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Short Report

Differential expression of FGF receptors during zebrafish fin regeneration

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ABSTRACT Teleost fins can be structural and functionally restored after severe injuries or partial amputation through a process of epimorphic regeneration, which requires an acute temporal and spatial control of basic cell events such as proliferation, and differentiation. FGF family of growth factors have been shown to regulate cell growth, survival, migration and differentiation. Through these abilities, FGFs induce and/or direct patterning in animal development and regeneration. Pleiotropic effects of FGFs are executed by activation of specific membrane receptors. In order to gain insights into the role of FGF signaling during teleost fin regeneration, we undertook the study of the expression of the four described FGF receptors (FGFRs). Preliminary results are presented, showing different patterns of immunoreactivity for each receptor during outgrowth regeneration stages, that are suggestive of particular functions for each of them.

Teleost fins are composed of a species-specific number of branched rays. These are sustained by a segmented dermal bone called lepidotrichia, which is, in turn, composed of two concave, facing bones, named hemi-lepidotrichia. Lepidotrichia are immersed in a loose, vascularized, and innervated connective tissue, and are surrounded by a multilayered epidermis (Montes *et al.*, 1982; Becerra *et al.*, 1983). At their distal tip, two palisades of actinotrichia parallel hemi-lepidotrichia inner faces. Actinotrichia are rigid, but non-mineralized, collagenic rods that not only support the ray tip, but are also though to have some morphogenetic role (Santamaría *et al.*, 1996a). Rays are connected to each other by the soft inter-ray region, which lacks skeletal elements.

When severely damaged or partially amputated, fins are able of complete self-restoration through a process of epimorphic regeneration. This involves the formation of a blastema at the stump of each ray, in contact with the wound epithelium, which is supposed to direct, at least in part, the regenerative process (Geraudié and Singer, 1992). Proliferation of distal blastema provides new cells, that differentiate proximally into fibroblast-like connective cells, and proximo-laterally into LFCs (Lepidotrichia Forming Cells), (Santamaría and Becerra, 1991). Meanwhile, epidermal proliferation causes the skin expansion needed for accompanying the new ray outgrowth (Santamaría *et al.*, 1996b).

Such a complex process requires precise co-ordination between cell proliferation, cell differentiation, morphogenesis and pattern formation. Cell proliferation and differentiation must be controlled by a series of specific mitogenic and anti-mitogenic signals that drive multiple pathways within the cells. One of these signals might be fibroblast growth factors (FGFs). These are involved in mammalian wound healing and vertebrate embryonic development (Szebenyi and Fallon, 1999). They are also implicated in urodele amphibian limb regeneration (Zenjari *et al.*, 1997), and recently, Poss *et al.*, (2000) have described *FGF* expression at the distal epidermal cap during initial steps of zebrafish fin regeneration.

FGF signaling is carried out through activation of specific membranal protein-kinase receptors. Four specific high-affinity FGF receptors have been described, that are present in all vertebrate taxa (Szebenyi and Fallon, 1999). In order to study the role played by FGF signaling in teleost fin regeneration, we undertook the study of FGF receptors expression in such process.

FGF receptors 1, 2, 3 and 4 were immunohistochemically detected in parallel tissue sections from mature and regenerated teleost fins. For this purpose, mature, non-regenerated as well as ten day regenerated zebrafish (*Brachydanio rerio*) caudal fins were collected and histologically processed for light microscopy. Immunohistochemical detection of the four FGF receptors was carried out according to standard protocols, and using commercial antibodies against each of the molecules. Unspecific immunoglobulins from the same species of the anti-FGFR antibodies, as well as peptide-neutralized antibodies, were used as specificity control. Peptide neutralization was performed for each of the anti-FGFR antibodies, by incubating overnight each anti-FGFR antibody with an excess of the peptide used in antibody production (ratio antibody:peptide was 1:5).

FGF and its receptors are highly conserved molecules, not only among vertebrate, but even among animal kingdom. This particularity allowed us the use of antibodies to detect their presence in teleost fin tissues. The used antibodies had been obtained immunizing donor animal against a synthetic peptide corresponding to a highly conserved region of FGF receptor molecule. The company guarantees antibody specificity and peptide neutralization assay confirmed such premise. The different staining patterns yielded by each anti-FGF receptor antibody constitute another proof for antibody specificity.

The four FGF receptors have been found in caudal fins, displaying different patterns in mature and regenerating fin. This is indicative of a distinct regulation of these molecules during regeneration with respect to quiescent ray, and is, so, suggestive of a role of FGF receptors in regeneration. Furthermore, differential expression of each receptor suggests each of them may be implicated in different functions.

Curiously, none of the receptors was found specifically in highly proliferative regions during fin regeneration. This would have been

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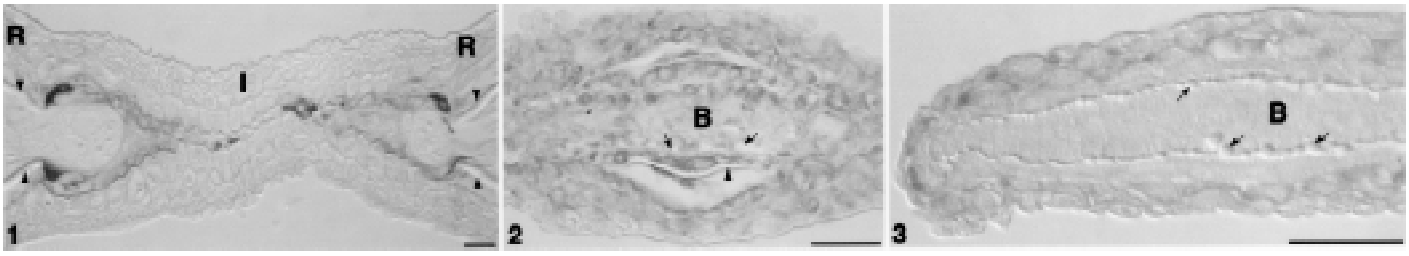

Fig. 1. Transversal sections through ten day-regenerated caudal fins *immunostained for FGF-R1* **(1)**, *FGF-R3* **(2)** *and FGF-R4* **(3)**. *Lepidotrichia (arrow heads), ray area (R), inter-ray (I), actinotrichia (arrows), blastema (B). Bars, 50 μm.*

an expected result since the most studied action of FGFs is induction of cell proliferation. On other side, Poss *et al.* (2000) have shown *FGFR1* mRNA expression at proliferative blastemal cells. We have detected FGFR1 protein only at the transition area intra-inter ray. At this location, some proliferation occurs, in relation to the lateral expansion of the regenerating ray (Santamaría *et al.*, 1996a). This is in concordance with Poss' hypothesis of FGFR1 implication in the control of cell proliferation during fin regeneration. Although we have not found FGFR1 immunoreactivity at the blastema, it might be possible that in these quickly proliferating cells the receptor is subject to a rapid turnover that prevents its detection by immunohistochemistry. FGFR1 is the only receptor we have found at the mature mesenchymal tissue. Poss and co-workers did not find *FGFR1* mRNA expression in mature rays. This suggests the receptor could be accumulated in mesenchymal cells, ready to being activated in response to injuries. This assertion will need further experimentation, but similar situations have been found in other systems, in which FGFR1 has been described to be expressed in quiescent tissues, where it participates in inmovilizing FGFs accumulated at the extracellular matrix (Szebenyi and Fallon, 1999).

The expression pattern found for FGFR4 also seems to link this receptor to the control of cell proliferation in regenerating fins, since it was only detected in regenerating rays, and only at the epidermal areas in contact with a proliferative mesenchyma.

FGFR2 and, specially, FGFR3 expression patterns suggest these are implicated in skeletal reconstruction. FGFR3 immunoreactivity is strongly enhanced in cells directly contacting actinotrichia and lepidotrichia, and disappears proximally, where these structures are already rebuilt. In mature rays, FGFR3 can be detected at the lateral borders of lepidotrichia, where lepidotrichia remodeling occurs. FGFR2 appears when lepidotrichia are partially reconstructed, in the outer LFCs. During lepidotrichia regenerative reconstruction, inner LFCs migrate along the edge of the young lepidotrichia and colonize the outer dermal bone surface (Santamaría and Becerra, 1991). There, they go on synthesizing lepidotrichial matrix, surrounded by a different environment (now they are not it contact with the mesenchyma, but with the epidermis).Those are the cells we found to present FGFR2 immunoreactivity. All these data indicate that the two different lepidotrichia synthesis mechanisms could be regulated by two different FGF receptors. This suggest an implication for FGF and its receptors in fin skeletal differentiation. Implication of FGF signaling in endochondral skeletal development have been previously described (Naski and Ornitz, 1998), but this is the first time a role is suggested for FGF signaling in dermal skeleton formation.

RECEPTOR	EXPRESSION IN MATURE RAY	EXPRESSION IN REGENERATED RAY	FIGS.
FGFR1	Epidermis and mesenchyma	Mesenchymal intra to inter-ray transition area	1
FGFR2	No mesenchymal immunoreactivity Certain phenotipically distingishable epidermal cells	Strong in outer LFCs No epidermal staining	
FGFR3	Some mesenchymal cells surrounding the lepidotrichia lateral edge Slight at the epidermis of the ray area	Distal blastema and epidermis. Acquires strong intensity in cells that contact actinotrichia and lepidotrichia. Disappears when these are restored	2
FGFR4	Not detected	Distal epidermis, in contact with a proliferative blastema	3

References

BECERRA, J, G.S. MONTES, S.R.R. BEXIGA, and L.C.U. JUNQUEIRA (1983). Structure of the tail fin in teleosts. *Cell Tissue Res.* 230: 127-137.

GERAUDIÉ, J., and M. SINGER (1992). The fish fin regenerate. *Monographs in Dev. Biol.,* 23: 62-72.

MONTES, G.S., J. BECERRA, O.M.S. TOLEDO, M.A. GORDILHO, and L.C.U. JUNQUEIRA (1982). Fine structure and histochemistry of the tail fin ray in teleosts. Histochemistry, *75*:363-376.

NASKI, M.C., and D.M. ORNITZ (1998). FGF signaling in skeletal development. *Front. Biosci.* 3: D781-D794.

POSS, K.D., J. SHEN, A. NECHIPORUK, G. MCMAHON, B. THISSE, C. THISSE, and M.T. KEATING (2000) Roles for FGF signaling during zebrafish fin regeneration. *Dev. Biol.* 222: 347-358.

SANTAMARÍA, J.A., and J. BECERRA (1991) Tail fin regeneration in teleosts: cell-extracellular matrix interaction in blastemal differentiation. *J. Anat.* 176: 9-21.

SANTAMARÍA, J.A., M. MARÍ-BEFFA, L. SANTOS-RUIZ, and J. BECERRA (1996b) Incorporation of Bromodeoxyuridine in regenerating fin tissue of the goldfish *Carassius auratus*. *J. Exp. Zool.* 275: 300-307.

SANTAMARÍA, J.A., L. SANTOS-RUIZ, and J. BECERRA (1996a) An antiserum against ceratotrichia (Selachian) recognizes actinotrichia in teleost regenerating fins. *Int. J. Dev. Biol.* Suppl.1: S175-S176.

SZEBENYI G., and J.F. FALLON (1999) Fibroblast growth factors as multifunctional signaling factors. *Int. Rev. Cytol.* 185: 45-106.

ZENJARI, C., B. BOILLY, H. HENDERMARCK, and Y. BOILLY-MARER (1997) Nerve-blastema interactions induce fibroblast growth factor-1 release during limb regeneration in *Pleurodeles waltii. Dev. Growth Differ.* 39: 15-22.