Gene Expression Patterns 8 (2008) 486–493

Contents lists available at ScienceDirect

Gene Expression Patterns

journal homepage: www.elsevier.com/locate/gep

uPARAP expression during murine lung development

Leah Smith [1], Teresa E. Wagner [1], Isham Huizar, Lynn M. Schnapp *

Division of Pulmonary and Critical Care Medicine, Department of Medicine, Harborview Medical Center, University of Washington, Box 359640, 325 9th Ave, Seattle, WA 98104, USA

ARTICLE INFO

Article history:
Received 17 November 2007
Received in revised form 7 June 2008
Accepted 24 June 2008
Available online 28 June 2008

Keywords:
Lung development
uPARAP
Endo 180
Collagen
Extracellular matrix

ABSTRACT

Lung remodeling requires active collagen deposition and degradation. Urokinase plasminogen activator receptor-associated protein (uPARAP), or Endo 180, is a cell-surface receptor for collagens, which leads to collagen internalization and degradation. Thus, uPARAP-mediated collagen degradation is an additional pathway for matrix remodeling in addition to matrix remodeling mediated by matrix metalloproteinases and cathepsins. Using immunohistochemistry, we demonstrate extensive uPARAP expression in the mesenchyme throughout murine lung development. By immunofluorescence, we demonstrate significant overlap of uPARAP expression with collagen IV expression, but minimal overlap with collagen I expression in the developing murine lung. Finally, we compared lung development between wild-type and uPARAP$^{-/-}$ mice, and found no significant histologic differences, indicating the presence of alternative collagen degradation pathways during murine lung development.

1. Results and discussion

Urokinase plasminogen activator receptor-associated protein (uPARAP), or Endo180, is a newly described cell-surface receptor that binds and internalizes collagens, including types I, IV, and V, targeting them for lysosomal degradation (Behrendt et al., 2000; Engelholm et al., 2001, 2003; Kjoller et al. 2004). A member of the mannose receptor family, uPARAP is expressed on macrophages and mesenchymal cells, including chondrocytes and some fibroblasts (Howard et al., 2004; Sheikh et al., 2000). Mesenchymal cells that lack uPARAP are unable to internalize collagen (Curino et al., 2005; East et al., 2003; Engelholm et al., 2003; Kjoller et al., 2004; Madsen et al., 2007; Mousavi et al., 2005). In addition to its role in collagen internalization and degradation, uPARAP contributes to the adhesion and migration of collagen in vitro (Engelholm et al., 2003). uPARAP may also play a role in human gingival re-epithelialization and remodeling after injury (Honardoust et al., 2006). In addition, uPARAP is expressed in tumor stromal cells and has a role in tumor progression (Curino et al., 2005; Sulek et al., 2007).

During lung development, the matrix undergoes active remodeling during the formation and differentiation of airways, vasculature, and mature alveoli. In particular, during the saccular stage (E17.5 to post-natal day 5 (PN5)) and alveolar stage (PN5–PN30), the lung interstitium undergoes extensive remodeling with a decrease in the interstitial space, maturation and narrowing of the blood–air barrier and thinning of the newly formed alveolar septa. Such remodeling requires active collagen synthesis and degradation. Much focus has been paid to extracellular pathways of collagen degradation, including matrix metalloproteinases (MMPs) and cathepsins. Despite the known role of MMPs in matrix remodeling, knockouts of MMPs to date (MMP-2, -3, -7, -8, -9, -11, -12, -28) show no lung abnormalities and no or minor developmental phenotypes (reviewed in Parks and Shapiro, 2001 and Greenlee et al., 2007). The one notable exception is MMP-14 (MT1–MMP), in which the knockout mice have severe skeletal abnormalities due to impaired collagen turnover (Holmbeck et al., 1999). The mice also have abnormalities in alveolarization, demonstrating a role for collagen degradation in alveolar formation (Atkinson et al., 2005). In contrast to these other pathways of degradation, uPARAP-mediated collagen internalization and degradation allows cells to recycle collagen components, a key feature of cell housekeeping. This makes uPARAP an attractive candidate for collagen turnover during normal development. While high levels of uPARAP mRNA have been found in fetal and adult total lung tissue (Wu et al., 1996), the expression pattern has not been examined in the developing murine lung. uPARAP is known to interact with collagens, including collagens I and IV, but the spatio-temporal relationship of uPARAP to these proteins has yet to be elucidated. We determined the spatio-temporal expression of uPARAP during murine lung development by immunohistochemistry and real-time PCR. We also examined lung development in uPARAP$^{-/-}$ mice to further investigate the role of uPARAP in the developing lung. Finally, we analyzed uPARAP$^{-/-}$ mice for differences in matrix synthesis and MMP expression during lung development.

* Corresponding author. Tel.: +1 206 341 5389; fax: +1 206 341 5392.
E-mail address: lschnapp@u.washington.edu (L.M. Schnapp).
[1] These authors are equally contributed.

1567-133X/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.gep.2008.06.006

uPARAP immunoreactivity was present at the earliest time point tested, (E12.5), the early pseudoglandular stage (E12.5–16.5), when branching morphogenesis occurs (Fig. 1A). uPARAP was detected on the majority of mesenchymal cells, but absent on airway epithelium. At mid-pseudoglandular stage (Fig. 1C–F), there was persistent mesenchymal immunoreactivity, however, the staining appeared more discontinuous along the mesenchymal cell-surface, with more prominent staining at areas of cell-matrix interactions (arrows, Fig 1F) and less staining at cell–cell junctions. By E16.5 (Fig 1I–J), uPARAP immunoreactivity was present in the layer beneath airway epithelium (arrowhead, Fig 1I) and became more pronounced during post-natal lung development (Fig. 2C–H). No uPARAP immunoreactivity was observed on epithelial cells at any stage of development. As controls, no uPARAP immunoreactivity was observed in lungs from uPARAP$^{-/-}$ mice or using isotype control antibody (Fig. 3 and not shown).

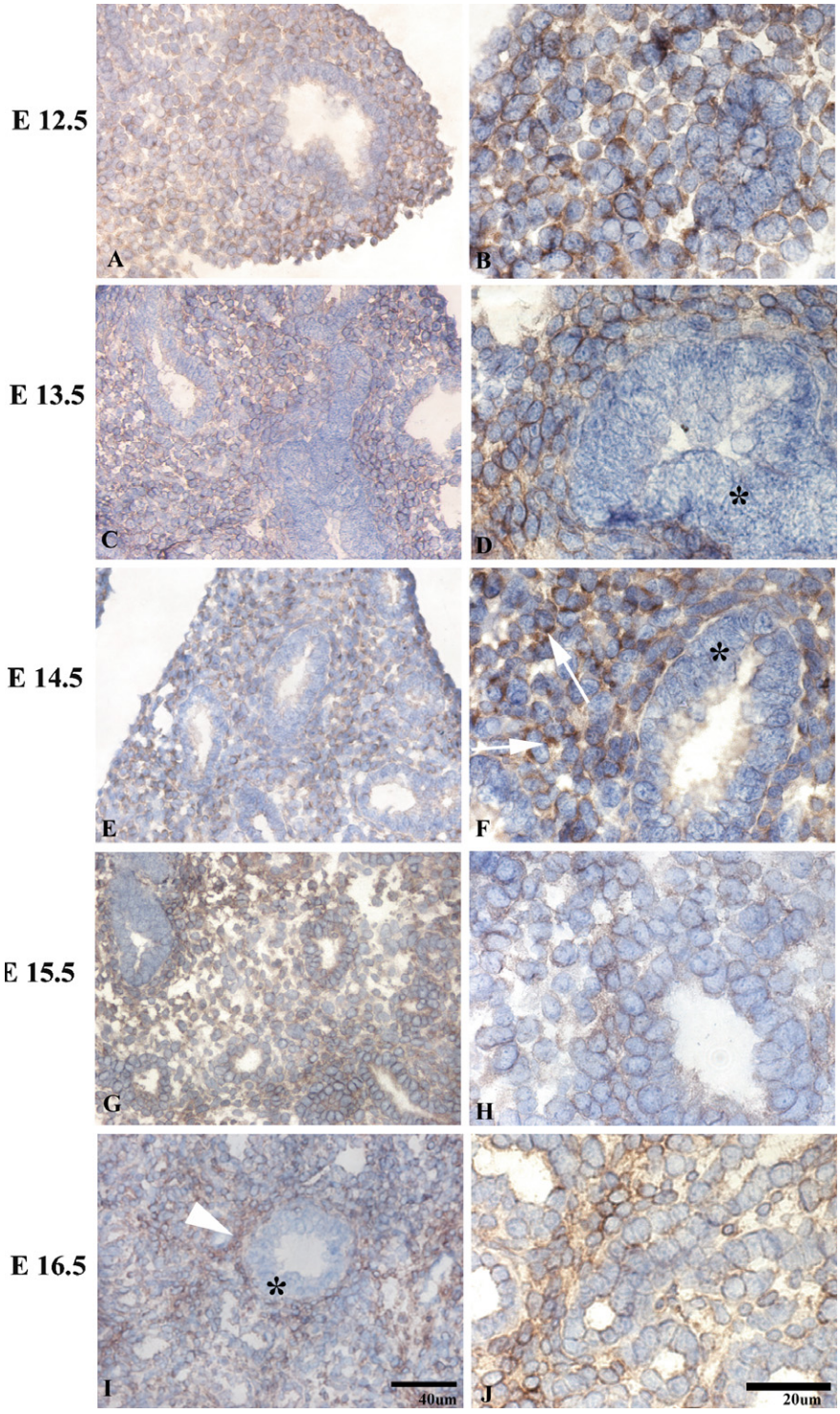

Fig. 1. Distribution of uPARAP in the pseudoglandular (A–H) and canalicular (I–J) stages. uPARAP immunoreactivity is seen as early as E12.5 (A and B). Epithelial staining is absent (∗).