Regulation of the neural niche by the soluble molecule Akhirin

Acharjee, Uzzal Kumar
Department of Developmental Neurobiology, Graduate School of Medical Sciences, Kumamoto University

Felemban, Athary Abdulhaleem
Department of Developmental Neurobiology, Graduate School of Medical Sciences, Kumamoto University

Riyadh, Asrafuzzaman M.
Department of Developmental Neurobiology, Graduate School of Medical Sciences, Kumamoto University

Ohta, Kunimasa
Department of Developmental Neurobiology, Graduate School of Medical Sciences, Kumamoto University

出版情報：Development, Growth & Differentiation (DGD). 58 (5), pp.463-468, 2016-05-01. Wiley
バージョン：
権利関係：

Regulation of the neural niche by the soluble molecule Akhirin

*Uzzal Kumar Acharjee[1,3], *Athary Abdulhaleem Felemban[1,2,4], M. Asrafuzzaman Riyadh[1,5], and Kunimasa Ohta[1,2,6,§]

[1]Department of Developmental Neurobiology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto 860-8556, Japan.

[2]Stem Cell-Based Tissue Regeneration Research and Education Unit, Kumamoto University, Kumamoto 860-8556, Japan.

[3]Program for Leading Graduate Schools HIGO (Health Life Science: Interdisciplinary and Glocal Oriented), Kumamoto University, Kumamoto 860-8556, Japan.

[4]Department of Biology, Faculty of Applied Science, Umm Al-Qura University, 21955, Makkah, Saudi Arabia.

[5]Department of Pathology and Laboratory Medicine, University of California, Davis, Sacramento, California 95817, USA.

[6]Japan Agency for Medical Research and Development (AMED), Tokyo 100-0004, Japan.

* Equal contribution

§ Correspondence should be addressed to: Kunimasa Ohta

Phone: (81)-96-373-5293

Fax: +81 96 373 5293

E-mail: ohta9203@gpo.kumamoto-u.ac.jp

Short title: Neural stem cell regulation by Akhirin

Key words: Akhirin, niche, neural stem cells, eye, spinal cord.

Abstract

Though the adult central nervous system has been considered a comparatively static tissue with little turnover, it is well established today that new neural cells are generated throughout the life. Neural stem/progenitor cells (NS/PCs) can self-renew and generate all types of neural cells. The proliferation of NS/PCs, and differentiation and fate determination of PCs are regulated by extrinsic factors such as growth factors, neurotrophins, and morphogens. Although several extrinsic factors that influence neurogenesis have already been reported, little is known about the role of soluble molecules in neural niche regulation. In this review, we will introduce the soluble molecule Akhirin and discuss its role in the eye and spinal cord during development.

Introduction

Focus on adult neurogenesis yields novel insight into the central nervous system (CNS) environment and new strategies for treating neurological disorders (Ming & Song 2011). Contrary to the notion that the CNS is relatively static with limited cell turnover, cells with stem cell like properties have been isolated from most neural tissues (Zhao *et al*. 2008). In addition to the brain, the eye and spinal cord are also important parts of the vertebrate CNS and contain neural stem/progenitor cells (NS/PCs) throughout life. For example, spinal cord ependymal cells that line dorsal central canal show NS/PCs potential (Hugnot 2010), while there are three candidate NS/PCs in the eye (Ohta *et al*. 2008).

Because of the fundamental roles of the eye and the spinal cord in vision and movement respectively, these organs have been a predominant focus of neuro-scientific researches on normal development and potential therapies for disorders. Therefore, our understanding of NS/PC behavior in the eye and the spinal cord can be

greatly improved if we consider cell-cell interactions, in addition to stem-cell maintenance, proliferation, and differentiation. Though it is well known that the adhesion molecules retain stem cells in the niche and thereby maintain its architecture and shape (Marthiens *et al*. 2010), little is known about how the neuronal niche is regulated by cell-adhesion molecules during development. Here, we introduce a secreted protein, Akhirin (AKH), isolated from chick E6 lens, which exerts heterophilic cell-adhesion activity and is expressed in the neural niche of the eye (Ahsan *et al*. 2005) and spinal cord (Felemban *et al*. 2014). In this review, we focus on the role of AKH as a niche molecule that regulates the proliferation and differentiation of NS/PCs.

NS/PCs in the eye and spinal cord

The retina and lens constitute basic and essential components of the eye and are formed from the eye field via biochemical and physiological processes. Several studies have identified a number of different cellular sources of NSs within the adult eye (Perron & Harris 2000). Based on their differential responsiveness to extrinsic factors, retinal NS/PCs are localized in the ciliary body epithelium, the iris pigmented epithelium, and Müller glia of the mouse eye (Ohta *et al*. 2008). In the developing chick eye, NS/PCs have been found in the peripheral marginal region of the retina, termed the ciliary marginal zone (Ahsan *et al*. 2005), where they contribute to postnatal growth of the retina.

The spinal cord is the caudal part of the CNS and transmits signals between the brain and the rest of the body. Multiple cell types have been identified in the central canal of the spinal cord to date, and include cuboidal, tanycytic, and radial classes of lumen-contacted ciliated ependymal cells (Hamilton *et al*. 2009). Ependymal cells

originate from radial glial cells—the neural stem cells in the developing CNS—and line the ventricular walls of the central canal of the spinal cord (Edward *et al*. 1990). Numerous studies have indicated that ependymal cells localized dorsal central canal show stem-cell activity both *in vivo* and *in vitro* (Namiki & Tator 1999; Sabourin *et al*. 2009; Barnabe`-Heider *et al*. 2010). The neural stem cell niche defines a microenvironment in which stem cells are retained after embryonic development for the production of new cells of the nervous system (Conover & Notti 2008; Zhao *et al*. 2008). Complex array of diffusible signaling molecules, including growth factors and neurotransmitters, influences the neural stem cell niche (Conover & Notti 2008). Thus, understanding the regulation of the neural niche in the vertebrate eye and spinal cord by extrinsic molecules is an important issue in developmental neurobiology.

Overview of AKH

Previously, our group isolated novel, secreted molecules from the embryonic day 6 (E6) chick lens using signal sequence trap cDNA screening (Klein *et al*. 1996; Mu *et al*. 2003). One such molecule was designated "Akhirin" (AKH), derived from the Bengali word "akhi" meaning "eye," based on the expression of this protein in the eye (Ahsan *et al*. 2005). AKH consists of one LCCL (Limulus factor C, Coch-5b2, and Lgl1) domain and two von Willebrand factor A (VWA) domains, having an open reading frame of 748 amino acid residues (Ahsan *et al*. 2005). AKH has relatively high homology to mouse vitrin (72.2%, Mayne *et al*. 1999) and chick cochlin (49%, Heller *et al*. 1998). No integrin-binding motif has been found in the AKH sequence (Ahsan *et al*. 2005; Fig. 1A).

Expression of AKH in the peripheral retina

In the vertebrate retina, different neuronal NS/PCs exist and their proliferation and differentiation are influenced by diverse signals (Clegg *et al*. 2000; Zhao *et al*. 2005). The development of the eye is controlled by a combination of intrinsic and extrinsic factors, then, a detailed characterization of AKH expression in the chick eye can potentially contribute to our understanding of the eye formation. In the chick peripheral retina, both AKH mRNA and protein are expressed through the ciliary epithelial layer at the embryonic stage; however, at the postnatal stage they accumulate in the presumptive ciliary marginal zone, where NS/PCs are localized (Ahsan *et al*. 2005; Fig. 1B). Co-localization of AKH mRNA and protein in the eye suggests that AKH is secreted from these cells and associated with other ECM components on cell surface to stabilize the matrix (Ahsan *et al*. 2005).

Heterophilic cell adhesion activity of AKH

As cell-cell adhesion plays a vital role in retinal development, several cell-adhesion molecules have been shown to act in chick retinal development (Jean *et al*. 1998). For instance, at embryonic and early postnatal stages of mouse, Müller cells and astrocytes in the retina and retinal ganglion cell axons express neural cell adhesion molecule (N-CAM) (Bartsch *et al*. 1990; Cole & Glaser 1984). Calcium-dependent cell-adhesion molecule Cadherins are associated with specific projections in the avian visual system (Redies 2000; Grunwald 1996; Wohrn *et al*. 1998) and specifically expressed in the inner nuclear layer (INL), ganglion cell layer (GCL), and the inner plexiform layer (IPL) of the retina (Honjo *et al*. 2000). In addition, integrins, which are the major cell-surface receptors of the ECM, have VWA domains in all their β subunits and can support cell-cell adhesion (Hynes 1992). During chick early development, integrin expression is found in the retina and in the apical membrane of

the retinal pigment epithelium (RPE) (Clegg *et al*. 2000; Rizzolo *et al*. 1994). These lines of evidences suggest that cell-cell adhesion plays an important role in the regulation of retinal development. On the basis of the two VWA domains in AKH, and cell aggregation tests using AKH-expressing transfectants with cell surface-trapped AKH proteins, it has been accepted that AKH has cell-adhesion activity (Fig. 1C). Further, both dissociated control cells and AKH transfectants adhered to immobilized AKH protein, which suggests a possible heterophilic cell-adhesion mechanism that is mediated by AKH (Ahsan *et al*. 2005).

Expression of AKH in the developing spinal cord

Signaling molecules and their pathways have long been implicated in pattern formation, cell fate specification, and body formation (Draper *et al*. 2003; Acharjee *et al*. 2015). During the development of the spinal cord, various molecules and morphogens are secreted from loci along the neural tube to initiate site-specific neurogenesis. AKH is transiently expressed in ependymal cells of the ventral central canal and regarded as a protein morphogen that controls the proliferation and differentiation of progenitors in the dorsal central canal of the mouse spinal cord (Felemban *et al*. 2014). AKH exhibits a dynamic expression pattern depending on the stage and the location of the tissue involved. For example, in mice the earliest expression of AKH is observed in the floor plate at E9.5, and around the central canal of the neural tube from E13.5 (Fig. 2A). Further, the AKH expression is restricted at the ventral central canal until the post-natal stage during which NSs originate (Felemban *et al*. 2014).

Chick AKH is also expressed around the central canal of the spinal cord (Fig. 2A), suggesting that AKH plays a role in stem-cell regulation during chick embryogenesis.

Furthermore, mouse cell-labeling studies show that self-renewing NSs reside in the spinal cord primordium and divide at intervals to generate neural progenitors (Forlani, *et al*. 2003; Mathis & Nicolas 2000; Delfino-Machin *et al*. 2005). Moreover, NSs have been isolated from the ependymal zone surrounding the central canal of the spinal cord (Weiss *et al*. 1996). A recent study demonstrated that NSs are the most dorsally located glial fibrillary acidic protein (GFAP)-positive cells lying ependymally (Sabourin *et al*. 2009). Since, AKH is a soluble molecule expressed in the ependymal cells of the ventral central canal (Fig. 2A), it could regulate stem cell proliferation and differentiation during development (Felemban *et al*. 2014). Because several molecules involved in the notch, Wnt, bone morphogenetic protein (BMP), and hedgehog signaling pathways are expressed in the central canal region (Hugnot 2010; Felemban *et al*. 2014), it is imperative to identify AKH partner molecules on account of its heterophilic properties (Ahsan *et al*. 2005).

Analysis of AKH loss-of-function in neural tube

AKH knockout mice (AKH-/-) are viable and fertile and possess significantly reduced the size of neural tubes compared with wild type (WT) mice (Felemban *et al*. 2014). To illustrate the impact of the functional removal of AKH during spinal-cord development, we examined related cellular proliferation and differentiation using immunohistochemistry. Our principal findings were that AKH-/- mice exhibited: 1) a reduction of BrdU-positive cells in the spinal cord; 2) a marked reduction in the distribution of nestin (NS/PCs) and GFAP (NSs) during embryonic development; and 3) a reduction in the expression of transcription factor Isl1/2, which is expressed in the motor neurons and neurons of the intermediolateral nucleus, and homeodomain neural progenitor gene Pax6, which is expressed in the central canal, though the

distribution of these cells is not disturbed. Together, these data indicate that AKH regulates NS/PCs differentiation into motor neurons and interneurons (Felemban *et al*. 2014).

Effects of AKH on spinal cord neurosphere formation

To understand the role of AKH in the control of NS/PCs, we performed *in vitro* culture of the spinal cord neurosphere and subsequent morphological analysis. We found that neurosphere size is significantly reduced in AKH-/- mice in comparison with the WT mice (Fig. 2B) (Felemban *et al*. 2014). Immunocytochemistry using different antibodies—CyclinD2 (Lukaszewicz & Anderson 2011), vimentin (the ependymal cell) (Bodega *et al*. 1994), and Ki67 (the proliferating cell) (Gerdes *et al*. 1983)—showed that CyclinD2 and Ki67 are expressed in the outer circumference of the neurospheres in WT mice, while in AKH-/- mice they are found throughout the neurosphere area (Felemban *et al*. 2014). Thus, the distribution of ependymal proliferation and differentiation markers in the AKH-/- spinal cord is disturbed, indicating that AKH is involved in NS/PCs regulation (Felemban *et al*. 2014).

Induction of AKH after spinal-cord injury

The spinal-cord ependymal niche region is composed of several site-specific cell types, which each express characteristic marker, different morphologies and contrasting functions (Hugnot 2010). Previous studies have shown that in the cell niche, ependymal cells of the spinal cord are normally quiescent in adult mice, but after spinal cord injury they are rapidly activated and proliferative, undergo multilineage differentiation to contribute astrocytes to the injured site, and display stem-cell properties (Hamilton *et al*. 2009; Felemban *et al*. 2014). Furthermore,

ependymal cells contribute to the regeneration of oligodendrocytes and remyelination after spinal cord injury (Meletis *et al*. 2008). Although the expression of AKH at the P30 spinal cord is very low or not observed around the central canal of the neural tube, the signal of AKH mRNA around the central canal is rapidly upregulated in ependymal cells after spinal cord injury (Fig. 2C). Thus, these results indicate that AKH controls cell proliferation and differentiation around the central canal as an immediate early response gene after injury (Felemban *et al*. 2014).

Conclusions

AKH is expressed in the ciliary marginal zone of the eye and the central canal of the spinal cord and has been implicated in neural niche regulation. AKH is additionally expressed in the subventricular zone of the mouse brain (data not shown), suggesting a crucial role in CNS development. As AKH exhibits heterophilic cell-adhesion activity, the identification of AKH's partner molecules and elucidation of their molecular interaction will enhance our understanding of neural niche regulation in the CNS.

Acknowledgements

We thank Naofumi Ito and Adil Ishtiyaq for their critical comments, and all members of our labs for their valuable help. This work was supported by the Precursory Research for Embryonic Science and Technology program of the Japan Science and Technology Agency (K.O.), and Grants-in-Aid for Scientific Research on Molecular Brain Science from the Ministry of Education Culture, Sports, Science, and Technology of Japan (K.O.), the Global COE Program (Cell Fate Regulation Research and Education Unit) (K.O.), and the Program for Leading Graduate Schools

“HIGO (Health Life Science: Interdisciplinary and Glocal Oriented)”, MEXT, Japan (U.K.A.).

References

Acharjee, U. K., Gejima, R., Felemban, A., Riyadh, M. A., Tanaka, H. & Ohta, K. 2015. Tsukushi expression is dependent on Notch signaling and oscillated in the presomitic mesoderm during chick somitogenesis. Biochem. Biophys. Res. Commun. 465, 625-630.

Ahsan, M., Ohta, K., Kuriyama, S. & Tanaka, H. 2005. Novel soluble molecule, Akhirin, is expressed in the embryonic chick eyes and exhibits heterophilic cell-adhesion activity. Dev. Dyn. 233, 95-104.

Barnabe-Heider, F., Goritz, C., Sabelstrom, H., Takebayashi, H., Pfrieger, F. W., Meletis, K. & Frisen, J. 2010. Origin of new glial cells in intact and injured adult spinal cord. Stem Cell 7, 470-482.

Bartsch, U., Kirchhoff, F. & Schachner, M. 1990. Highly sialylated N-CAM is expressed in adult mouse optic nerve and retina. Journal of Neurocytology 4, 550-565.

Bodega, G., Suarez, I. & Rubio, M. 1994. Ependyma: Phylogenetic evolution of glial fibrillary acidic protein (GFAP) and vimentin expression in vertebrate spinal cord. Histochemistry 102, 113-122.

Clegg, D. O., Mullick, L. H., Wingerd, K. L., Lin, H., Atienza, J. W., Bradshaw, A. D., Gervin, D. B. & Cann, G. M. 2000. Adhesive events in retinal development and function: The role of integrin receptors. Vertebrate eye development 141-156.

Cole, G. J. & Glaser, L. 1984. Inhibition of embryonic neural retinal cell substratum adhesion with a monoclonal antibody. J Biol Chem 259, 4031-4034.

Conover, J. C. & Notti, R. Q. 2008. The neural stem cell niche. Cell Tissue Res 331, 211-224.

Delfino-Machin, M., Lunn, J. S., Breitkreuz, D. N., Akai, J. & Storey, K. G. 2005. Specification and maintenance of the spinal cord stem zone. Development 132, 4273-4283.

Draper, B. W., Stock, D. W. & Kimmel, C. B. 2003. Zebrafish fgf24 functions with fgf8 to promote posterior mesodermal development. Development 130, 4639-4654.

Edward, M. A., Yamamoto, M. & Caviness, V. S. 1990. Organization of radial glia and related cells in the developing murine CNS. An analysis based upon a new monoclonal antibody marker. Neuroscience 36, 121-144.

Felemban, A., Song, X., Kawano, R., Uezono, N., Ito, A., Ahmed, G., Hossain, M., Nakashima, K., Tanaka, H. & Ohta, K. 2014. Akhirin regulates the proliferation and differentiation of neural stem cells in intact and injured spinal cord. Dev. Neurobiol. 75, 494-504.

Forlani, S., Lawson, K. A. & Deschamps, J. 2003. Acquisition of Hox codes during gastrulation and axial elongation in the mouse embryo. Development 130, 3807-3819.

Gerdes, J., Schwab, U. & Lemke, H. 1983. Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int. J. Cancer 31, 13-20.

Grunwald, G. B. 1996. Cadherin cell adhesion molecules in retinal development and pathology. Prog Retinal Eye Res. 15, 363-392.

Hamilton, L. K., Truong, M. K. V., Bednarczyk, M. R., Aumont, A. & Fernandes, K. J. L. 2009. Cellular organization of the central canal ependymal zone, a niche of latent neural stem cells in the adult mammalian spinal cord. NSC 164, 1044-1056.

Heller, S., Sheane, C. A., Javed, Z. & Hudspeth, A. J. 1998. Molecular markers for cell types of the inner ear and candidate genes for hearing disorders. Proc. Natl Acad. Sci. USA 15, 11400-11405.

Honjo, M., Tanihara, H., Suzuki, S., Tanaka, T., Honda, Y. & Takeichi, M. 2000. Differential expression of cadherin adhesion receptors in neural retina of the postnatal mouse. Invest. Ophthalmol. Vis. Sci. 41, 546-51.

Hugnot, J. P. 2010. The spinal cord neural stem cell niche. In: Sun, T., editor. Neural Stem Cells and Therapy. Intech, pp 71-92.

Hynes, R. O. 1992. Integrins: versatility, modulation, and signaling in cell adhesion. Cell 69, 11-25.

Jean, D., Ewan, K. & Gruss, P. 1998. Molecular regulator involved in vertebrate eye development. Mech. Dev. 76, 3-18.

Klein, R. D., Gu, Q., Goddard, A. & Rosenthal, A. 1996. Selection for genes encoding secreted proteins and receptors. Natl Acad. Sci. USA 93, 7108-7113.

Lukaszewicz, A. I. & Anderson, D. J. 2011. Cyclin D1 promotes neurogenesis in the developing spinal cord in a cell cycle-independent manner. Proc. Natl Acad. Sci. USA 108, 11632-11637.

Marthiens, V., Kazanis, I., Moss, L., Long, K. & ffrench-Constant, C. 2010. Adhesion molecules in the stem cell niche – more than just staying in shape? J. Cell Science 123, 1613-1622.

Mathis, L. & Nicolas, J. F. 2000. Different clonal dispersion in the rostral and caudal mouse central nervous system. Development 127, 1277-1290.

Mayne, R., Ren, Z. X., Liu, J., Cook, T., Carson, M. & Narayana, S. 1999. VIT-1: the second member of a new branch of the von Willebrand factor A domain superfamily. Biochem. Soc. Trans. 27, 832-835.

Meletis, K., Bernabe-Heider, F., Carlen, M., Evergren, E., Tomilin, N., Shupliakov, O. & Frisen, J. 2008. Spinal cord injury reveals multilineage differentiation of ependymal cells. PLoS Biol. 6, e182.

Ming, G. L. & Song, H. 2011. Adult neurogenesis in the mammalian brain: significant answers and significant questions. Neuron 70, 687-702.

Mu, H., Ohta, K., Kuriyama, S., Shimada, N., Tanihara, H., Yasuda, K. & Tanaka, H. 2003. Equarin, a novel soluble molecule expressed with polarity at chick embryonic lens equator, is involved in eye formation. Mech. Dev. 120, 143-155.

Namiki, J. & Tator, C. H. 1999. Cell proliferation and nestin expression in the ependymal of the adult rat spinal cord after injury. J Neuropathol. Exp. Neurol. 58, 489-498.

Ohta, K., Ito, A. & Tanaka, H. 2008. Neural stem/progenitor cells in the vertebrate eye. Develop. Growth Differ. 50, 253-259.

Perron, M. & Harris, W. A. 2000. Retinal stem cells in vertebrates. Bio. Essays 22, 685-688.

Redies, C. 2000. Cadherins in the central nervous system. Prog Neurobiol. 61, 611-648.

Rizzolo, L. J., Zhou, S. & Li, Z. Q. 1994. The neural retina maintains integrins in the apical membrane of the RPE early in development. Invest. Ophthalmol. Vis. Sci. 35, 2567-2576.

Sabourin, J-C., Ackema, K. B., Ohayon, D., Guichet, P-O, Perrin, F. E., Garces, A., Ripoll, C., Charite, J., Shimonneau, L., Kettenmann, H., Zine, A., Privat, A., Valmier, J., Pattyn, A. & Hugnot, J-P. 2009. A mesenchymal-like ZEB1(+) niche harbors dorsal radial glial fibrillary acidic protein-positive stem cells in the spinal cord. Stem Cells 27, 2722-2733.

Weiss, S., Dunne, C., Hewson, J., Wohl, C., Wheately, M., Peterson, A. C. & Reynolds, B. A. 1996. Multipotent CNS stem cells are present in the adult mammalian spinal cord and ventricular neuroaxis. J. Neurosci. 16, 7599-7609.

Wohrn, J. C. P., Puelles, L., Nakagawa, S., Takeichi, M. & Redies, C. 1998. Cadherin expression in the retina and retinofugal pathways of the chicken embryo. J Comp Neurol. 396, 20-38.

Zhao, X., Das, A., Soto-Leon, F. & Ahmad, I. 2005. Growth factor-responsive progenitors in the postnatal mammalian retina. Dev. Dyn. 232, 349-358.

Zhao, C., Deng, W. & Gage, F. H. 2008. Mechanisms and functional implications of adult neurogenesis. Cell 132, 645-660.

Figure legends

Fig. 1. (A) Structural motif identified in chick AKH and comparison with its orthologs. The values given for each domain represent the percentage amino acid identity with the corresponding domains in chick AKH. (B) mRNA expression of AKH in chick peripheral retina. CMZ: ciliary marginal zone. Re: retina. Note that the expression of AKH is accumulated to the CMZ at P1 retina. (C) Cell aggregation analysis showing that AKH-expressing transfectants formed large aggregates compared with control cells. Scale bar: 100 μm in A-C.

Fig. 2. (A) Expression of chick and mouse AKH in the spinal cord. Note that strong expression is found around the central canal (CC). (B) Expression of CyclinD2 (red), vimentin (green), and Ki67 (blue) in the spheres derived from WT and AKH-/- spinal cord. (C) Induction of AKH expression in the damaged spinal cord on day 1. Scale bars: 100 μm.