed mesenchymal cells and extracellular matrix with specific ultrastructural features (Colon and Conforti, 1993).

The villus cluster was not found in full-length villi by PDGFR-α labeling and histological analysis of serial sections. We could not detect signs of cell death in the villus mesenchyme by TUNEL labeling or standard histology, hence the loss of the villus cluster does not appear to result from cell death. The PDGFR-α positive cells distributed along the villus basement membrane may instead have detached from the cluster during villus elongation. Some PDGFR-α positive cells remain at this location in adult intestine. It is not known whether these cells represent quiescent mesenchymal stem or progenitor cells, or whether they exert a differentiated function.

**Fig. 7.** PDGFR-α−/− mice display a similar phenotype to PDGF-A−/− mice, but earlier during development. (A-D) Hematoxylin/Eosin staining at E16.5 revealed fewer and thicker villi in PDGFR-α−/− embryos (B, D) and reduced cellularity in the sub-villus mesenchymal compartment (indicated by hatched white lines and arrows in C,D). Villus clusters in C and D are indicated with a black hatched line and arrowhead. (E,F) Staining for SMA at E16 was increased in the mesenchymal villus core of in PDGFR-α−/− (F) compared with wild-type (E) intestine.
Villus morphogenesis in wild-type and PDGF-A\(^{-/-}\) mice

Based on our analyses of GI villus morphogenesis in normal and PDGF-A\(^{-/-}\) or PDGFR-\(\alpha\)\(^{-/-}\) mice, we hypothesize the following sequence of events in normal villus morphogenesis (Fig. 9A): (1) PDGF-A produced by the pseudostratified epithelium drives the proliferation of PDGFR-\(\alpha\) positive cells subjacent to the epithelium. (2) PDGFR-\(\alpha\) positive cells aggregate, exit the cell cycle and initiate expression of BMP-2 and -4. (3) The villus clusters trigger epithelial folding and mitogenic arrest of the epithelium at the tip of the fold, perhaps through signals mediated by BMP-2 and -4. PDGF-A expression becomes restricted to the proliferating epithelium at the bottom of the folds (the intervillus epithelium), at which site PDGF-A promotes proliferation and prevents premature differentiation of non-clustered PDGFR-\(\alpha\) positive cells. This is necessary to secure mesenchymal cell renewal. (4) During elongation of the villus, PDGFR-\(\alpha\) positive cells (possibly detached from the cluster) distribute along the basement membrane, at which location they remain. As the gut diameter and distance between villi increases, new villi form between already existing ones through the same sequence of events. In the PDGF-A\(^{-/-}\) intestine (Fig. 9B), PDGFR-\(\alpha\) positive cells are recruited to villus clusters, but become depleted during the initial rounds of cluster formation as they fail to undergo PDGF-A dependent renewal. As a result, new rounds of villus formation do not occur properly.

The proposed model would explain the lower abundance of villi in PDGF-A deficient intestine, but does not offer an immediate explanation for the abnormal villus shapes. It is possible that the premature SMA expression seen in PDGF-A\(^{-/-}\) and PDGFR-\(\alpha\)\(^{-/-}\) villi, possibly reflecting premature mesenchymal differentiation, leads to the formation of abnormal villus shapes. Alternatively, as the spatial arrangement and the size of the villi in the normal gut are well ordered (O’Connor, 1966) and probably a result of periodic patterning, low villus abundance per se may have secondary influences on villus shapes. Finally, although less likely, systemic effects on GI development may result from other defects in the PDGF-A\(^{-/-}\) mice, such as the lung emphysema.
Role of BMP-2 and BMP-4 in villus formation

The expression of BMPs by the villus cluster may suggest a role in periodic patterning of villi, similar to what has been suggested for the periodic patterning of feather anlagen (Jung et al., 1998). It is possible that BMP-2 and -4 expressed by the cluster induce differentiation and mitogenic arrest in nearby epithelium, hence limiting the epithelial proliferation (and epithelial stem cells) to the villus and prospective crypt regions. This idea lends support from the knockout of two transcription factors, in which downregulated BMP-2 and -4 expression has been correlated with increased epithelial proliferation in the intestine. First, a null mutation in the winged helix transcription factor Fkh-6/FoxL1 led to abnormal villus formation and to the distribution of proliferating epithelial cells outside the crypts (Kaestner et al., 1997). Fkh-6/-FoxL1−/− intestines showed perturbed mesenchymal aggregation and reduced expression of BMP-2 and -4 in RNase protection assays. It is an interesting possibility that Fkh-6/FoxL1 is essential for the formation of villus clusters. Second, targeted disruption of the homeobox transcription factor Nkx2-3, which is normally expressed by intestinal mesenchyme, led to lowered expression of BMP-2 and -4, increased epithelial proliferation and perturbed villus morphology (Pabst et al., 1999).

Similarities between the villus cluster and the dermal papilla

Skin appendages such as hairs and feathers involve the formation of mesenchymal clusters with critical morphogenetic properties (Kollar, 1970; Jahoda et al., 1984; Hardy, 1992). It has occurred to us that similarities exist between the villus clusters studied here and the dermal papillae of hair follicles. In both cases, the mesenchymal clusters constitute structures around which the epithelium folds. In the hair follicle this results in the embedding of the dermal papilla within an epithelial pocket located at the base of the follicle. In the gut, the epithelium folds around the mesenchymal cluster to form the prospective villus tip. Both mesenchymal clusters consist of postmitotic PDGFR-α and BMP-4 positive cells (this paper and Karlsson et al., 1999). However, in the hair follicle BMP-2 is not expressed by the dermal papilla, but is instead expressed by the adjacent follicular germinative epithelium (Karlsson et al., 1999). Nevertheless, the morphological and genetic similarities between villus clusters and dermal papillae suggest that similar signaling events and associated morphogenetic processes may be operating in the generation and periodic patterning of both ectodermal and endodermal epithelial derivatives.

Multiple roles of PDGF-A in development

PDGF-A null mice have previously been shown to develop a number of phenotypes that reflect impaired proliferation and spreading of progenitor cells. These phenotypes include lung emphysema due to failure of proliferation and distal spreading of PDGFR-α positive alveolar smooth muscle progenitors along the peripheral branches of the respiratory tract (Boström et al., 1996; Lindahl et al., 1997), hypomyelination of the central nervous system due to failure of proliferation and distant spreading of PDGFR-α positive oligodendrocyte progenitors (Calver et al., 1998; Fruttiger et al., 1999), skin and hair abnormalities due to failure of proliferation of PDGFR-α positive dermal mesenchymal progenitors (Karlsson et al., 1999) and male spermatogenic arrest due to deficient postnatal formation of Leydig cells (Gnnesi et al., 2000). We present here the first evidence for a role of PDGF-A in the development of an appropriately sized mucosal lining of the GI-tract. Our study also reveals key events in the morphogenesis of GI villi and highlights villus formation as a useful model system for the study of epithelial-mesenchymal interaction. Together with the previously established roles for PDGF-A in the formation of lung alveoli and PDGF-B in kidney glomeruli (Boström et al., 1996; Levêché et al., 1994; Soriano, 1994), the present study implicates a function for PDGFs in promoting the generation of cells involved in the morphogenesis of folded epithelial sheaths.

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