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Short Report

Sfrp1 demarcates the anterior neural plate and promotes eye development in the chick embryo

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ABSTRACT Secreted frizzled related proteins (SFRPs) are a new class of signaling molecules that antagonize the activity of Wnt proteins. However, the full range of SFRP activities and mechanisms of action are not fully understood. In our search for molecules which may participate in the development of the eye, we have isolated the chick homologue of *Sfrp1*. Here we show that in chick, *Sfrp1* has a widespread and dynamic expression in the most anterior portion of the embryo. At later stages of development, *Sfrp1* continues to be expressed with a pattern that is consistent with a function in cell fate determination and differentiation in both the eye and the neural tube. On the basis of this expression pattern, we have analyzed possible roles of *Sfrp1* in the development of chick embryos. With *in vitro* and *in vivo* experiments, we show here that *Sfrp1* has a dual function in the development of the chick retina: it promotes axon outgrowth and increases neuronal differentiation, while inhibiting cell proliferation.

WNT proteins regulate cell fate and cell behavior in a wide variety of biological processes. During nervous system development, Wnt signaling has been implicated in the induction of posterior neural tissue in both *Xenopus* and mammalian embryos. WNT proteins are also required for midbrain development and for controlling the proliferation of precursor cells of the neural crest, dorso-lateral neural tube and hippocampus. In both fly and vertebrate, WNT proteins have been further implicated in modulating growth cone behavior and during cerebellar development they act as retrograde synaptogenic factors (Patapoutian and Reichardt, 2000). Wnt signaling is initiated by binding of WNT proteins to transmembrane Frizzled (Fz) receptors and involves the cytoplasmic accumulation and the consequent nuclear translocation of beta-catenin, a protein involved in the activation of Wnt target genes. In the extracellular space, Wnt signaling is further controlled by a number of soluble molecules, including the expanding family of Secreted Frizzled Related Proteins (SFRPs), a new class of molecules that, as their name indicates share structural homology to the extracellular cystein rich domain (CRD) of the Fz receptors (Wodarz and Nusse, 1998).

In search for molecules that may participate in the development of the eye, we have isolated the chick homologue of *Sfrp1*. By means of whole mount in situ hybridization using a digoxigenin-labeled anti-sense probe generated from a 2Kb cDNA fragment (1040-3065 bp; Accession number: AJ404652), we have determined that, in chick, *Sfrp1* has a widespread and dynamic expression since blastula stage. During early primitive streak formation, its expression is restricted to the anterior primitive streak with a pattern which overlaps with that of *Otx2* and is complementary to that of *cWnt8c* (Esteve *et al.*, 2000). During neural plate formation c*Sfrp1* mRNAs are abundantly localized only to the anterior domain of the neural tissue, including the prospective eye field (Fig. 1). As neural tube closes, the expression of *Sfrp1* extends with a rostro-caudal gradient to the ventral neural tube. At later stages of development, *Sfrp1* continues to be expressed with a pattern that is consistent with a function in cell fate determination and differentiation in both the eye and the neural tube.

Based on this expression pattern, we have set out the hypothesis that *Sfrp1* might be necessary for the establishment of the antero-posterior axis of the embryo, as already shown in *Xenopus* and mouse embryos for other members of this family (Borello *et al.*, 1999; DeRobertis *et al.*, 2001). In addition, we have addressed the question of whether *Sfrp1* plays a role in the specification and differentiation of the neural retina.

To address these issues, we have generated a source of soluble SFRP1 protein by transfecting MDCK cells with a pcDNA3.1 eukaryotic expression vector containing the full coding sequence of *cSfrp1* or with the vector alone. Isolated clones with the highest yields of protein secretion into the culture medium were selected as source of soluble molecule. Conditioned medium containing soluble SFRP1 protein was added to either dissociated or explant cultures derived from E5 chick embryos. After one day of culture, the effects of SFRP1 addition were evaluated by immunocytochemical staining with cell proliferation (bromo-deoxy-uridine incorporation and phospho-histone H3 expression) and cell differentiation (Beta-tubulin-III, neurofilament and Islet1/2 expression)

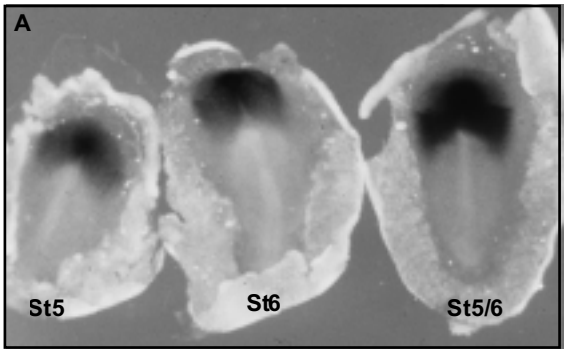

Fig. 1. Expression pattern of c*Sfrp1* during neural induction. *Dorsal view of embryos of St 5-6 hybridized in toto with a digoxigenin labeled probe against Sfrp1. Note the strong expression in the anterior neural plate.*

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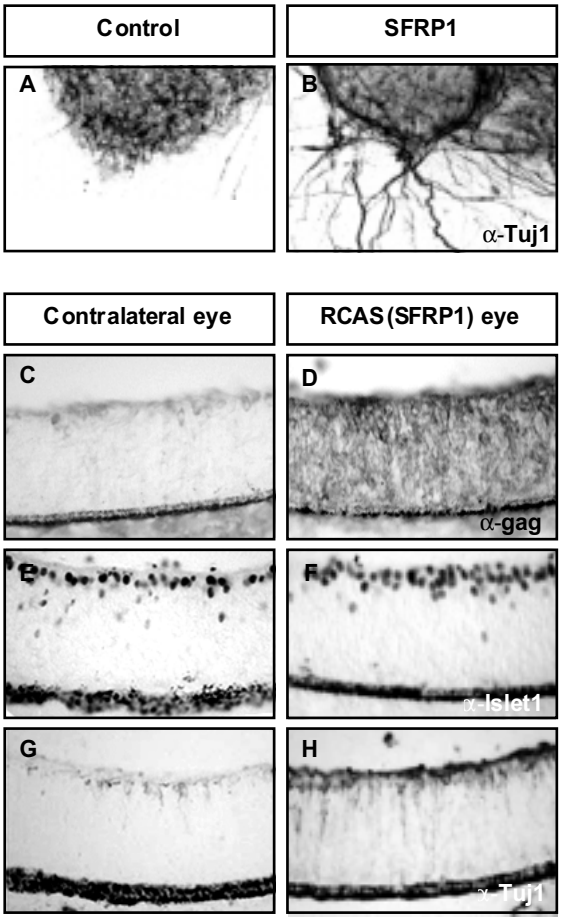

Fig. 2. SFRP1 promotes axon outgrowth and cell differentiation in the chick retina. (A,B). *Explant cultures from E5 chick retinas were grown in defined medium in the presence (B) or absence (A) of partially purified SFRP1. After 24 hours cultures were fixed and immunostained with anti-Tuj1 antibodies. Note how SFRP1 favors neurite outgrowth.* **(C-H)** *Frontal cryostat sections from retinas of E6 embryos infected with RCAS-SFRP1viruses at stage 10. Adjacent sections from infected (D,F,H) and contralateral uninfected (C,E,G) central retinas were immunostained with antibodies against the p27 gag viral protein (C,D); Islet1/2 (E,F) and Tuj1 (G,H). Note how the number of ganglion cells is increased in gag-positive retina. Note also the thickness of the fiber layer in the infected retinas (H) as compared to control tissue (G). Retinas are oriented with the pigmented epithelium on the bottom and the fiber layer on the top.*

markers. The results we have obtained *in vitro* clearly indicated that *Sfrp1* has a dual function in the development of the chick retina. It promotes axon outgrowth and increases neuronal differentiation, while inhibiting cell proliferation. Thus, in culture, addition of SFRP1 decreased the number of BrdU-positive cells to about a fourth of those observed in controls. This effect was associate with a clear increase of about 60 to 100% in cell differentiation. More strikingly SFRP1 appeared to have a strong neurite promoting activity which was evident in both retina explants embedded in collagen gel matrix (Fig. 2 A,B) and dissociated retinal cultures, independently of the substrate over which retinal cells were plated.

Furthermore, we have generated a replication competent RCAS retroviral vector to over-express *Sfrp1* in the developing embryo in ovo. Alkaline phosphatase(AP)-RCAS virus was also prepared and used as control. Concentrated viruses were injected into the optic vesicles of stage HH10-11 embryos. Four or five days post infections (E6-7), embryos were harvested and fixed. Analysis of infected embryos confirmed our previous results. Thus, in infected, gag-positive retinas (Fig. 2 C,D), Islet1/2- or Tuj1-positive cells were not only more densely packed in the retina ganglion cell layer but were also more frequent in the ventricular zone (Fig. 2 D,F,H), as compared to equivalent retinal regions of uninfected contralateral eyes or eyes of RCAS-AP infected embryos (Fig. 2 C,E,G).

Consistent with the idea that *Sfrp1* might contribute to antero-posterior axis formation, we observed that widespread infection with RCAS-Sfrp1 at early stages of embryo development consistently induced shortening of the body axis and absence of the tail, effects that were never observed in RCAS-AP treated embryos.

Conclusions

In addition to confirming an important role for SFRPs in the formation of the vertebrate body axis, our data reveal new roles for SFRP1 during chick eye development. Whether cSFRP1 exerts these activities through the Wnt signaling pathway or through independent signaling mechanisms remains to be established.

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