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Review Article

SIGNIFICANCE OF FGF-2 DURING TAIL REGENERATION IN LIZARDS: A REVIEW

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Abstract: Among reptiles, lizards have retained remarkable power of regenerating their tails as part of their adaptation. The healing of the wound is a crucial event which differentiates the regenerating and non-regenerating tissues due to presence of signaling molecules that guide the wound for the regeneration of the lost tail. This crucial event includes signals like FGFs, especially FGF-2, and Wnt proteinsVEGF, EGF etc. FGF-2 is released by nerve endings at the AEC and might be involved in dedifferentiation of cells and formation of blastema. Cross talks between FGF-2 and other signaling molecules,during initial stages of blastema formation, lead to the pathways that regenerate the lost tail in lizards. FGF-2 is involved in reshuffling of the extracellular matrix by activating MMPs, angiogenesis, spinal cord regeneration and muscle regeneration. Inhibition of FGF-2 or its receptors before or during blastema formation hampers or stops the regeneration process.

Key words: Fibroblast growth factor-2, Lizard tail

INTRODUCTION

Some invertebrate groups and few vertebrate groups show the ability to regrow the lost tissue or appendage. It has also been seen that as the complexity of the organisms increases, the power to regenerate organs decreases. In vertebrates, the regeneration occurs in fishes, amphibians, reptiles and to some extent in mammals. There have been extensive studies on amphibians to know the cellular and molecular mechanisms of regeneration [1-7]. Among the reptiles, the lizards have retained the ability to regenerate their tails, although they do not form the replica of the original tail but the regenerated tail compensates for the function of the lost tail [8,9]. Since the reptiles are closer to mammals in the phylogeny[10] the mechanism of regeneration in reptiles may give us better understanding of the similar process in mammals. Among the reptiles, lizards possess a remarkable ability to regenerate their tails as part of their adaptation process. However, all the lizards do not regenerate their tails and regenerative capacity is shown by few groups like scincids (skinks), gekkos, lacertids and anoles [8]. Most of these species can regenerate throughout their life.

The regeneration of any lost appendage,in amphibians and reptiles alike, occurs in a very precise manner which involves three critical stages *viz.*formation of wound epithelium (WE), formation of blastema (BL) and differentiation. In mammals, any loss of structure results in the healing of the wound and not the regrowth of the lost appendage or organ. The distinguishing ability of amphibians and lizards to regenerate their lost limbs and/or tails is a result of differential expression of many genes and the cross-talk between several signaling molecules like FGFs, VEGF, EGF, NGF, Wnt proteins etc. Of all the molecules which differentiate a regenerating tissue

from a normal wound tissue, fibroblast growth factor-2 (FGF-2 or basic FGF) merits a special mention as it is one of the first molecules that can be seen in both types of tissues but has a key role in a regenerating tissue, as has been noticed in the regenerating limbs in amphibians [11]. Similar studies have been performed in the lizards also*i.e.*elucidating the role of FGF-2 in regenerating tails. This review summarizes some of these studies.

FGF-2 and formation of blastema in lizards: After the loss of the tail, the wound is open with exposed dermis, adipose tissue, nerves, spinal cord etc. Immediately a clot is formed at the distal end of the spinal cord and the integument surrounds the wound [12]. The cells from the surrounding epidermis rush to the wound site and cover up the wound. At the wound site in a regenerating tissue, a thick layer of epithelial cells is formed below the clot. The distal part of this mass of cells is called Apical Epithelial Cap (AEC). The proliferation of cells in the AEC leads to formation of a structure called blastema, which is wider than long. However, as the proliferation continues, the blastema becomes longer and the cells start differentiating. The AEC is maintained in the blastema/regenerating tissue at the distal-most part as it is a source of two very important signal molecules viz. Wintless protein (Wnt) and Fibroblast Growth Factors (FGFs).

Some scientists are of the opinion that the blastema is a collection of dedifferentiated cells [10,13] while some believe that it carries undifferentiated cells [14,15]. During the formation of blastema, first of all the healing of the wound takes place as it takes place in other animals including mammals. But the distinctive feature is that some cells are induced to re-enter the cell cycle precisely [1]. These cells then proliferate and differentiate to form the lost structure [16]. In amphibians, the resident fibroblasts undergo dedifferentiation to contribute to formation of blastema and seem to be the earliest target for the signals that initiate formation of blastema [17]. These signals are provided by either AEC or nerves. In lizards, the type of cells that contribute to the formation of blastema need to be elucidated.

FGF signaling is a crucial event in the proliferation, developmental patterning and differentiation during regeneration [18]. If the FGF-2 signal is inhibited by using the antiFGF-2, the time taken to form the blastema is delayed, clearly indicating that an optimum level of FGF-2 is required for proper formation of blastema [19,20].Similar study by Yadav et al. [19] also suggests that extraneous FGF-2 enhances the progress of tail regeneration in *Hemidactylus flaviviridis* in initial stages *viz.* during blastema formation and proliferation. The post blastema stages do not seem to respond to the presence of FGF-2 and are not affected by the absence of FGF-2 either.The differentially expressed genes in the AEC in lizards include *fgfr13* [21] which suggests that FGF-2 is not synthesized *denovo* within the AEC instead it is supplied to the regenerating tissue by nerve endings.

FGF-1 and FGF-2 both have been found to be localized in wound epidermis whereas FGF-1 is also found in higher levels in the cells of blastema, 16 days post-amputation [22]. However, the FGF-2 levels decrease in the scar tissues as compared to actively regenerating tissue in due course.

Specific functions of FGF-2 during blastema formation: FGFs are neurotrophic molecules and are supplied by the nerve endings terminating in the blastema in regenerating tissues. They have been localized in apical epidermal cap (AEC) of the blastema, along with their receptors [5]. FGF-2 is also involved in the nerve regeneration during epimorphosis as it stimulates proliferation of neural progenitors and also maintains their undifferentiated state [11]. Supply of nerves to a regenerating tissue is an important step as has been shown byEndo et al. [23]. They deviated nerves to the wound site and observed that a blastema like structure is formed. However, it degenerated in later stages of wound healing. Further, they grafted a skin flap from the opposite side of the limb concomitant along with nerve deviation. This ectopic blastema continued to grow and formed the entire limb. This experiment conveys that along with FGF-2, there are other signals that come through the nerves and contribute to the maintenance of blastema and their interaction with the signals from the AEC ultimately results in the regeneration of the lost limb or tail.

FGF-2 is involved in several other events during regeneration like angiogenesis, smooth muscle cell growth, wound healing and tissue repair [24,25]. Along with cell proliferation and dedifferentiation of cells at wound site, during blastema formation there

is extensive rearrangement of extracellular matrix with the help of enzymes like matrix metalloproteinases (MMPs). One of the inducer for activity of MMPs is FGF-2 [26].

Further, FGF-2 signals the proliferation and chemotaxis of ependymal cells lining the central canal of the spinal cord in tail stump. These ependymal cells proliferate and migrate towards the AEC and form an ependymal tube which is the precursor of future spinal cord [27]. If the drug SU5402, an inhibitor of FGF receptors, is used then it blocks the extension of ependymal tube, suggesting an important role of FGF-2 in the initial stages of regeneration of spinal cord as well. Studies by Alibardi & Loviku [28] have shown that the regenerating spinal cord and nerves of lizards contain high levels of FGF-2 and FGF-1.

Wound healing: are there differences between scar tissue and regenerative tissue?: There are some critical differences between a tissue whose wound will heal and form a scar and another tissue which will enter regeneration process. In embryo/ fetus, the scar-free healing shows less inflammatory response and low levels of TGF-β [29,30]. The tissues which show massive inflammatory response lead to scars whereas those destined for regeneration show mild signs of inflammation, as hypothesized by Alibardi [31]. So, the early steps in the healing of the wound determine whether the tissue will follow the regeneration process or not.

FGFs (especially FGF-2 and FGF-1) are present in both the types of wounds viz., the wounds that will form a scar and the wounds that will regenerate the lost appendage. However, in the wounds that will form scar, the FGFs disappear after 7-14 days post-amputation while they remain in the AEC in the blastema which are destined to regenerate [32]. This was confirmed by studies wherein antagonists to FGFR1 slow down or completely inhibit the regeneration if administered during or before formation of blastema [19,20,33].

Similarly,Alibardi and Lovicu [28] have shown, in ***Lampropholis guichenoti*,** by immunolocalization that FGF-2 is localized in wound, AEC, differentiating muscles, spinal ganglia, regenerating nerves and spinal cord.While blastema cells show high levels of FGF-1 but they have relatively lower FGF-2 levels. Further, transcriptome studies byHutchins et al. [21] and Vitulo et al. [34] have shown that FGFs and their receptors are upregulated in regenerating tail while they are absent in scarring limb.

Further studies have revealed that in mice denervation hampers the regeneration of the digits as the FGF-2 signaling pathways are blocked. The FGF-2 may be localized in the regenerating tissue but it is absent in these tissues after denervation [35]. Along with Vascular Endothelial Growth Factor A (VEGFA), FGF-2 induces proliferation of endothelial cells to form capillaries in mammals [36].Thus, FGF-2 signaling is an important factor in the regeneration of tail in lizards or epimorphic regeneration in other animals. However, after the blastema is formed and it starts growing, the cells get engaged in the proliferation and differentiation and thus, the later stages of regeneration are not dependent on the presence of FGF-2 [19,20].

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