Isolation and RT-PCR-based characterization of the caudal neural plate in the chick embryo

Abstract

Objective and Aim:

During vertebrate neurogenesis, the caudal neural plate (CNP)—consisting of a stem zone—remains open. It adopts with the extension of the body to form the developing spinal cord. In the caudal¬ most part of the stem zone, the cell population has a dual neural/mesenchymal fate, depending on whether they move to the cranial part of the stem zone or gastrulate to form the underlying presomitic mesoderm. Due to FGF signaling in presomitic mesoderm, the cells residing in the CNP are in a proliferative state. When the cells migrate cranially to the closing part of the neural tube, they differentiate into neurons. Here, they are exposed to retinoic acid (RA), secreted from the somites. The aim of this study was to characterization of the caudal neural plate and evaluation of their cells after isolation.

Methods

The hen' s fertilized eggs were incubated for about 28-33h at 35-37° c and 50-60% humidity until the embryos reached to stages 8-9 according to Hamburger-hamilton developmental stage table.Then the embryos were removed from egg' s yolk and the caudal neural plate was isolated and subjected to PCR to study their gene expression profile. For the primary culture, the CNP explants were transferred into 24¬ well culture dishes and cultured up to 11 days in DMEM/F12+Glutamax +10%FBS + 1%NEAA+1%Pen/Strep medium.

Results

The caudal neural plate cells proliferated in culture condition and acquired the neural phenotype in the culture condition.They also expressed markers including Sox2, Delta1 and Brachyury, Pax3, BMP4 by RT-PCR method.

Conclusion

The CNP explant¬ derived cells showed a neural progenitor/mesenchymal fate by expressing Sox2, Delta1 and Brachyury genes**.**They can acquire neural phenotype and identity when separated from the underlying mesoderm and cultured in vitro.

Key Word*:* caudal neural plate, Chick embryo, Neural tube