CRE EXPRESSION IN THE NODE AND NOTOCHORD IN TRANSGENIC MICE DRIVEN BY FOXA2 MINIMAL NOTOCHORD ELEMENT
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Abstract
Introduction
Gene targeting to cause gene inactivation by homologous recombination in embryonic stem (ES) cells has yielded remarkable advances in understanding specific gene function in mammalian development. However, embryonic lethality which results from a mutation of a gene encoding an important gene for early embryogenesis is an obstacle to studies of gene function in later stages of development. To circumvent this limitation, a number of strategies have been developed in order to inactivate a gene in a temporal- and/or spatial-specific manner (Lewandoski, 2001). The Cre/loxP system is one of the conventional strategies facilitating excision of DNA sequences located between two loxP sites by the Cre recombinase of Bacteriophage P1. Therefore, generation of different tissue-specific Cre mice, which can be used for conditional gene inactivation in various tissues in mice, is a very useful tool for studying gene function in different tissues in mice.

Foxa2 is a winged-helix transcription factor, which is essential for development of the node and the notochord. In mouse, Foxa2 expression can be found in the node, notochord, floor plate and gut epithelium. The enhancer element responsible for Foxa2 expression in the node and notochord has previously been localized to a 520bp fragment, the minimal notochord element (mNE). We have utilised this mNE of Foxa2 to generate transgenic mice expressing the Cre recombinase (Cre) gene in the node and notochord. Here we report the generation and expression analysis of these mNE-Cre transgenic mice. The recombinase activity was examined by crossing mNE-Cre mice with the reporter strain, Z/EG. Enhanced green fluorescent protein (EGFP) signal was detected in the node and the notochord from E8.0 and continued until E9.5. Immunostaining carried out on Cre/Z/EG compound heterozygous embryo sections showed that the expression of the EGFP signal was specific in the notochord. No recombination was observed outside the node and notochord. The mNE-Cre transgenic mice provide an important tool for conditional gene manipulation in the node and the notochord.

Method

The 10.8 kb gene construct was isolated by restriction enzyme digestion with NotI and SalI) and purified from vector sequences. It was then microinjected into the pronuclei of fertilized C57BL/6 x CBA F1 hybrid eggs to generated transgenic mice. mNE-Cre founder mice were identified by polymerase chain reaction (PCR) performed on tail biopsies using Cre-specific primers 5'-GGACATGTTCAGGGATCGCCAGGCG-3' and 5' -GCATAACCGTAGAACACATGCTTG- that amplify a 268bp fragment.

Transgenic mNE-Cre founders were crossed with Z/EG homozygous reporter mice. Embryos were harvested at different stages (E8.0 to E9.5). The yolk sac of the embryos were digested by proteinase K and the DNA extracted from it was used for genotyping.

Results

To direct the expression of Cre recombinase (Cre) to the developing notochord, we have generated transgenic mice expressing a mNE-Cre gene construct in which the Cre gene was placed under the control of the mNE and linked to an IRES-lacZ reporter. Five mNE-Cre independent transgenic founder lines were generated. Two founders transmitted the transgene through the germline. Due to the presence of the lacZ reporter gene in the construct, the expression pattern of the transgene in the transgenic mouse can be detected by breeding the mNE-Cre transgenic males with wild type mice. Embryos were collected at various time points (from E7.5 to E9.5) and stained for β-galactosidase (β-gal) activity. The β-gal staining results demonstrated that the mNE-Cre transgene was expressed from E7.5 to E9.5 specifically in the notochord.

Discussion

The Cre activity was detected at E7.5 and E8.0 as shown by the lacZ staining. However, no EGFP signal could be detected at these two stages (data not shown). This may be due to the low expression level of mNE-Cre at E7.5 and E8.0, resulting in an EGFP signal below the limitations of detection. In addition, there may be a time-lag between the expression of Cre and the subsequent recombination and activation of GFP expression in the Cre/Z/EG heterozygotes. Therefore, even though mNE-Cre is expressed in the node and the notochord, GFP signal cannot be detected under the microscope and failed to be captured by digital camera. Immunostaining on E9.5 embryo sections using anti-GFP antibody confirmed that the mNE-Cre expression mouse lines have notochord specific expression.

In conclusion, we have shown that the mNE-Cre transgenic mice described here are a very useful resource for conditional gene manipulation in the notochord of mice.