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Original research article

Dynamics of actinotrichia regeneration in the adult zebrafish fin

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ABSTRACT

The skeleton of adult zebrafish fins comprises lepidotrichia, which are dermal bones of the rays, and actinotrichia, which are non-mineralized spicules at the distal margin of the appendage. Little is known about the regenerative dynamics of the actinotrichia-specific structural proteins called Actinodins. Here, we used immunofluorescence analysis to determine the contribution of two paralogous Actinodin proteins, And1/2, in regenerating fins. Both proteins were detected in the secretory organelles in the mesenchymal cells of the blastema, but only And1 was detected in the epithelial cells of the wound epithelium. The analysis of whole mount fins throughout the entire regenerative process and longitudinal sections revealed that And1-positive fibers are complementary to the lepidotrichia. The analysis of *another longfin* fish, a gain-of-function mutation in the potassium channel *kcnk5b*, revealed that the long-fin phenotype is associated with an extended size of actinotrichia during homeostasis and regeneration. Finally, we investigated the role of several signaling pathways in actinotrichia formation and maintenance. This revealed that the pulse-inhibition of either TGFβ/Activin-βA or FGF are sufficient to impair deposition of Actinodin during regeneration. Thus, the dynamic turnover of Actinodin during fin regeneration is regulated by multiple factors, including the osteoblasts, growth rate in a potassium channel mutant, and instructive signaling networks between the epithelium and the blastema of the regenerating fin.

1. Introduction

The zebrafish caudal fin provides a valuable model system to study mechanisms of adult organ regeneration in vertebrates. After fin amputation, the zebrafish is able to swim and to restore the original size and shape of the appendage within approximately three weeks. This phenomenon depends on appropriate wound healing, creation of blastema progenitor cells and on their progressive redifferentiation, as recently reviewed (Jazwinska and Sallin, 2016; Tornini and Poss, 2014; Wehner and Weidinger, 2015).

The fin is a non-muscularized flattened appendage, which is used for propulsion while swimming. As opposed to the tetrapod limb, which is entirely supported by the endoskeleton, the fin fold is stabilized by skeletal elements of dermal origin, called rays, whereas the endoskeleton is confined only to the base of the fin (Grandel and Schulte-Merker, 1998; Thorogood, 1991; Witten and Huysseune, 2007). The zebrafish caudal fin typically contains 16–18 main occasionally bifurcated rays, which are connected by soft interray tissue (Akimenko et al., 2003; Mari-Beffa and Murciano, 2010; Pfefferli and Jaźwińska, 2015). Each ray contains a pair of segmented parenthesis-shaped bones, called lepidotrichia, which are deposited by osteoblasts underneath the stratified epidermis. The tips of the rays lack bones and are solely supported by unsegmented brush-like spicules, named actinotrichia (Becerra et al., 1983; Witten and Huysseune, 2007). While lepidotrichia are dermal structures, actinotrichia are considered to be of ectodermal origin, as they are formed in the early embryonic fin fold, before mesenchyme recruitment, and they precede the differentiation of rays in various fish species (Dane and Tucker, 1985; Feitosa et al., 2012; Geraudie, 1977; Grandel and Schulte-Merker, 1998; Heude et al., 2014; Kemp and Park, 1970; Thorogood, 1991; Wood and Thorogood, 1984; Zhang et al., 2010). In the mature zebrafish fin, both skeletal structures occupy the epidermal-mesenchymal interface along the proximal-distal axis of the appendage, whereas the interior space of the fin is filled with vascularized and innervated mesenchymal tissue (Akimenko et al., 2003; Mari-Beffa and Murciano, 2010; Pfefferli and Jaźwińska, 2015). After adult fin amputation, the stump forms a new outgrowth, which is initially supported by actinotrichia before lepidotrichia formation (Duran et al., 2011; Mari-Beffa et al., 1989). Actinotrichia play at least two functions in the fin: providing mechanical support to the fin fold during swimming and acting as a substrate for the migration of mesenchymal cells during morphogenesis (Bhadra and Iovine, 2015; Duran et al., 2011; van den Boogaart et al., 2012; Wood and Thorogood, 1984; Zhang et al., 2010). The attachment of mesenchymal cells to the actinotrichial migratory substrate requires

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Hemicentin 2 and Fibulin 1 matrix proteins (Feitosa et al., 2012).

Actinotrichia are composed of multiple collagenous and non-collagenous matrix proteins, which form a supramolecular aggregate, historically referred to as elastoidin (Galloway, 1985; Geraudie and Meunier, 1980; Mari-Beffa et al., 1989). By electron microscopy, actinotrichia exhibit a regular cross-banding, with a periodicity varying