Cell Type-specific Effect of Hypoxia and Platelet-derived Growth Factor-BB on Extracellular Matrix Turnover and Its Consequences for Lung Remodeling*

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Hypoxia is associated with extracellular matrix remodeling in several inflammatory lung diseases, such as fibrosis, chronic obstructive pulmonary disease, and asthma. In a human cell culture model, we assessed whether extracellular matrix modification by hypoxia and platelet-derived growth factor (PDGF) involves the action of matrix metalloproteinases (MMPs) and thereby affects cell proliferation. Expression of MMP and its activity were assessed by zymography and enzyme-linked immunosorbent assay in human lung fibroblasts and pulmonary vascular smooth muscle cells (VSMCs), and synthesis of soluble collagen type I was assessed by enzyme-linked immunosorbent assay. In both cell types, hypoxia up-regulated the expression of MMP-1, -2, and -9 precursors without subsequent activation. MMP-13 was increased by hypoxia only in fibroblasts. PDGF-BB inhibited the synthesis and secretion of all hypoxia-dependent MMP via Erk1/2 mitogen-activated protein (MAP) kinase activation. Hypoxia and PDGF-BB induced synthesis of soluble collagen type I via Erk1/2 and p38 MAP kinase. Hypoxia-induced cell proliferation was blocked by antibodies to PDGF-BB or by inhibition of Erk1/2 but not by the inhibition of MMP or p38 MAP kinase in fibroblasts. In VSMCs, hypoxia-induced proliferation involved Erk1/2 and p38 MAP kinases and was further increased by fibroblast-conditioned medium or soluble collagen type I via Erk1/2. In conclusion, hypoxia controls tissue remodeling and proliferation in a cell type-specific manner. Furthermore, fibroblasts may affect proliferation of VSMC indirectly by inducing the synthesis of soluble collagen type I.

The incidences of chronic inflammatory lung diseases are increasing worldwide (1, 2). The pathogenesis of such diseases, including sleep apnea, chronic obstructive pulmonary disease, emphysema, fibrosis, and asthma are characterized by exten-sive modifications of the extracellular matrix (ECM)2 and of local insufficient oxygen supply, hypoxia (3–5). Hypoxia has been identified as an important regulator of cell proliferation, ECM synthesis, and organ function and is assumed to result from increased tissue mass or insufficient vascularization (6).

Locally restricted hypoxia increases the secretion of pro-inflammatory cytokines and growth factors, including interleukin-6 and -8, transforming growth factor-β (TGF-β), and platelet-derived growth factor-BB (PDGF-BB) via hypoxia-sensitive transcription factors and feedback on hypoxia-related tissue remodeling (3, 7, 8). The local turnover and the composition of the ECM, especially its collagen content, are key factors in the understanding of tissue remodeling (9, 10). ECM is degraded by matrix metalloproteinases (MMPs), which are controlled by the tissue inhibitors of MMP (TIMPs) (11, 12). The initial step of ECM degradation involves active collagenases, especially MMP-1, -8, -13, and -18, which digest intact collagens followed by further digestion by gelatinases, mainly MMP-2 and MMP-9 (10, 13). In such conditions, other cell types can invade and integrate into the area of the degraded ECM, where they will form their own cell type-specific bed of ECM (9, 10). In this context, hypoxia enhances the expression and action of MMP-1, -2, -3, -7, -9, -13, and -14 (MT1-MMP) apparently in a cell type- but also species-specific manner (11–13). As the enzymatic activity of MMP can be inhibited by TIMP, the effect of hypoxia on TIMP expression is of some importance (11, 12). Furthermore, TIMPs seem to be essential to regulating the conversion of pro-MMP-2 into active MMP-2 and need the formation of a complex consisting of pro-MMP-2, TIMP-2, and MT1-MMP, which leads to cleavage and activation of pro-MMP-2, subsequently affecting cell proliferation and differentiation (14).

The effect of hypoxia or one of the associated cytokines on airway remodeling has been studied, but less attention has been paid so far to the feedback mechanism of the cytokines examined on hypoxia or on the interaction of the cell types involved.

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‡ The abbreviations used are: ECM, extracellular matrix; APMA, 4-aminophenylmercuric acetate; Erk, extracellular signal-regulated kinase; HIF, hypoxia-inducible factor; MAP, mitogen-activated protein; MMP, matrix metalloproteinase; OO-Hy, cis-9-octadecenoyl-N-hydroxylamide-oleoyl-N-hydroxy-lamide; PDGF, platelet-derived growth factor; TIMP, tissue inhibitor of matrix metalloproteinase; VSMC, vascular smooth muscle cells; TGF, transforming growth factor; FCS, fetal calf serum; BSA, bovine serum albumin; Ab, antibody; mAb, monoclonal antibody.
In this study, we assessed the effect of hypoxia (3% O₂), PDGF-BB, and their combination on the synthesis and activity of MMP, TIMP, total collagen deposition, and soluble collagen type I synthesis by human lung fibroblasts and pulmonary vascular smooth muscle cells (VSMCs). We further studied the underlying intracellular signaling pathway and the feedback mechanism of these factors on proliferation, as well as the interaction of the two cell types.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—Primary human lung cell lines were prepared from patients undergoing lobectomy or pneumonectomy for surgical therapy after written consent and approval by the Ethics Committee of the Faculty of Medicine, University Hospital, Basel, Switzerland (M75/97). Primary human pulmonary VSMC lines were established from lung arteries, as described previously (15), and were grown in minimal essential medium supplemented with 5% fetal calf serum (FCS), 20 mM HEPES buffer (all Biochrom, Basel, Switzerland) and 1× amino acid mix (Invitrogen). No antibodies or anti-mycotics were added at any time. VSMC were characterized by morphology and positive staining for smooth muscle cell actin. Primary human lung fibroblasts were grown from the same lung biopsies (16) and were grown in RPMI 1640 supplemented with 10% FCS, 8 mM l-glutamine, 20 mM HEPES buffer and 1× amino acid mix. All cells were grown to confluence and were expanded by trypsinization and used for experiments until the fourth passage.

**Cell Culture Conditions**—Cells were seeded onto 24-well culture plates (Falcon, Basel, Switzerland) and grown until 80% confluence. Before stimulation with PDGF or exposure to hypoxia (3% O₂), the cells were serum-deprived for 48 h with their specific growth medium containing 0.1% FCS. To avoid auto-stimulation, the medium was exchanged every 12 h. The cells were stimulated with 1–20 ng/ml PDGF-BB (human recombinant, R & D Systems) and/or incubated under hypoxic or normoxic conditions for 12, 24, or 48 h. Normoxia was 21% O₂, 74% N₂, and 5% CO₂. Hypoxia was 3% O₂, 93% N₂, and 5% CO₂ (11). The effect of the MMP inhibitor O0-Hy (catalog number 44244; Calbiochem, Lucern, Switzerland) and of neutralizing polyclonal anti-PDGF-BB antibodies (AB-220-NA; R & D Systems) on proliferation (48 h) was also investigated. PD98059, a p38 MAP kinase inhibitor, and SB203580, an inhibitor of the two cell types.

**Measurement of TIMP-1 and TIMP-2**—Total TIMP-1 and TIMP-2 were measured in cell culture medium samples containing 10 µg/ml total protein by ELISA (Amersham Biosciences).

**Immunoblotting**—For protein analysis, 10⁵ cells were lysed with 100 µl of Laemmli buffer, and the protein concentration was adjusted to 5 µg/µl before size fractionation by electrophoresis in SDS-polyacrylamide gels (4–15%, 40 V, 90 min, 4 °C) as described earlier (19). Protein loading was controlled by Ponceau staining after transfer onto nylon membranes. Unspecific binding was blocked by 3% skim milk in TBS-T (25 mM Tris-base, pH 7.4, 0.15 M NaCl, 0.05 M KCl, 0.05% Tween 20; 30 min, room temperature), and the expression of TIMP-1 and -2 and MMP-1 and -13 was analyzed using specific polyclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) and subsequent incubation with species-specific horseradish peroxidase-conjugated antibodies (Dako). Specific protein bands were visualized by ECL and documented by scanning (19).

**Determination of Soluble Collagen Type I**—The concentration of soluble collagen type I was determined in 50-µl cell culture medium by ELISA (11). Samples were dried, dissolved in water (50 µl), and coated overnight (60 °C) onto polystyrene plates. Unspecific binding was blocked by 4% BSA in PBS-T (1 h, 37 °C), plates were washed with PBS-T (2×), and collagen type I antibodies (mAb339, Chemicon) were added (0.1% BSA, 20 h, 4 °C). Following three washes with PBS-T, a peroxidase-conjugated goat anti-mouse IgG was added (dilution 1:5000/492 nm). Purified human MMP-1 and MMP-13, respectively, were used to standardize MMP concentrations (18).

**Reverse Transcription-PCR**—Total RNA was isolated using the RNAeasy kit (Qiagen, Basel, Switzerland). One microgram of total RNA was reverse-transcribed using M-MLV reverse transcriptase (Promega, Madison, WI). PCR was performed by denaturation (3 min, 94 °C) followed by 20–25 cycles of denaturation (30 s, 94 °C), primer annealing (30 s, 58 °C), and extension (1 min, 72 °C) followed by a final extension (10 min, 72 °C). PCR products were analyzed by electrophoresis (1.5% agarose gel, ethidium bromide). Primers used for COL1A1 were forward (5’-CCC CTG GCG CTG TCG TGT GTC CGT CTG-3’) and reverse (5’-GGG CGG CAA GGT CTC CAG CAG AA-3’) (product 201 bp) and for β-actin forward (5’-GTA CTG TGC TAT CCA GGC TGT GC-3’) and reverse (5’-TCA GGC AGC TCG TAG CTC TTC TC-3’) (product 350 bp).
Cell Proliferation—Cells were seeded (10^4 cells/ml) in 24-well plates and allowed to attach overnight and then arrested by serum starvation (0.1% FCS, 24 h) and exposed to hypoxia and/or PDGF-BB for 3 days before being manually counted (Neubauer hematocytometer).

Protein Determination—The content of total protein of each sample was determined with a standard Bradford assay (Bio-Rad) using BSA (Sigma) as the standard of known concentrations.

**Statistics**—Means ± S.E. were calculated from results obtained from cultures of primary human VSMCs or fibroblasts established from lung tissue biopsies from at least four different patients. Determinations were always made in triplicate. Statistical analysis was performed using analysis of variance and Student’s t test.

**RESULTS**

**Hypoxia Induces and PDGF-BB Inhibits the Secretion of Pro-MMP-2 and Pro-MMP-9**—The morphology or viability of VSMCs or human lung fibroblasts was not affected by hypoxia (up to 72 h) or PDGF-BB (1–20 ng/ml), as assessed by immunostaining or trypan blue exclusion staining. Fibroblasts and VSMCs (Fig. 1A) secreted pro-MMP-2 and pro-MMP-9 under normoxia (48 h). Gelatinolytic activity was completely inhibited in the presence of the metal chelators Na₂EDTA (20 mM) (Fig. 1A). Gelatinolytic activity in eight fibroblast and eight VSMC lines was quantified using a computer-assisted image analysis program and revealed that the ratio of pro-MMP-2 to pro-MMP-9 activity was 40:1 for both cell types. However, the results on pro-MMP-9 secretion were not further quantified or statistically analyzed due to the fact that only minor amounts of pro-MMP-9 could be detected.

In fibroblasts (Fig. 1B) and VSMCs (Fig. 1C), hypoxia significantly increased the secretion of pro-MMP-2 by ~38% (p < 0.02) within 48 h, and the addition of PDGF-BB significantly reduced this effect (p < 0.05). In fibroblasts, inhibition of p38 (but not Erk1/2 MAP kinase) dose-dependently reduced hypoxia-induced pro-MMP-2 secretion. In contrast, Erk1/2 MAP kinase counteracted the inhibitory effect of PDGF-BB on hypoxia-induced pro-MMP-2 secretion, whereas p38 MAP kinase inhibition had no effect, and the control substance SB202474 (negative Erk1/2 inhibitor) was also without significant effects under all tested conditions (Fig. 1B).

Surprisingly, the signaling cascade was different in VSMCs, as inhibition of Erk1/2 and p38 MAP kinases were reducing the stimulatory effect of hypoxia on pro-MMP-2 secretion (Fig. 1C). However, only inhibition of Erk1/2 MAP kinase reduced
the inhibitory effect of PDGF-BB on hypoxia-dependent pro-MMP-2 release; the control SB202474 was without significant effects under all tested conditions (Fig. 1C).

**PDGF-BB Increases TIMP-1 Production Preferentially by Fibroblasts, and Pro-MMP-2 Is Complexed with TIMP-2—** PDGF-BB significantly increased TIMP-1 secretion by both cell types, whereas hypoxia had an additional increase but insignificant effect (Fig. 2A). In fibroblasts, the effect of PDGF-BB on TIMP-1 expression was mediated via Erk1/2 and did not involve p38 MAP kinase; the Erk1/2 control substance SB202474 was without significant effect under all tested conditions (Fig. 2A). Fibroblasts secreted significantly more TIMP-1 per cell than VSMCs, and the basic effects of hypoxia and of PDGF-BB were similar in both cell types (Fig. 2A). The release of TIMP-2 from non-stimulated fibroblasts and VSMCs was not significantly affected by hypoxia or PDGF-BB within 48 h (Fig. 2B).

When cell culture samples were treated with 4-aminophenylmercuric acetate (APMA, 1 mM) for 24 h prior to gelatin zymography, we observed that pro-MMP-2 was partially converted into active MMP-2 (Fig. 2C). Quantification of gelatinolytic activity revealed that the sum of the intensities of the two gelatinolytic bands after APMA treatment was almost identical to the intensity of the initial pro-MMP-2 band. Neither hypoxia nor PDGF-BB modified the effect of APMA under normoxia in either cell-type (Fig. 2C).

**PDGF-BB Inhibits Hypoxia-induced MMP-1 Synthesis—** As determined by ELISA, fibroblasts and VSMCs constitutively secreted MMP-1 within 48 h, and hypoxia significantly increased the release of MMP-1, which was abrogated by PDGF-BB, but PDGF-BB had no effect on the constitutive expression of MMP-1 in either cell type (Fig. 3A). Overall MMP-1 release by fibroblasts was significantly higher (11.7 ± 1.5 versus 8.1 ± 1.3 pg/µg protein, p < 0.01) than in VSMCs. Similar results were obtained by immunoblotting. Here we demonstrated that an anti-PDGF-BB antibody and Erk1/2 MAP kinase inhibition counteracted the effect of PDGF-BB on hypoxia-induced MMP-1 expression in fibroblasts (Fig. 3A). Furthermore, our data indicate that the stimulatory effect of hypoxia on MMP-1 expression involves p38 AMP kinase but not Erk1/2 or PDGF-BB (Fig. 3B). Similar results were obtained with VSMCs (data not shown).

**Hypoxia Stimulates Hypoxia-inducible Factor (HIF)-1α Expression—** HIF-1α is a hypoxia-associated transcription factor, and our data show that 24 h of hypoxia up-regulates HIF-1α expression in fibroblasts. PDGF-BB alone did not induce HIF-1α expression and did not alter the stimulatory effect of hypoxia (Fig. 3B). HIF-1α expression was only down-regulated in the presence of the Erk1/2 MAP kinase inhibitor SB203580 but not by its inactive control substance (SB202474) or the p38 MAP kinase inhibitor (PD98059) (Fig. 3B). Similar results were obtained with VSMCs (data not shown).
Hypoxia Increases MMP-13 in Fibroblasts but Not in VSMCs—

Hypoxia almost doubled the release of MMP-13 by lung fibroblasts compared with normoxia (1.8 ± 0.24 versus 0.97 ± 0.14 pg/μg total protein, p < 0.01) within 48 h (Fig. 3, B and C). Although PDGF-BB did not affect the release of MMP-13 from fibroblasts under normoxia, it abolished the hypoxia-induced increase in MMP-13 (p < 0.01) (Fig. 3, B and C). Interestingly, MMP-13 secretion by VSMCs was neither affected by hypoxia nor by PDGF-BB (Fig. 3C). Assessing the underlying signaling pathways, we observed that only p38 MAP kinase inhibition abrogated the stimulatory effect of hypoxia, whereas Erk1/2 PDGF-BB significantly increased cell numbers by 33.8 ± 2.5% (p < 0.01) as compared with hypoxia, but this increase was not significant compared with PDGF-BB alone (p = 0.81) (Fig. 5B).

The inhibition of Erk1/2 dose-dependently inhibited the mitogenic effect of hypoxia, as did both cell types (Fig. 5). In contrast, inhibition of p38 MAP kinase did not affect hypoxia-induced fibroblast proliferation (Fig. 5A), whereas it partially inhibited the stimulatory effect of hypoxia on VSMCs (Fig. 5B). When the two MAP kinases were inhibited, we observed an additive anti-proliferative effect in VSMC (Fig. 5B) but not in fibroblasts (Fig. 5A). Neutralizing antibodies to PDGF-BB also

**FIGURE 3.** A, the effect of hypoxia (black bars) and PDGF-BB (striped bars) on MMP-1 expression by fibroblasts and VSMC within 48 h was assessed by ELISA. Each bar represents the mean ± S.D. of triplicates in six independent experiments. B, a representative immunoblot demonstrates the role of Erk1/2 and p38 MAP kinase in the hypoxia-induced expression of MMP-1, MMP-13, and hypoxia-inducible factor-1α (HIF-1α) as well as on the antagonizing effect of PDGF-BB in human lung fibroblasts. PD98059 inhibits Erk1/2 MAP kinase, SB203580 inhibits p38 MAP kinase and SB202474 is a negative control for SB203580. Ab, neutralizing antibody; control, protein extract from untreated cells before stimulation. C, hypoxia- and PDGF-BB-induced MMP-13 expression by fibroblasts but not by VSMC within 48 h. Each bar represents the mean ± S.D. of triplicate determinations from six independent experiments. Similar results were obtained with two additional cell lines.
Cell Type-specific ECM Modulation by Hypoxia

hypoxia activates the phosphorylation of Erk1/2 and p38 MAP kinase in human pulmonary fibroblasts and VSMCs (19); therefore, we further analyzed the underlying signaling cascade. Erk1/2 signaling mediated the proliferative effect of hypoxia and PDGF-BB in fibroblasts and VSMCs, whereas p38 MAP kinase was only relevant for VSMC proliferation. Erk1/2 has been shown to mediate mitogenic signals of various stimuli, including PDGF-BB (26) and hypoxia (27). Our data did not confirm the involvement of p38 MAP kinase in the mitogenic effect of PDGF-BB, as it was described in rat myofibroblasts, which could either be due to a species-specific or cell differentiation-dependent effect (28). In this context, it is interesting to note that p38 MAP kinase mediates proliferation of VSMC in response to PDGF-BB (29), even more in an organ- and cell type-specific manner (30).

An additional pro-mitogenic factor for VSMCs was soluble collagen type I, the effect of which was mediated via Erk1/2. In this context, it has been reported that collagen type I may affect myofibroblast differentiation and VSMC response to different stimuli (31) and that hypoxia-induced proliferation and vascularization is increased by collagen type I (32). Interestingly, soluble collagen type I was exclusively produced by fibroblasts under hypoxia and/or PDGF-BB but with a stimulus-specific signaling cascade. PDGF-BB (but not hypoxia) induced COL1A1 mRNA synthesis via Erk1/2, in agreement with similar observations in rat stellate cells (33). Hypoxia has been shown to increase COL1A1 expression in fibroblasts via TGF-β and p38 MAP kinase (11, 34), which in turn may affect the ability of VSMCs to form new vessels (35). Our experiments indicate that both Erk1/2 and p38 MAP kinase are involved in this particular effect. Erk1/2 signaling might be induced by PDGF-BB, which we documented to mediate the proliferative effect of hypoxia. This increase of soluble collagen type I may be partly explained by down-regulation of collagenase activity under hypoxia and in combination with enhanced levels of PDGF-BB or TGF-β, eventually leading to angiogenesis, hypertrophy, or hyperplasia (36).

MMP and their inhibitors (TIMPs) are the major regulators of extracellular matrix turnover in the lung and also have been shown to affect proliferation of both cell types. As observed earlier, hypoxia increased the expression of most MMP, and this effect was counteracted by PDGF-BB in both cell types (11, 19). Similar to our findings, increased MMP-1 (11, 12), MMP-13 (37), pro-MMP-2, and pro-MMP-9 were reported under hypoxic conditions. Increased expression of MMP-1 was demonstrated in the lungs of patients with chronic obstructive pulmonary disease (37, 38), whereas in asthma, several studies indicate a down-regulation (38). Earlier, we reported that hypoxia, together with TGF-β, increased MMP-1 synthesis (11). Here, we showed that the stimulatory effect of hypoxia on MMP-1 involved p38 MAP kinase and the inhibitory effect of PDGF-BB involved Erk1/2. This was confirmed by experiments following APMA treatment of the cells, which indicated that MMP-2 is not involved in complex with TIMP-2, even under hypoxia. In this respect, TIMP-2 not only inhibits the enzymatic action of active MMP-2, but it is also essential for binding and presenting the inactive proMMP-2 to its activators, such as MT-MMP-1 (14).

DISCUSSION

In this study, we provide evidence that hypoxia and PDGF-BB modulate the effects of each other on tissue remodeling in a cell type-specific pattern in human lung cells. Gelatinases, MMP-1, and TIMP-1 and -2 are increased by hypoxia in fibroblasts and VSMCs, and PDGF-BB counteracts this effect. In contrast, MMP-13 and soluble collagen type I are only expressed by fibroblasts. Collagen type I contributes to hypoxia and PDGF-BB-dependent proliferation of VSMCs. Hypoxia-induced proliferation involves Erk1/2 in both cell types and also p38 MAP kinase in VSMCs.

Hypoxia has been shown to stimulate tissue remodeling during embryogenesis and wound repair as well as in tumorigenesis and other hypoxia-associated diseases (3, 6, 20, 21). Tissue remodeling is the result of modified fibroblast proliferation (22, 23) and of a significant modification of the local ECM (24). These events subsequently increase proliferation of VSMC and therefore affect neovascularization (23, 25). Our experiments confirmed the mitogenic effect of hypoxia and revealed an additive effect when combined with PDGF-BB on both fibroblasts and VSMCs. In an earlier study, we provided evidence that
dose-dependently reduced cell proliferation with a significant effect only in fibroblasts (Fig. 5A) but not in VSMC (Fig. 5B). Furthermore, the addition of soluble collagen type I to the growth medium of VSMC increased the mitogenic effect of hypoxia significantly, an effect that was also down-regulated in the presence of an Erk1/2 inhibitor (Fig. 5B). Inhibition of MMP neither altered proliferation of fibroblasts (Fig. 5A) nor that of VSMCs (Fig. 5B).

FIGURE 4. A, the effect of hypoxia and PDGF-BB on the release of soluble collagen type I within 48 h was determined by ELISA in the cell culture medium obtained from human lung fibroblasts (n = 6). Each bar represents the mean ± S.D. Experiments in each cell line were performed in triplicates. B, the inducing effect of hypoxia and PDGF-BB on collagen type I 1 chain (COL1A1) was confirmed on the mRNA level in three cell lines and the effect of Erk1/2 and p38 MAP kinase was assessed by reverse transcriptase-PCR. β-actin gene expression was used as a housekeeping gene, and data are displayed as a representative PCR product analysis. PD98059 inhibits Erk1/2 MAP kinase, SB203580 inhibits p38 MAP kinase, and SB202474 is a negative control for SB203580.

FIGURE 5. A, the effect of hypoxia and PD98059 on the release of soluble collagen type I was exclusively produced by fibroblasts under hypoxia and/or PDGF-BB but with a stimulus-specific signaling cascade. PDGF-BB (but not hypoxia) induced COL1A1 mRNA synthesis via Erk1/2, in agreement with similar observations in rat stellate cells (33). Hypoxia has been shown to increase COL1A1 expression in fibroblasts via TGF-β and p38 MAP kinase (11, 34), which in turn may affect the ability of VSMCs to form new vessels (35). Our experiments indicate that both Erk1/2 and p38 MAP kinase are involved in this particular effect. Erk1/2 signaling might be induced by PDGF-BB, which we documented to mediate the proliferative effect of hypoxia. This increase of soluble collagen type I may be partly explained by down-regulation of collagenase activity under hypoxia and in combination with enhanced levels of PDGF-BB or TGF-β, eventually leading to angiogenesis, hypertrophy, or hyperplasia (36).
However, TIMP-2 expression was not affected by hypoxia in our settings, and therefore its contribution to hypoxia-dependent pathologies might be regulated by other cell types. Similarly, TIMP-1 expression was only marginally increased by hypoxia, but when combined with PDGF-BB, TIMP-1 levels were significantly increased over the effect of PDGF-BB alone. Erk1/2 was the main mediator of the PDGF-BB-dependent increase, which is in agreement with other studies, but the underlying signaling pathway may depend on the cell type or stimulus used (39). The clinical implication of hypoxia and/or PDGF on TIMP-1 secretion by human lung fibroblasts and VSMCs is that TIMP-1 may be associated with lung fibroproliferative diseases, as indicated in animal models of lung fibrosis (40, 41), and in the sputum of pulmonary fibrosis patients (42). With respect to the regulation of MMP secretion by collagen, we have previously shown that collagen type IV (but not gelatin or collagen type I) stimulated pro-MMP-2 secretion in pulmonary fibroblasts and VSMCs under normoxia. Furthermore, collagen type IV had a synergistic effect with hypoxia on pro-MMP-2 expression and MMP-2 production in both cell types (19).

MMP-13 was only produced by fibroblasts, and as with MMP-1, this effect was inhibited by the proliferative stimulus PDGF-BB. Based on immunoblotting, our data suggest that MMP-13 expression by hypoxia is mediated via p38 MAP kinase, whereas the inhibitory effect of PDGF-BB involves the activation of Erk1/2, which is in agreement with the findings of Krejci et al. (43). The same signaling pathway seems to control the stimulating effect of hypoxia on pro-MMP-2 and pro-MMP-9 expression by both cell types (14, 43). Furthermore, the pro-MMP-2 we determined in the cell culture medium was bound to TIMP-2, as we demonstrated by the partial conversion of pro-MMP-2 into active MMP-2 by APMA and the stability of the MMP-2 band after prolonged APMA treatment. This complex is essential to the activation of pro-MMP-2 and possibly pro-MMP-9 by infiltrating inflammatory cells (45).

We also found that the expression of the HIF-1α in fibroblasts and VSMCs is up-regulated 24 h after exposure to hypoxic cell culture conditions and that this effect involves Erk1/2 MAP kinase but neither PDGF-BB nor p38 MAP kinase. The role of Erk1/2 MAP kinase in HIF-1α expression has been shown by others (46, 47). However, from our data, HIF-1α cannot be involved in the inhibitory effect of PDGF-BB on MMP-1 and MMP-13. For the same reason, we can exclude the involvement of HIF-1α on the synergistic effect of PDGF-BB and hypoxia on COL1A1 expression.
In summary, our results demonstrate that the net effect of hypoxia on extracellular matrix and cell proliferation should not be studied without taking into account that growth factors and cytokines may significantly modulate or even reverse the effects of hypoxia. Furthermore, this modulatory effect of growth factors is cell type-specific, and it seems that, in several pathologies, the effect of hypoxia is irreversible. We postulate that hypoxia triggers the proliferation and accumulation of extracellular matrix deposition in the lung, that both processes are augmented by growth factors such as PDGF-BB or TGF-β isoforms, and that their synergistic net effect contributes to the progression of pulmonary diseases, such as sleep apnea syndrome, fibrosis, emphysema, asthma, and lung carcinoma.

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