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Short Report

Pattern of cell proliferation and cell death in early chick embryos and their control by growth factors

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ABSTRACT The mechanisms underlying neural and epithelial specification in the blastoderm are under constant study. Growth factors of several kinds participate in this specification process but also they affect the proliferation of blastoderm cells and apoptosis. In this work we analyze the distribution of cell death and the pattern of cell proliferation in early chick embryos and evaluated how these cellular processes are governed by growth factors.

Introduction

Fibroblast growth factors (FGFs) and the bone morphogenetic proteins (BMPs) are molecules that participate in the early steps of the formation of the neural and epithelial tissues. FGF signaling is required for the formation of neural cells (Wilson *et al.,* 2000; Streit *et al.,* 2000) and the application of an ectopic source of FGF in epithelial committed tissue elicis the formation of the neural plate (Alvarez *et al.,* 1998; Storey *et al.,* 1998). On the other hand, FGFs are also very well known proliferative molecules in a great variety of cells and tissues. Despite this dual function in development (neural inducers and mitotic molecules), no relationship between the two processes has been established. BMPs are molecules that function in the early embryo as epithelial inducers and in this way avoid other cells in the blastoderm becoming neural (Sasai and De Robertis, 1997). In the same way as the FGFs, besides their participation in differentiation processes, BMPs are involved in the proliferation of cells and tissues and also in the activation of the process of cell death (Hogan, 1996). There is little data available about the location and number of cells that suffer cell death at the stages when neural induction is taking place, although this mechanism occurs in the blastoderm in this period of development (Sanders *et al.,* 1997). This lack of information in the pattern of cell death impairs the knowledge of its role in the early formation of the neural plate. Cell proliferation has not been determined in early chick embryos, neither the proliferative characteristics of the dividing cells nor the possible existence of proliferative regions and/or centers in the blastoderm. Here we analyze the distribution of cell death and cell proliferation in the initiation of the neural induction and the control of these processes by members of the FGF and BMP family of growth factors.

Material and Methods

Chick embryos were obtained routinely and prepared for detection of cell death using the TUNEL technique with a kit for the immunohistochemical detection of DNA fragmentation (Roche). For that, PFA fixed embryos were washed in PBT before the TUNEL reaction mixture was performed at 37ºC for 3 h with shaking. Once stopped and washed, embryos were blocked with 4% Normal Goal Serum (Sigma) in PBT for 1 h and then incubated with anti-fluorescein antibody (Roche) conjugated with peroxidase and cell death was detected with DAB. To detect the proliferation, a hole in the culture media was made and filled with BrdU solution. After 2-8 h embryos were processed according to the manufacture's protocol (Roche) and the incorporation of BrdU detected by binding to Anti-BrdU antibody (Sigma) conjugated with peroxidase in a similar protocol to that described to the TUNEL technique. Growth factors were applied with acrylic beads soaked with recombinant proteins as described in Alvarez *et al.,* 1998. We used BMP4 from R&D systems at concentrations ranging between 0.1 to 1 mg/ml, and one or two beads were implanted on one side of the embryo.

Results

Most of the cells that suffer apoptotis are located at the periphery of the embryo, in the margin between the area opaca and the area pellucida. The region with intense cell death embraces the anterior region of the embryo forming a crecent-shaped area (Fig. 1A). There is scattered cells death are in other regions of the embryo but the presence of this apoptotic center defines clearly the region where the delimitation between the neural and epithelial tissues is taking place. The incorporation of BrdU in embryos at the same stages also showed a precise localization of the proliferating cells (Fig. 1B). Most cells that incorporate BrdU in a four- hour period were localized in the anterior marginal zone of the blastoderm. Therefore, the patterns of cell death and proliferation during these stages of development are very similar, and both coincide in time and space.

We have evaluated whether BMPs can induce additional cell death in the chick embryo by overexpression of BMP4 (Fig. 1C). After the application of the growth factor and performance of the TUNEL technique, apoptotic cells were detected surrounding the bead(s). Therefore, BMPs are able to induce cell death in chick blastoderms and they probably control in some way the elimination of cells in the periphery of the embryo. When the same experiment was performed but this time studing the incorporation of BrdU, an increase in the proliferation in the periphery of the embryo was also observed (Fig. 1D).

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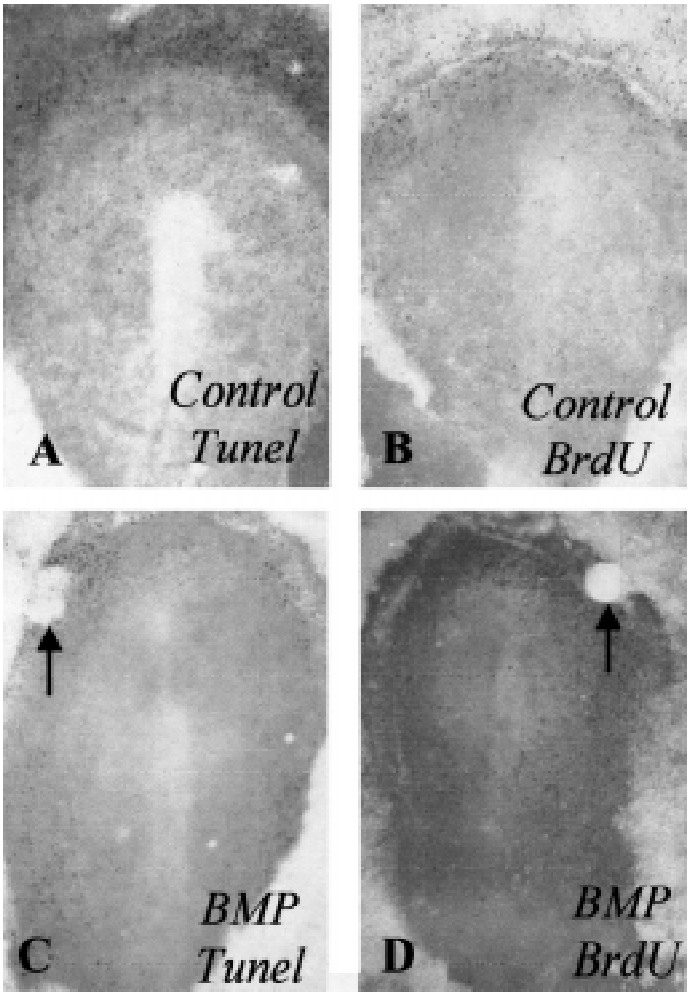

Fig. 1. Control embryos to which the TUNEL technique has been applied (A) or the incorporation of BrdU was evaluated (B). *Both death cell and proliferating cells are present in the anterior peripherial region of the embryo.* **(C,D)** *Embryos were treated with beads (arrows) soaked with BMP4 at 0.2 mg/ml at the periphery of the blastoderm (germinal crescent). After four hours of treatment with the growth factor, the embryos were processed by TUNEL or checked for the incorporation of BrdU. As can be seen the growth factor induces both apoptosis and proliferation in the blastoderm cells.*

Discussion

Here we show that the region where the BMPs are produced occurs the most intense area of cell death and proliferation. This result is clear evidence that BMPs are molecules that not only specify the epithelial territory in the blastoderm, but also govern the balance between the cells that proliferate and contribute to the future embryo and those that are eliminated.

The elimination of cells in this peripherial region could be explained by the fact that it is the border territory that will separate the neural plate of the epithelial sheet. This border region probably has a very fine balance of signals to delimit the two territories, and cells that cannot interpret this information correctly are eliminated. In support of this possibility, regions between two kinds of tissues are clearly involved in extensive processes of cell death. Therefore, we propose that BMPs not only specify epithelial tissue in the chick blastoderm (Gallego-Díaz *et al.,* submitted) but that they also control the proliferative activity and the entrance of cells in the apoptotic program in the periphery at the embryo.

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