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O-3 The Expression and Function of FGF-8 in Limb Development and Regeneration of Mexican Axolotl, *Ambystoma mexicanum*

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INTRODUCTION

Urodeles have a remarkable regeneration ability to restore the missing part faithfully during the whole span of life. In the process of regeneration, many kinds of factors are suggested to be produced from nerve, wound epidermis, or mesenchymal tissue of blastema, and they are presumed to play crucial roles for the successful limb regeneration. Many kinds of FGFs such as FGF-2, -4 ,and -8 have been reported to play important signaling roles in the vertebrate limb development. *Fgf-2* is expressed in the mesenchymal tissue and epidermis of the developing limb, and *Fgf-4* is expressed in the posterior half of apical ectodermal ridge (AER). Especially, *Fgf-8* begins to be expressed at the presumptive AER of the pre-limb bud ectoderm, and continuously expressed in the AER during the period of limb bud outgrowth (Crossley *et al.*, 1996). Moreover, these FGFs can substitute for AER's role as has been well demonstrated by the restoration of basic gene expression pattern such as *Shh* and of skeletons with exogenous FGF supply in AER-removed limb bud (Mahmood *et al.*, 1995). Interestingly, these FGFs are well known to induce ectopic limb from the flank region of chick embryo (Vogel *et al.*, 1996). Because the FGF-8 produced from AER is a crucial signaling molecule in limb development, and the apical ectodermal cap (AEC) of urodele limb regenerate derived from the wound epidermis is speculated to be a functional equivalent of AER, it is intriguing to know if the *Fgf-8* expression pattern in a urodele species, axolotl, is similar to amniotic counterparts. Thus, it needs to be examined whether the general role of FGF-8 is able to apply to the limb development and regeneration of urodele amphibian. In the present study, the cloning and characterization of a *Fgf-8* homologue was carried out in an urodele species, Mexican axolotl (*Ambystoma mexicanum*), and the expression pattern of axolotl *Fgf-8* was examined in embryonic development. Next, the expression pattern and the function of FGF-8 were analyzed in the developing and the regenerating limb of axolotl. In addition, the effect of retinoic acid (RA) on the expression pattern of *Fgf-8* was examine in the regenerating limb since RA is a well-known pattern modifier in limb regeneration.

MATERIALS & METHODS

An *Fgf-8* cDNA of axolotl was cloned through the screening of an axolotl embryo cDNA library. Using the above clone as a probe, the temporal and spatial expression patterns of *Fgf-8* were examined in developing embryos, and in normal and RA-treated regenerating larval limbs by whole mount *in situ* hybridization. Furthermore, to know the function of FGF-8 in limb regeneration, FGF-8 was applied locally into the normal and denervated regenerating limbs of axolotl, and the blastema growth was accessed in terms of *Msx-1* expression and morphology after FGF-8 bead implantation.

RESULTS & DISCUSSION

The 1.27-kb axolotl cDNA cloned in this study contained an open reading frame encoding 212 amino acid residues with 84.5%, 88%, and 76% a.a. identities to those of *Xenopus*, chick, and mouse, respectively. In developing embryos, *Fgf-8* was expressed in neural fold, spinal cord, midbrain-hindbrain junction, tail and limb buds, somites, pharyngeal clefts, primordia of maxilla, mandible, and eyes. In the developing axolotl limb, *Fgf-8* began to be expressed in the epidermis of prospective forelimb region at prelimb bud stage before hatching and in the epidermis of the budding limb after hatching. At pre-digit stage, a strong expression was detected in the mesenchymal tissue of the limb bud, but not in the epidermis. In the regenerating limb, *Fgf-8* expression was noted in the wound epidermis and the distal mesenchymal tissue of stump at wound healing to blastema formation stages of regeneration. These data

suggest that *Fgf-8* is involved in the organogenesis of various craniofacial structures and the initiation and outgrowth of limb development and regeneration.

RA treatment caused a modification in the *Fgf-8* expression profile of regenerating limbs. In RA-treated limbs, the duration of *Fgf-8* expression was longer than in normal regenerating limb, and higher level of *Fgf-8* expression by RA in the blastema was maintained during the blastema formation stage. In the limb development and regeneration, *Fgf-8* expression domain was not restricted to epidermis unlike amniotes, instead it was mainly detected in mesenchymal tissue. Therefore, the expression domain of *Fgf-8*, at least in limb development and regeneration, suggests that the regulatory mechanism of *Fgf-8* expression in axolotl might be somewhat different between urodeles and other species.

Administration of FGF-8 protein by bead implantation in the early phase of limb regeneration accelerated the blastema formation, and substituted partially nerve factor(s) in the denervated limb regenerates.

Thus, axolotl FGF-8 appears to be in important signaling molecule during embryogenesis and limb development and regeneration. Moreover, unlike amniote, the unique expression of *Fgf-8* in the mesenchymal tissue of the regenerating axolotl limb suggests it might be causally related to the remarkable regeneration capacity of urodele.

SUMMARY

From the present study, following conclusions can be drawn: 1. Like in other species, axolotl FGF-8 is proposed to play a similar role in the early phase of limb development. However, the mechanism of its expression might be somewhat different from amniotes considering its characteristic mesenchymal expression. 2. In the regenerating axolotl limbs, *Fgf-8* expression profile suggests that it is involved in wound healing, dedifferentiation, and blastema formation. 3. Exogenously supplied FGF-8 can accelerate blastema formation and concomitantly increase the *Msx-1* expression level at the early stage of limb regeneration. Furthermore, it can partially substitute for nerve factor(s) as has been indicated by the induction of blastema formation in the denervated regenerates after FGF-8 application. 4. The unique expression feature of *Fgf-8* in the mesenchymal tissue of the regenerating axolotl limb might be casually related to its remarkable regeneration capacity of urodele.

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