

# **Mechanisms of blood vessels development in the branchial arches region**

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## **Abstract**

### **Aortic arches development in the course of Pelobates fuscus metamorphosis**

Larval development of Spade-foot Toad *Pelobates fuscus* (Amphibia, Anura) is modified compared to tetrapod ancestors. However, it is still possible from its metamorphosis to reconstruct changes, which have occurred during the transition of vertebrates onto the dry land. Transformations of aortic arches during the metamorphosis have been studied by the corrosion casting of the vascular system with Mercox. Corrosion casts were subsequently studied with a scanning electron microscope.

Aim of this study is to compare development of aortic arches of *Pelobates fuscus* with other anuran species and contribute to the findings about the transformation of the aortic arches during the transition of the vertebrates onto the dry land.

Aortic arches, which in larval period supply the gills, are considerably transformed due to the reduction of the gills and transition on the breathing of atmospheric oxygen. Aortic arches are in *Pelobates* suddenly transformed after a long period of gradual preparation and reduction of gill blood vessels. Among main changes occurring during the metamorphosis, belong disappearance of ductus Botalli, which cause the direct shunting of the blood to the lungs and skin. Further ductus caroticus is reduced. Also the fifth aortic arch disappears as it loses its function and yields to the developing breathing muscle of the adult frog. The fourth aortic arch becomes the main blood stem for the body, as it is the only arch not losing connection to the dorsal aorta. Lungs of *Pelobates* are well developed even in stages when they do not participate on the air breathing.

It is probable that vertebrates were already preadapted to the transition onto the dry land and have i.e. lungs well developed. Therefore we assume that the final transformation occurred relatively fast as in the course of *Pelobates* metamorphosis. It is supposed that the branching pattern of the aortic arches of permanently water-dwelling piscine ancestors and of primitive tetrapods capable of spending longer periods on land, had been similar as in the *Pelobates* premetamorphic or metamorphosing larvae. Their ductus caroticus and ductus Botalli were not interrupted, the fifth aortic arch was still complete and these structures have reduced after the longer period spends on the dry land.

### **Influence of Sonic hedgehog (Shh) onto the blood vessel development in the branchial arches region**

Branchial arches region and its blood vessels are extensively transformed in the embryonic development. Aim of this study is to investigate mechanisms of the branchial arches region development and to study which way a morphogen Sonic hedgehog (Shh) participate on the formation and remodeling of branchial arches and their blood vessels.

Influence of Shh was evaluated based on the changes caused by its inhibition *in vivo*. Shh function was inhibited with an anti-Shh antibody, which was produced to the embryo from the applied hybridoma cells. Shh signaling cascade was also inhibited by cyclopamine.

Results show that Shh is important for *de novo* formation of the blood vessels in the branchial arches region. Further Shh is necessary for stabilization of the vessel wall, mainly for anterior cardinal vein. Shh also affects later vessel development and transformation, which includes i.e. fusion of the dorsal aorta, branching of the internal carotid artery and outflow tract development. Short time inhibition of Shh has minor effect on the apoptosis and proliferation activity of the branchial arches region mesenchymal cells.

We assume that Shh signals directly to the blood vessels endothelial cells, as Shh receptor *ptc1* is also expressed in endothelial cells and its signal is reduced with Shh inhibition. In studied stages Shh does not affect distribution of the smooth muscle cells in the vessel wall or the expression of the vascular endothelial growth factor receptor (VEGFR2). Morphogen Shh is therefore one of the important factors, which affect not only formation of luminized vessels, but also their stability and remodeling in the region of branchial arches.