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Original and regenerating lizard tail cartilage contain putative resident stem/progenitor cells

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ABSTRACT

Regeneration of cartilaginous tissues is limited in mammals but it occurs with variable extension in lizards (reptiles), including in their vertebrae. The ability of lizard vertebrae to regenerate cartilaginous tissue that is later replaced with bone has been analyzed using tritiated thymidine autoradiography and 5BrdU immunocytochemistry after single pulse or prolonged-pulse and chase experiments. The massive cartilage regeneration that can restore broad vertebral regions and gives rise to a long cartilaginous tube in the regenerating tail, depends from the permanence of some chondrogenic cells within adult vertebrae. Few cells that retain tritiated thymidine or 5-bromodeoxy-uridine for over 35 days are mainly localized in the inter-vertebral cartilage and in sparse chondrogenic regions of the neural arch of the vertebrae, suggesting that they are putative resident stem/progenitor cells. The study supports previous hypothesis indicating that the massive regeneration of the cartilaginous tissue in damaged vertebrae and in the regenerating tail of lizards derive from resident stem cells mainly present in the cartilaginous areas of the vertebrae including in the perichondrium that are retained in adult lizards as growing centers for most of their lifetime.

Contents

1. Introduction

During tail regeneration in lizards a massive cartilaginous tube replaces the vertebrae of the original tail (Quattrini, 1954; Werner, 1967; Alibardi and Sala, 1981; Alibardi and Meyer-Rochow, 1989; Bellairs d' and Bryant, 1985; McLean and Vickaryous, 2011; Fisher et al., 2012; Gilbert et al., 2013; Lozito and Tuan, 2015; Fig. 1A). The origin of the new cartilaginous cells is still uncertain, as the new chondroblasts might be derived from the multiplication of cells from the original vertebrae of the stump or they might derive by metaplasia from other connective cells that accumulate over the tail stump to form the regenerative blastema.

Preliminary studies indicated that sparse putative stem cells are present in the tail stump, identified by their long retention of nuclear tritiated thymidine and 5-Bromo-deoxy-uridine. These cells are likely at the origin of the outstanding regenerative ability of the tail in lizards (Alibardi, 2014). However, a large regenerative ability in amniotes, like in anamniotes, is likely related to their previous development and the active somatic growth still

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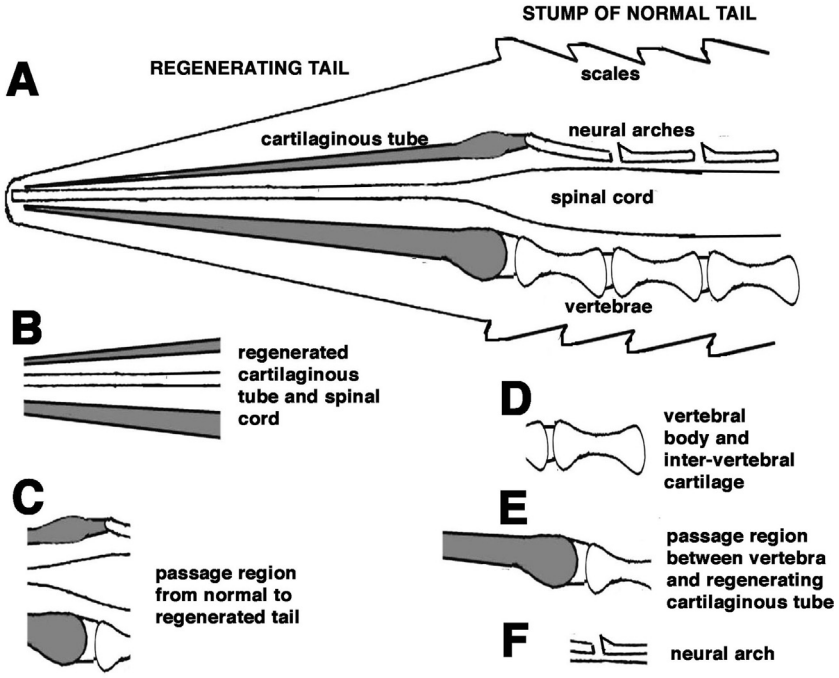

Fig. 1. Schematic drawing of normal tail stump in continuity with the regenerating tail (A). The different regions analyzed in the present study are indicated below (B–F), and serve as orientation and referring anatomical areas for the following figures (see text).

present during adulthood. Lizards, like in other reptiles, grow at different rate during most of their lifespan (Avery, 1994), and it is likely that these reptiles maintain numerous stem cell niches (Weissman, 2000) in body regions where growth is still active (indefinite growth), including in their vertebrae and long bones. Therefore the regenerative ability of their organs during adulthood may simply reflect an over-stimulation of these resident stem cells after injury, that give rise to a larger number of transient amplifying cells in comparison to those that are normally produce in uninjured conditions. Stem cell niches (Weissman, 2000) may instead be reduced in mammals to crucial regions involved with cell replacement (gut, skin, bone marrow) or reproduction (gonads), since in most other organs growth is completely ceased after maturity (definite growth).

Among other tissues the skeletal system grows continuously in lizards and this process is probably related to the presence of progenitor/stem cell niches located in various growing centers of different bones, including the vertebrae. The growth of vertebrae and long bones, and their recovering capability after injury in lizards, likely requires the presence of stem/progenitor cells in their inter-vertebral cartilages, epiphyses and perichondrium/periosteum (Pritchard and Ruzicka, 1950; Haynes, 1969; Alibardi, 2015). In the present study we have analyzed the presence of long label retaining cells in the caudal vertebrae of lizards that may explain the broad repairing process of vertebrae after injuries or tail regeneration (Alibardi, 2010). The study was done using autoradiography for tritiated thymidine labeled cells and through immunohistochemistry for the detection of 5Bromo deoxyuridine-labeled cells at progressive periods from the administration of these cell proliferation markers.

2. Materials and methods

2.1. Sampling, fixation and embedding

The present material was collected and prepared in previous studies using different lizard species and histochemical, autoradiographic and immunohistochemical methods were utilized to detect general glycosamino-glycans, collagen and proliferating cells. The same tissues were here specifically analyzed for details concerning the vertebrae structure and the localization of proliferating cells. Details on the employed methods are reported in the cited papers below, and here we briefly outline these methods.

Normal and regenerating tails at the elongation stage (5–10 mm) from eight wall lizards (*Podarcis sicula*) were fixed and studied histologically as previously presented (Alibardi and Sala, 1981). This study gave detailed histological and histochemical information on the type of bone and cartilaginous tissues present in normal vertebrae of the tail connected to the cartilaginous tube of the regenerated tail. Briefly, the tissues (regenerated tail of 5–10 mm and tail stumps of 2–3 mm in length) were fixed in buffered formaldehyde 10%, dehydrated and embedded in wax. Using a rotative microtome, sections of 7–10 μm in thickness were collected on slides, de-waxed and stained with Haematoxylin-Eosin, Alcian blue 8GX, Alcian Blue-PAS reaction for detecting acidic glycosaminoglicans (Alcian Blue) and glycoproteins/collagen (Periodic Acid of Shiff, PAS) (see details in Alibardi and Sala, 1981).

2.2. Autoradiography

For the autoradiographic study on proliferating cells, five green anole lizards (*Anolis carolinensis*) with regenerating tails of 5–7 mm were injected with tritiated thymidine (10–15 μCi/gBody Weight), and the animals were sacrificed 4 h later to collect the tail tissues (see details in Alibardi 1995). Other garden skinks (*Lampropholis delicata*) with regenerating tails of 3–5 mm in length were injected with tritiated thymidine (10–12 μCi/gBody Weight) in a single pulse, and the lizards were left to regenerate their tails with no further injection (chase) for 12 days ($n = 5$) and 20 days ($n = 5$) before sampling (see details in Alibardi, 1995). The specific procedures adopted for the present autoradiographic detection both for light and electron microscopy have been reported in the original paper cited above. The samples from both lizard species were here re-utilized for the present study, focusing the analysis mainly on the