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SDF-1α/CXCR4 signaling mediates digit tip regeneration promoted by BMP-2

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ABSTRACT

Previously we demonstrated that BMP signaling is required for endogenous digit tip regeneration, and that treatment with BMP-2 or -7 induces a regenerative response following amputation at regeneration-incompetent levels (Yu et al., 2010, 2012). Both endogenous regeneration and BMP-induced regeneration are associated with the transient formation of a blastema, however the formation of a regeneration blastema in mammals is poorly understood. In this study, we focus on how blastema cells respond to BMP signaling during neonatal digit regeneration in mice. First, we show that blastema cells retain regenerative properties after expansion in vitro, and when re-introduced into the amputated digit, these cells display directed migration in response to BMP-2. However, in vitro studies demonstrate that BMP-2 alone does not influence blastema cell migration, suggesting a requirement of another pivotal downstream factor for cell recruitment. We show that blastema cell migration is stimulated by the cytokine, SDF-1α, and that SDF-1α is expressed by the wound epidermis as well as endothelial cells of the blastema. Blastema cells express both SDF-1α receptors, CXCR4 and CXCR7, although the migration response is inhibited by the CXCR4-specific antagonist, AMD3100. Mice treated with AMD3100 display a partial inhibition of skeletal regrowth associated with the regeneration response. We provide evidence that BMP-2 regulates *Sdf-1α* expression in endothelial cells but not cells of the wound epidermis. Finally, we show that SDF-1α-expressing COS1 cells engrafted into a regeneration-incompetent digit amputation wound resulted in a locally enhanced population of CXCR4 positive cells, and induced a partial regenerative response. Taken together, this study provides evidence that one downstream mechanism of BMP signaling during mammalian digit regeneration involves activation of SDF-1α/CXCR4 signaling by endothelial cells to recruit blastema cells.

Introduction

The digit tip is unique in mammals in that it possesses the ability to undergo an epimorphic regenerative response following amputation. The mouse digit tip exhibits a differential regeneration response depending on the amputation position: amputation through the distal level of the terminal phalanx undergoes faithful regeneration, while proximal transection fails to regenerate (Borgens, 1982; Han et al., 2008; Reginelli et al., 1995; Zhao and Neufeld, 1995). This level-specific regeneration property is also observed in human finger tips (Douglas, 1972; Illingworth, 1974). Thus, the mouse digit represents an excellent model system to study mammalian regeneration and is clinically relevant (Allan et al., 2006; Muneoka et al., 2008).

Most of our understanding about the regeneration of tetrapod vertebrate structures has been established from studies using urodele amphibians. For example, newts and salamanders can fully regenerate missing structures such as the tail, jaws, spinal cord, gills, lens, and limbs throughout their entire lifespan (Brockes and Kumar, 2002; Chernoff et al., 2003; Echeverri and Tanaka, 2002). Limb regeneration is accomplished by the formation of an undifferentiated cell population called the blastema at the amputation wound. The blastema is formed underneath the wound epidermis by the migration and dedifferentiation of cells derived from injured stump tissues including dermis, nerves, and skeletal elements during the healing response (Brockes, 1997; Bryant et al., 2002; Thornton, 1968). Based on histological studies the blastema appears to be a homogenous population of mesenchymal cells, however, a recent study shows that it is in fact heterogeneous and composed of different progenitor cell populations, some of which maintain lineage limitations (Kragl et al., 2009). The final stages of limb regeneration involve the transition from blastema cell proliferation to morphogenesis and differentiation to accurately replace only limb structures removed by amputation.

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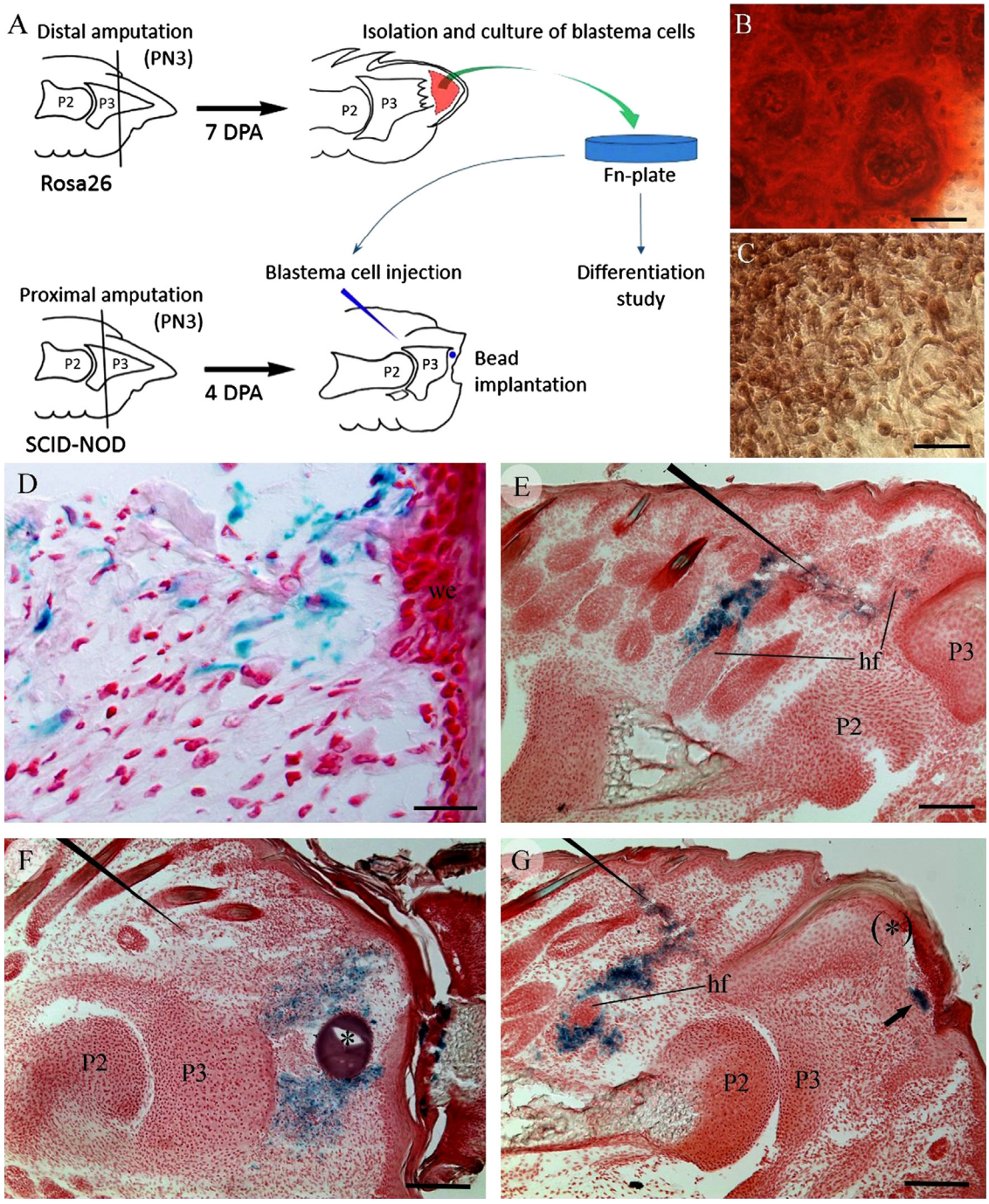

Fig. 1. In vitro analysis of neonatal mouse digit blastema cells. A: Schematic diagram showing the overall procedure of digit amputation, isolation and culture of blastema cells, cell injection and bead implantation. B and C: Blastema cells were induced to differentiate by culturing in osteogenic medium for 17 days. Alizarin red S staining was used to detect osteocytes. Blastema cells were induced to differentiate into osteocytes in osteogenic medium (B), but not in MSC medium (C). D–G: Blastema cells isolated from Rosa26 mice digits were injected into the proximally amputated digits of SCID-NOD mice. Some injected blastema cells (blue) remained at the apical injection site associated with the wound epidermis (we; D), however the majority of injected cells were found aggregated around proximal hair follicles (hf; E). When a BMP-2-soaked bead (*) was implanted underneath the wound epidermis, the injected blastema cells remained at the injection site associated with the BMP-2 bead (F). In control studies involving BSA soaked bead (*), the majority of blastema cells were associated with the hair follicle (G) and a few cells remained at the apical mesenchyme (arrow). The bead position in G is out of the plane of section but approximated by the asterisk. We note that the blastema cells found associated with the hair follicles in E and G were not localized along the injection trajectory (long arrow heads in E–G). Distal is toward the right in D–G. Scale bars: 100 μm in B and C; 50 μm in D; 200 μm in E–G.

Mouse digit tip regeneration is also associated with blastema formation. The blastema of the regenerating digit tip shows high mitotic activity and re-activation of development-relevant genes such as *Msx*, *Dlx*, and *Bmps* (Fernando et al., 2011; Lehoczky et al., 2011; Muneoka et al., 2008). Like the urodele limb blastema, the regenerating mouse digit gives rise to a blastema that is heterogeneous and contains multiple cell types including fibroblasts, osteoprogenitor cells, and endothelial progenitor cells (Fernando et al., 2011; Muneoka et al., 2008). Recent studies provide evidence that the regenerated digit tip forms from cells that are lineage restricted and that cells derived from a number of different tissue types participate in regeneration but display no plasticity (Lehoczky et al., 2011; Rinkevich et al., 2011; Takeo et al., 2013; Wu et al., 2013). The cellular heterogeneity of the digit blastema coupled with the organized differentiation of regenerating tissues suggests that active and directed cell migration is likely to play a critical role during the regenerative response. Indeed, cell

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