Migration and gelatinases in cultured fetal, adult, and hyperoxic alveolar epithelial cells

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Buckley, S., B. Driscoll, W. Shi, K. Anderson, and D. Warburton. Migration and gelatinases in cultured fetal, adult, and hyperoxic alveolar epithelial cells. Am J Physiol Lung Cell Mol Physiol 281: L427–L434, 2001.—Alveolar epithelial cell (AEC) migration mediated by matrix metalloproteinases (MMPs) is required for lung development and repair after injury such as hyperoxia. Of specific interest in lung remodeling are the gelatinases, which are upregulated in AEC after hyperoxia. We correlated migration and gelatinase production in AEC cultured from fetal, adult, and hyperoxic rats. Fetal AEC (19–20 days) had higher MMP-2 and MMP-9 gelatinase expression than adult AEC, with fivefold higher MMP-9 activity, and were migratory through gelatin, responding to epidermal growth factor, keratinocyte growth factor, and fibroblast growth factor-10. MMP-2 and MMP-9 expression and migratory activity could be detected from the time of plating. In contrast, adult AEC migrated and expressed MMP-2 and MMP-9 proteins only after 48 h of culture. AEC from hyperoxic rats were significantly more migratory through gelatin than control adult AEC, with significantly higher MMP-9 activity. Inhibition of MMPs with doxycycline reduced the migration of AEC from hyperoxic rats to the level of control adult AEC. Fibronectin-cultured “hyperoxic” AEC acquired a temporary capacity for migration similar to the A549 lung cancer cell line, which is both highly migratory and invasive and is derived from the AEC type 2 lineage. These data suggest that MMP activity is associated with a migratory phenotype in fetal, hyperoxic, and transformed AEC in vitro, and we speculate that MMPs may play a key mechanistic role in AEC migration in vivo during lung development and repair.

matrix metalloproteinase-9; matrix metalloproteinase-2; hyperoxic injury and repair; lung remodeling

A CAPACITY FOR MIGRATION in alveolar epithelial cells (AEC) is necessary for remodeling the developing lung and effecting repair after lung injury. During development, epithelial cell migration is observed during the glandular stage of organogenesis (13). In the recovery phase of acute lung injury, reepithelialization of the denuded alveolar basement membrane is achieved by migration and proliferation of the alveolar epithelial progenitor cells. Estimations of the cell kinetics of alveolar epithelial repair suggest that the alveolar epithelial type 2 cell (AEC2) has the proliferative capacity to restore high proportions of damaged cells within a few hours (14). It has been inferred that, after hyperoxic injury, the acquisition of a migratory phenotype by the normally quiescent AEC2 would further increase efficiency of reepithelialization, and in vivo and in vitro studies support this hypothesis. In vivo studies using the pneumotoxicant monocrotaline, which inhibits AEC2 mitosis, show that AEC2 migration and coverage of the exposed basal lamina proceed rapidly after diffuse alveolar type 1 cell injury, even in the absence of AEC2 division (27). In vitro models also suggest that migration, in addition to proliferation, plays a dominant role in the process of reepithelialization in respiratory epithelia (15, 23, 28).

Extracellular matrix (ECM) degradation, which facilitates cell migration, is mediated by matrix metalloproteinases (MMPs). The MMPs are a family of zinc-dependent proteases that function at neutral pH and cooperatively hydrolyze most proteins in the ECM. MMPs are secreted as latentzymogens, requiring specific proteolytic activation (2). Because MMP gene expression can be regulated by cytokines (19), it is not surprising that MMPs are reportedly elevated in the lung after acute lung injury (10, 21) and, specifically, by subacute hyperoxia (20, 22, 25). Injury-induced gelatinase in lung has been specifically attributed to various cell types, including AEC2 after hyperoxia (20) and macrophages after lipopolysaccharide injury (10). We hypothesized that MMP activity, specifically gelatinase activity, would correlate with AEC migration in AEC, speculating that the in vitro gelatinase and migratory profile would reflect the remodeling capacity of the AEC in vivo.

To compare AEC from lungs undergoing presumptive active remodeling with AEC from control lungs, we isolated AEC from 19- to 20-day-old fetal rats and rats subjected to hyperoxia and compared these with AEC from adult rats. Our model of hyperoxia, consisting of 48 h of >90% oxygen exposure, induces consistent lung damage with minimal mortality and has a reproducible and well-characterized 72-h in vivo repair period (4).
From the large family of MMPs, we chose MMP-2 and MMP-9 (gelatinases A and B) for our study, since they are reported to be elevated in AEC in another model of hyperoxia (20). The AEC-derived lung cancer cell line A549 was used as a positive control in migration studies with adult AEC, since adult AEC are relatively nonmigratory. Epidermal growth factor (EGF) was used to stimulate adult AEC migration (18). EGF, keratinocyte growth factor (KGF), and fibroblast growth factor-10 (FGF-10) were also tested as stimulants of migration in fetal AEC, and since fibroblasts can contaminate fetal AEC cultures, we also assayed 19- to 29-day fetal fibroblasts in parallel with fetal AEC. Conditioned media from control AEC and AEC from hypoxic rats were assayed for active MMP-9, and doxycycline was used to assess the effects of MMP inhibition on hyperoxia-induced AEC migration.

The data presented herein show that cultured AEC exhibit a characteristic MMP-9 and migratory profile in accord with the developmental age of the animal and the current role of the cell in vivo. The hyperoxia-induced MMP-9 measured in vitro parallels the time frame of in vivo repair in our model and resolves after the animal has recovered, suggesting its in vivo relevance. The observation that AEC from hypoxic rats grown on fibronectin can invade and migrate with an efficiency similar to A549 cancer cells grown on gelatin, albeit temporarily, leads to the speculation that, in vivo, fine regulation of AEC migration is required during alveolar remodeling events.

**METHODS**

**Oxygen treatment and recovery.** Adult male Sprague-Dawley rats were exposed to short-term hyperoxia as described previously. Briefly, rats were placed in a 90 × 42 × 38-cm Plexiglas chamber and exposed to humidified 90% oxygen for 48 h, with the 48-h oxygen exposure time inducing lung damage with minimal mortality. Control rats were kept in room air. In some experiments, the oxygen-exposed rats were allowed to recover in vivo for various times before AEC isolation. At the end of the exposure/recovery period, the rats were anesthetized by an intraperitoneal injection of pentobarbital sodium. After complete exsanguination by saline perfusion via the pulmonary artery, the lungs were lavaged to remove macrophages. The lavaged lungs were then used for AEC2 isolation and culture.

**Isolation and culture of adult AEC.** AEC2 were isolated from lavaged lungs by elastase digestion, followed by differential adherence on IgG plates as described by Dobbs et al. (7). The cells were plated at 2 × 10^5 cells/cm^2 in DMEM with 10% FCS, 0.2 ml/cm^2, on plastic. Immunostaining of attached cells after 24 h of culture with a surfactant protein (SP) C antibody confirmed that ~95% of the attached cells were positive for SP-C. Fresh isolates were ~90% SP-C positive. The antibody to SP-C was kindly provided by Jeffrey Whitsett from the Children’s Hospital Medical Center of Cincinnati (Cincinnati, OH).

**Isolation of fetal AEC and fibroblasts.** Fetal AEC at the canalicular stage, 19–20 days gestation, were isolated by a modification of the method of Post et al. (24) using trypsin and collagenase digestions followed by three differential adherence steps to remove fibroblasts and differential centrifugation. Immunostaining with an antibody to vimentin (Sig-

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**Measurement of MMP-2 and MMP-9 gelatinase by SDS-PAGE zymography.** Denatured but nonreduced concentrates of conditioned medium corrected for cell number and concentration factor (usually ~10-fold) were electrophoresed through commercially prepared 1 mg/ml 10% gelatin gels (Novex, San Diego, CA) under nonreducing conditions. Conditioned medium from NIH/3T3 cells, which contains both MMP-9 and MMP-2, was used as a zymogram control. The gels were renatured for 30 min and then developed overnight at 37°C using Novex reagents in accordance with the manufacturer’s instructions. The gels were stained for 30 min with 0.5% Coomassie brilliant blue 250R, destained, and dried at room temperature. Representative zymograms were scanned for Figs. 1–6 with Adobe PhotoDeluxe Business Edition software.

**Measurement of MMP-9 activity.** MMP-9 activity was quantitated in conditioned medium from cultured “hyperoxic” and control adult AEC. The cells corresponding to the conditioned medium were lysed, and the protein was measured so results could be normalized. MMP-9 activity was measured in the cell supernatants using the Biotrak assay system from Amersham Pharmacia Biotech (Little Chalfont, UK) according to the manufacturer’s exact instructions. The assay uses the pro form of a detection enzyme that can be activated by captured active MMP-9 into an active detection enzyme through a single proteolytic event and thus measures active MMP-9. The assays were done in 96-well plates, and the color product was detected using a plate reader; MMP-9 was calculated relative to standards provided with the kit. Active MMP-9 levels thus obtained were corrected for concentration factor and are expressed as nanograms of MMP per milligram of cellular protein.

**Measurement of cell migration.** Cells were assayed for migration immediately after isolation and after 24, 48, and 72 h of culture. The fetal cells were not assayed at 72 h because of the increasing presence of fibroblasts. Two different migration systems were used, each giving comparable results. One method used inserts within the wells of a tissue culture multwell plate (Becton Dickinson, Lincoln Park, NJ), and the other method, requiring much fewer cells, used the disposable “Chemotx” migration chamber with a 96-well format (Neuroprobe, Cabin John, MD). The Chemotx plates, which were not provided sterile, were sterilized with ethylene oxide before use. Both systems involved membranes containing pores of 8-μm diameter and 10^5 pores/cm^2 and were routinely coated with gelatin at 5 μg/cm^2 on the lower surface. In some experiments, the filters were coated with fibronectin at 5 μg/cm^2.
AEC were removed from culture flasks by dissociation with trypsin and DNase to ensure a single-cell suspension. The cells were washed two times in PBS to remove all traces of serum, counted, and plated at 2 cells/pore in DMEM and 0.1% BSA on filters atop either DMEM (unstimulated migration) or DMEM with growth factor (stimulated migration). Because control adult AEC exhibit relatively low chemotaxis even when stimulated, A549 lung cancer cells were used as control cells in the establishment of the migration assays, with 150 μg/ml laminin as the chemotactic stimuli (19). A549 cells were obtained from American Type Culture Collection (Manassas, VA). EGF and KGF were obtained from PeproTech (Rocky Park, NJ), and FGF-10 was from R&D Systems (Minneapolis, MN). After 24 h of incubation, the inserts were washed with PBS, and the cells adherent to the upper surface were gently removed using a Q-tip. After microscopic examination confirmed complete removal of cells from the upper surface, the lower surface of the filter was Wright stained. The Chemotx filter unit was read in a plate reader at 600 nm, with individual readings corrected for substrate blanks. When using the insert system, the membranes were excised from the inserts, and the color was eluted in 0.1 M HCl and was spectrophotometrically measured at 600 nm (11).

A linear relationship between cell number and dye intensity was confirmed by serial dilution. All samples in an individual experiment were done in quadruplicate for the insert system and sextuplicate for the Chemotx system. One filter for each condition was fixed and immunostained for cytokeratin to confirm epithelial phenotype, although the relatively low density of migrated cells made it easy to distinguish any fibroblasts by morphology alone. Fibroblasts were the major contaminant, at ≈5% for adult AEC, 5–10% for hyperoxic AEC2, and ≈10% for fetal AEC, especially after 2 days of culture.

Western blotting of proteins. Western analysis was performed on cell lysates as described by Bui et al. (4), using 20 μg protein/lane. Equal loading was confirmed by blotting the lower half of the blots with an antibody to actin. Proteins of interest were detected using horseradish peroxidase-linked secondary antibodies and the enhanced chemiluminescence system following the manufacturer’s instructions (Amersham, Arlington Heights, IL). Antibodies to MMP-9 and MMP-2 were goat polyclonal antibodies from Santa Cruz Biotechnology (Santa Cruz, CA). Antibody to actin was from ICN (Irvine, CA). Secondary antibodies were from Sigma (St. Louis, MO).

Blots were scanned with Adobe PhotoDeluxe Business Edition software.

Inhibition of MMP-9. The effective dose of doxycycline required to inhibit gelatinase production was predetermined by titration with conditioned medium from AEC from hyperoxic animals. Doxycycline (50 μg/ml; Sigma) was added to the cells after attachment when the serum-containing medium was withdrawn. This dose inhibited MMP-9 expression and partially inhibited MMP-2 expression as measured by zymography. Higher doses of doxycycline were toxic.

RESULTS

MMP-2 and MMP-9 protein expression are induced in adult AEC with time in culture, whereas in fetal AEC, expression is detected in the fresh isolate and early in culture. The expression of MMP-2 and MMP-9 proteins was examined in freshly isolated and cultured AEC from control rats, rats subjected to 48 h of >90% hyperoxia with no recovery period, and 19- to 20-day fetal rats by Western analysis. MMP-2 and MMP-9 were detected as single bands corresponding to the proenzyme forms, ~72 and 92 kDa, respectively (Fig. IA). Gelatinase levels were very low in the fresh isolate and during early culture of adult AEC, were maximally expressed after 48 h of culture, and decreased to lower levels after 72 h. Under our culture conditions, adult AEC after 48 h of culture still expressed significant SP-C levels and thus still had AEC2 characteristics. Lysates of hyperoxic AEC had slightly increased MMP expression compared with control lysates. The timed induction of MMP protein expression seen in cultured AEC suggests that the “late window” for cell migration reported in cultured AEC (16, 18) may in fact correlate with in vitro MMP production.

In contrast, expression of MMP-2 and MMP-9 protein in fetal AEC was detected immediately upon isolation and did not increase with time in culture (Fig. IB). AEC from hyperoxic rats and fetal AEC secrete increased MMP-9 compared with control adult AEC. Conditioned medium from cultured AEC isolated from remodeling or normal lungs equalized for concentration...
factor and cell number was collected over the 24- to 48-h culture period. Zymography (Fig. 2A) demonstrated that 19- to 20-day fetal AEC (lane 3) produce both active and latent MMP-2 and MMP-9 gelatinases in greatly increased quantities compared with adult AEC (lane 1), with MMP-9 levels also increased in AEC from hyperoxic lungs (lane 2). Conditioned medium was also assayed from fetal fibroblasts over the 48- to 72-h culture period, since fibroblasts contaminate at levels >10% by this stage. Fetal fibroblasts (19–20 days) secrete less MMP-2 and MMP-9 than fetal AEC. MMP-2 was the predominant gelatinase in the conditioned medium obtained from cultures of the AEC2-derived lung cancer cell line A549.

AEC from hyperoxic rats secrete significantly more active MMP-9 than control AEC. Because zymography demonstrated that the increase in MMP-9 secretion by AEC after hyperoxia was modest, we looked for a difference in activation between control and hyperoxic AEC. MMP-9 activity was quantitated in conditioned medium from AEC cultures using the Amersham Biotrak activity assay. AEC from hyperoxic animals secreted twofold more active MMP-9 than control AEC (2.1 ± 0.7 ng/mg protein for hyperoxic AEC2 compared with 1.1 ± 0.05 ng/mg protein for control adult AEC, P < 0.05 by Student’s t-test, n = 4 animals/group).

Fetal AEC (19–20 days) secreted fivefold more active MMP-9 (5.25 ± 0.25 ng/mg cellular protein) than adult AEC (Fig. 2B).

In vivo recovery from hyperoxia is associated with increased MMP-9 gelatinase in cultured AEC. AEC were isolated from hyperoxic rats that had been allowed to recover in vivo for various times ranging from 0 to 72 h. Conditioned serum-free medium was collected for 24 h after attachment, concentrated, and loaded on a zymogram gel (Fig. 3). MMP-9 was induced as the animals recovered in vivo, was maximal after 48 h of recovery, and returned to control levels after 72 h of in vivo recovery. We have shown previously that hyperoxic lungs examined after varying recovery periods showed peak epithelial repair after 48 h of in vivo recovery as measured by in situ bromodeoxyuridine uptake and immunostaining for proliferating cell nuclear antigen and mitotic cyclins. Concurrent cultures of AEC confirmed that a similar proliferative repair process was occurring in vitro as demonstrated by increases in mitotic cyclin expression and downregulation of autocrine transforming growth factor-β secretion (3, 4). Thus the temporal induction of MMP-9 seen in AEC cultured from recovering rats exactly paralleled in vivo repair in our model of hyperoxic injury.

AEC from hyperoxic rats are significantly more migratory than control AEC, with fibronectin further enhancing migration. We compared stimulated and unstimulated cell migration of hyperoxic and control adult AEC, both freshly isolated and cultured for var-

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**Fig. 2.** A: MMP-9 levels are increased in fetal AEC and AEC from hyperoxic rats compared with control AEC. Concentrates of conditioned medium from AEC collected for 24 h after attachment and corrected for concentration factor and cell number were electrophoresed through a 10% Tris-glycine gel containing 1% gelatin. The gel was renatured, developed, and Coomassie stained. Absence of stain denotes gelatinolytic activity. Both latent and active MMP-2 and MMP-9 are highly expressed in conditioned medium from 19- to 20-day fetal AEC (lane 3; ~72- and ~68-kDa rat MMP-2 and ~92- and 88-kDa rat MMP-9). MMP-9 gelatinolytic activity in conditioned medium from hyperoxic AEC (lane 2) presents as a broader band than control conditioned medium (lane 1). Fetal fibroblasts from the same animals from which the fetal AEC were obtained were included (lane 4), since they can contaminate fetal AEC cultures, and the AEC2-derived A549 cancer cell line, secreting predominantly MMP-9, was also assayed from fetal fibroblasts over the 48- to 72-h culture period, since fibroblasts contaminate at levels >10% by this stage. Fetal fibroblasts (19–20 days) secrete less MMP-2 and MMP-9 than fetal AEC. MMP-2 was the predominant gelatinase in the conditioned medium obtained from cultures of the AEC2-derived lung cancer cell line A549.

**Fig. 3.** MMP-9 gelatinase is induced in AEC isolated from rats during the in vivo recovery period after hyperoxic injury. Rats were allowed to recover in vivo for various times after 48 h of hyperoxia. AEC were isolated, and conditioned media were collected for 24 h after attachment. Aliquots of concentrated conditioned media were analyzed by gelatin zymography. Lane 1, 24-h in vivo recovery; lane 2, 48-h in vivo recovery; lane 3, 72-h in vivo recovery; lane 4, 96-h in vivo recovery; lane 5, NIH/3T3 cell zymography control. MMP-9 is induced in AEC during the in vivo recovery period after hyperoxic injury.
ious times, measuring migration through gelatin-coated filters containing 8-μm pores with 25 ng/ml EGF as stimulant (16, 18). Cultured adult AEC are relatively nonmigratory, so we used A549 cells with 150 μg/ml laminin as an attractant (18) and a positive control. As reported by others, we found no significant migration in the fresh isolate or over the period 24–48 h postplating (16, 18), so the data presented represent migration during the 48- to 72-h culture period (Fig. 4A). Unstimulated AEC from hyperoxic rats were found to be significantly more migratory through gelatin (P < 0.025) than unstimulated control adult AEC over the period 48–72 h postplating, although the magnitude of the response to EGF was not increased by hyperoxia. Although KGF does not elicit a significant migratory response in control AEC (18), we assessed its effect on AEC from hyperoxic rats. We also tested FGF-10 as a possible chemoattractant for control and hyperoxic AEC, since it is chemotactic for epithelial cells in developing lung (1). We found no significant stimulation of migration of control or hyperoxic AEC with either KGF or FGF-10 (data not shown).

Because the AEC from hyperoxic animals showed increased migration through gelatin, we then tested migration through a substrate more representative of in vivo conditions, choosing fibronectin as better representing the provisional matrix on which reepithelialization occurs after hyperoxic injury (Fig. 4B). AEC from hyperoxic rats exhibited significantly higher migration through fibronectin than control adult AEC (P < 0.025), achieving migration similar to stimulated A549 cell migration on gelatin (see Fig. 4A). The migration of both hyperoxic and control adult AEC through fibronectin was significantly greater than through gelatin (P < 0.01 and 0.05, respectively, by Student’s t-test; n = 4 animals/group).

Fetal AEC migrate immediately upon isolation and respond to EGF, FGF-10, and KGF in contrast to adult AEC. We measured stimulated migration of 19- to 20-day fetal AEC in freshly isolated cells and after 24 and 48 h of culture, testing EGF, KGF, and FGF-10 as logical candidates for chemoattractants for fetal AEC (1, 6, 18). Fetal AEC, in contrast to adult AEC, migrated significantly toward EGF immediately upon isolation (P < 0.005) and up until 48 h of culture (P < 0.01). KGF and FGF-10, growth factors found to be ineffective in stimulating migration of adult AEC, were both significantly stimulatory in the fresh isolate of fetal AEC and in early culture (each P < 0.05) but less so than EGF (Fig. 5). The experiments with fetal AEC were discontinued after the 48- to 72-h time period because of the increasing presence of fibroblasts, and, as a control, fetal fibroblasts were assayed for migration in parallel with the AEC over the 48- to 72-h period. They showed a different migratory profile, with a higher response to EGF and KGF than the AECs.

Doxycycline inhibits MMP-9 production and hyperoxia-induced migration in cultured AEC. Hyperoxic and control adult AEC were cultured in the absence of serum with and without 50 μg/ml doxycycline, which inhibits production of mammalian collagenases and gelatinases (12). Successful inhibition was confirmed by zymography. When migration through gelatin was measured, doxycycline was found to significantly lower hyperoxia-induced migration (P < 0.05 by Student’s t-test; n = 3–4 animals/group; Fig. 6) while having no significant effect on control AEC (data not shown). This suggests a functional correlation between migration and MMP activity in hyperoxic AEC, given the caveats...
that doxycycline could have other effects in the cell in addition to inhibition of MMP production.

**DISCUSSION**

Cell migration is facilitated by ECM degradation and thus by MMPs, a family of zinc-dependent proteases that function at neutral pH to cooperatively hydrolyze most proteins in the ECM (2). This study focuses on the gelatinases MMP-2 and MMP-9, which degrade basement membrane collagens and gelatins. A temporary upregulation of gelatinases in the lung has been reported during glandular organogenesis (13), hyperoxia (20, 22), and alveolar wound repair (23). Cell migration also plays a critical role in these processes and is seen both during lung morphogenesis (1) and during regeneration of the alveolar basement membrane after hyperoxia (14). Migration and gelatinases have been linked in bronchial epithelial cells during wound repair (17), but the relationship between MMPs and migration has not been studied in AEC. In the present study, we have correlated the in vitro migratory profiles and gelatinase levels of AEC from control lungs and lungs undergoing remodeling using adult cultured control AEC as opposed to hyperoxic rat AEC and 19- to 20-day fetal rat AEC. We also compared the A549 human cancer cell line as an invasive and migratory positive control derived from the AEC2 lineage. In our established, reproducible model of hyperoxic lung injury, the in vivo and in vitro repair/remodeling period has been thoroughly characterized with respect to the AEC2, with good correlation between in vivo and in vitro results (4). Additionally, an in vitro culture system allows us to reproducibly measure the migration of a specific cell type under controlled conditions that have previously been well described (16, 18) and enables us to work with a highly pure population of AEC.

The adult AEC, which is normally quiescent both in vivo and in primary culture, is relatively nonmigratory and constitutively secretes relatively low amounts of MMP-2 and MMP-9. Interestingly, cultured AEC isolated from rats after 48 h of hyperoxia with no in vivo recovery are significantly more migratory through gelatin than AEC from adult control rats, with further increased migration through fibronectin, a major component of the healing alveolar basement membrane after hyperoxia. AEC from hyperoxic rats also secrete significantly increased levels of active MMP-9 compared with control adult AEC. The inhibition of hyperoxia-induced migration by doxycycline, which strongly inhibits MMP-9 production in AEC, suggests a link between gelatinase and migration in AEC, given the caveat that doxycycline could have other effects on the cell in addition to MMP inhibition. MMPs other than the gelatinases could well contribute to this doxycycline-inhibitable migration. It may be that all AEC...
cultured from hyperoxic rats are more migratory than control AEC or perhaps hyperoxia selects a more migratory population. The peak of MMP-9 expression in AEC isolated during the recovery period after hyperoxic exposure occurs at the time of maximal in vivo epithelial repair in our model (3, 4). Although measured in vitro, the induction of MMP activity in AEC cultured from hyperoxic rats together with the concomitant increase in migration is consistent with the role of AEC in the lung during alveolar basement membrane repair after hyperoxic injury. A similar induction of MMP-9 during upper airway repair has been reported in human bronchial epithelial cells both in vivo and in culture, where cellular MMP-9 expression paralleled epithelial cell migration during remodeling of the provisional ECM (17, 28). In contrast, MMP-9 is not expressed by intact or injured tracheal epithelium, and matrilysin and not MMP-9 facilitates reepithelialization of wounded mouse tracheal epithelial cells (8). The selection of MMPs expressed in AEC during alveolar remodeling can evidently vary in response to the mode of damage in pathological conditions or gestational cues during lung development. Dunsmore et al. (8) measured MMP expression in lungs from diverse pathological conditions other than hyperoxia that involve alveolar remodeling and found no changes in gelatinase expression in AEC, although increased expression of matrilysin was noted in AEC from cystic fibrosis lungs. During the epithelial remodeling phase of alveolarization, specific MMPs are expressed by AEC in a timely manner, with MMP1, matrix type 1 MMP, and MMP-2 being expressed early in development and with MMP-9 together with activated MMP-2 being expressed mainly in the late stage (9).

Gelatinase protein expression in cultured control AEC increases with time in culture, receding again after 72 h, which may explain why primary cultures of adult AEC have a limited window of migration, with maximal migration occurring after 48 h of culture (19). Although this phenomenon could be a cultural artifact, the absence of MMP expression in fresh isolates of adult AEC compared with expression seen in fresh isolates of fetal AEC suggests that there are intrinsic differences between adult and fetal AEC gelatinase that are unrelated to culturing, consistent with in vivo biology. Cessation of gelatinase protein expression after 72 h in culture is accompanied by a reduced capacity for migration and a phenotypic change toward a larger, flattened cell phenotype, suggesting a link between MMP expression and transdifferentiation. This “intermediate” phenotype of AEC is also seen in vivo near the end of the hyperoxic recovery period (14) just preceding transformation into type 1 AEC.

Fetal AEC, at the gestational age studied (19–20 days, pseudoglandular), migrate immediately upon isolation and secrete high levels of MMP-2 and MMP-9, a profile consistent with an active remodeling function and quite different from the sessile phenotype of adult AEC. Unlike adult AEC, fetal AEC migrate immediately upon isolation, ruling out culture effects and suggesting in vivo priming by signals within the developing lung. KGF is a potent growth factor for both adult and fetal AEC (6, 26) and is present at increased levels in the lung during alveolar remodeling (5). Although the adult AEC did not migrate toward KGF, 19- to 20-day fetal AEC migrated toward both KGF and FGF-10, growth factors that promote lung development. KGF enhances maturation of late-gestational fetal rat lung epithelial cells by potently stimulating surfactant synthesis (6), whereas FGF-10, present in the mesoderm of developing lung, regulates endoderm proliferation and bud outgrowth (1). Thus, in cultured fetal AEC, the abundant gelatinase secretion and migration in response to specific growth factors known to promote lung maturation in vivo are consistent with a cell participating in lung development. In vivo studies of developing rabbit lung confirm increased expression of the gelatinases by AEC during lung maturation (9).

The invasive A549 lung cancer cell line, which is derived from the AEC lineage, migrates and invades by constitutively producing large amounts of active MMP-2 (29), strongly suggesting that increased migration is an important functional consequence of dysregulation of gelatinase production in AEC. The observation that fibronectin-cultured hyperoxic AEC can temporarily assume a migratory phenotype similar to A549 lung cancer cells on gelatin stresses the importance of tight regulatory control of gelatinase production in the resolution phase of the AEC injury response. The catalytic activity of MMPs is highly regulated, since they are expressed as inactive zymogens requiring specific activators, and their activity is further regulated by tissue inhibitors of metalloproteinases (2). The mechanism by which invasive lung cancer cells derived from AEC2 circumvent these regulatory controls is as yet unknown. Although the contribution of the other members of the MMP family to AEC migration also remains to be determined, our data suggest that gelatinase production by fetal and hyperoxic AEC is associated with a migratory phenotype in vitro, and we speculate that MMPs facilitate AEC migration during development and repair in vivo.

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