A study of the forkhead transcription factors Foxf1 and Foxf2 in mouse embryogenesis

Jeanette Astorga

Department of Cell and Molecular Biology, Göteborg University, Box 462, SE-405 30 Göteborg, Sweden

ABSTRACT

The subject of this thesis is analysis of targeted mouse mutants for two forkhead transcription factors, Foxf1 and Foxf2, in order to understand their roles in embryogenesis. *FoxF* genes are expressed in lateral and extraembryonic mesoderm, starting at gastrulation, and later in derivatives of the splanchnic mesoderm. *Foxf1* dominates during early stages of development. *Foxf2* is widely expressed in neural crest cells, whereas *Foxf1* has a restricted expression in cranial neural crest. *Foxf1* null mutants are completely avascular in extraembryonic structures and die at mid-gestation. Hedgehog (Hh) proteins (Sonic and Indian) secreted by the endoderm are required for vasculogenesis in the yolk sac mesoderm. *Foxf1* was shown to be a key target gene for the vasculogenic activity of Hh. The murine allantois lacks endoderm, and in this tissue vasculogenesis was shown to be independent of Hh, but to still require Foxf1. Bone morphogenetic protein 4 (Bmp4) was shown to be a target of Foxf1 that induces formation of a vascular plexus from mesodermal progenitor cells. Inhibition of Bmp signaling blocked vasculogenesis, whereas exogenous Bmp4 rescued the avascular phenotypes of $Foxf1^{-/-}$ allantois, as well as of $Smo^{-/-}$ yolk sac, in which Hh signaling is inactivated. Both *FoxF* genes are important for gut development and exhibit non-allelic non-complementation: $Foxf2^{-/-}$ and $Foxf1^{-/+}$; $Foxf2^{-/+}$ compound heterozygotes die at birth with similar, but non-identical, intestinal defects. Malformations included megacolon due to lack of innervation of the posterior gut (Hirschsprung's disease); muscle layer hypoplasia; luminal obstruction; and tissue disintegration. Tissue dissociation was shown to result from a deficiency of extracellular matrix collagens, owing to a cell autonomous dependence on FoxF proteins for collagen production by intestinal fibroblasts. Resistance to apoptosis and persistent proliferation in epithelial cells of the villi suggested ectopic activation of the Wnt/β-catenin/Tcf pathway, which was confirmed by demonstrating constitutive nuclear localization of epithelial β-catenin and mesenchymal overexpression of *Wnt5a* in *FoxF* mutants. Expression of *Bmp4* was reduced in the mutants and explant cultures demonstrated an inverse relationship between Bmp signaling and *Wnt5a* expression. *FoxF* genes have non-overlapping expression patterns in cranial neural crest cells of the developing maxilla, with *Foxf1* in dental mesenchyme and *Foxf2* expressed in the palatal shelves. Foxf2 mutants are born with a cleft palate, which was shown to result from reduced proliferation and collagen content in the palatal mesenchyme. TGFβ is a mesenchymal mitogen and an activator of collagen synthesis during palatogenesis. A substantial reduction in the level of phosphorylated Smad2/3 in $Foxf2^{-/-}$ palatal shelves indicated that decreased TGFβ signaling is responsible for the cleft palate malformation.

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