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Wnt-1 immunodetection in the regenerating tail of lizard suggests it is involved in the proliferation and distal growth of the blastema

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ABSTRACT

Lizard tail regeneration depends from growth of the apical tip and autonomous regeneration of a new spinal cord, cartilaginous tube and muscles. The presence of embryonic signaling pathways is likely involved and we have focused on immunolocalization of Wnt1 protein in regenerating tissues, a protein promoting proliferation and tumorigenesis. Western blot indicates some immunoreactive bands in the expected range at 46 and 33 kDa in the regenerating tail. Immunolocalization indicates that Wnt1 is prevalently detected in the apical wound epidermis, blastema, and ependyma ampulla of the regenerating tail while it lowers in other tissues of more proximal regions close to the original tail stump. Although a gradient for Wnt1 was not detected, the higher immunofluorescence present in the apical region of the blastema and around the regenerating spinal cord indicates that the protein could be secreted from the apical wound epidermis and the ependyma and might influence cell proliferation in the blastema, the distal-most growing center of the new tail. The present observations suggest the involvement of the Wnt pathway to direct the process of tail regeneration in lizard. The stimulation of proliferation of epidermal and mesenchymal cells in the apical blastema by Wnt proteins remains to be experimentally validated.

1. Introduction

The regeneration of the tail in lizards represents a remarkable example of organ regeneration among amniotes (Simpson, 1970; Bellairs and Bryant, 1985; Alibardi, 2010, 2014). Most of the new tail is regenerated within 40 days post-amputation following autotomy (a natural mechanism of release of the tail). Tail loss by autotomy or amputation determines the activation of the regenerative program which remains poorly known in molecular terms (Alibardi, 2014; Hutchins et al., 2014; Liu et al., 2015; Lozito and Tuan, 2016).

Microscopic studies have shown that mesenchymal cells from different tissues in the tail of lizards accumulate on the surface of the tail stump and give rise to the blastema, covered by a stratified (wound) epidermis (Hughes and New, 1959; Simpson, 1965; Cox, 1969; McLean and Vickaryous, 2011; Gilbert et al., 2013, 2015; Fig. 1). These cells derive from a reserve of stem cells that are sparse in many tissues of the tail but also from the dedifferentiation from injured cells of the stump, although the relative contribution of the two processes in the formation of the regenerative blastema is not known (Alibardi, 2014, 2015a, 2015b, 2016).

The coexistence of an embryonic organ, the blastema, with adult tail tissues in an amniote, the lizard, is an exceptional case of immune tollerance which, together a low inflammation, favors tissue regeneration. As an organ with embryonic characteristics, it is likely that in the tail blastema developmental signaling pathways are re-activated like the Fibroblast Growth Factors pathway (FGFs, Alibardi and Lovicu, 2010; Alibardi, 2012, 2015b, 2016). One of the main signaling pathways operating during development and regeneration is the Wnt-pathway, which is involved in numerous cellular processes (Clevers, 2006; Kawakami et al., 2006; Wizenman, 2012). These pathways contribute to determine different morphogenetic process such as axial formation in the embryo, nervous system development, limb development, but also the induction of numerous types of malignant proliferations leading to different forms of cancers. The latter pathological process indicates that in developmental and physiological conditions the Wnt pathway is under a strict regulation.

Recent transcriptome studies have indicated that some Wnt-genes are up-regulated during tail regeneration in lizards (Hutchins et al., 2014; Vitulo et al., 2016), but the localization of these proteins remains unknown. As part of a research program dealing with the

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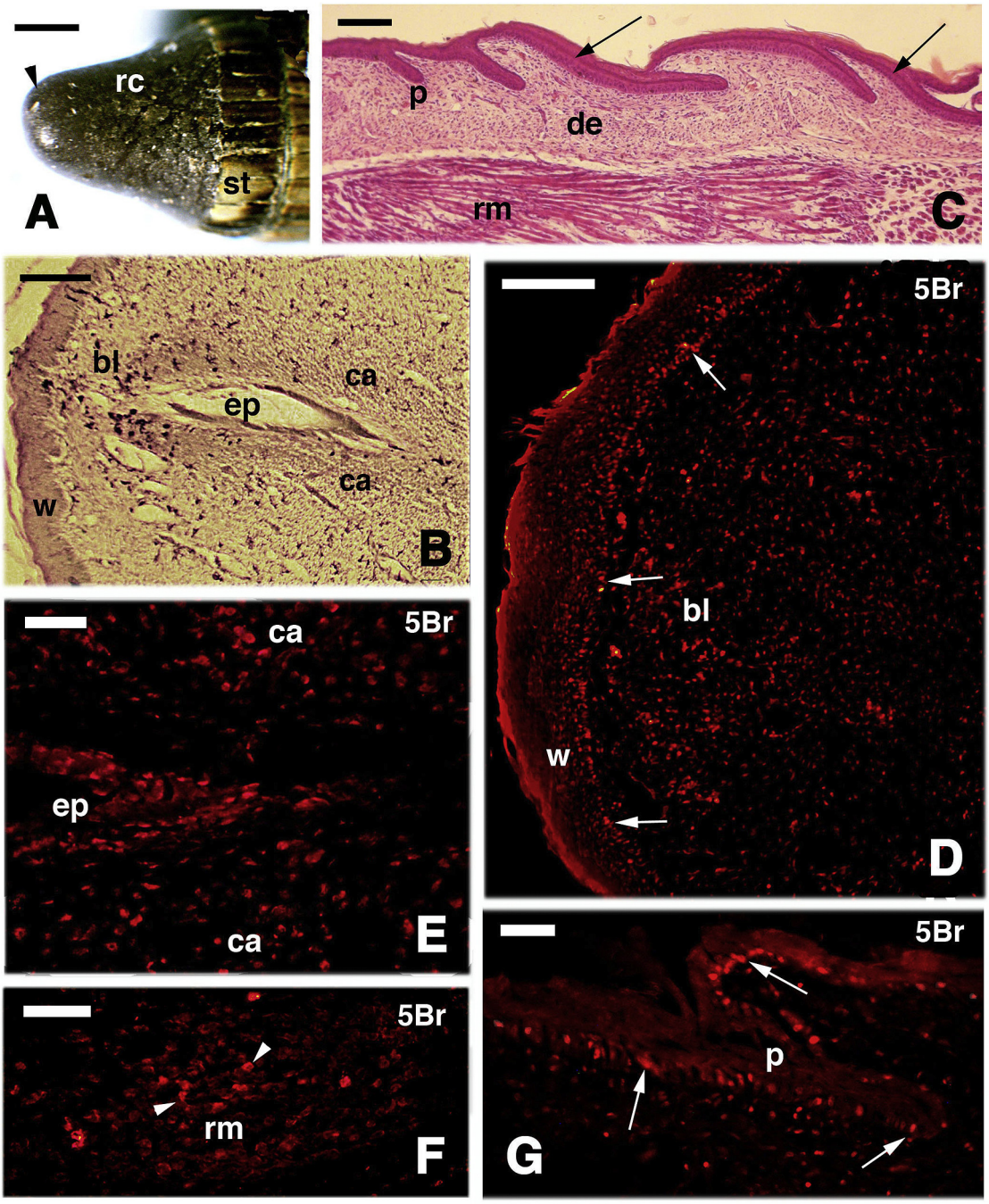

Fig. 1. Macroscopic aspect (**A**), histological structure (**B, C**), and 5BrdU-immunofluorescence (5Brd, **D–G**) of the regenerating tail. **A**, regenerating cone at 16 days post-amputation. The arrowhead points to the apical blastema. Bar, 1 mm **B**, detail of the apical blastema showing the wound epidermis and the ependymal ampulla surrounded by the forming cartilaginous tube. Bar, 50 μm. **C**, epidermal pegs of the regenerating scales become longer in proximal regions where also the new beta-layer is forming (arrows). Bar, 50 μm. **D**, apical blastema showing sparse labeled cells and those in the wound epidermis (arrows on the basal cells). Bar, 50 μm. **E**, ependymal ampulla with numerous labeled cells, also present in the surrounding cartilaginous tissue. Bar, 20 μm. **F**, regenerating myotome with labeled myoblasts (some indicated by arrowheads). Bar, 20 μm. **G**, elongated epidermal peg of regenerating scale showing numerous labeled nuclei in the basal layer (arrows). Bar, 20 μm. **Legends**: bl, blastema; ca, regenerating cartilage; de, forming dermis; ep, ependymal ampulla; rc, regenerated cone; rm, regenerated muscles; p, epidermal peg; st, tail stump (normal tail); w, wound (regenerating) epidermis.

study of the molecular mechanisms determining tail regeneration in lizards, the present account reports the presence of Wnt1-like immunoreactivity in the regenerating tail of lizards using western blotting and light immunocytochemistry. The prevalent localization of this signaling protein in the apical blastema suggests that it is correlated with cell proliferation and maintenance of a growing blastema.

2. Materials and methods

2.1. Animals and fixation

A total of ten adult wall lizards (*Podarcis muralis*) of both sexes were utilized for the study on tissue regeneration in the amputated tails, as previously indicated (Alibardi, 2012). The lizards were col-

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