Chapter 89

A possible role of neuroglobin in the retina after optic nerve injury: a comparative study of zebrafish and mouse retina

Kayo Sugitani[1], Yoshiki Koriyama[2], Kazuhiro Ogai[3], Keisuke Wakasugi[4], Satoru Kato[5]

[1]Department of Clinical Laboratory Science, Graduate School of Medical Science, Kanazawa University, 5-11-80 Kodatsuno, Kanazawa, 920-0942, Japan

[2]Graduate School and Faculty of Pharmaceutical Sciences, Suzuka University of Medical Science, Suzuka, 513-8670, Japan.

[3]Wellness Promotion Science Center, Institute of Medical, Pharmaceutical and Health Science, Kanazawa University, 5-11-80 Kodatsuno, Kanazawa, 920-0942, Japan

[4]Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, 3-8-1 Komaba, Meguro-ku, 153-8902, Japan

[5]Department of Molecular Neurobiology, Graduate School of Medicine, Kanazawa University, 13-1 Takara-machi, Kanazawa, 920-8640, Japan.

Corresponding author: Kayo Sugitani, Department of Clinical Laboratory Science, Graduate School of Medical Science, Kanazawa University, 5-11-80 Kodatsuno, Kanazawa 920-0942, Japan, Phone: +81 76 265-2599; E-mail: sugitani@staff.kanazawa-u.ac.jp

Abstract Neuroglobin (Ngb) is a new member of the family of heme proteins and is specifically expressed in neurons of the central and peripheral nervous systems in all vertebrates. In particular, the retina has a 100-fold higher concentration of Ngb than do other nervous tissues. The role of Ngb in the retina is yet to be clarified. Therefore, to understand the functional role of Ngb in the retina after optic nerve injury (ONI), we used 2 types of retina, from zebrafish and mice, which have permissible and non-permissible capacity for nerve regeneration after ONI, respectively. After ONI, the Ngb protein in zebrafish was upregulated in the amacrine cells within 3 days, whereas in the mouse retina, Ngb was downregulated in the retinal ganglion cells (RGCs) within 3 days. Zebrafish Ngb (z-Ngb) significantly enhanced neurite outgrowth in retinal explant culture. According to these results, we designed an overexpression experiment with the mouse Ngb (m-Ngb) gene in RGC-5 cells (retinal precursor cells). The excess of m-Ngb actually rescued RGC-5 cells under hypoxic conditions and significantly enhanced neurite outgrowth in cell culture. These data suggest that mammalian Ngb has positive neuroprotective and neuritogenic effects that induce nerve regeneration after ONI.

89.1. Introduction

In 2000, neuroglobin (Ngb) was discovered as a new member of the globin superfamily predominantly expressed in neurons (Burmester et al. 2000) and it contains hexacoordinated heme Fe atoms. Mammalian Ngb has shown high affinity for O_2 and might be involved in the alleviation of various types of oxidative stresses, elimination of reactive oxygen species (Li et al. 2008; Li et al. 2011), and in preservation of mitochondrial function via prevention of apoptosis (Brittain et al. 2010; Raychaudhuri et al. 2010). Furthermore, Wakasugi et al. (2005) proposed a new function of Ngb as a regulator protein in signal transduction where it inhibits the

dissociation of GDP with the α-subunit of a G protein. It is well known that retina contains the highest concentration of Ngb among various nervous tissues (Schmidt et al. 2003; Burmester et al. 2009). Fish retinal ganglion cells (RGCs) can survive and regenerate their axon after optic nerve injury (ONI), whereas mouse RGCs cannot survive and fail to regenerate after ONI (Kato et al. 2013). In the present study, we examined in detail the changes of Ngb expression in zebrafish and mice after ONI. After ONI, opposite responses in retinal Ngb levels could be seen: upregulation of Ngb in the fish retina and downregulation of Ngb in the mouse retina. On the basis of these results, we tried to achieve overexpression of mouse Ngb in RGC-5 cells, a retinal precursor cell line, to induce nerve regeneration in the mammalian retina after ONI.

89.2. Stimulation of neurite sprouting by z-Ngb in the zebrafish retina after ONI

In a previous study (Kamioka et al. 2013), we reported that the level of z-Ngb mRNA in the zebrafish retina increased 3 days after ONI and returned to the control levels by 20 days after ONI. The cellular localization of z-Ngb mRNA was in amacrine cells. Immunohistochemical analysis further supported this finding regarding z-Ngb: immunoreactivity of z-Ngb in the control retina could be barely seen in the inner retina (Fig. 89.1, zebrafish 0 d). The ←Fig. 1 immunoreactivity of z-Ngb increased in the amacrine cells in the inner nuclear layer and inner plexiform layer 3 days after ONI (Fig. 89.1, zebrafish 3 d). In particular, immunoreactivity of amacrine cells became conspicuously stronger than that of control retinas. Addition of z-Ngb into the zebrafish retinal explant cultures induced a significant neurite outgrowth in a naïve (intact) retina (Sugitani, unpublished data). On the other hand, the z-Ngb protein did not protect zebrafish ZF4 cells from cell death caused by hydrogen peroxide exposure (Kamioka et al.

2013). The reason being that z-Ngb has a cell membrane-penetrating domain but not a cell-protecting domain (Wakasugi et al. 2005). Thus, the z-Ngb protein that is upregulated in the amacrine cells after ONI is easily secreted and translocated into the damaged ganglion cells to induce neurite sprouting at such an early stage (3 days) of optic nerve regeneration (Kato et al. 2013).

89.3. Neuroprotective and neurite sprouting effects of mouse Ngb in the retina after ONI

The structure of m-Ngb comprises a monomer of 151 amino acid residues with a molecular mass of 17 kDa. The m-Ngb exhibited a very high homology with human Ngb (94% identity). Although m-Ngb has no cell membrane-penetrating activity, it exerts a cell-protecting effect through its GDP anchor protein (Wakasugi et al. 2005). Immunohistochemical analysis revealed that strong signals of the m-Ngb protein can be seen in the control retina: the tissue localization is limited to the ganglion cells (Fig. 89.1, mouse 0 d). Lechauve et al. (2013) recently showed this kind of strong immunoreactivity in rat RGCs. After ONI, m-Ngb signals disappeared from the mouse retina after 3 days (Fig. 89.1, mouse 3 d). To further explore the role of m-Ngb in mouse retina, we performed an overexpression experiment with the m-Ngb gene using murine retinal precursor cells, RGC-5 cells (Krishnamoorthy et al. 2001). The m-Ngb overexpression ←Fig. 2 certainly enhanced cell viability of RGC-5 cells under hypoxia-reperfusion conditions compared to mock or control cells (Fig. 89.2a). Furthermore, the overexpressed m-Ngb induced the growth of significantly long neurites in RGC-5 cells in culture (Fig. 89.2b). These data suggest that m-Ngb is involved in dual neuroprotective and neuritogenic mechanisms. In the case of lens injury and advanced glaucoma, Ngb protein is certainly upregulated in the Muller cells and inner

nuclear cells (Lechauve et al. 2013; Rajendram and Rao 2007). In the case of acute ONI, production of m-Ngb cannot catch up to the excess amount of oxygen radicals. If we overcome this disadvantage, Ngb might become a key molecule for therapeutic regeneration of mammalian central neurons, for example, in the form of a chimeric Ngb protein with a cell membrane-penetrating module from z-Ngb (Kamioka et al. 2013).

89.4. Conclusions

In this study, we compared Ngb expression in the retina before and after ONI (Table 1). Fish Ngb, upregulated in amacrine cells after ONI, might be released from amacrine cells followed by translocation into neighboring RGCs, and may induce nerite sprouting in damaged RGCs at the early stage of optic nerve regeneration. In contrast, mammalian Ngb downregulated immediately after ONI. Mammalian Ngb has been known to have beneficial effects: neuroprotective and neuritogenic. Thus, a successful method for the maintenance of high levels of Ngb expression in the retina after ONI may protect neural cells from cell death and might induce neurite outgrowth in damaged RGCs.

←Table 1

Acknowledgements This work was supported by Grants-in-Aid for Scientific Research to K.S. (No. 23618006) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

Brittain T, Skommer J, Raychaudhuri S et al (2010) An antiapoptotic neuroprotective role for Neuroglobin. Int J Mol Sci 11:2306–2321

Burmester T, Hankeln T (2009) What is the function of neuroglobin? J Exp Biol 212:1423–1428

Burmester T, Weich B, Reinhardt S et al (2000) A vertebrate globin expressed in the brain. Nature 407:520-523.

Kamioka Y, Fujikawa C, Ogai K et al (2013) Functional characterization of fish neuroglobin: zebrafish neuroglobin is highly expressed in amacrine cells after optic nerve injury and can translocate into ZF4 cells. Biochim Biophys Acta 1834:1779-1788

Kato S, Matsukawa T, Koriyama Y et al (2013) A molecular mechanism of optic nerve regeneration in fish: the retinoid signaling pathway. Prog Retin Eye Res 37:13-30

Krishnamoorthy RR, Agarwal P, Prasanna G, et al (2001) Characterization of a transformed rat retinal ganglion cell line. Brain Res Mol Brain Res 86:1–12

Lechauve C, Augustin S, Roussel D et al (2013) Neuroglobin involvement in visual pathways through the optic nerve. Biochim Biophys Acta 1834:1772-1778

Li RC, Morris MW, Lee SK et al (2008) Neuroglobin protects PC12 cells against oxidative stress. Brain Res 1190:159–166

Li W, Wu Y, Ren C et al (2011) The activity of recombinant human neuroglobin as an antioxidant and free radical scavenger. Proteins 79:115–125

Rajendram R, Rao NA (2007) Neuroglobin in normal retina and retina from eyes with advanced glaucoma. Br J Ophthalmol 91:663-666

Raychaudhuri S, Skommer J, Henty K et al (2010) Neuroglobin protects nerve cells from apoptosis by inhibiting the intrinsic pathway of cell death. Apoptosis 15:401–411

Schmidt M, Giessl A, Laufs T et al (2003) How does the eye breathe? Evidence for neuroglobin-mediated oxygen supply in the mammalian retina. J Biol Chem 278:1932–1935

Wakasugi K, Kitatsuji C, Morishima I (2005) Possible neuroprotective mechanism of human neuroglobin. Ann N Y Acad Sci 1053:220-230

Figure Legends

Fig. 89.1. Immunohistochemical staining of zebrafish and mouse retina with an anti-neuroglobin (anti-Ngb) antibody

The panel (zebrafish) 0 d: very weak zebrafish Ngb (z-Ngb) signals can be seen in the control (intact) retina. 3 d: z-Ngb expression clearly increased in the amacrine cells in the inner nuclear layer and the inner plexiform layer 3 days after optic nerve injury. The panel (mouse) 0 d: m-Ngb signals can be seen in the control (intact) retina. 3 d: m-Ngb expression clearly decreased in the ganglion cell layer 3 days after optic nerve injury. The scale bar is 20 μm. INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer.

Fig. 89.2. Effects of Ngb overexpression on (a) cell viability and (b) neurite outgrowth in RGC-5 cells

a) Overexpression of mouse Ngb (m-Ngb) increased cell viability under oxidative stress (hypoxic conditions for 24 hours) compared with the control cells and mock-transfected cells (**$P < 0.01$: decreased relative to the control without oxidative stress, *$P < 0.05$: decreased relative to the control without oxidative stress, $^{++}P < 0.01$: increased relative to the control with oxidative stress).

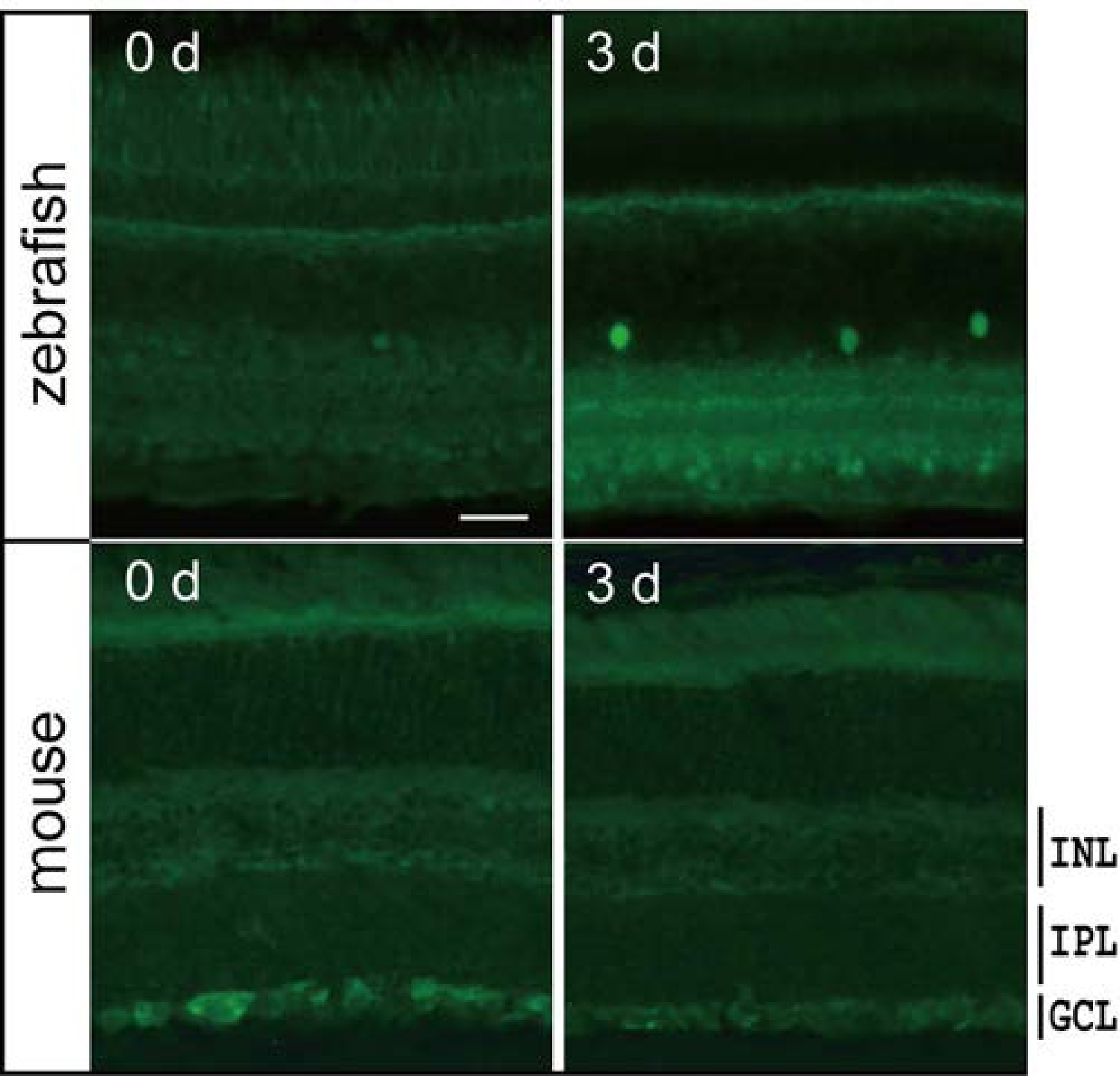
b) Overexpression of m-Ngb increased the length of neurite outgrowth compared with the control or mock-transfected cells (**$P < 0.01$ increased relative to the control or mock).

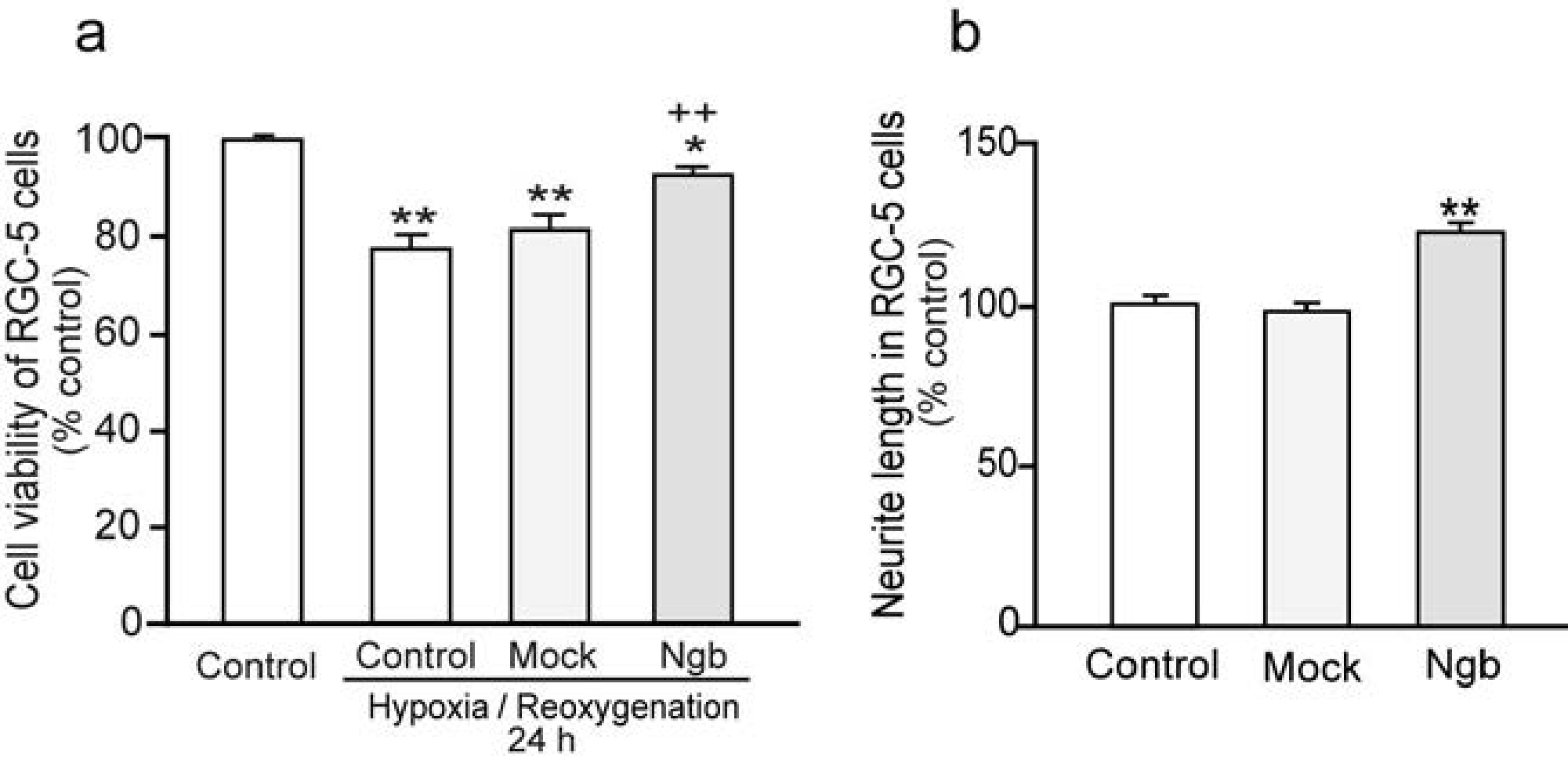
Differences between the groups were analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's multiple-comparison test.

Tables

Table 89.1. Comparison of Ngb expression in mouse and zebrafish after optic nerve injury (ONI).

	Ngb expression after ONI	Localization in retina	Function
Mouse Ngb	Decreases	Retinal ganglion cells	● Enhances cell viability ● Neurite outgrowth
Zebrafish Ngb	Increases (~20 days)	Amacrine cells	● Neurite outgrowth


zebrafish
mouse
0 d
3 d
0 d
3 d
INL
IPL
GCL


a
Cell viability of RGC-5 cells
(% control)
100
80
60
40
20
0
**
**
++
*
Control
Control
Mock
Ngb
Hypoxia / Reoxygenation
24 h
b
Neurite length in RGC-5 cells
(% control)
150
100
50
0
**
Control
Mock
Ngb