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Gene expression pattern

Irx1 and *Irx2* expression in early lung development

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Abstract

We describe a comparative lung expression analysis of the murine *Irx1* and *Irx2* genes. At embryonic day 8.5 (E8.5), the *Irx1* and *Irx2* expression starts in the foregut region, where the laryngo-tracheal groove will form. The expression is prominent in the lung epithelium during glandular development. It declines at the end of the canalicular phase. We further compare the *Irx1* and *Irx2* expression domains to *Gli1*, *2*, *3* and *Mash1*. Their homologues in *Drosophila melanogaster* are known as regulative partners of the *iroquois* complex. The *Irx* and *Gli* genes are coexpressed in the developing lungs at the same time. Their transcripts are not localised in the same cells but adjacent to each other in either mesenchymal or epithelial structures. It is thought that the lung development is regulated by the mesenchymal/epithelial interactions.

Keywords: Mouse; Trachea; *Irx1*; *Irx2*; *iroquois*; *Gli1*; *Gli2*; *Gli3*; *Mash1*; Lung development; Lung epithelium; Lung buds; Glandular period; Canalicular period

1. Results

In early mammalian development, the endoderm gives rise to a number of derivatives, such as the lung, the liver and the pancreas. The lung buds develop from the foregut of the caudal pharyngeal region and consist of an epithelial layer of endoderm surrounded by mesoderm (Hogan and Yingling, 1998). During the glandular (up to embryonic day 10.0, E10.0) and canalicular stages of lung development (E10.0–E15.5) the buds grow out and branch in a highly stereotyped pattern to form two main bronchi, multiple bronchioles and terminal buds (Ten Have-Opbroek, 1991).

Herein, we describe the expression pattern of two members of the murine *iroquois* gene family, *Irx1* and *Irx2* (Bosse et al., 1997) during lung development. We found *Irx2* mRNA expression in the anterior foregut region to be starting at about E8.5 (Fig. 1B). This coincides with the first steps of laryngo-tracheal groove formation, which is followed by the appearance of the tracheal diverticulum (Kaufmann and Bard, 1999). The proximal part of the tracheal diverticulum will form the trachea and the distal end divides into two lung buds. At E10.5, *Irx2* expression is exclusively visible in the epithelium of the newly formed lung buds but not in the surrounding mesenchyme (Fig. 1D).

During the canalicular period, *Irx2* mRNA is still restricted to the epithelium. Fig. 2B,D,F shows *Irx2* expression in the lung buds starting from E11.5. The *Irx2* transcripts concentrate to the distal tips of the branching buds. However, while the two buds separate, the *Irx2* expression covers the entire epithelium of the separation zone. In the newly formed buds it is again focused on the distal tips (Fig. 2B,H). A striking change of *Irx2* expression occurs at E13.5 when the terminal bronchi form. In all five lobes of the lung, there is a dramatic decrease of the *Irx2* expression. Only single *Irx2* expressing buds at the margin of the lobes can be detected by whole mount staining (Fig. 2D,J). In radioactive in situ experiments, this late expression is confined to the epithelial layer (data not shown). *Irx2* expression is no longer visible in the lungs at the end of the canalicular period (E14.5).

Irx1 lung expression is very similar to that of *Irx2*. There are two main differences, however. At E8.5, *Irx1* expression in the foregut is much broader than that seen for *Irx2* (Fig. 1A). Later, *Irx1* transcripts, like *Irx2*, become confined to the prospective lung area and restricted to the epithelium (Fig. 2G,I). The *Irx1* gene activity is not found in the surrounding mesenchyme (Figs. 1C, 2G,I). Fig. 2A,C shows the *Irx1* expression in the canalicular period and monitors the branching into multiple bronchioles at E12.5 and E13.5. Another difference between *Irx1* and *Irx2* expression is the fact that *Irx1* is still prominent throughout the lung at E13.5 (Fig. 2C). One day later, the transcript is concentrated at the rim whereas the expression starts to decline in the centre of the five lobes (Fig. 2E). The activity

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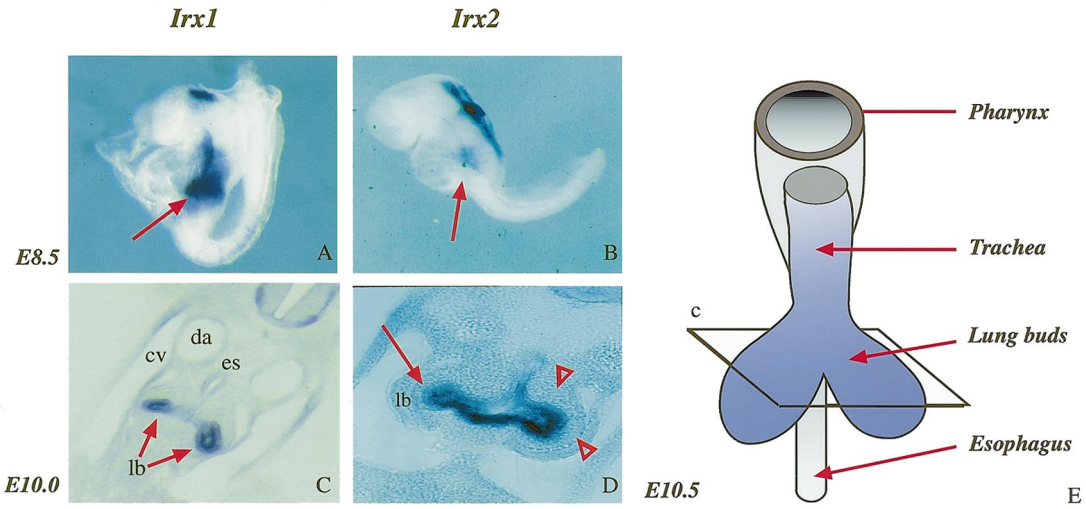

Fig. 1. Expression pattern of *Irx1* and *Irx2* at embryonic stages E8.5 and E10.0–E10.5; (A,B) Lateral view of whole mount stained embryos. (C,D) Vibratome sections of stained embryos. (E) Drawing of the mouse lung at the developmental stage E10.5 and indicating the plane of section of (C). (A) *Irx1* expression in the midbrain and wide spread in the foregut region (arrow). The centre of expression is dorsal to the developing heart at the side of lung induction. (B) *Irx2* expression in the CNS with the highest transcript concentration in the hindbrain. Weak but distinct expression in the foregut at the dorsal–caudal side of the heart (arrow). (C) Vibratome section (30 μm) of an *Irx1* stained embryo at E10.0. Plane of section is shown in (E). Specific expression is seen in the epithelium of the two main bronchi (arrow). (D) Frontal section (20 μm) of an *Irx2* stained embryo at E10.5. The transcript is located in the epithelium (arrow) and not in the surrounding mesenchyme (arrowhead). cv, cardial vein; da, dorsal aorta; es, oesophagus; lb, lung bud.

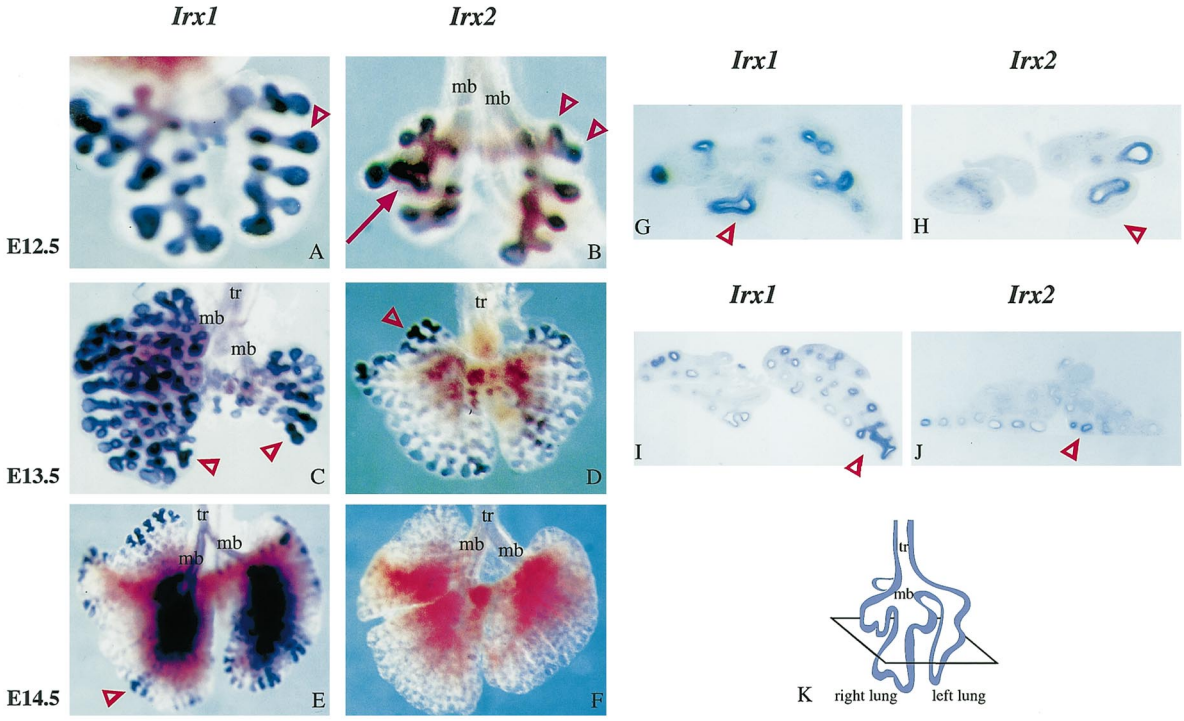

Fig. 2. Overlapping lung expression of *Irx1* and *Irx2* in the canalicular phase of lung development. Pictures (A–F) show dissected lungs of whole mount stained embryos, pictures (G–J) show cross-sections of 40 μm and in (K) the plane of section is indicated in the schematic drawing. (A,B) The segmental bronchi of embryonic stage E12.5 show a high concentration of the *Irx1* and *Irx2* transcripts in the distal tips (arrowhead) without any difference from the asymmetry of the lung. Expression of *Irx2* covers the complete separation zone of forming lung buds (arrow). (C,D) Strong expression of *Irx1* and *Irx2* in all lobes at E13.5. *Irx2* expression starts to decline in the centre of the lung bud but is prominent in the distal buds (arrowhead). (E, F) Expression of *Irx2* fades out at the end of the canalicular phase while *Irx1* is still present. (G) Expression of *Irx1* in the lung epithelium (arrow) at E12.5. (H) Expression of *Irx2* in the lung epithelium (arrow) at E12.5. (I) Expression of *Irx1* in the lung epithelium (arrow) at E13.5. (J) Expression of *Irx2* in the lung epithelium (arrow) at E13.5. mb, main bronchi; tr, trachea.

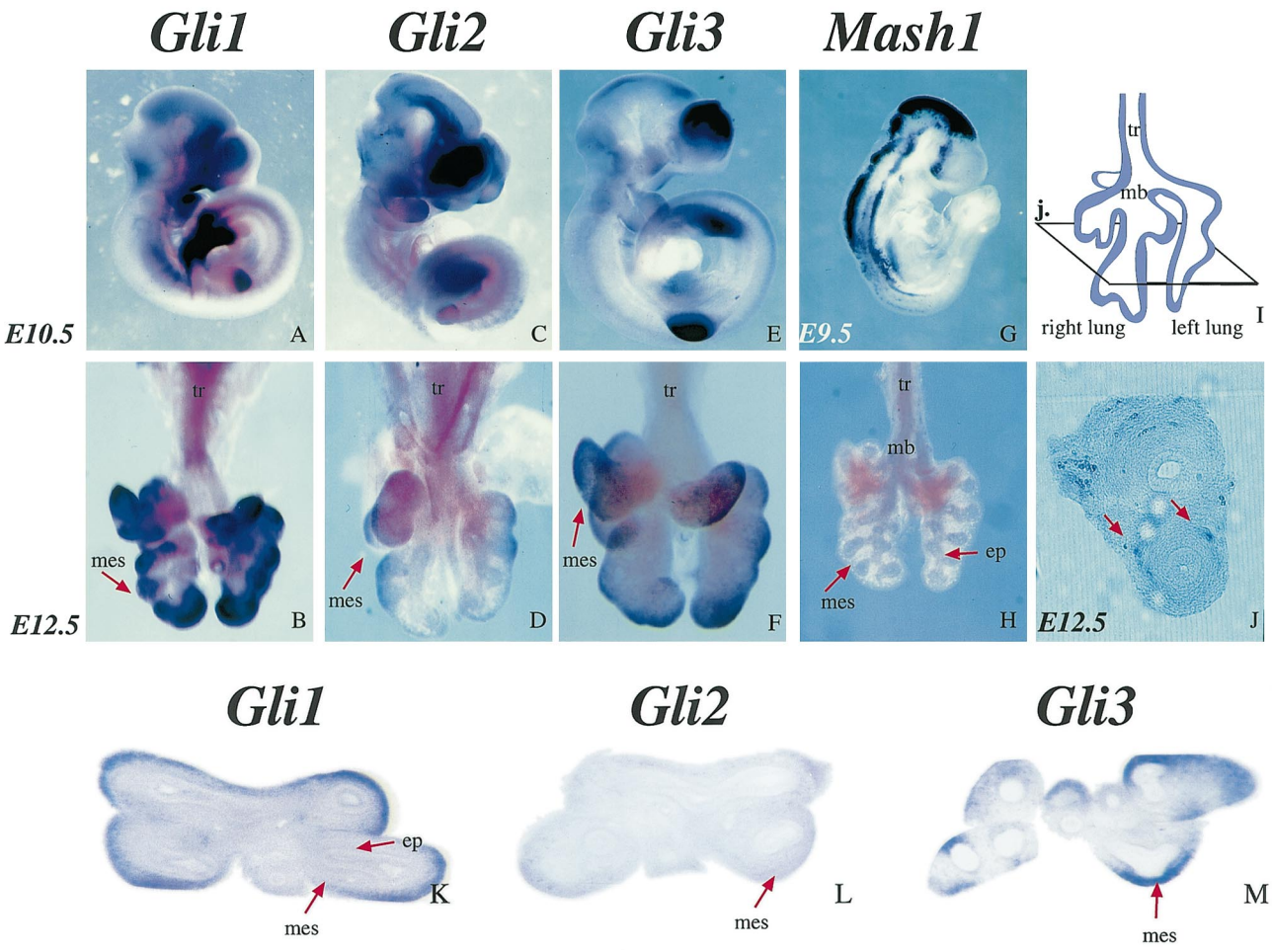

Fig. 3. Expression of the three *Gli* genes and *Mash1* in mice. (A,B) Overview of *Gli1* expression at E10.5 and in the dissected lung at E12.5. The *Gli1* transcript covers a broad mesenchymal area. It gradually declines from the tip to the centre of the lung. (C,D) Overview of *Gli2* expression at E10.5 and in the dissected lung at E12.5. In the mesenchyme, the *Gli2* transcript is distributed in a diffuse manner at the outer rim of the lobes. (E,F) Overview of *Gli3* expression at E10.5 and in the dissected lung at E12.5. The *Gli3* expression nicely represents the surrounding mesenchyme. (G,H) Overview of *Mash1* expression at E9.5 and in the dissected lung at E12.5. Due to the weak expression of *Mash1* in the lung the staining intensity cannot be compared to *Gli* or *Irx* expression. (I) Schematic drawing indicating the plane of the lung section shown in (J). (J) Vibratome cross-section (20 μm) shows single cells expressing *Mash1* in the lung at E12.5 (see arrows). (K) Gene expression is concentrated in the mesenchyme and not in the epithelium in the cross-section (40 μm) of a *Gli1* stained lung at E12.5. (L) Cross-section (40 μm) of a *Gli2* stained lung at E12.5. The weak expression is seen in the mesenchyme. (M) Cross-section (40 μm) of a *Gli3* stained lung. The strong expression is visible in the loose mesenchyme at E12.5. ep, epithelium; mb, main bronchi; mes, mesenchyme; tr, trachea.

of *Irx1* stops in the developing lung with the beginning of the alveolar phase.

Araucan, one of the prepattern genes of the *iroquois* complex, is regulated by *cubitus interuptus* (*ci*) and regulates itself the gene activity of the *achaete scute-complex* (*AS-C*) (Gomez-Skarmeta et al., 1996). The murine homologues of *ci* and *AS-C* are the *Gli* and *Mash* genes, respectively (Guillemot and Joyner, 1993; Grindley et al., 1997). Upstream as well as downstream factors in the *iroquois* regulation cascade in *Drosophila melanogaster* are expressed in the developing mouse lung. As shown in Fig. 3B,D,F,K,L,M all three *Gli* genes are expressed in the overlapping domains of the lung mesenchyme. At the same time, the *Irx1* and *Irx2* genes are active in the distal part of the epithelium (Figs. 1 and 2). The branching morphogenesis of the lung depends upon interactions between the epithelium and the mesoderm (Spooner and Wessells, 1970). *Mash1*-positive cells can be detected on cross-sections (Fig. 3J). They are located in the proximity of the *iroquois*-positive epithelium and belong to pulmonary neuroendocrine cells and their precursors (Ito et al., 2000).

In the same space of time, *Irx* and *Gli* genes are coexpressed in the developing lungs in adjacent tissues, whereas *Mash1* transcripts can be found at the same time in the developing lungs but solely in single cells.

2. Materials and methods

2.1. In situ hybridization

In situ hybridizations were performed on mouse embryos

or lungs from E8.5 to E14.5. Embryos were collected by time matings of NMRI mice, fixed 4 h to overnight in 4% paraformaldehyde/PBS. The lungs were dissected from dehydrated embryos. Whole mount in situ hybridizations were done using digoxygenin labelled UTP and NBT/BCIP as a substrate for alkaline phosphatase activity according to Wilkinson (1992). Probes used were *Irx1* and *Irx2* (Bosse et al., 1997), *Gli1* (A.L. Joyner), *Gli2* (A.L. Joyner), *Gli3* (U. Rüther) and *Mash1* (F. Guillemot).

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