Available online at www.sciencedirect.com
SCIENCE DIRECT
Developmental Biology 291 (2006) 67–82
DEVELOPMENTAL BIOLOGY
www.elsevier.com/locate/ydbio

Evidence that autocrine signaling through Bmpr1a regulates the proliferation, survival and morphogenetic behavior of distal lung epithelial cells

Maxwell C. Eblaghie [a,1], Mary Reedy [a], Tim Oliver [a], Yuji Mishina [b], Brigid L.M. Hogan [a,*]

[a] *Department of Cell Biology, Duke University Medical Center, Durham, NC 27710, USA*
[b] *Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, USA*

Received for publication 24 August 2005; revised 22 November 2005; accepted 1 December 2005
Available online 18 January 2006

Abstract

Lung development requires reciprocal epithelial/mesenchymal interactions, mediated by signaling factors such as Bmps made in both cell populations. To address the role of Bmp signaling in the epithelium, we have exploited the fact that Bmp receptor type Ia (Alk3) is expressed in the epithelium during branching morphogenesis. Deletion of *Bmpr1a* in the epithelium with an *Sftpc-cre* transgene leads to dramatic defects in lung development. There is reduced epithelial proliferation, extensive apoptosis, changes in cell morphology and extrusion of cells into the lumen. By E18.5, there are fewer Type II cells than normal, and the lung contains large fluid-filled spaces. If cell death is prevented by making embryos homozygous null for the proapoptotic gene, *Bax*, the epithelial cells that are rescued can apparently differentiate, but normal morphogenesis is not restored. To determine whether Bmps made by the epithelium can function in an autocrine manner, mesenchyme-free endoderm was cultured in Matrigel™ with Fgfs. Under these conditions, the mutant epithelium fails to undergo secondary budding. Abnormal development was also seen when *Bmp4* was specifically deleted in the epithelium using the *Sftpc-cre* transgene. Our results support a model in which Bmp signaling primarily regulates the proliferation, survival and morphogenetic behavior of distal lung epithelial cells.

Keywords: Bmpr1a; Alk3; Bmp4; Sftpc; Conditional mutant; Mouse embryo; Lung; Epithelium; Proliferation; Survival

Introduction

Bone morphogenetic proteins (Bmps) constitute a large family of evolutionarily conserved, intercellular signaling proteins that are dynamically expressed in both epithelial and mesenchymal cells during embryonic development. They function through conserved Type I and Type II transmembrane receptors and Smad-dependent and -independent pathways, to regulate a range of biological processes, including cell proliferation, apoptosis, differentiation and cell shape, in a highly context-dependent manner (Massague, 2000; Chen et al., 2004; Aubin et al., 2004; Kishigami and Mishina, 2005). Given this complexity, it is a major challenge to define the primary actions of Bmps during the growth and morphogenesis of organs such as the kidney, intestine or lung that involve intimate and reciprocal interactions between distinct mesodermal and epithelial cell populations. One way to tackle this problem genetically is to study the effect of conditionally deleting individual genes in the Bmp signaling pathway in specific cell populations during development. Here, we use this approach to dissect out the role of Bmp signaling mediated through Bmp receptor 1a (Alk3) in epithelial cells of the embryonic mouse lung.

Mouse lung development begins on embryonic day (E) 9.5 when two primary buds arise in the ventrolateral foregut (Cardoso, 2000; Warburton et al., 2000). Each bud consists of an inner endodermal epithelium surrounded by mesoderm and mesothelium. During the pseudoglandular stage (E9.5–16.0), the buds undergo repetitive and stereotypic rounds of branching and outgrowth, to give rise to a tree-like organ. This branching morphogenesis is orchestrated by reciprocal interactions between the epithelium and mesenchyme. These,

* Corresponding author. Fax: +1 919 684 8592.
E-mail address: b.hogan@cellbio.duke.edu (B.L.M. Hogan).
[1] Current address: Department of Ophthalmology, Duke University Eye Center, Durham, NC 27710, USA.

0012-1606/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2005.12.006

in turn, are mediated by a network of signaling pathways, including those downstream of Bmps, Fgfs, Wnts and retinoic acid, as well as extracellular matrix proteins (Cardoso, 2000; Cardoso et al., 1996, 1997; Lebeche et al., 1999; Liu et al., 2004; Malpel et al., 2000; Park et al., 1998; Warburton et al., 2000). A complete cell lineage of the developing lung is not yet available (Perl et al., 2002). However, it appears that during the pseudoglandular phase, a population of undifferentiated, multipotent progenitor cells exists in the distal tips of the epithelial branches. This population gives rise to the differentiated Clara, ciliated and neuroendocrine cells of the proximal airways (intralobular bronchi and bronchioles). During the canalicular stage (E16.0–17.5), the distal epithelium narrows, develops a tight association with blood vessels and gives rise to numerous terminal saccules that are the precursors of the alveoli containing mature Type I and Type II cells. The development of the alveoli and their septation continues for ~20 days after birth.

Previous studies have identified a number of Bmp and Bmp receptor genes that are expressed in the embryonic and early postnatal mouse lung. The patterns of expression can be highly dynamic. For example, during the pseudoglandular and canalicular stages, the expression of *Bmp4* is high in both the distal tips of the epithelium and in the mesenchymal stroma adjacent to the more proximal developing airways (Weaver et al., 2003). At this stage, *Bmp4* expression is upregulated in the mesenchyme by Sonic hedgehog and in the epithelium by FGFs (Bitgood and McMahon, 1995; Chuang and McMahon, 2003; Chuang et al., 2003; Hyatt et al., 2002; Weaver et al., 2000). Just before birth, *Bmp4* expression declines in the epithelium of the terminal saccules but is expressed in the endothelium of the capillaries. Less is known about the transcription of other *Bmp* genes in the developing lung. *Bmp7* is expressed predominantly throughout the endoderm at E11.5–13.5 but at lower levels in the epithelium and mesenchyme by E15 (Bellusci et al., 1996; Lyons et al., 1995; Takahashi and Ikeda, 1996). *Bmp5* is expressed in the mesenchyme from E10.5 through at least E16.5 (King et al., 1994), and lung abnormalities ("cysts") have been reported in both homozygous null and, to a lesser extent, heterozygotes on some genetic backgrounds (Green, 1968). *Bmp3* is expressed in the bronchiolar epithelium at midgestation (Takahashi and Ikeda, 1996). Null *Bmp3* mutants on a mixed genetic background have no lung phenotype, but on the C57Bl/6 inbred background abnormalities develop perinatally (Daluiski et al., 2001 and K. Lyons, personal communication). *Bmp6* was detected in the adult lung by both Northern blot (Ozkaynak et al., 1992) and protein analysis (Wall et al., 1993; Rosendahl et al., 2002), but no lung phenotype has been reported in null mutants. These dynamic expression patterns suggest that Bmps have the potential to act locally within the cell population in which they are made (autocrine signaling) or between two different populations (paracrine signaling).

All of the three Type I transmembrane receptors known to mediate signaling by Bmps (Bmpr1a/Alk3; Bmpr1b/Alk6; Acvr1/ActR-1/Alk2) are potentially involved in embryonic mouse lung development. *Bmpr1a* is strongly expressed in both the epithelial and mesenchymal populations. By contrast, *Bmpr1b* transcripts are highest in the proximal endoderm with much lower levels in the distal epithelium and mesenchyme (Dewulf et al., 1995 and Supplemental Figs. 1A–F). *Acvr1*, which can potentially mediate signaling from Bmp7, appears to be localized to the mesenchyme of the embryonic lung (Verschueren et al., 1995). Importantly, these differences in spatial localization imply that Bmpr1a is the major Type I receptor in the distal epithelium mediating signaling from Bmps, including Bmp4, produced either in the epithelium or the mesenchyme.

Current ideas about the role of Bmp signaling in the epithelium of the embryonic lung during the pseudoglandular and canalicular stages have focused on two distinct processes: cell proliferation and cell differentiation. One hypothesis is that Bmp4, together with Fgfs and Wnts, promotes the proliferation of the distal progenitor cells and helps maintain them in an undifferentiated, multipotent state (Weaver et al., 2000; Liu et al., 2003). This activity of Bmp4 would be analogous to its proposed function in maintaining the undifferentiated state of pluripotent mouse embryonic stem (ES) cells (Ying et al., 2003; Qi et al., 2004). Two lines of evidence support this model. First, adding Bmp4 to organ cultures of the whole embryonic lung promotes branching morphogenesis and increases the number of peripheral epithelial buds (Bragg et al., 2001). Second, transgenic expression of the Bmp antagonists, noggin or gremlin, in the epithelium leads to the generation of lungs that are smaller than normal and with bronchial epithelial cell types (ciliated and Clara cells) extending almost to the periphery (Lu et al., 2001; Weaver et al., 1999). This "proximalized" phenotype could result from the reduced proliferation of distal epithelial cells relative to bronchial cells and/or from a higher than normal probability of distal progenitors differentiating into proximal cell types. However, not all experimental data support this model. Significantly, transgenic overexpression of *Bmp4* in the epithelium also leads to smaller lungs and to a reduction in epithelial cell proliferation (Bellusci et al., 1996). Moreover, addition of exogenous Bmp4 protein to mesenchyme-free endoderm cultured in vitro with Fgfs inhibits proliferation, secondary budding and differentiation (Weaver et al., 2000; Hyatt et al., 2002, 2004). These findings suggest that Bmps function in a highly dose-dependent manner and/or elicit different responses through different pathways.

Faced with these complexities, we have turned for clarification to the technique of Cre-lox conditional gene deletion. We use a *cre* transgene that is expressed specifically in the epithelium of the developing lung to delete floxed alleles of *Bmpr1a* and *Bmp4*. In addition, we use in vitro culture of mesenchyme-free, *Bmpr1a*-deficient endoderm to ask whether Bmps made by this population of cells can function in an autocrine manner. Taken together, our results support a model in which Bmp4 made in the epithelium acts through Bmpr1a to promote the proliferation, survival and morphogenetic behavior of the distal endoderm.