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Gene expression pattern

Endoglin expression in early development is associated with vasculogenesis and angiogenesis

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Abstract

Endoglin is an auxiliary receptor for the transforming growth factor-β family of cytokines and is required for angiogenesis and heart development. Endoglin expression during mouse embryogenesis was analysed by monitoring β-galactosidase expression from a *lacZ* reporter cassette inserted downstream of the endoglin promoter. Expression was first detected at 6.5 days post-coitum (dpc) in the amniotic fold and developing allantois. Between 7.5 and 8.5 dpc, endoglin was expressed in endothelial cells of the yolk sac, dorsal aorta and primitive heart tube, and from 9.5 to 13.5 dpc in endothelial cells throughout the developing vasculature. Interestingly, this pattern of endoglin expression is almost identical to that reported for Alk1.

Keywords: Endoglin; Alk1; Transforming growth factor-β; Vasculogenesis; Angiogenesis; Vascular endothelium; Heart development; Hereditary haemorrhagic telangiectasia

The transforming growth factor-β (TGFβ) family of cytokines regulates a wide range of cellular processes, including proliferation, differentiation, apoptosis and migration. Endoglin is an auxiliary receptor for this family of cytokines and has been shown in vitro to interact with type II receptors for both TGFβ and activin (Barbara et al., 1999). Mice lacking endoglin have a similar phenotype to mice lacking either TGFβ1, or one of the TGFβ receptors (Alk1, Alk5 or TBRII), which all die at mid-gestation from defects in yolk sac vascular development. This suggests that endoglin is involved in TGFβ signalling during developmental angiogenesis (Dickson et al., 1995; Li et al., 1999; Arthur et al., 2000; Oshima et al., 1996). In humans, mutations in the endoglin gene are associated with the autosomal dominant disease, hereditary haemorrhagic telangiectasia type 1 (HHT1), which is characterised by vascular malformations (McAllister et al., 1994). Mutations in Alk1, a TGFβ type I receptor, are associated with the related disease HHT2 (Johnson et al., 1996).

1. Results and discussion

Expression from the endoglin promoter was first detected at 6.5 days post-coitum (dpc) in extraembryonic ectoderm in the region of the future amniotic fold (Fig. 1A). At 7 dpc, endoglin is expressed in the amnion (Fig. 1B), and at 7.5 dpc, expression is seen in the allantois and the cardiogenic plate (Fig. 1C,D). Expression in the primitive endothelial cells of the yolk sac can be seen from 8.5 dpc (Fig. 2). During early organogenesis at 9.5 dpc, endoglin expression is found in endothelial cells throughout the developing vasculature and is particularly strong in the endocardium, but entirely absent from the myocardium. High levels of expression can be seen in the capillary plexus surrounding the forebrain, midbrain and hindbrain, but not in the neural epithelium itself. A similar expression pattern is seen in endothelial precursors of the hyaloid vessels surrounding the optic vesicle. In the trunk region of the embryo, endoglin is expressed in the endothelium of the developing arteries, veins and associated capillary networks (Fig. 2). Between 12.5 and 13.5 dpc, endoglin is expressed throughout the vascular endothelium, irrespective of whether it is derived by angiogenesis (e.g. within neural tissue) or vasculogenesis (e.g. in the perineural vascular plexus). It is abundant in the endothelial cells of the choroid plexus in the brain, and in the developing vascular endothelium surrounding the spinal cord and dorsal root ganglion, but absent in epithelial cells. Endoglin is expressed strongly in the endocardium, the vascular components of the liver, lung and gut, as well as in the differentiating mesenchyme of the developing limb, tooth

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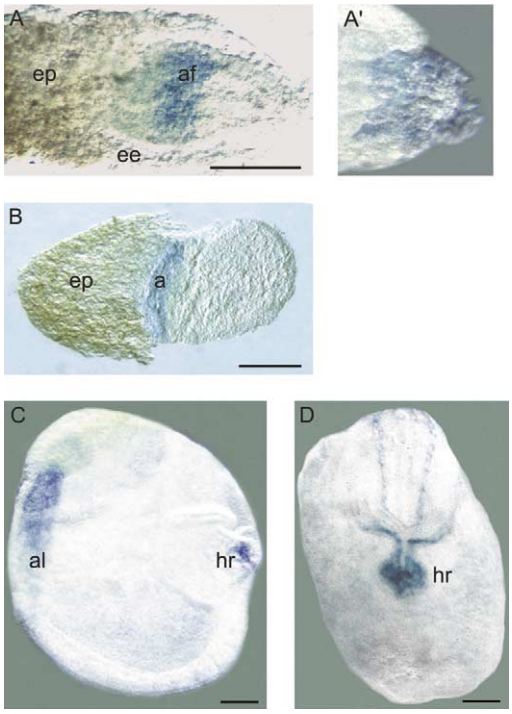

Fig. 1. Expression of endoglin in early embryogenesis. Expression is detected from age 6.5 dpc in extraembryonic ectoderm in the region of the future amniotic fold (A), and can be seen in an embryo dissected to reveal this region (A′). (B) At 7 dpc, endoglin is expressed in the amnion. (C,D) At 7.5 dpc, expression is seen in the allantois and precardiogenic mesodermal cells. a, amnion; af, region of future amniotic fold; al, allantois; ee, extraembryonic endoderm; ep, ectoplacental cone; hr, heart-forming region. Scale bar, 100 μm.

bud and ear (Fig. 3). Although endoglin is expressed strongly throughout the vascular endothelium at 9.5 dpc, it is clear that by 12.5 dpc, expression is more variable and is strongest in capillaries, weakest in veins and intermediate in arteries (Fig. 3). Interestingly, the pattern of endoglin expression from 6.5 to 13.5 dpc is strikingly similar to that of Alk-1 (Roelen et al., 1997), in agreement with the involvement of both genes in the same signalling pathway. There is also considerable overlap between endoglin expression and TGFβ1 and TBRII expression in mesenchyme associated with angiogenesis and regions of epithelial–mesenchymal interaction (Lawler et al., 1994; Akhurst et al., 1990), whilst there is much less overlap with Alk5, TGFβ2 and 3, or ActivinRIIB expression (Iseki et al., 1995; Mariano et al., 1998; Schmid et al., 1991; Manova et al., 1995). The expression pattern of endoglin is consistent with a role in TGFβ1 signalling throughout vasculogenesis, angiogenesis and heart development.

2. Experimental procedures

Embryos were heterozygous for a mutation in the endoglin gene which included the insertion of a *lacZ* reporter cassette downstream of the endoglin promoter as previously described (Arthur et al., 2000). Endoglin expression was detected using XGAL in mice of the NIH strain, which exhibited no vascular defects when a single copy of the endoglin mutation was present.

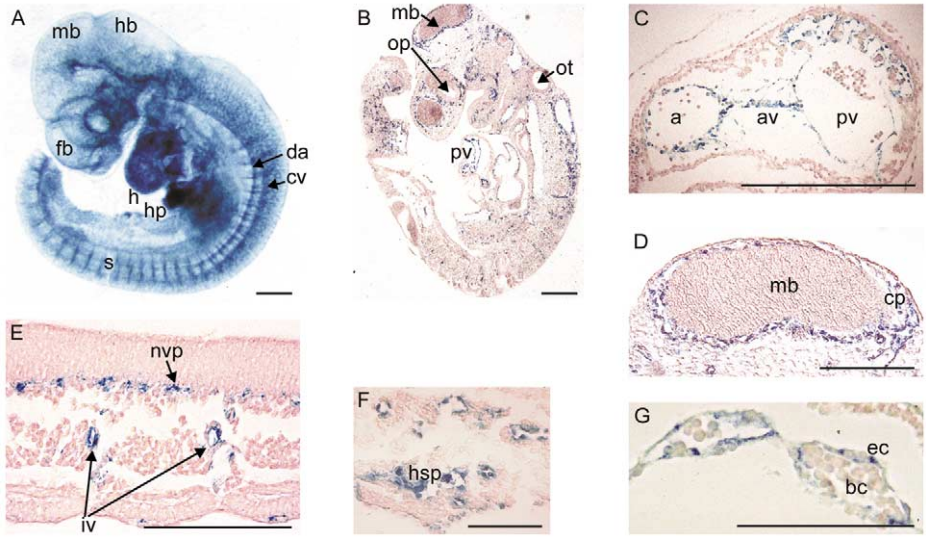

Fig. 2. Endoglin expression in early organogenesis. At 9.5 dpc, endoglin is expressed throughout the developing vasculature seen in: (A), whole-mount; and (B), sagittal sections. (C) It is strongest in endocardium of the atrioventricular canal, the ventricle and atrium of the primitive heart. Expression is seen in the endothelial cells of: (D), meningeal capillary plexus; (E), intersomitic vessels; and (F), hepatic primordia. (G) Expression in the endothelial cells of the yolk sac can be seen from 8.5 dpc. a, atrial chamber; av, atrioventricular canal; bc, primitive red blood cells; cp, capillary plexus; cv, cardinal vein; da, dorsal aorta; ec; endothelial cells; fb, forebrain; h, heart; hb, hindbrain, hp hepatic primordium; hsp, hepatic sinusoid primordia; iv, intersomitic vessels; mb, midbrain; nvp, neural vascular plexus; op, optic vesicle; ot, otic vesicle; pv, primitive ventricle; s, somite. Sections (B–D) are sagittal, whilst (E–G) are transverse. Scale bar, 250 μm for (A–D); and 100 μm for (E–G).

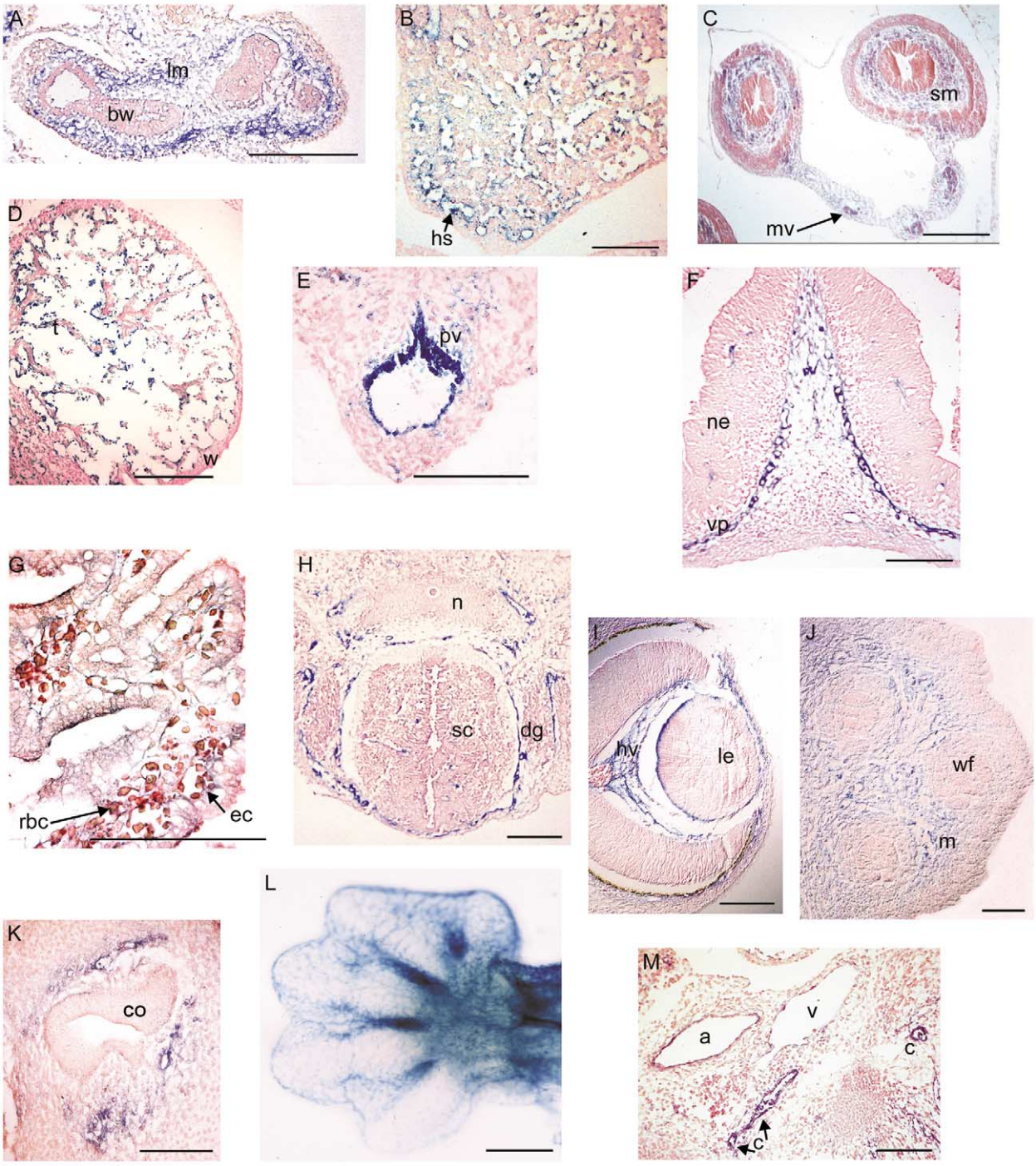

Fig. 3. Endoglin expression during organogenesis from 12.5 to 13.5 dpc. Endoglin expression can be seen in the mesenchymal cells undergoing vasculogenesis around the primitive lung bronchi (A) and in the endothelial lining of the hepatic sinusoids (B). Endoglin is expressed in developing submucosal and mesenteric vasculature of the gut (C); in the endocardium of the trabeculated left ventricle (D); and in the heart valves, for example, the pulmonary valve (E). Expression is also high in the meningeal capillaries, e.g. surrounding the choroidal fissure (F), as well as within the choroid plexus (G), and vessels around the spinal cord (H). The hyaloid vessels of the eye, including fine branches intimately associated with the lens epithelium (I), and the differentiating mesenchyme of the whisker follicles (J) and ear (K) also express endoglin. (L) The vascularised interdigital region of the paw shows strong endoglin expression. (M) Endoglin expression is strongest in capillaries, intermediate in arteries and weakest in veins. a, artery; bc, blood cells; bw, bronchial wall; c, capillaries; co, cochlea; dg, dorsal root ganglion; ec, endothelial cells; hs, hepatic sinusoids; hv, hyaloid vessels; le, lens; lm, lung mesenchyme; m, mesenchyme; mv, mesenteric vessels; n, notochord; ne, neural epithelium; pv, pulmonary valve; rbc, red blood cell; sc, spinal cord; sm, submucosal layer; t, trabeculae; v, vein; vp, vascular plexus; w, wall of left ventricle; wf, whisker follicle. (A), (B), (D–F), (H), (L) and (M) are E12.5 embryos, whilst (C), (G) and (I–K) are E13.5 embryos. All are transverse sections, except: (J), sagittal; and (L), whole-mount. Scale bar, 250 μm, except for (G) where it is 100 μm.

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