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Review Article

Reaching the brain: Advances in optic nerve regeneration

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ABSTRACT

The optic nerve has been widely used to investigate factors that regulate axon regeneration in the mammalian CNS. Although retinal ganglion cells (RGCs), the projection neurons of the eye, show little capacity to regenerate their axons following optic nerve damage, studies spanning the 20th century showed that some RGCs can regenerate axons through a segment of peripheral nerve grafted to the optic nerve. More recently, some degree of regeneration has been achieved through the optic nerve itself by factors associated with intraocular inflammation (oncomodulin) or by altering levels of particular transcription factors (Klf-4, -9, c-myc), cell-intrinsic suppressors of axon growth (PTEN, SOCS3), receptors to cell-extrinsic inhibitors of axon growth (Nogo receptor, LAR, PTP-σ) or the intracellular signaling pathway activated by these receptors (RhoA). Other regulators of regeneration and cell survival continue to be identified in this system at a rapid pace. Combinatorial treatments that include two or more of these factors enable some retinal ganglion cells to regenerate axons from the eye through the entire length of the optic nerve and across the optic chiasm. In some cases, regenerating axons have been shown to innervate the appropriate central target areas and elicit postsynaptic responses. Many discoveries made in this system have been found to enhance axon regeneration after spinal cord injury. Thus, progress in optic nerve regeneration holds promise not only for visual restoration but also for improving outcome after injury to other parts of the mature CNS.

Contents

1. [Introduction]

The optic nerve is an integral part of the central nervous system (CNS) by virtue of its embryonic origin and cellular composition, and like most CNS pathways in mature mammals, it cannot regenerate if injured. Consequently, victims of traumatic or ischemic nerve injury or degenerative conditions such as glaucoma suffer irreversible losses of

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vision. Because of its unique accessibility, relatively simple anatomy, and functional importance, the optic nerve has become one of the principal systems for investigating factors that suppress or enable axon regeneration in the CNS.

The axons that course through the optic nerve derive from multiple subtypes of retinal ganglion cells (RGCs) that are tuned to distinct features of the visual world (Roska and Meister, 2014). These axons project to brainstem nuclei that relay image-forming information on to the cortex (dorsal lateral geniculate nucleus, dLGN, and superior colliculus, SC), mediate orienting responses (SC), or regulate circadian entrainment (suprachiasmatic nucleus, SCN), the pupillary light reflex (olivary pretectal nucleus, OPN), retinal image stabilization (nuclei of the accessory optic system), and other functions.

2. Developmental regulation of intrinsic growth capacity: role of Klf transcription factors

During the initial formation of visual projections, RGCs extend axons rapidly in vivo or when placed in culture (Chen et al., 1995; Goldberg et al., 2002a; So et al., 1981), and can regenerate injured axons in vivo, at least over short distances (Chen et al., 1995). The capacity for rapid axonal growth and regeneration are both lost in the early postnatal period at about the time when RGCs increase dendritic growth in the retina and synaptic inputs expand (Fig. 1A). Cell culture experiments reveal that this loss is triggered by contacts between RGCs and amacrine cells, one of the principal types of retinal inter-neurons (Fig. 1B: Goldberg et al., 2002b). These results suggest that the developmental decline in RGCs' regenerative ability is related in part to a diminished intrinsic capacity for rapid axonal growth and is triggered by non cell-autonomous factors.

The developmental decline in RGCs' capacity for rapid axon growth is associated with numerous changes in gene expression (Moya et al., 1988; Wang et al., 2007). Of particular interest are the changes that occur in Krüppel-like factor (Klf) transcription factors (Fig. 1C). Diminished growth potential is associated with increased expression of Klf-4 and -9 which, when overexpressed in cell culture, suppress neurons' capacity for axon growth (Moore et al., 2009). At the same time, Klf-6 and -7, which are positive regulators of axon growth in culture (Moore et al., 2009) and of optic nerve regeneration in vivo in zebrafish (Veldman et al., 2007), show a decline in expression (Fig. 1D). Genetic deletion of the *klf4* gene or acute knock-down in mature mice promotes axon regeneration following optic nerve injury, in part by de-repressing signalling through the Jak-STAT pathway (Moore et al., 2009; Qin et al., 2013). Knock-down of Klf-9 has even more dramatic effects on regeneration (Apara et al., 2014). Importantly, manipulating Klf family member expression also enhances axon regeneration in spinal cord projection neurons in vivo (Blackmore et al., 2012). These results suggest that the pathways regulated by KLF transcription factors may be important for the regulation of regenerative potential throughout the adult mammalian CNS.

3. Axon regeneration in a peripheral nerve and CNS environment

Although mature mammalian RGCs are normally unable to regenerate axons through the optic nerve itself, a modest number of RGCs exhibit robust axon regeneration when given the opportunity to grow

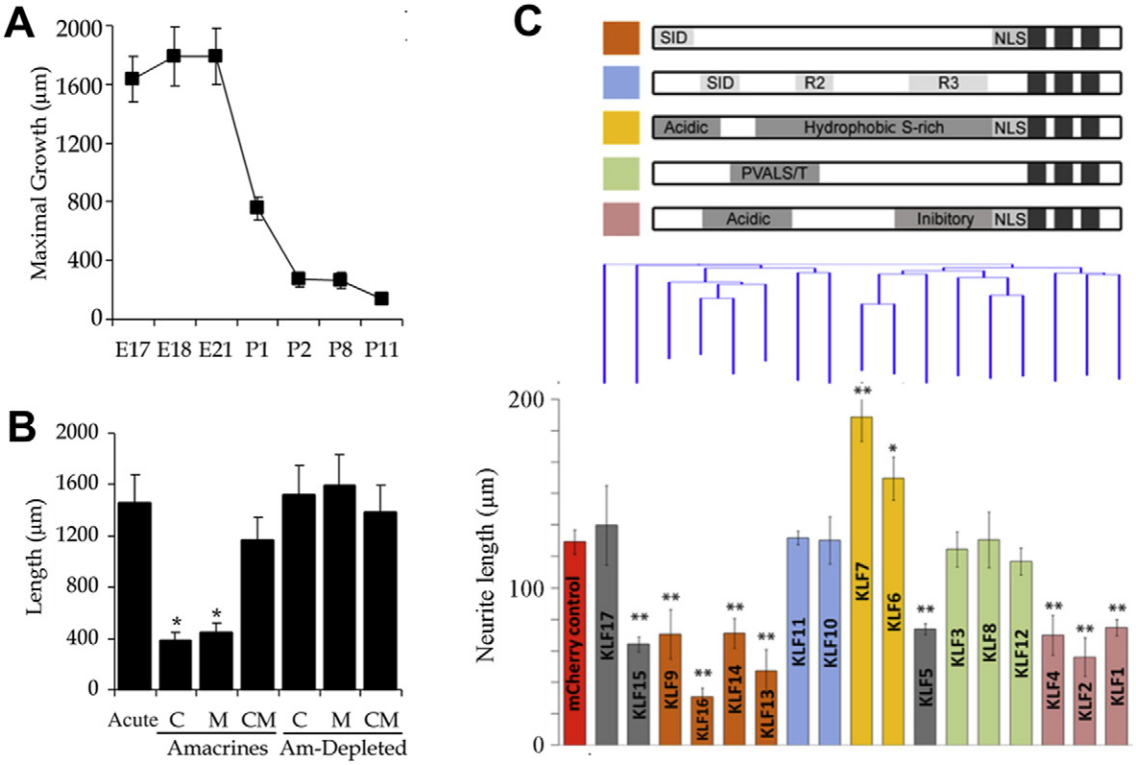

Fig. 1. Amacrine cells and Krüppel-like family (KLF) transcription factors contribute to the developmental decline in retinal ganglion cells' intrinsic growth potential. (A) When placed in culture, RGCs show a dramatic decline in rate of axon growth between embryonic day 17 (E17) and postnatal day 11 (P11). RGCs were isolated by immunopanning and cultured under permissive conditions. (B) Amacrine cells regulate RGCs' growth state. E20 RGCs were either acutely purified (left bar, positive control) or aged 3–4 days with components of immunopurified amacrine cells or of the remaining retinal cells (Am-depleted). Incubation was carried out in the presence of whole cells (C), cell membranes (M), or conditioned medium (CM) from these cell populations. RGCs were then replated and evaluated for axon outgrowth after 3 days. *P < 0.05 compared to positive controls. (C) Role of Klf transcription factors in regulating axon growth. *Top*: Klf family members grouped according to structural domains. *Bottom*: P5 cortical neurons were transfected with individual KLFs and grown for 3 days on laminin. Graph shows average neurite length. *P < 0.05, **P < 0.01 compared to transfection control (red). (Goldberg et al., 2002b; Moore et al., 2009).