## MEMBERS OF THE FIBROBLAST GROWTH FACTORS INDUCE ECTOPIC NEURAL PLATE FORMATION IN CULTURED CHICK EMBRYOS

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Neural cells that will form the neural plate arise from the epiblast cell layer under the influence of signals produced by organizer centers such as the blastopore dorsal lip in amphibia and its counterpart in amniotes that is the Hensen´s Node (HN). Experimental evidence for such affirmation comes mainly from classical manipulation experiments in which the dorsal lip or the HN is transplanted to ectopic places and a new neural axis is induced in the host embryo (Spemann and Mangold, 1924; Waddington, 1932). Nevertheless, the molecular mechanism by which signals produced by the HN produce neural induction is still poorly understood. Several putative molecules have been proposed as candidates for such neural induction, including transcription factors expressed in the HN and secreted molecules that can diffuse along the embryo to induce the formation of the neural plate. Recently, it has been shown that several members of the fibroblast growth factors (FGFs) family are able to induce neural cells when assayed in Xenopus animal caps cultures that otherwise would form mesoderm cells (Kengaku and Okamoto, 1995; Lamb and Harland, 1995 ) suggesting that FGFs can participate in normal neural induction. Using chick embryo cultures (New, 1955) and targeted over-expression with heparin acrylic beads as described by Cohn et al., 1995, we are currently studying the neural inducing ability of this family of growth factors and their role in the antero-posterior patterning of the nervous system.

METHODS

For targeted expression of FGFs, chick embryos at early neurulating stage of development (stage 4 of Hamburger and Hamilton, 1951) were prepared for New culture as described previously (Alvarez and Schoenwolf, 1991). Heparin acrylic beads (Sigma) were soaked with FGF-2 or FGF-4 (R&D Systems) during two hours and placed in the periphery of the embryo. After the implantation the embryos were allowed to develop for 24 hours and then recorded and fixed. Alternatively, beads soaked with PBS implanted in the same positions were used as controls to ensure that the bead itself do not produce major alterations in development and that the effect obtained is specific of the FGF used. After fixation embryos were processed for immunohistochemistry or in situ hybridization for several mesodermal and neural markers according to techniques used routinely in our laboratory.

RESULTS

FGFs released for the bead were able to induce ectopic tissues derived from the epiblast cell layer that protrude dorsally and that resemble small neural tubes (Fig. 1). This ectopic tissue resembles neuroepithelium in histological sections and it is positive for several neural markers (not shown). This result shows that FGFs transdeterminate still uncommitted epiblast cells from the normal epidermal fate to neural fate, probing that FGF can induce new neural cells. To ascertain whether this neural induction is mediated by newly formed axial mesoderm or FGF is acting directly over epiblast cells, we conducted experiments with FGF soaked beads and analyzed the expression pattern for several axial mesodermal markers after 24 hours of development, including not-1 a high specific marker for notochordal cells. After the application of FGF to the chick embryo ectopic tissue developed near the bead, but there are not new notochordal cells near the new neural plate formed (Fig. 1). These results were confirmed with other mesodermal markers, including Sonic hedgehog (data not shown).

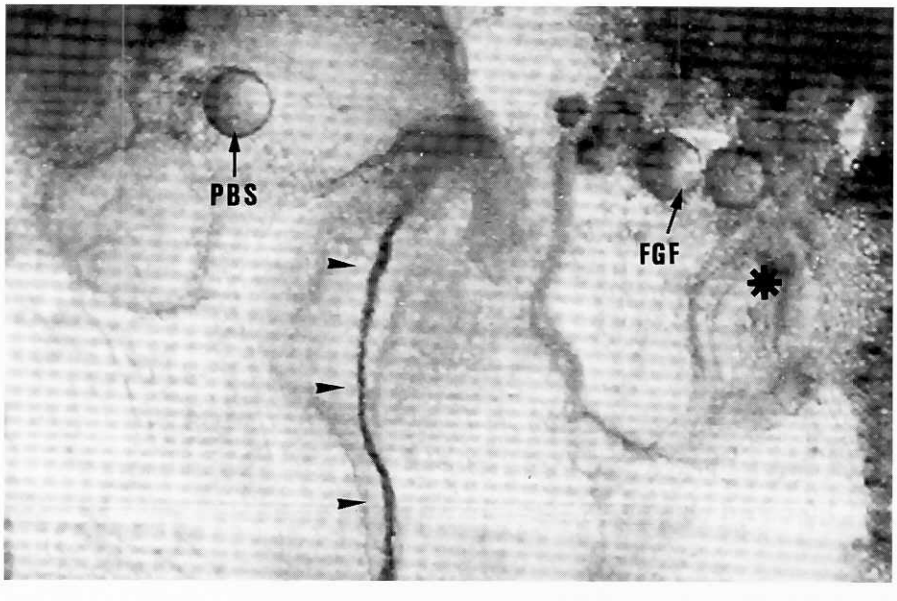


Figure 1. Dorsal view of a chick embryo treated with two FGF-2 soaked beads on the right side and a PBS soaked bead in the left side at Stage 4. After 24 hours in culture the embryo developed an almost normal neural tube. On the side treated with FGF an ectopic small neural tube-like structure developed (asterisk). The embryo was processed for the detection of not-1, a specific marker for notochord cells. The embryo shows in the midline a normal expression of not-1 corresponding to normal notochord (arrowheads). No positive cells for not-1 were found on the experimental side underneath of the ectopic neural tissue or either on the control side treated with a PBS soaked bead.

We conclude that FGF is able to induce new neural plate cells in early chick embryos in our experimental conditions. Moreover the mechanisms of induction by FGF is not mediated by mesoderm and it is different of the mechanism of induction mediated by the transplantation of the HN to ectopic places. In the former experimental system the formation of extra neural tissue always involve the induction of new axial mesoderm cells. Therefore FGFs can change the fate of epiblast cells to neural cell without involvement of mesoderm cells. This capability of transdetermination by FGF can be expanded to other cells including neuroepithelial cells that change the positional information as viewed by several markers (not shown) and mesoderm and endoderm cells that develop extra hearts (not shown). We are currently studying how this neural induction is carried out.

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