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Short Report

Interactions between FGFs and BMPs in the control of programmed cell death in the developing limb

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Programmed cell death (PCD) has a major role in vertebrate limb morphogenesis. In the developing chick embryo four areas of PCD, the anterior necrotic zone (ANZ), the posterior necrotic zone (PNZ), the opaque patch (OP) and the interdigital necrotic zones (INZ) are responsible for sculpturing the shape of the limb. There is abundant evidence showing that BMPs are responsible for triggering apoptosis in the limb mesoderm (Macías *et al.*, 1997). However, BMPs are at the same time the signals acounting for chondrogenesis (Zou *et al.*, 1997; Pizette and Niswander, 2000). Local application of BMPs into the undifferentiated limb mesoderm causes apoptosis (Fig. 1A). However, the same treatment applied into the prechondrogenic mesenchyme results in massive chondrogenesis (Fig. 1 B,C). Thus, the stage of differentiation of the mesodermal target tissue appears to have a major influence between the two opposite effects of BMPs. FGFs are the signals responsible for maintaining the limb mesoderm in an undifferentiated and proliferating state (Martin, 1998). It has been shown that FGFs protect the limb mesoderm from the apoptotic influence of BMPs (Gañan *et al.*, 1996), thus acting as survival factors. In a recent study (Montero *et al.*, 2001) we have confirmed that exogenous administration of FGFs into the limb mesoderm has an initial inhibitory effect on cell death. This effect is appreciable during the first 10-12 h after the treatment (Fig. 1D). However, this initial anti-apoptotic effect is later followed by a dramatic increase in apoptosis (Fig. 1E) which can be inhibited by administration of BMP-antagonists (Fig. 1F). Together, these findings suggest that FGFs inhibit cell death but at the same time make the cells sensitive to the apoptotic effect of BMPs. In accordance with this interpretation, blocking FGF-signalling by administration of a specific FGF inhibitor (SU5402) abolishes interdigital cell death (Fig. 1G) and results in syndactylous limb. Several genes, including *MSX-2*, and *Snail* has been implicated in the apoptotic promoting effects of FGFs. Here we have

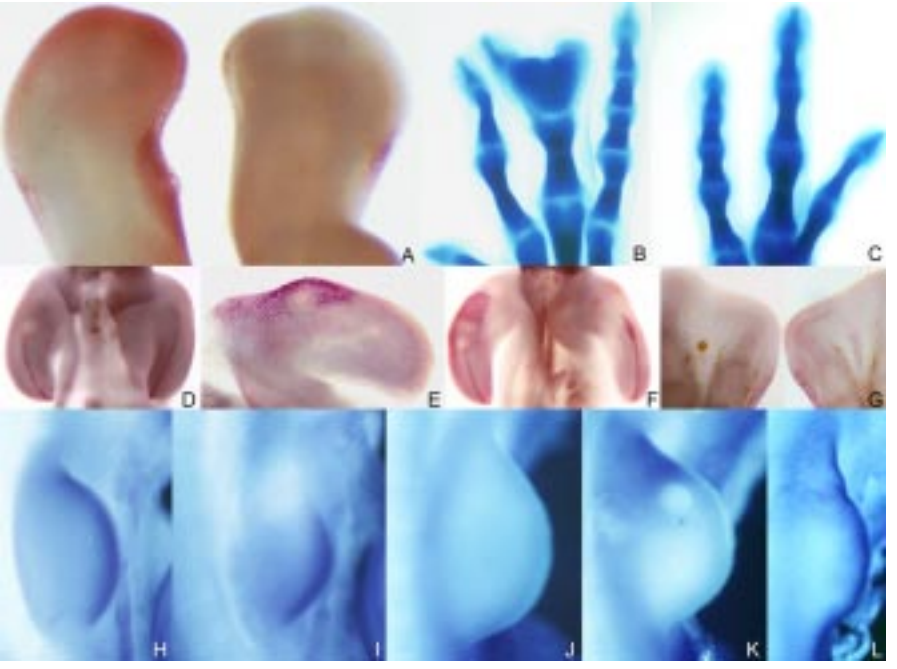

Fig. 1. (A) *Neutral red staining of stage 26 embryo showing induced cell death after 8/10 hours of BMP7 soaked bead implantation.* **(B,C)** *Autopode stained with alcian green showing chondrogenesis induced three days after BMP7 soaked bead application at the tip of digit 3 in a stage 28 embryo. Note increased chondrogenesis at the tip of the digit with respect to a control limb in 1C.* **(D,E)** *Cell death vital stained with neutral red 10/12 hours after application of an FGF2-bead into the anterior mesenchyme of a stage 20 limb bud. Note that cell death is inhibited in ANZ (1D). However 20/24 hours after bead implantation cell death is highly induced in the ANZ (1E).* **(F)** *Neutral red staining of a limb treated with both FGF-2 and Noggin. The FGF2-bead was placed in a proximal position on a stage 20 wing bud. Noggin, was placed close but in a more distal position. Note that Noggin blocks the ectopically induced cell death by FGF.* **(G)** *Neutral red stainig of limb autopods. On the left side, the third interdigit was treated for 24 h with Su5402, a specific inhibitor of FGFs, and interdigital cell death appears inhibited in comparison with its contralateral untreated limb at the right side.* **(H-K)** *Whole mount in situ hybridisation showing FGFR 1 (H-I) and 3 (J-K) expression in stage 20/22 control limb buds (H and J) and after 8 hours of BMP7 treatment in the anterior mesenchyme (I and K). FGFR1 expression is inhibited in the experimental limb bud (I) with respect to the control (H). On the other hand, FGFR3 expression is highly induced in treated mesenchyme (K) with respect to the contralateral limb bud (J).* **(L)** *FGFR2 expression after BMP7 bead implantation in the anterior mesenchyme of stage 20 limb buds. Note that FGFR2 is overexpressed after 8 hours of BMP7 exposure.*

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explored whether BMPs are able to modulate the effects of FGFs. Local application of BMPs (BMP-2; BMP-4 and BMP-7) in the subridge mesoderm of the limb bud has an intense an rapid (2hr) effect inhibiting the expression of *FGF-8* in the AER, also the expression of the type 1 FGF receptor is downregulated (Fig. 1 H,I). However, the expression of *FGFR2* (Fig. 1L) and *FGFR3* (Fig. 1 J,K) are upregulated by BMPs. Taken together all those findings reveal the occurrence of an interaction between FGFs and BMPs in the control of limb outgrowth. On the one hand, FGFs promote growth, inhibit differentiation, and make the mesodermal sensitive to the apoptotic effects of BMPs. On the other hand BMPs inhibit the expression of FGFs and promote the expression of the type 2 and 3 FGF receptors. It is important to note that FGF receptor 3 has been implicated in the differentiation of cartilage inhibiting cell proliferation (Sahni *et al.,* 1999) and in the onset of apoptosis in different systems (Legeai-Mallet *et al.,* 1998). Thus, FGFs and BMPs may cooperated both in apoptosis and in chondrogenesis.

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