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REVIEW

The expression and function of midkine in the vertebrate retina

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The functional role of midkine during development, following injury and in disease has been studied in a variety of tissues. In this review, we summarize what is known about midkine in the vertebrate retina, focusing largely on recent studies utilizing the zebrafish (*Danio rerio*) as an animal model. Zebrafish are a valuable animal model for studying the retina, due to its very rapid development and amazing ability for functional neuronal regeneration following neuronal cell death. The zebrafish genome harbours two *midkine* paralogues, *midkine-a* (*mdka*) and *midkine-b* (*mdkb*), which, during development, are expressed in nested patterns among different cell types. *mdka* is expressed in the retinal progenitors and *mdkb* is expressed in newly post-mitotic cells. Interestingly, studies of loss- and gain-of-function in zebrafish larvae indicate that midkine-a regulates cell cycle kinetics. Moreover, both *mdka* and *mdkb* are expressed in different cell types in the normal adult zebrafish retina, but after light-induced death of photoreceptors, both are up-regulated and expressed in proliferating Müller glia and photoreceptor progenitors, suggesting an important and (perhaps) coincident role for these cytokines during stem cell-based neuronal regeneration. Based on its known role in other tissues and the expression and function of the *midkine* paralogues in the zebrafish retina, we propose that midkine has an important functional role both during development and regeneration in the retina. Further studies are needed to understand this role and the mechanisms that underlie it.

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Abbreviations
ALK, anaplastic lymphoma kinase; Ascl1a, ascl1a achaete-scute complex-like 1a; BrdU, bromodeoxyuridine; CMZ, ciliary marginal zone; GCL, ganglion cell layer; HC, horizontal cell; hpf, hours post-fertilization; hpl, hours post-lesion; Id2a, inhibitor of DNA binding 2a; INL, inner nuclear layer; Insm1a, insulinoma-associated 1a; LRP1, low density lipoprotein receptor-related protein 1; mdka, midkine-a; mdkb, midkine-b; ONL, outer nuclear layer; Ptn, pleiotrophin; Ptp99A, protein tyrosine phosphatase 99A; RPTPβ/ζ, receptor protein tyrosine phosphatase β/ζ

Introduction

The retina is a complex neural circuit that converts photons of light into electrical impulses, which encode and process the images cast on the retina (Dowling, 2012). The retina has a long history of serving as a model tissue for discovering the mechanisms that govern brain development (Agathocleous and Harris, 2009), for investigating synaptic function (Wei and Feller, 2011) and for developing therapeutic approaches to treating brain injury and disease (Seiler and Aramant, 2012). Among vertebrates, retinal structure and function are evolutionarily very highly conserved. The retina is a laminated tissue, formed by the orderly arrangement of six classes of neuronal cell bodies, their processes and synaptic interconnections, and one specialized type of glial cell, the Müller glia, a radial glial cell that is unique to the retina and functions, in part, similarly to astrocytes in other CNS regions (Figure 1). Neuronal cell types consist of two classes of

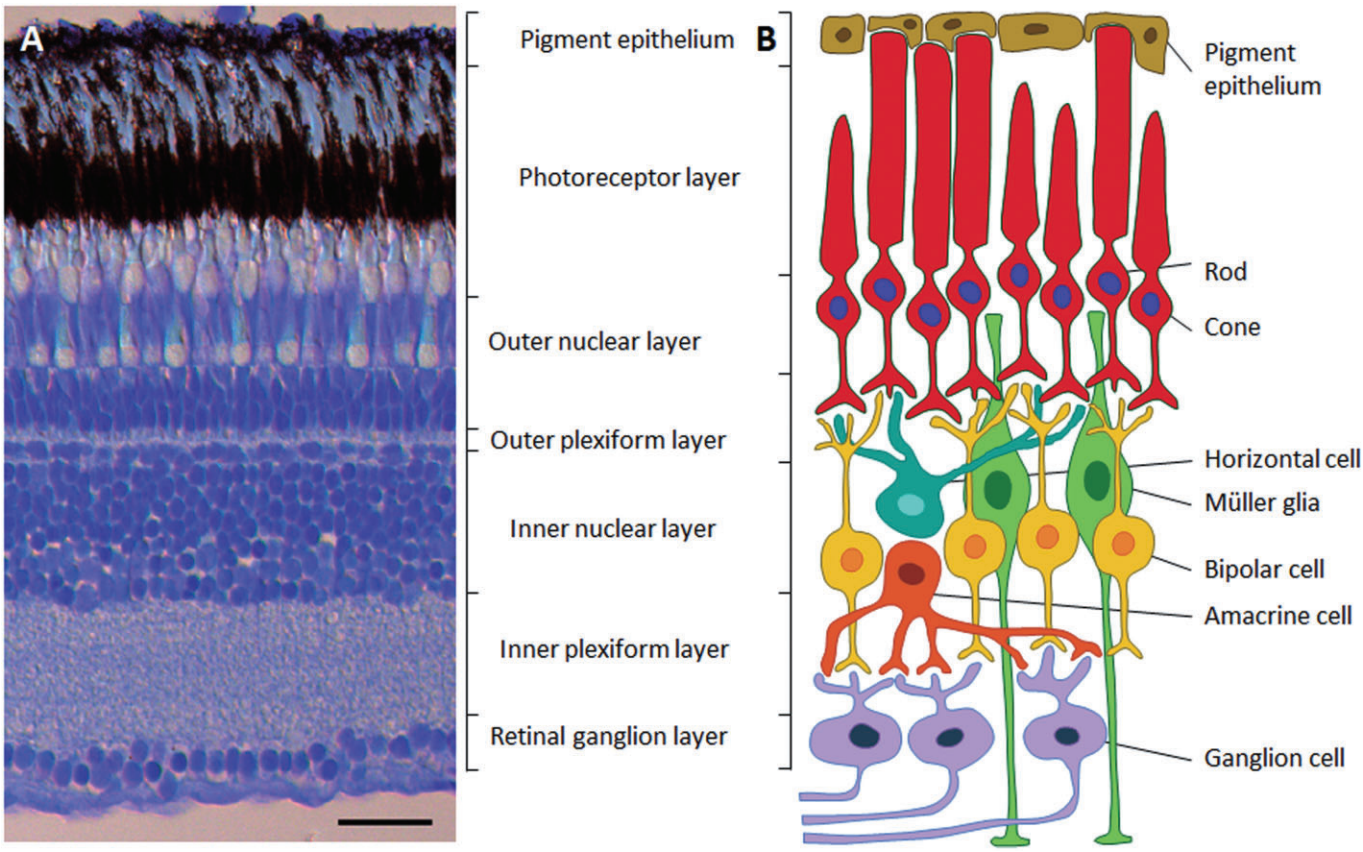

Figure 1

Structure of the retina. A: Microphotograph of a cross-section through the retina of an adult zebrafish, showing the different cellular and synaptic retinal layers. B: Diagram of the neural circuit of the retina, showing the six neuronal cell types and the two supporting cell types (Müller glia and retinal pigmented epithelium). In A, the scale bar = 25μm.

photoreceptors (rods that detect low light intensities and cones that encode colour and function during daytime vision), three classes of interneurons [horizontal cells (HCs), bipolar cells and amacrine cells], and ganglion cells, which send axons out of the eye to visual centres in the brain.

Midkine and pleiotrophin (Ptn) are the only two members of a family of small heparin-binding neurotrophic factors. Both cytokines perform multiple functions in developing and adult tissues, including the brain, and also in a variety of tissues following injury. Midkine was first discovered as a retinoic acid-inducible gene in embryonal carcinoma cells (Kadomatsu *et al.*, 1988). In the mouse, midkine is highly expressed during mid-gestation and is associated with epithelial–mesenchymal interactions (Mitsiadis *et al.*, 1995). In the developing brain, both midkine and Ptn are localized to the processes of radial glia, upon which neural progenitors both migrate and differentiate (Kadomatsu and Muramatsu, 2004; Muramatsu, 2010), supporting an important role of these cytokines in mouse neural development. Also within the nervous system, protective and reparative roles have been attributed to midkine (Muramatsu, 2011). For example, midkine knockout mice show a delay in axonal degeneration and regeneration in injured peripheral nerves (Sakakima *et al.*, 2009) and midkine expression is up-regulated following ischaemia in the rat retina (Miyashiro *et al.*, 1998) and brain (Ishikawa *et al.*, 2009), perhaps protecting against the effects of ischaemia within these tissues (Ooboshi, 2011). Similarly, Ptn plays a neuroprotective role in the nigro-striatal pathway, in Parkinson's disease (Marchionini *et al.*, 2007) and also following neurotoxicity induced by drugs of abuse (Gramage *et al.*, 2010). Specifically, Ptn knockout mice are more vulnerable to the amphetamine-induced damage in the dopaminergic neurons of the substantia nigra and their axons in the striatum (Gramage *et al.*, 2010; see Herradón and Pérez-García, 2013). Midkine and Ptn are up-regulated in spinal motor neurons after injury (Sakakima *et al.*, 2004), in response to exposure to drugs of abuse, in both animal models and humans (Ezquerra *et al.*, 2007; Flatscher-Bader and Wilce, 2008) and in the degenerating substantia nigra of Parkinson's disease patients (Marchionini *et al.*, 2007). These and other studies provide evidence for a role for both midkine and Ptn in ameliorating the effects or effecting repair after injury to nervous tissue.

In contrast to mammals, which have a single unique *midkine* gene, the zebrafish genome encodes two *midkine* paralogues, *midkine-a* (*mdka*) and *midkine-b* (*mdkb*). These paralogues share 68% amino acid identity and are likely to result from a genome duplication event during the evolution-

ary history of teleosts (Winkler *et al.*, 2003). In the zebrafish brain, the expression of *mdka* and *mdkb* is differentially regulated during development (see below) and in adulthood. Interestingly, both paralogues are up-regulated during regeneration of multiple tissues and organs (Lien *et al.*, 2006; Schebesta *et al.*, 2006; Calinescu *et al.*, 2009a; Fujisawa *et al.*, 2011; Grotek *et al.*, 2013; Parente *et al.*, 2013), including the retina, suggesting that in fish, as in mammals, these cytokines also function to modify or repair tissue-specific injuries.

Role of midkine during retinal development

The retina develops from a neuroepithelial sheet comprised of undifferentiated progenitor cells undergoing active proliferation. As for all complex organs, cellular differentiation in the retina is governed by complex signalling events (see Agathocleous and Harris, 2009). In zebrafish, neuronal differentiation in the retina is amazingly rapid (Stenkamp, 2007). Retinal progenitors first begin to withdraw from the cell cycle and initiate programmes of differentiation around 28–32 h post-fertilization (hpf; Schmitt and Dowling, 1996; Hu and Easter, 1999). The initial differentiation begins within a nasoventral patch. This is then followed by waves of differentiation that, analogous to the hands on a clock face, sweep circumferentially from ventronasal to ventrotemporal domains (Easter and Malicki, 2002). During retinal morphogenesis in zebrafish, the expression of *mdka* and *mdkb* are differentially regulated (Calinescu *et al.*, 2009a; Figure 2). Starting at about 30 hpf (Calinescu *et al.*, 2009a), *mdka* is expressed in the mitotic retinal progenitors, though the level of expression appears greatest at the ciliary marginal zone (CMZ; Figure 2), which contains the retinal stem cell niche (Raymond *et al.*, 2006). As retinal morphogenesis proceeds, *mdka* expression is down-regulated in cells that exit the cell cycle. Starting at about 72 hpf and persisting through about 120 hpf, *mdka* is transiently expressed in Müller glia. The final adult pattern emerges between 72 and 120 hpf and consists of the exclusive expression of *mdka* in HCs (cf. Figures 2 and 3A). In contrast, *mdkb* expression temporally lags behind that of *mdka*, and *mdkb* is expressed in newly post-mitotic cells within the inner nuclear and ganglion cell layers (GCLs). This expression pattern persists into and throughout adulthood (Calinescu *et al.*, 2009a; Figure 3B).

The cellular expression of *mdka* in the embryonic retina suggests that during retinal development, midkine-a (Mdka) may govern aspects of retinal neurogenesis. With this as an underlying hypothesis, Luo *et al.* (2012) used reverse genetics approaches to investigate the function of Mdka during early retinal development in the zebrafish. This study found that Mdka regulates proliferation among retinal progenitors by governing cell cycle kinetics (Luo *et al.*, 2012). When Mdka synthesis is blocked with *mdka*-targeted morpholino-oligonucleotides, retinal progenitors fail to exit the cell cycle and neuronal differentiation is delayed, although the underlying neurogenic programme within the retina is initiated at the correct developmental stage. This delay in neuronal differentiation following Mdka loss-of-function results from the increased length of the cell cycle, largely a consequence of an increase in the duration of the S-phase. The delay of neuronal differentiation results in a mild microphthalmia. Complementing the results from Mdka loss-of-function, overexpression of Mdka in a line of transgenic fish carrying an inducible *mkda* allele results in an acceleration of the cell cycle, an excess number of retinal progenitors and a mild macrophthalmia. This acceleration of the cell cycle does not lead to premature cell cycle exit, however. Expression levels of several core cell cycle regulators remain unchanged after

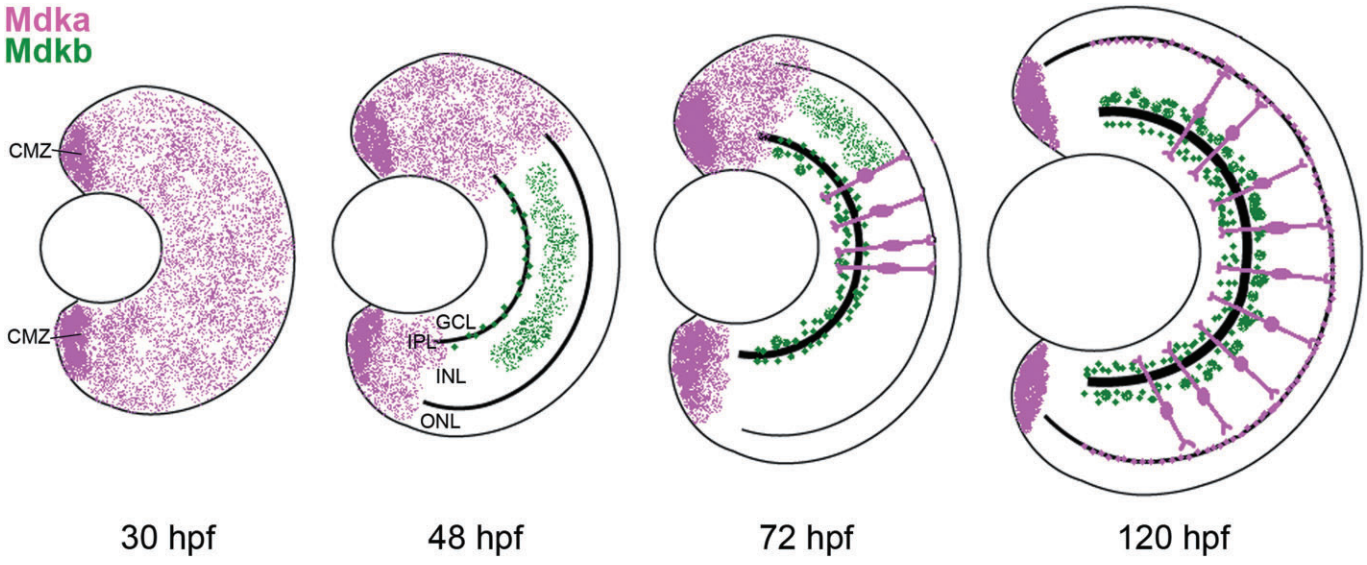

Figure 2

Expression of *mdka* and *mdkb* during retinal development. *mdka* (pink) is expressed in proliferating retinal progenitors, whereas *mdkb* (green) is expressed in newly post-mitotic cells. Between 72 and 120 hpf, *mdka* is transiently expressed in Müller glia.

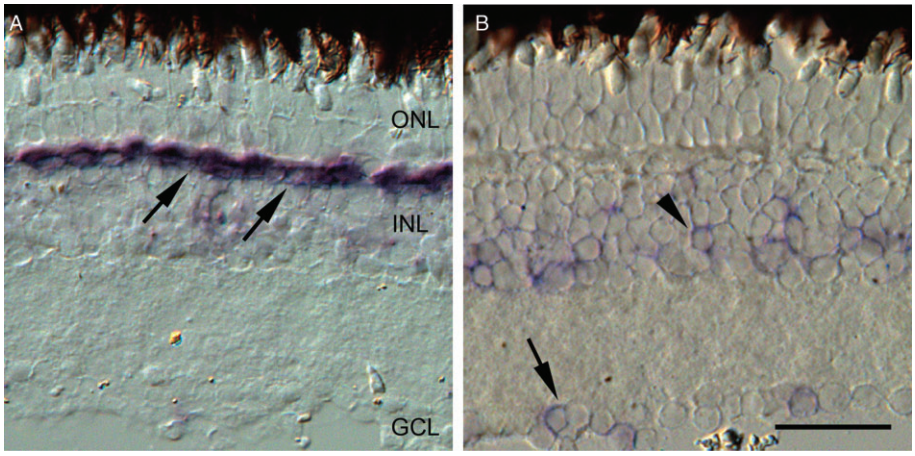

Figure 3

Expression of *mdka* and *mdkb* in the adult zebrafish retina. A: *In situ* hybridization showing the expression of *mdka* in horizontal cells (arrows). B: *In situ* hybridization showing the expression of *mdkb* in amacrine cells (arrowhead) in the inner tier of the inner nuclear layer and ganglion cells (arrow). GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer. Scale bar = 25μm.

Mdka loss- or gain-of-function, though the possibility exists that Mdka could regulate the translation, duration or phosphorylation state of these proteins.

The retinal phenotypes observed following Mdka loss- and gain-of-function were reminiscent of those observed following loss- and gain-of-function of the putative transcription factor-binding protein, Id2a (inhibitor of DNA binding 2a) (Uribe and Gross, 2010). Id2a loss-of-function lengthens the cell cycle, delays neuronal differentiation and leads to microphthalmia (Uribe and Gross, 2010), whereas Id2a gain-of-function shortens the cell cycle, accelerates the S- to M-phase transition and leads to a mild macrophthalmia. Testing for potential epistatsis between Mdka and Id2a found that Id2a functions downstream of Mdka in a shared signalling pathway. Injecting *id2a* mRNA into embryos is sufficient to rescue the Mdka loss-of-function and restores the normal timing of cellular differentiation, and systematically altering Mdka levels induces corresponding changes in the expression of *id2a* (Luo *et al.*, 2012). Based on these data, it was concluded that Mdka and Id2a reside in a shared signalling pathway that functions to govern cell cycle kinetics and neuronal differentiation in the vertebrate retina. There were slight differences in the retinal phenotypes in embryos following alterations in the expression of Mdka and Id2a, respectively; however, the basis for these phenotypic differences awaits further clarification.

Unlike *mdka*, the function of *mdkb* during retinal development is completely unexplored. Ubiquitous Mdkb gain-of-function results in the absence of eyes or severely reduced eye size (Winkler and Moon, 2001; Lim *et al.*, 2013) and is likely to be due to secondary effects resulting from the development defects in the forebrain. In future studies, it will be important to develop tissue-specific approaches to knockdown or overexpress Mdkb in the retina in order to examine its functional role in the developing retina.

The function of midkine in the developing mammalian retina has yet to be investigated. The evidence that midkine may be functionally important, however, is based on a screen for transcripts expressed in CNS progenitors in the mouse (Livesey *et al.*, 2004). In this study, *midkine* was identified as a member of the core set of transcripts enriched in retinal progenitors. Given the functional role of Mdka in the zebrafish retina, one can hypothesize that in the developing retina (and brain) of mammals, midkine may also play an important functional role governing neurogenesis.

Interestingly, Ptn, the other member of this family of heparin-binding growth factors, is expressed in the post-natal rat retina and functions to determine the fates of late-born neurons (Roger *et al.*, 2006). Ptn is expressed in retinal progenitors within the outer neuroblastic layer and post-mitotic cells within the inner nuclear layer (INL). The overexpression of Ptn diminishes the genesis of rod photoreceptors and promotes the genesis of bipolar cells.

Finally, *miple*, the *Drosophila* orthologue of vertebrate *midkine/pleiotrophin*, plays a role in the development of the invertebrate eye (Muñoz-Soriano *et al.*, 2013). The compound eye of *Drosophila* is a highly organized structure composed of about 750 ommatidia, each containing a cluster of eight photoreceptor cells (Hsiao *et al.*, 2012). Each cluster of photoreceptors develops from a precluster of five cells that, as the photoreceptors differentiate, undergo a precise 90° rotation, creating a mirror image between the dorsal and ventral ommatidia along the dorsal–ventral midline (Wolff and Ready, 1993; Mlodzik, 1999). Miple regulates this ommatidial rotation by binding to the midkine receptor Ptp99A (protein tyrosine phosphatase 99A) (Muñoz-Soriano *et al.*, 2013), the *Drosophila* orthologue of PTPζ (see below).

The expression of midkine in adult zebrafish retina

In the retina of the adult zebrafish, as in the developing retina, the expression of *mdka* and *mdkb* is differentially regulated. *mdka* is expressed exclusively in HCs, whereas

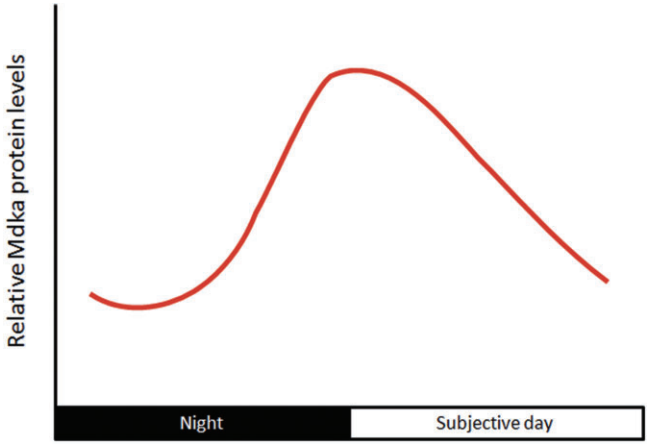

Figure 4

Circadian regulation of Mdka levels in the adult zebrafish retina. The expression of Mdka increases in anticipation of light onset and decreases throughout the daylight hours, reaching a minimum during the night.

mdkb is more broadly expressed in cells within the inner nuclear and GCLs (Calinescu *et al.*, 2009a; Figure 3). In the midst of studies determining the adult expression of these midkine paralogues, it was discovered that the expression of *mdka* (and possibly of *mdkb*) is regulated by circadian rhythms (Calinescu *et al.*, 2009b). Circadian rhythms are biological oscillations entrained by the environmental light/dark cycle, and the regulatory and metabolic networks that direct the body's adjustments to variations of external and internal environment (Anderson *et al.*, 2013). Using both *in situ* hybridization and Western blot analysis, it was discovered that both Mdka mRNA and protein reach peak levels shortly after light onset, and the levels of both decrease as the day progresses, reaching a minimum several hours after the onset of darkness (Figure 4). It was confirmed that this pattern of expression is governed by circadian rhythms, because the cyclical expression of both mRNA and protein persists for at least 48 h in the complete absence of light. The expression of *mdkb* might also be governed by circadian rhythms, but the data supporting this conclusion were less consistent than for Mdka (Calinescu *et al.*, 2009b). It is unknown if the expression of midkines in other brain regions of zebrafish is similarly governed by circadian rhythms, but this possibility must be considered when interpreting the significance of experimentally induced changes in levels of expression.

The function of midkine in neuroprotection and retinal regeneration

It is well established that midkine is involved in the protection and repair in a variety of neural tissues (Muramatsu, 2010). The protective role of midkine in the CNS was first demonstrated in the retina, where midkine promotes photoreceptor survival following constant light exposure (Unoki *et al.*, 1994). In rats, an intraocular injection of midkine is sufficient to rescue photoreceptors that would otherwise be killed by exposure to constant light. This rescue of the photoreceptors is both anatomical and functional (Masuda *et al.*, 1995). Interestingly, the normal levels of endogenous midkine in the rat retina are up-regulated after pressure-induced retinal ischaemia (Miyashiro *et al.*, 1998). The fact that midkine is up-regulated after retinal damage and prevents light-induced death of photoreceptors leads to the hypothesis that in the injured nervous system, midkine might play an important role in neuroprotection.

Although basic retinal structure and function are evolutionarily conserved among all vertebrates, there exist striking differences between vertebrate species in their ability to regenerate retinal neurons following injury (Karl and Reh, 2010). The mammalian retina has a negligible ability for neuronal regeneration, but retinas of non-mammalian vertebrates, amphibians and fish (and birds to a lesser extent), can fully regenerate the retina and restore function (Hitchcock and Raymond, 2004). For zebrafish, any lesion that kills retinal neurons is sufficient to induce complete neuronal regeneration (see Hitchcock *et al.*, 1992; Fausett *et al.*, 2008; Sherpa *et al.*, 2008; Montgomery *et al.*, 2010). This regenerative neurogenesis relies upon Müller glia, which also serves as the intrinsic retinal stem cell. Regardless of the nature of the injury, the response of Müller glia and their progeny is relatively stereotyped (Raymond *et al.*, 2006). At about 24 h post-lesion (hpl), Müller glia re-enter the cell cycle. Progeny from individual Müller glia then divide rapidly to form neurogenic clusters that envelope the parent Müller glia. This proliferative phase peaks at about 72–80 hpl (Figure 5). Retinal progenitors within neurogenic clusters then migrate to the sites of cell death, exit the cell cycle and differentiate to replace the missing neurons (Hitchcock *et al.*, 1992; Fausett and Goldman, 2006; Bernardos *et al.*, 2007; Thummel *et al.*, 2008; Nelson and Hyde, 2010). Each type of regenerated neuron then re-establishes its characteristic synaptic connections (Hitchcock and Cirenza, 1994; Hitchcock, 1997).

One of the most utilized injury paradigms is a photolytic lesion that selectively kills photoreceptors (Vihtelic *et al.*, 2006; Taylor *et al.*, 2012; Thomas *et al.*, 2012; Figure 5). The advantage of this approach is that light-induced damage selectively kills only photoreceptors and leaves other neuronal classes undamaged. The selective death of photoreceptors serves as a model for aspects of human photoreceptor dystrophies. As for all retinal injuries in zebrafish, the death of photoreceptors (Figure 5A–D) stimulates Müller glia to de-differentiate, enter the cell cycle and give rise to photoreceptor progenitors that migrate to the depleted outer nuclear layer (ONL) (Figure 5E). These progenitor cells then exit the cell cycle and differentiate into rod and cone photoreceptors. Interestingly, the order of photoreceptor regeneration (cone regeneration precedes rod regeneration) recreates the temporal pattern of photoreceptor genesis during retinal development (Raymond *et al.*, 2006).

Relatively little is known about the molecular mechanisms that govern the ability of Müller glia to de-differentiate and re-enter the cell cycle, though this is presently being intensely investigated (Qin *et al.*, 2009; Calinescu *et al.*, 2009a; Craig *et al.*, 2010; Meyers *et al.*, 2012; Nelson *et al.*,

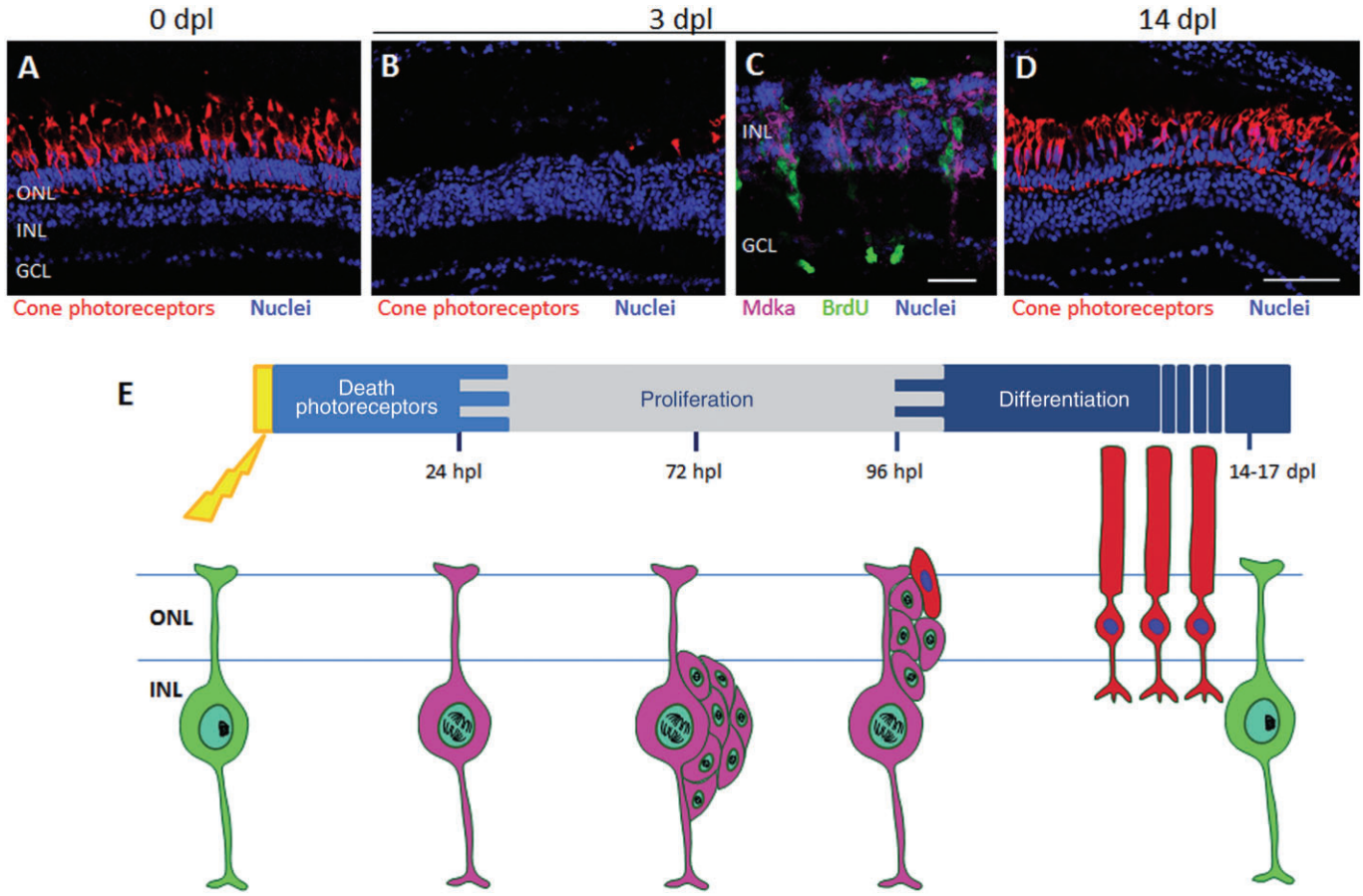

Figure 5

Midkine expression in zebrafish retinal regeneration. A: Cone photoreceptors are immunolabelled (red signal) in an unlesioned retina. B: Photoreceptors are nearly completely killed following exposure to a photolytic lesion. C: *In situ* hybridization showing that *mdka* is expressed in proliferating photoreceptor progenitors (pink label) following photoreceptor death. The *mdka* message co-localizes with antibody staining for bromodeoxyuridine (BrdU, green signal). D: Fourteen days following the onset of a photolytic lesion, rod (not shown) and cone photoreceptors (red signal) are regenerated. E: Timeline of the different processes that occur in the adult zebrafish retina after light-induced damage and the proliferative response of Müller glia. The pink colour represents the onset and duration of *mdka* expression in Müller glia and photoreceptor progenitors. Scale bar = 25 μm (C) and 50μm (A, B and D). GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; dpl: days post-lesion.

2012; 2013; Ramachandran *et al.*, 2012; Wan *et al.*, 2012). Studies to date indicate that dying neurons are likely to signal to Müller glia their imminent demise, and key transcriptional regulators and signalling pathways within Müller glia govern their ability to re-enter the cell cycle. For example, it was recently discovered that dying photoreceptors synthesize TNF-α (Nelson *et al.*, 2013), which serves as a potential paracrine signal to Müller glia (see also Rattner and Nathans, 2005; Zhou *et al.*, 2012). Similarly, recent studies have shown that induction of the transcription factors ascl1a achaete-scute complex-like 1a (Ascl1a) (Ramachandran *et al.*, 2010), Stat3 (Nelson *et al.*, 2012) and Insm1a (insulinoma-associated 1a) (Ramachandran *et al.*, 2012) are required for Müller glia to re-enter the cell cycle.

Though they have yet to be studied experimentally, midkines are hypothesized to also be important in neuronal regeneration in the zebrafish retina. The presence of *midkines* in the zebrafish retina was first discovered in a microarray screen for genes whose expression is induced by photoreceptor death and during photoreceptor regeneration (Calinescu *et al.*, 2009a). The microarray data were validated both by qRT-PCR and *in situ* hybridizations. Following photoreceptor death, *mdka* expression expands from its restricted expression in HCs (Figure 3A) to cells throughout the INL and ONL (Figure 5C). Similarly, *mdkb* expression expands from the inner tier of the INL to cells throughout this cellular layer, including HCs (not shown). As shown by *in situ* hybridization, this broadening of the expression within the INL appears to be at a relatively low level. Markedly, however, the expression of both midkine paralogues is induced at high levels in Müller glia that have re-entered the cell cycle and in dividing photoreceptor progenitors (Figure 5C,E). This *de novo* induction of *mdka* and *mdkb* in Müller glia suggests an important role for these cytokines in photoreceptor regeneration. As discussed above, separate functions have been identified for Mdka and Mdkb in the developing brain of zebrafish (Liedtke and Winkler, 2008; Luo *et al.*, 2012). The fact that both paralogues of *midkine* have coincident expression in the

proliferating Müller glial suggests that both cytokines may govern common cellular functions during neuronal regeneration. Further studies are needed to understand the function of these midkines during retinal regeneration.

Midkine receptors in the retina

As a growth factor, midkine exerts its function through binding with a variety of receptors (see Sakamoto and Kadomatsu, 2012), including anaplastic lymphoma kinase (ALK) (Stoica *et al.*, 2002), low density lipoprotein receptor related protein 1 (LRP1) (Muramatsu *et al.*, 2000), integrins (Muramatsu *et al.*, 2004), Notch2 (Huang *et al.*, 2008), and cell surface proteoglycans such as syndecan, glypican (Kurosawa *et al.*, 2001), neuroglycan-C (Ichihara-Tanaka *et al.*, 2006) and the receptor protein tyrosine phosphatase β/ζ (RPTP β/ζ) (Maeda *et al.*, 1999).

Although all the proposed midkine receptors are expressed in the vertebrate retina (Table 1), at present it remains unclear which receptor, or combination of receptors (see below), transduce midkine function in this tissue. One possibility is that, as in other tissues, ALK is involved in the midkine-induced proliferation (Reiff *et al.*, 2011). Also, a recent study has shown that in the developing mouse retina, *notch2* is expressed in progenitors and Müller glia (Zhu *et al.*, 2013), an expression pattern that is reminiscent of that for *mdka* in the developing zebrafish. In addition, in zebrafish the cellular expression of *mdka* and *notch2* are similar during retinal regeneration (Cameron *et al.*, 2005; Calinescu *et al.*, 2009a). Based on these data, it would be reasonable to evaluate the potential functional relationship between Mdka and either ALK or Notch2 both during development and regeneration in the zebrafish retina. Interestingly, in mice it has been shown that RPTPβ/ζ (receptor protein tyrosine phosphatase β/ζ) inhibits the proliferation of oligodendrocyte precursor cells and promotes their development into mature oligodendrocytes (Lamprianou *et al.*, 2011). Whether or not Mdka regulates cell cycle kinetics in the developing retina through binding RPTPβ/ζ is still unclear. A potential complexity is that midkine receptors may function as components in a molecular complex. For example, RPTPβ/ζ and LRP6 can be co-immunoprecipitated (Muramatsu *et al.*, 2004). Clearly, initiatives are needed to identify and functionally characterize midkine receptors and additional downstream signalling pathways in both the developing and regenerating retina.

Conclusions

As in other regions of the mammalian brain, retinal neurons that are lost through diseases or injury are not replaced. In distinct contrast, in the zebrafish, spontaneous stem cell-based regeneration occurs to replace lost neurons and restore lost function. In zebrafish, Müller glia can re-enter the cell cycle and regenerate all neuronal types – a process in which midkine may play a central role. A better understanding of this regulated reprogramming by Müller glia could provide

Table 1

Receptors for midkine and their expression patterns in retina

Receptor	Known expression pattern in retina	References
ALK	Neural layer of E11.5 and E13.5 mouse retina	Vernersson *et al.*, 2006.
Notch2	Mouse Müller glia	Roesch *et al.*, 2008;
	Mouse E9.5 optical vesicle, E11.5 and E14.5 retinal progenitors, presumptive future Müller glial cells at P6, adult Müller glia	Zhu *et al.*, 2013;
	Weak expression in adult zebrafish retina but up-regulated after lesion	Cameron *et al.*, 2005.
LRP1	RGC in normal rat retina	Shi *et al.*, 2008.
Integrins	Developing chick retina (α4; β1; α6 β1 integrins)	Leu *et al.*, 2004; Cann *et al.*, 1996; de Curtis *et al.*, 1991)
	Mouse RGC and undifferentiated retinal neuroblasts during axon extension and migration (α4 β1 integrin)	Hikita *et al.*, 2003.
	Tiger salamander retina (α1–6 integrins)	Sherry and Proske, 2001.
Syndecan-3 (N-Syndecan)	Transiently expressed in the neural fibres at early post-natal stages and in the axons of RGCs in rat retina	Inatani *et al.*, 2002.
Glypican	Optic nerve, NFL of the optic cup and weakly in the INL at E16 rat retina	Karthikeyan *et al.*, 1994.
NGC	NFL and IPL of post-natal rat retina	Inatani *et al.*, 2000.
RPTP β/ζ	Chick Müller glia culture	Shock *et al.*, 1995;
	Mouse E13 retina	Horvat-Bröcker *et al*, 2008;
	Mouse E13 retina (precursor cells) and mature retina (RGC, HC, NFL, IPL, OPL)	Klausmeyer *et al.*, 2007.

ALK, anaplastic lymphoma kinase; HC, horizontal cell; INL, inner nuclear layer; IPL, inner plexiform layer; LRP1, low-density lipoprotein receptor-related protein 1; NFL, nerve fibre layer; NGC, neuroglycan C; OPL, outer plexiform layer; RGC, retinal ganglion cells; RPTP β/ζ, receptor protein tyrosine phosphatase β/ζ.

insights into reprogramming potential stem cells in the mammalian retina and brain. It is not too speculative to infer that midkine, or drugs acting on midkine signalling pathways, may have therapeutic use for stimulating neuronal regeneration in the mammalian CNS. Key to this approach will be knowledge of which receptor or combination of receptors mediate midkine function in the brain. An immediate goal will be to link the intrinsic neuronal regeneration in zebrafish and the molecular biology of midkine signalling so that potential therapeutic approaches for treating neuronal degeneration in the human retina can be developed.

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Conflict of interest

We have no conflicts of interest to declare.

References

Agathocleous M, Harris WA (2009). From progenitors to differentiated cells in the vertebrate retina. Annu Rev Cell Dev Biol 25: 45–69.

Anderson G, Beischlag TV, Vinciguerra M, Mazzoccoli G (2013). The circadian clock circuitry and the AHR signaling pathway in physiology and pathology. Biochem Pharmacol 85: 1405–1416.

Bernardos RL, Barthel LK, Meyers JR, Raymond PA (2007). Late-stage neuronal progenitors in the retina are radial Müller glia that function as retinal stem cells. J Neurosci 27: 7028–7040.

Calinescu AA, Vihtelic TS, Hyde DR, Hitchcock PF (2009a). Cellular expression of midkine-a and midkine-b during retinal development and photoreceptor regeneration in zebrafish. J Comp Neurol 514: 1–10.

Calinescu AA, Raymond PA, Hitchcock PF (2009b). Midkine expression is regulated by the circadian clock in the retina of the zebrafish. Vis Neurosci 26: 495–501.

Cameron DA, Gentile KL, Middleton FA, Yurco P (2005). Gene expression profiles of intact and regenerating zebrafish retina. Mol Vis 11: 775–791.

Cann GM, Bradshaw AD, Gervin DB, Hunter AW, Clegg DO (1996). Widespread expression of beta1 integrins in the developing chick retina: evidence for a role in migration of retinal ganglion cells. Dev Biol 180: 82–96.

Craig SE, Thummel R, Ahmed H, Vasta GR, Hyde DR, Hitchcock PF (2010). The zebrafish galectin Drgal1-l2 is expressed by proliferating Müller glia and photoreceptor progenitors and regulates the regeneration of rod photoreceptors. Invest Ophthalmol Vis Sci 51: 3244–3252.

de Curtis I, Quaranta V, Tamura RN, Reichardt LF (1991). Laminin receptors in the retina: sequence analysis of the chick integrin alpha 6 subunit. Evidence for transcriptional and posttranslational regulation. J Cell Biol 113: 405–416.

Dowling JE (2012). The Retina: An Approachable Part of the Brain, Revised edn. Belknap Press Harvard University Press: Cambridge, MA.

Easter SS Jr, Malicki JJ (2002). The zebrafish eye: developmental and genetic analysis. Results Probl Cell Differ 40: 346–370.

Ezquerra L, Pérez-García C, Garrido E, Díez-Fernández C, Deuel TF, Alguacil LF *et al.* (2007). Morphine and yohimbine regulate midkine gene expression in the rat hippocampus. Eur J Pharmacol 557: 147–150.

Fausett BV, Goldman D (2006). A role for alpha1 tubulin-expressing Müller glia in regeneration of the injured zebrafish retina. J Neurosci 26: 6303–6313.

Fausett BV, Gumerson JD, Goldman D (2008). The proneural basic helix-loop-helix gene ascl1a is required for retina regeneration. J Neurosci 28: 1109–1117.

Flatscher-Bader T, Wilce PA (2008). Impact of alcohol abuse on protein expression of midkine and excitatory amino acid transporter 1 in the human prefrontal cortex. Alcohol Clin Exp Res 32: 1849–1858.

Fujisawa K, Terai S, Hirose Y, Takami T, Yamamoto N, Sakaida I (2011). Senescence marker protein 30 (SMP30)/regucalcin (RGN) expression decreases with aging, acute liver injuries and tumors in zebrafish. Biochem Biophys Res Commun 414: 331–336.

Gramage E, Rossi L, Granado N, Moratalla R, Herradón G (2010). Genetic inactivation of pleiotrophin triggers amphetamine-induced cell loss in the substantia nigra and enhances amphetamine neurotoxicity in the striatum. Neuroscience 170: 308–316.

Grotek B, Wehner D, Weidinger G (2013). Notch signaling coordinates cellular proliferation with differentiation during zebrafish fin regeneration. Development 140: 1412–1423.

Herradón G, Pérez-García C (2013). Targeting midkine and pleiotrophin signaling pathways in addiction and neurodegenerative disorders: recent progress and perspectives. Br J Pharmacol. doi:10.1111/bph.12312 [Epub ahead of print].

Hikita ST, Cann GM, Wingerd KL, Mullick LH, Wayne WC, Webb SW *et al.* (2003). Integrin alpha4beta1 (VLA-4) expression and activity in retinal and peripheral neurons. Mol Cell Neurosci 23: 427–439.

Hitchcock PF (1997). Tracer coupling among regenerated amacrine cells in the retina of the goldfish. Vis Neurosci 14: 463–472.

Hitchcock PF, Cirenza P (1994). Synaptic organization of regenerated retina in the goldfish. J Comp Neurol 343: 609–616.

Hitchcock PF, Raymond PA (2004). The teleost retina as a model for developmental and regeneration biology. Zebrafish 1: 257–271.

Hitchcock PF, Lindsey Myhr KJ, Easter SS Jr, Mangione-Smith R, Jones DD (1992). Local regeneration in the retina of the goldfish. J Neurobiol 23: 187–203.

Horvat-Bröcker A, Reinhard J, Illes S, Paech T, Zoidl G, Harroch S *et al.* (2008). Receptor protein tyrosine phosphatases are expressed by cycling retinal progenitor cells and involved in neuronal development of mouse retina. Neuroscience 152: 618–645.

Hsiao HY, Johnston RJ, Jukam D, Vasiliauskas D, Desplan C, Rister J (2012). Dissection and immunohistochemistry of larval, pupal and adult Drosophila retinas. J Vis Exp 69: e4347.

Hu M, Easter SS (1999). Retinal neurogenesis: the formation of the initial central patch of postmitotic cells. Dev Biol 207: 309–321.

Huang Y, Hoque MO, Wu F, Trink B, Sidransky D, Ratovitski EA (2008). Midkine induces epithelial-mesenchymal transition through Notch2/Jak2-Stat3 signaling in human keratinocytes. Cell Cycle 7: 1613–1622.

Ichihara-Tanaka K, Oohira A, Rumsby M, Muramatsu T (2006). Neuroglycan C is a novel midkine receptor involved in process elongation of oligodendroglial precursor-like cells. J Biol Chem 281: 30857–30864.

Inatani M, Tanihara H, Oohira A, Otori Y, Nishida A, Honjo M *et al.* (2000). Neuroglycan C, a neural tissue-specific transmembrane chondroitin sulfate proteoglycan, in retinal neural network formation. Invest Ophthalmol Vis Sci 41: 4338–4346.

Inatani M, Honjo M, Oohira A, Kido N, Otori Y, Tano Y *et al.* (2002). Spatiotemporal expression patterns of N-syndecan, a transmembrane heparan sulfate proteoglycan, in developing retina. Invest Ophthalmol Vis Sci 43: 1616–1621.

Ishikawa E, Ooboshi H, Kumai Y, Takada J, Nakamura K, Ago T *et al.* (2009). Midkine gene transfer protects against focal brain ischemia and augments neurogenesis. J Neurol Sci 285: 78–84.

Kadomatsu K, Muramatsu T (2004). Midkine and pleiotrophin in neural development and cancer. Cancer Lett 204: 127–143.

Kadomatsu K, Tomomura M, Muramatsu T (1988). cDNA cloning and sequencing of a new gene intensely expressed in early differentiation stages of embryonal carcinoma cells and in mid-gestation period of mouse embryogenesis. Biochem Biophys Res Commun 151: 1312–1318.

Karl MO, Reh TA (2010). Regenerative medicine for retinal diseases: activating endogenous repair mechanisms. Trends Mol Med 16: 193–202.

Karthikeyan L, Flad M, Engel M, Meyer-Puttlitz B, Margolis RU, Margolis RK (1994). Immunocytochemical and in situ hybridization studies of the heparan sulfate proteoglycan, glypican, in nervous tissue. J Cell Sci 107: 3213–3222.

Klausmeyer A, Garwood J, Faissner A (2007). Differential expression of phosphacan/RPTPbeta isoforms in the developing mouse visual system. J Comp Neurol 504: 659–679.

Kurosawa N, Chen GY, Kadomatsu K, Ikematsu S, Sakuma S, Muramatsu T (2001). Glypican-2 binds to midkine: the role of glypican-2 in neuronal cell adhesion and neurite outgrowth. Glycoconj J 18: 499–507.

Lamprianou S, Chatzopoulou E, Thomas JL, Bouyain S, Harroch S (2011). A complex between contactin-1 and the protein tyrosine phosphatase PTPRZ controls the development of oligodendrocyte precursor cells. Proc Natl Acad Sci U S A 108: 17498–17503.

Leu ST, Jacques SA, Wingerd KL, Hikita ST, Tolhurst EC, Pring JL *et al.* (2004). Integrin alpha4beta1 function is required for cell survival in developing retina. Dev Biol 276: 416–430.

Liedtke D, Winkler C (2008). Midkine-b regulates cell specification at the neural plate border in zebrafish. Dev Dyn 237: 62–74.

Lien CL, Schebesta M, Makino S, Weber GJ, Keating MT (2006). Gene expression analysis of zebrafish heart regeneration. Plos Biol 4: e260.

Lim J, Yao S, Graf M, Winkler C, Yang D (2013). Structure-function analysis of full-length midkine reveals novel residues important for heparin binding and zebrafish embryogenesis. Biochem J 451: 407–415.

Livesey FJ, Young TL, Cepko CL (2004). An analysis of the gene expression program of mammalian neural progenitor cells. Proc Natl Acad Sci U S A 101: 1374–1379.

Luo J, Uribe RA, Hayton S, Calinescu AA, Gross JM, Hitchcock PF (2012). Midkine-A functions upstream of Id2a to regulate cell cycle kinetics in the developing vertebrate retina. Neural Develop 7: 33.

Maeda N, Ichihara-Tanaka K, Kimura T, Kadomatsu K, Muramatsu T, Noda M (1999). A receptor-like protein-tyrosine phosphatase PTPzeta/RPTPbeta binds a heparin-binding growth factor midkine. Involvement of arginine 78 of midkine in the high affinity binding to PTPzeta. J Biol Chem 274: 12474–12479.

Marchionini DM, Lehrmann E, Chu Y, He B, Sortwell CE, Becker KG *et al.* (2007). Role of heparin binding growth factors in nigrostriatal dopamine system development and Parkinson's disease. Brain Res 1147: 77–88.

Masuda K, Watanabe I, Unoki K, Ohba N, Muramatsu T (1995). Functional Rescue of photoreceptors from the damaging effects of constant light by survival-promoting factors in the rat. Invest Ophthalmol Vis Sci 36: 2142–2146.

Meyers JR, Hu L, Moses A, Kaboli K, Papandrea A, Raymond PA (2012). β-catenin/Wnt signaling controls progenitor fate in the developing and regenerating zebrafish retina. Neural Develop 7: 30.

Mitsiadis TA, Muramatsu T, Muramatsu H, Thesleff I (1995). Midkine (MK), a heparin-binding growth/differentiation factor, is regulated by retinoic acid and epithelial-mesenchymal interactions in the developing mouse tooth, and affects cell proliferation and morphogenesis. J Cell Biol 129: 267–281.

Miyashiro M, Kadomatsu K, Ogata N, Yamamoto C, Takahashi K, Uyama M *et al.* (1998). Midkine expression in transient retinal ischemia in the rat. Curr Eye Res 17: 9–13.

Mlodzik M (1999). Planar polarity in the Drosophila eye: a multifaceted view of signaling specificity and cross-talk. EMBO J 18: 6873–6879.

Montgomery JE, Parsons MJ, Hyde DR (2010). A novel model of retinal ablation demonstrates that the extent of rod cell death regulates the origin of the regenerated zebrafish rod photoreceptors. J Comp Neurol 518: 800–814.

Muñoz-Soriano V, Ruiz C, Pérez-Alonso M, Mlodzik M, Paricio N (2013). Nemo regulates cell dynamics and represses the expression of miple, a midkine/pleiotrophin cytokine, during ommatidial rotation. Dev Biol 377: 113–125.

Muramatsu H, Zou K, Sakaguchi N, Ikematsu S, Sakuma S, Muramatsu T (2000). LDL receptor-related protein as a component of the midkine receptor. Biochem Biophys Res Commun 270: 936–941.

Muramatsu H, Zou P, Suzuki H, Oda Y, Chen GY, Sakaguchi N *et al.* (2004). alpha4beta1- and alpha6beta1-integrins are functional receptors for midkine, a heparin-binding growth factor. J Cell Sci 117: 5405–5415.

Muramatsu T (2010). Midkine, a heparin-binding cytokine with multiple roles in development, repair and diseases. Proc Jpn Acad Ser B Phys Biol Sci 86: 410–425.

Muramatsu T (2011). Midkine: a promising molecule for drug development to treat diseases of the central nervous system. Curr Pharm Des 17: 410–423.

Nelson CM, Hyde DR (2010). Müller glia as a source of neuronal progenitor cells to regenerate the damaged zebrafish retina. Adv Exp Med Biol 723: 425–430.

Nelson CM, Gorsuch RA, Bailey TJ, Ackerman KM, Kassen SC, Hyde DR (2012). Stat3 defines three populations of Müller glia and is

required for initiating maximal müller glia proliferation in the regenerating zebrafish retina. J Comp Neurol 520: 4294–4311.

Nelson CM, Ackerman KM, O'Hayer P, Bailey TJ, Gorsuch RA, Hyde DR (2013). Tumor necrosis factor-alpha is produced by dying retinal neurons and is required for Muller glia proliferation during zebrafish retinal regeneration. J Neurosci 33: 6524–6539.

Ooboshi H (2011). Gene therapy as a novel pharmaceutical intervention for stroke. Curr Pharm Des 17: 424–433.

Parente V, Balasso S, Pompilio G, Verduci L, Colombo GI, Milano G *et al*. (2013). Hypoxia/reoxygenation cardiac injury and regeneration in zebrafish adult heart. Plos ONE 8: e53748.

Qin Z, Barthel LK, Raymond PA (2009). Genetic evidence for shared mechanisms of epimorphic regeneration in zebrafish. Proc Natl Acad Sci U S A 106: 9310–9315.

Ramachandran R, Fausett BV, Goldman D (2010). Ascl1a regulates Müller glia dedifferentiation and retinal regeneration through a Lin-28-dependent, let-7 microRNA signalling pathway. Nat Cell Biol 12: 1101–1107.

Ramachandran R, Zhao XF, Goldman D (2012). Insm1a-mediated gene repression is essential for the formation and differentiation of Müller glia-derived progenitors in the injured retina. Nat Cell Biol 14: 1013–1023.

Rattner A, Nathans J (2005). The genomic response to retinal disease and injury: evidence for endothelin signaling from photoreceptors to glia. J Neurosci 25: 4540–4549.

Raymond PA, Barthel LK, Bernardos RL, Perkowski JJ (2006). Molecular characterization of retinal stem cells and their niches in adult zebrafish. BMC Dev Biol 6: 36.

Reiff T, Huber L, Kramer M, Delattre O, Janoueix-Lerosey I, Rohrer H (2011). Midkine and Alk signaling in sympathetic neuron proliferation and neuroblastoma predisposition. Development 138: 4699–4708.

Roesch K, Jadhav AP, Trimarchi JM, Stadler MB, Roska B, Sun BB *et al*. (2008). The transcriptome of retinal Muller glial cells. J Comp Neurol 509: 225–238.

Roger J, Brajeul V, Thomasseau S, Hienola A, Sahel JA, Guillonneau X *et al*. (2006). Involvement of Pleiotrophin in CNTF-mediated differentiation of the late retinal progenitor cells. Dev Biol 298: 527–539.

Sakakima H, Yoshida Y, Kadomatsu K, Yuzawa Y, Matsuo S, Muramatsu T (2004). Midkine expression in rat spinal motor neurons following sciatic nerve injury. Brain Res Dev Brain Res 153: 251–260.

Sakakima H, Yoshida Y, Yamazaki Y, Matsuda F, Ikutomo M, Ijiri K *et al*. (2009). Disruption of the midkine gene (Mdk) delays degeneration and regeneration in injured peripheral nerve. J Neurosci Res 87: 2908–2915.

Sakamoto K, Kadomatsu K (2012). Midkine in the pathology of cancer, neural disease, and inflammation. Pathol Int 62: 445–455.

Schebesta M, Lien CL, Engel FB, Keating MT (2006). Transcriptional profiling of caudal fin regeneration in zebrafish. ScientificWorldJournal 6: 38–54.

Schmitt EA, Dowling JE (1996). Comparison of topographical patterns of ganglion and photoreceptor cell differentiation in the retina of the zebrafish, Danio rerio. J Comp Neurol 371: 222–234.

Seiler MJ, Aramant RB (2012). Cell replacement and visual restoration by retinal sheet transplants. Prog Retin Eye Res 31: 661–687.

Sherpa T, Fimbel SM, Mallory DE, Maaswinkel H, Spritzer SD, Sand JA *et al*. (2008). Ganglion cell regeneration following whole-retina destruction in zebrafish. Dev Neurobiol 68: 166–181.

Sherry DM, Proske PA (2001). Localization of alpha integrin subunits in the neural retina of the tiger salamander. Graefes Arch Clin Exp Ophthalmol 239: 278–287.

Shi Z, Rudzinski M, Meerovitch K, Lebrun-Julien F, Birman E, Di Polo A *et al*. (2008). Alpha2-macroglobulin is a mediator of retinal ganglion cell death in glaucoma. J Biol Chem 283: 29156–29165.

Shock LP, Bare DJ, Klinz SG, Maness PF (1995). Protein tyrosine phosphatases expressed in developing brain and retinal Muller glia. Brain Res Mol Brain Res 28: 110–116.

Stenkamp DL (2007). Neurogenesis in the fish retina. Int Rev Cytol 259: 173–224.

Stoica GE, Kuo A, Powers C, Bowden ET, Sale EB, Riegel AT *et al*. (2002). Midkine binds to anaplastic lymphoma kinase (ALK) and acts as a growth factor for different cell types. J Biol Chem 277: 35990–35998.

Taylor S, Chen J, Luo J, Hitchcock P (2012). Light-induced photoreceptor degeneration in the retina of the zebrafish. Methods Mol Biol 884: 247–254.

Thomas JL, Nelson CM, Luo X, Hyde DR, Thummel R (2012). Characterization of multiple light damage paradigms reveals regional differences in photoreceptor loss. Exp Eye Res 97: 105–116.

Thummel R, Kassen SC, Enright JM, Nelson CM, Montgomery JE, Hyde DR (2008). Characterization of Müller glia and neuronal progenitors during adult zebrafish retinal regeneration. Exp Eye Res 87: 433–444.

Unoki K, Ohba N, Arimura H, Muramatsu H, Muramatsu T (1994). Rescue of photoreceptors from the damaging effects of constant light by midkine, a retinoic acid-responsive gene product. Invest Ophthalmol Vis Sci 35: 4063–4068.

Uribe RA, Gross JM (2010). Id2a influences neuron and glia formation in the zebrafish retina by modulating retinoblast cell cycle kinetics. Development 137: 3763–3774.

Vernersson E, Khoo NK, Henriksson ML, Roos G, Palmer RH, Hallberg B (2006). Characterization of the expression of the ALK receptor tyrosine kinase in mice. Gene Expr Patterns 6: 448–461.

Vihtelic TS, Soverly JE, Kassen SC, Hyde DR (2006). Retinal regional differences in photoreceptor cell death and regeneration in light-lesioned albino zebrafish. Exp Eye Res 82: 558–575.

Wan J, Ramachandran R, Goldman D (2012). HB-EGF is necessary and sufficient for Müller glia dedifferentiation and retina regeneration. Dev Cell 22: 334–347.

Wei W, Feller MB (2011). Organization and development of direction-selective circuits in the retina. Trends Neurosci 34: 638–645.

Winkler C, Moon RT (2001). Zebrafish mdk2, a novel secreted midkine, participates in posterior neurogenesis. Dev Biol 229: 102–118.

Winkler C, Schafer M, Duschl J, Schartl M, Volff JN (2003). Functional divergence of two zebrafish midkine growth factors following fish-specific gene duplication. Genome Res 13: 1067–1081.

Wolff T, Ready DF (1993). Pattern formation in the Drosophila retina. In: Bate M, Martinez Arias A (eds). The Development of Drosophila Melanogaster. Cold Spring Harbor Press: Cold Spring Harbor, pp. 1277–1326.

Zhou WT, Ni YQ, Jin ZB, Zhang M, Wu JH, Zhu Y *et al*. (2012). Electrical stimulation ameliorates light-induced photoreceptor degeneration in vitro via suppressing the proinflammatory effect of microglia and enhancing the neurotrophic potential of Müller cells. Exp Neurol 238: 192–208.

Zhu MY, Gasperowicz M, Chow RL (2013). The expression of NOTCH2, HES1 and SOX9 during mouse retinal development. Gene Expr Patterns 13: 78–83.