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In vivo analysis of morphogenesis of choroid plexus in transgenic zebrafish

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Background

The choroid plexus is a specialized structure for production of cerebrospinal fluid (CSF). Its development has been studied mainly by traditional histological techniques. In result our understanding of this process lacks a continuity and accuracy of the *in vivo* observations.

Materials and methods

We generated a range of random transgenics by means of enhancer trapping using the *Tol2* transposon that carries the *EGFP* gene controlled by a partial *keratin8* promoter. It inserts randomly and reveals activity of enhancers in tissue-specific manner in stable transgenic lines. (Parinov et al., 2004; Choo et al.2006). One of these lines, ET33 expressed GFP in the roof plate, dorsal interneurons and meninx. The injection of the transposase mRNA into ET33 embryos resulted in transposition of Tol2 in germ cells and many novel transgenic lines including ET33E20 ("Gateways") were generated. Gateways embryos demonstrate the GFP expression pattern mainly restricted to the brain and spinal cord, similar to that of several genes located close to the insertion site that we cloned in an attempt to define which genes expression is recapitulated by GFP expression in Gateways. Interestingly, the early GFP expression is localized to sites, where several cranial blood vessels develop later on. GFP also appears in the roof plate and in groups of cells at the midline in contact with brain ventricles, including the choroid plexus. Using the enhancer trap lines we studied the morphogenesis of the choroid plexus of IV ventricle using a combination of histology, immunohistochemistry and *in vivo* analysis during normal development.

Results

The time-lapse movies of choroid plexus formation in Gateways for the first time illustrated this process *in vivo* in detail. Based on this we defined three phases of choroid plexus morphogenesis. Our comparative *in vivo* analysis of mutants affecting major developmental signaling pathways, including Notch and Hedgehog at the Gateways background revealed a role of these pathways during formation of the choroid plexus.

Conclusion

The transgenic embryos of zebrafish represent living markers that allow studying details of brain morphogenesis *in vivo*. These transgenics expand possibilities to monitor *in vivo* effect of genetic, toxicological and other experiments performed on embryos of vertebrates.