Matrix GLA protein modulates branching morphogenesis in fetal rat lung

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Submitted 10 December 2003; accepted in final form 7 January 2004

Gilbert, Kirk A., and Stephen R. Rannels. Matrix GLA protein modulates branching morphogenesis in fetal rat lung. Am J Physiol Lung Cell Mol Physiol 286: L1179–L1187, 2004; 10.1152/ajplung.00188.2003.—The regulation of matrix γ-carboxyglutamic acid protein (MGP) expression during the process of lung branching morphogenesis and development was investigated. MGP mRNA expression was determined over an embryonic and postnatal time course and shown to be developmentally regulated. Immunohistochemical analysis revealed increased staining for MGP in peripheral mesenchyme surrounding distal epithelial tubules. Fetal lung explants were used as an in vitro growth model to examine expression and regulation of MGP during branching morphogenesis. MGP mRNA expression over the culture interval mimicked the in vivo time course. Explants cultured in the presence of antibodies against MGP showed gross dilation and reduced terminal lung bud counts, accompanied by changes in MGP, sonic hedgehog, and patched mRNA expression. Similarly, antifibronectin antibody treatment resulted in explant dilation and reduced MGP expression, providing evidence for an interaction with MGP and fibronectin. Conversely, intraluminal microinjection of anti-MGP antibodies had no effect either on explant growth or MGP expression, supporting the hypothesis that MGP exerts its effects through the mesenchyme. Taken together, the results suggest that MGP plays a role in lung growth and development, likely via temporally and spatially specific interactions with other branching morphogenesis-related proteins to influence growth processes.

MAMMALIAN LUNG BRANCHING MORPHOGENESIS is a complex growth process involving temporal and spatial expression of numerous gene products from diverse gene families. From the initial formation of the rudimentary lung bud, a continual signaling interaction occurs between the epithelial and mesenchymal compartments to ensure proper growth and differentiation of the developing lung. The end result is a highly branched organ capable of efficient gas exchange over impressively large epithelial and endothelial surface areas. The cellular and molecular regulation of lung growth is an area of intense research, and, although the molecular regulation of lung branching morphogenesis has been the subject of recent reviews (9, 43), the process is still incompletely understood.

Matrix γ-carboxyglutamic acid (GLA) protein (MGP) is a vitamin K-dependent extracellular matrix protein originally isolated from bovine bone and subsequently found in many soft tissues, with highest expression observed in lung (16). Interestingly, MGP accumulates in the extracellular matrix of only cartilage and bone (18, 33), suggesting that its broad tissue distribution warrants important, yet undefined, roles for the protein. MGP belongs to a limited family of proteins that contain posttranslationally modified GLA residues, with the blood-coagulating proteins being the most well-characterized members of this family. The γ-carboxylase responsible for converting glutamate to GLA requires vitamin K hydroquinone as a cofactor, and vitamin K antagonists such as warfarin impair protein function by preventing the recycling of oxidized vitamin K epoxide back to its reduced hydroquinone form (15).

Results from numerous studies suggest that MGP is involved in cell growth and differentiation. MGP is expressed in vitro in a large variety of cells, including vascular smooth muscle cells (14, 30, 42), osteoblasts (1, 7), chondrocytes (1, 7, 44), fibroblasts (7), kidney epithelial cells (6), pulmonary epithelial cells (34), breast epithelial cells (11), and cardiac myocytes (16). In most cell culture models, MGP expression increases with increasing cell density, with the highest rates of expression and secretion occurring in postconfluent, nondividing cells (6). During early development, in situ hybridization reveals that MGP expression is restricted to the epithelial-mesenchymal border in developing lung and limb buds (26). High MGP expression is observed in embryonic kidney and calvaria, with peak mRNA and protein levels occurring during the perinatal period in many developing organs, such as kidney, lung, and spleen (47). Taken together, the cell-tissue profile, location, and time course of expression of MGP underscore the complexity of its function and lend support to its potential regulatory role in cellular processes of growth and differentiation.

Although present in most vertebrate tissues examined, the physiological function of MGP is not completely understood. Recently, it has been demonstrated that mice lacking MGP develop extensive vascular and cartilaginous calcification to such an extreme that null mutant animals die within 2 mo postpartum due to rupture of the thoracic or abdominal aorta (27). The extensive arterial calcification, osteopenia, and growth plate inhibition present in these MGP−/− animals suggest that one of MGP’s primary roles may be as an inhibitor of tissue calcification. Indeed, virally driven MGP overexpression in cultured embryonic chick chondrocytes results in blocked mineralization of these cells (44). Similarly, in vivo overexpression of MGP in developing chick limb bud causes inhibition of cartilage mineralization, delayed chondrocyte maturation, and inhibition of endochondral bone ossification (44).

Given the increasing evidence that MGP is an important regulator of growth and differentiation and that MGP is highly expressed in the lung, we sought to further define the role of MGP in the process of lung development. This study provides...
a detailed description of lung MGP expression over a time course of pre- and postnatal periods of lung growth and development. In addition, fetal lung explants were used as a model of lung branching morphogenesis to specifically address MGP’s involvement in this process.

METHODS

Isolation of fetal lung explants. Timed pregnant adult Wistar rats were purchased (Charles River Laboratories) and housed according to institutional policies. Pregnant rats were euthanized with 13–14% CO

coitum, and embryonic (E) 13- to 14-day fetal rat pups were aseptically dissected from the uterine decidua into ice-cold, sterile PBS. With the aid of a dissecting stereomicroscope, lung bud rudiments were dissected free from the embryos and placed on cell culture inserts (0.4 μm size; Falcon) in six-well tissue culture plates (Falcon). Lung explants were cultured in BGJb medium (Fitton-Jackson Modification; Life Technologies) supplemented with 5% carbon-stripped fetal calf serum. Typical culture conditions consisted of three to five explants per well. The medium was changed at 24-h intervals, and lung explants were photographed on a daily basis over the culture period. MGP’s involvement in this process.

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Lung RNA isolation. Total RNA was isolated from fetal lung explants based on the method of Chomczynski and Sacchi (12). Briefly, lung explants were homogenized in 0.75 ml of Tri-Reagent (Molecular Research), with manual disruption of the tissue by using conical-shaped pestles designed to fit 1.5-ml microcentrifuge tubes. Samples were homogenized on ice according to the Tri-Reagent protocol. RNA was quantified by spectrophotometry and stored at −80°C for subsequent use in RT-PCR; DNase digestion of total RNA samples was performed before spectrophotometric quantitation.

RT-PCR analysis. Total RNA (0.1–1 μg) was reverse-transcribed for 1 h at 42°C by using poly(dT) primers, 500 μM deoxynucleotides, 10 mM dithiothreitol, 1× RT buffer, and an RNase H− reverse transcriptase (Superscript H−; Invitrogen) in a total volume of 20 μl. After RT, samples were variably diluted for subsequent PCR amplification; dilutions were empirically based on relative abundance of the respective gene product in fetal lung. MGP mRNA expression was quantified by competitive PCR, as described previously (17). Both endogenous MGP and an MGP cDNA mimic amplified by competitive PCR, as described previously (17). Both endogenous MGP and an MGP cDNA mimic amplified by competitive PCR, as described previously (17). Both endogenous MGP and an MGP cDNA mimic amplified by competitive PCR, as described previously (17).

GAPDH used to normalize expression of these genes. Gene-specific primers included the following: Shh forward primer 5′-GGAAAA-CACGAGCAAGC-3′ from mouse sequence base pairs 28–47; Shh reverse primer 5′-CCACGAGTTCCTCTCCTTCC-3′ from base pairs 335–316 to give a 390-base pair product for Shh amplification; Ptc forward primer 5′-TGGATGCCCAGTGGT-3′ from base pairs 505–486 to give a 371-base pair product for PTC amplification; BMP-4 forward primer 5′-CTCTCTGAGCTTCCAC-3′ from mouse sequence base pairs 266–285; BMP-4 reverse primer 5′-CTGGGATGTCTCCAGATT-3′ from base pairs 750–731 to give a 486-base pair product for BMP-4 amplification; FGF-10 forward primer 5′-GCCACATACATTTGTCACC-3′ from base pairs 556–537 to give a 554-base pair product for FGF-10 amplification; Ptc reverse primer 5′-AACAAGTCTGGGTGGAG-3′; GAPDH reverse primer 5′-CAAAAGTGGAGATGGAAG-3′; and EFTu forward primer 5′-GACTGGTTGGTGAGAC-3′ and EFTu reverse primer 5′-GAACGACGATGTCATTCC-3′.

Lung protein isolation and Western blot analysis. Proteins (20–25 μg) were separated by SDS-PAGE on 15% SDS-polyacrylamide gels, followed by transfer to nitrocellulose membranes with the use of a Transblot apparatus (Bio-Rad Laboratories). Immunodetection of transferred proteins was performed by standard Western blot procedure. Brieﬂy, nonspeciﬁc binding sites were blocked with blotto (5% nonfat dry milk and 0.05% Tween-20 in PBS) for 1 h. Membranes were incubated in primary antibody (1:2,000 dilution in blotto) for 2 h at room temperature and then washed extensively. A subsequent incubation in a solution containing the biotinylated secondary antibody (Vector Laboratories) was performed for 1 h, followed by another round of extensive washing. Immunodetection was visualized by using enhanced chemiluminescence detection reagents (ECL; Amersham) and short exposure to X-ray film.

Immunohistochemistry. For experiments aimed at in situ localization of specific proteins, lungs were ﬁxed and parafﬁn embedded for histological processing into 4-μm tissue sections. Sections were mounted on glass slides, deparafﬁnized, and rehydrated. Antigen retrieval was accomplished by microwave heating in 10 mM citric acid (pH 6.0). Sections were then incubated in blocking solution containing 10% goat serum in PBS Tween-20. Primary antibody incubation was performed overnight at 4°C, followed by a series of washes in Tris-buffered saline. Detection of primary antibody was performed by using a Vectastain avidin-biotin complex kit (Vector Laboratories). Vector VIP was the substrate chromogen with methyl green used as a counterstain. Parallel sections were stained with hematoxylin-eosin to visualize general morphology.

Antibody treatment of fetal lung explants. Two synthetic peptides corresponding to speciﬁc rat MGP protein sequences were used to generate anti-MGP antibodies. The ﬁrst sequence was a 12-residue peptide located in the propeptide-like region of rat MGP and contains the sequence NH2–FPNRRNANTFI–COOH. This region binds the vitamin K-dependent γ-carboxylase required for conversion of glutamic acid residues to γ-carboxylated GLA residues. The antibodies generated with this peptide have been termed the “midpeptide antibody.” The second peptide sequence was a 12-residue peptide located at the extreme C-terminal portion of rat MGP and contains the sequence NH2–YNFRQRQGK–COOH. The antibodies generated with this peptide were termed the “C-terminal antibody.” Affinity-puriﬁed antibodies isolated from rabbits challenged with these peptide immunogens were routinely used in explant cultures at a 1:100 to 1:200 dilution of 1 mg/ml antibody, for a final concentration of 5–10 ng/μl. Overnight incubation of anti-MGP antibodies with the respective peptide used for generating the antibodies before their use in explant culture or immunohistochemistry was performed to successfully block anti-MGP antibody effects and/or immunostaining.
The fibronectin antibody was a mouse monoclonal antibody (EPS; sc-8422, Santa Cruz Biotechnology; stock concentration 200 μg/ml) raised against a recombinant protein corresponding to fibronectin of human origin; dilution of this antibody for use in explant culture experiments was 1:100, resulting in a final concentration of 2 ng/μl.

Microinjection of antibodies into the lung lumen. A microinjector system (Narishige, MO-202) was mounted to a stereoscope for injection of nanoliter amounts of undiluted MGP antibodies or PBS (control) into the lumen of lung explants positioned on cell culture inserts. Micropipettes were drawn by using a Narishige PC-10 device, and injection pressures were generated with a Femtolet (Eppendorf) system. Injection volumes were precalibrated, and both control and experimental solutions were initially colorized with Coomassie blue to visualize fluid distribution. Subsequent experiments were performed without the addition of Coomassie blue. Beginning on day 2 of culture, ~50–100 nl of antibody were injected directly into the trachea and distal luminal spaces, resulting in an even distribution of solution throughout the lumen of the whole lung or individual lobes. It was estimated that the local exposure of antibody was as much as four times that used for external, mesenchymal application. After microinjections, cell culture inserts were replaced, and explants were incubated at 37°C for the remainder of the culture interval.

Quantitation of explant morphology. To assess morphological changes in fetal lung explants, terminal lung buds were counted with the aid of a stereo microscope and statistically compared across culture conditions. Terminal lung buds were defined as acinar structures separated by distinct septae located at the periphery of the growing explants.

Data analysis. Quantitation of morphogenesis was as described above. For some experiments, such as immunostaining or in situ analysis of specific tissue sections or specimens, results were assessed by using a qualitative approach. PCR experiments were repeated at least three times for statistical comparisons. Significant differences in gene expression or morphological measurements resulting from various treatment protocols were determined by using Student’s t-test. Results were considered significant at values of P < 0.05.

RESULTS

MGP expression in developing lung. MGP mRNA levels were determined in rat lung from gestational day 13 (E13) through the perinatal period and in the adult by using a competitive PCR assay, as previously described (17) (Fig. 1). MGP mRNA was found in early lung primordia at E15 and continued to rise during gestation, with a small peak occurring just before birth. Postnatally, levels dropped slightly and were steadily maintained until a sharp increase was observed during the alveolarization interval, beginning in the second postnatal week. MGP mRNA was most highly expressed in adult lung. These results demonstrate that MGP message expression is developmentally regulated, with higher levels of expression occurring during periods of lung development traditionally characterized by low-cell proliferation and increased differentiation.

In addition to overall changes in MGP expression during lung development, site-specific localization of protein was determined. Fetal lungs were prepared as described for immunohistochemical localization of MGP protein by using the C-terminal anti-MGP antibody (Fig. 2). MGP immunostaining was associated predominantly with the mesenchymal cells surrounding epithelial tips of distal buds (Fig. 2, A and C). This staining pattern was compared with that of pro-surfactant protein (SP)-C in adjacent sections, where pro-SP-C was found in the epithelium of early embryonic lungs (Fig. 2, B and D).

Later in gestation, MGP protein became more widely distributed in the lung. By E20, additional staining was observed in the bronchial epithelium, along with continued staining in the mesenchyme (Fig. 2, E and F). As the mesenchyme became more differentiated in early postnatal lung, MGP was localized to airway epithelial cells and vascular walls, as well as light deposition in the surrounding parenchyma (Fig. 2, H and M). In the adult, light distribution throughout the parenchyma and airway cells was still evident, with more intense staining lining the airways (Fig. 2, O and P). This pattern of distribution in adult lung is consistent with other reports (26, 27). No staining was observed in the absence of primary antibody or if anti-MGP antibody was preincubated with a blocking peptide (Fig. 2, G and N).

To further investigate MGP regulation in the developing lung, particularly during early branching morphogenesis, an in vitro explant model was used. Explants remain in the pseudoglandular phase of lung development during culture. This model provides an ideal system in which to examine specific regulators of lung growth and differentiation by using reagent-addition protocols. Increased MGP mRNA expression over the culture interval mimics the early embryonic in vivo time course (17). A stereotypic morphological pattern of explant growth was observed in control cultures, characterized by a high degree of distal epithelial branching (Fig. 3A). Interfering with MGP function by addition of anti-MGP antibodies to the culture medium revealed a completely different explant morphology after 4 days in culture. Compared with control cultures, anti-MGP antibody treatment induced a grossly dilated morphology characterized by elongated epithelial tubules (Fig. 3A). By the end of the culture interval, lung branching, as assessed by terminal lung bud count, was significantly reduced...
in anti-MGP antibody-treated explants by using either the midpeptide or C-terminal peptide-derived antibodies (Fig. 3C). Both of these antibodies were specific for MGP, as assessed by Western blotting of purified MGP isolated from rat femurs (Fig. 3B). In contrast to the external application of anti-MGP antibody, explant growth was not altered when antibodies were microinjected directly into the lumen of specific lobes of intact explants (Fig. 4), suggesting that endogenous MGP exerts its effects primarily through the mesenchyme.

A series of experiments demonstrated the dynamic nature of the anti-MGP antibody-induced dilation of fetal lung explants. The presence of antibody throughout the culture interval resulted in marked dilation of distal airways compared with untreated lungs (Fig. 5, A and B). When C-terminal antibody...
was externally applied after 1 or 2 days of culture, distal bud dilation was evident, whereas morphology of medial structures was comparable to controls (Fig. 5, C and D, respectively). Conversely, when antibody was withdrawn after 1 or 2 days in culture, normal terminal branching and bud formation resumed, with the more medial structures remaining dilated (Fig. 5, E and F, respectively). Blocking peptide was also added to explant cultures following anti-MGP antibody withdrawal to block any residual antibody that may have remained in the tissue (Fig. 5, G and H). The resulting morphology was similar to antibody withdrawal in the absence of blocking peptide, as seen in Fig. 5, E and F. Peptide alone had no effect on gross explant morphology and growth (Fig. 5I). Preincubation of anti-MGP antibody with its immunogenic peptide completely blocked the antibody-induced dilation (Fig. 5J). Thus addition or withdrawal of anti-MGP antibody at different times during the culture interval altered only the most distal morphology. These results suggest that MGP expression and function are both site specific and time dependent, requiring cyclic deposition with each new generation of epithelial branching.

Based on our results demonstrating altered branching with MGP antibody treatment (Figs. 3 and 5), we examined the expression of some genes known to play a role in lung branching morphogenesis in antibody-treated explants (Table 1). Anti-MGP antibody treatment caused a significant downregulation of MGP mRNA expression (Table 1 and Fig. 6). Antibody treatment also significantly downregulated the expression of Shh and Ptc mRNA, whereas the expression of Bmp-4 and Fgf-10 was not significantly different from that of controls (Table 1). Interestingly, antifibronectin antibody treatment of fetal explants caused a similar dilated morphology as that seen with anti-MGP (Fig. 6), reflected by a 21% decrease in terminal bud counts (56.75 ± 4.66 in control vs. 56.75 ± 1.38 in fibronectin antibody-treated explants; Fig. 6B). Combined treatment with both anti-MGP and antifibronectin antibodies resulted in an even greater reduction in terminal bud counts. Additionally, antifibronectin antibody also caused a similar decrease in MGP mRNA expression as that seen with anti-MGP antibody (Fig. 6C); however, the combination of antifibronectin and anti-MGP antibodies did not further reduce MGP mRNA expression. These results suggest that these two proteins coordinately contribute to regulation of branching morphogenesis in the developing lung.

DISCUSSION

The present results identify MGP as a potential regulatory factor in the process of lung growth and development. The time course of expression during the pre- and postnatal periods
sugests that MGP is developmentally regulated, with peak expression occurring during periods of relatively low cellular proliferation and increased cellular differentiation. The prenatal rise in mRNA expression occurs at a time when the proximal airway epithelium loses the ability to respond to distal mesenchymal signals, suggestive of a terminally differentiated phenotype (36). Similarly, the increase in MGP expression occurring in the second postnatal week coincides with alveolar differentiation and lung maturation. The time course of expression that we observed is similar to that reported in a limited study in the mouse (26), in which MGP mRNA was expressed peripherally at the epithelial-mesenchymal border early in gestation and became more restricted to proximal airways later in development. The high levels observed in the adult are consistent with a proposed role of the lung in airways later in development. The high levels observed in the adult are consistent with a proposed role of the lung in airways later in development. The high levels observed in the adult are consistent with a proposed role of the lung in airways later in development.

Table 1. Gene expression analysis following anti-MGP antibody treatment of fetal explants

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MGP mRNA</th>
<th>Bmp-4 mRNA</th>
<th>Fgf-10 mRNA</th>
<th>Shh mRNA</th>
<th>Ptc mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100±5.2</td>
<td>100±9.3</td>
<td>100±6.0</td>
<td>100±10.9</td>
<td>100±7.3</td>
</tr>
<tr>
<td>Anti-MGP</td>
<td>46.4±5.5*</td>
<td>90.6±3.7</td>
<td>92.5±3.7</td>
<td>63.7±7.2†</td>
<td>80.9±8.3‡</td>
</tr>
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Values are means ± SE. Explants were cultured for 4 days in the absence (Control) or presence of anti-matrix γ-carboxyglutamic acid protein (MGP) antibody (Anti-MGP). Control values were averaged across 8 experiments for each given gene product and normalized to 100%. Competitive PCR analysis of MGP mRNA expression following 4 days of culture resulted in a downregulation of MGP expression. Semi-quantitative PCR was used to determine message expression of 4 genes involved in branching morphogenesis, bone morphogenetic protein-4 (Bmp-4), fibroblast growth factor-10 (Fgf-10), sonic hedgehog (Shh), and patched (Ptc), and normalized against the expression of the control gene elongation factor Tu. *P < 0.0001; †P < 0.01; ‡P < 0.05.
MGP is tightly associated with fibronectin and a member of the BMP protein family, subsequently identified as BMP-2 (39). Indeed, more recent reports have shown that MGP binds strongly with BMP-2 in ligand-binding studies (41) and that BMP-2 co-precipitates with MGP in transfected human embryonic kidney 293 cells (45), suggesting that MGP may regulate or contribute to the functional role of BMP-2 through their physical interaction. BMPs are members of the TGF-β superfamily and function in many diverse cellular processes during development, including cellular differentiation and tissue morphogenesis. Several BMP antagonists, some of which act as BMP binding proteins, limit the activity or availability of BMPs and thus regulate BMP function. In this respect, one of the roles of MGP may be to limit the availability of these proteins by binding and sequestering the protein in specific locations during growth and differentiation.

Although BMP-2 expression has not been reported in lung, a closely related family member, BMP-4, plays an important role in lung branching morphogenesis, where its expression is limited to the epithelial tips of distal lung buds and surrounding mesenchyme during lung development. It is hypothesized that lung BMP-4 functions to inhibit local epithelial proliferation at distal bud tips, forming a suppression zone at the branch point and forcing lateral growth (4). The regulation of BMP-4 expression or function in early lung development is not completely understood. Exogenous addition of BMP-4 to E11.5 mouse lung explant cultures actually enhanced lung branching morphogenesis (5, 37), an effect that was blocked by adenoviral-induced overexpression of the BMP-4 antagonist gremlin (37). Lung branching was not affected in explants, however, when BMP-4 was microinjected into the lung lumen, thereby restricting exposure to the luminal epithelium (5). Targeted epithelial misexpression of Bmp-4 using the SP-C enhancer/promoter produced normal mouse lungs at E11.5 and E12.5, but, by E15.5, transgenic lungs were significantly smaller and less branched than normal (4). These studies underscore the importance of tightly regulated spatial and temporal expression of Bmp-4 for normal lung branching to occur. A recent hypothesis suggests that BMP-4 signaling in regions of high expression (such as at the bud tips) could induce inhibitors of BMP-4 that, in turn, modulate or inhibit local BMP activity, allowing bud induction to occur (13). Thus it is possible that MGP may act as a binding protein to BMP-4, limiting its inhibitory capacity. The reduced branching observed in the presence of anti-MGP antibodies is consistent with this hypothesis.

Interestingly, MGP expression in a mouse chondrocyte cell line (MC615) is reduced in the presence of either BMP-2 or BMP-4 (40). In osteogenic sarcoma cells, however, MGP expression is unaltered by BMP-4, except in the presence of retinoic acid (RA), where the cytokine further enhanced the RA-induced expression of MGP (50). These studies suggest a cell type-specific regulatory interaction with MGP and BMP-4 that likely is dependent on the presence of additional molecular regulators. In our model system, anti-MGP antibody treatment did not significantly alter overall BMP-4 expression, but site-specific or cell type-specific changes in expression remain to be determined. It is of interest to note that RA has been reported to repress endogenous MGP expression in a cell-type-specific manner through RA receptor binding to a response element located on the MGP promoter (22). Other studies, however, demonstrated elevated MGP expression in the presence of RA (7). It is known that RA, an important morphogen in the control of lung branching morphogenesis (10), exerts its effects on gene expression in a concentration-dependent manner. It is also likely that RA-induced changes in gene expression alter both the spatial and temporal balance of specific regulatory proteins, which, in turn, affect the local growth characteristics of the developing lung. Indeed, BMP-4 expression patterns become much more diffuse, with severely attenuated expression at the epithelial tips in explants cultured in the presence of RA (28). We speculate that MGP and BMP-4 may be integral components in a regulatory complex of proteins in the cleft region and that paracrine factors such as RA may contribute to the regulation of expression at specific sites.

The effects of anti-MGP antibody treatment on expression of Shh and Ptc are likely the result of the antibody-induced...
diffuse mesenchymal expression of MGP and fibronectin (8, 25), suggesting that these two matrix proteins work coordinately to regulate complex cell-matrix interactions. The establishment of a suppression zone, or cleft region, during branching morphogenesis is, in part, accomplished by spatially specific deposition of several ECM proteins, resulting in very local decrease in cellular proliferation, morphogenetic stabilization, and a remodeling of the ECM (29). Overexpression of antisense fibronectin RNA in Madin-Darby canine kidney cells severely attenuated branching morphogenesis, decreased cell growth rates, and reduced cell migration (21). Conversely, vascular smooth muscle cells incubated with an active fragment of fibronectin resulted in accelerated phenotypic changes accompanied by increased expression of MGP (20). The decrease in MGP expression and branching observed with antifibronectin antibodies in the present study (see Fig. 6) is consistent with a regulatory interaction between the two proteins. Overall, these data suggest that TGF-β1 activation may be an important initial step in orchestrating cleft formation, and, based on the present results with anti-MGP and antifibronectin antibody treatments and reduced fetal lung branching, it is reasonable to suggest that MGP and fibronectin play important downstream roles in this process. Indeed, it has been suggested that ECM proteins may be able to, in turn, modulate the local activity of TGF-β1 and keep it sequestered in specific regions so that it is available to surrounding cells at the appropriate time (35). It is important to emphasize that normal branching patterns are mediated by very specific spatial and temporal patterns of expression. These protein-protein interactions are presently under investigation to further define the role of MGP in fetal lung growth and differentiation.

ACKNOWLEDGMENTS

We thank Jing Zhou and Dr. Carolyn Lloyd for excellent technical assistance in these studies.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grant RFA HL-62869.

REFERENCES


