HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm

Original research https://doi.org/10.1016/j.apjtm.2017.10.027

Expression of vimentin and glial fibrillary acidic protein in central nervous system development of rats

Hua Luo[1], Xiao-Qiong Wu[2], Min Zhao[3], Qiong Wang[4], Geng-Pan Jiang[4], Wei-Jun Cai[5], Ming-Ying Luo[3]✉

[1]*Xiangtan Medicine & Health Vocational College, Xiangtan, Hunan, 411104, China*

[2]*Department of Anatomy & Neurobiology, Xiangya School of Medicine, Central South University, Changsha, Hunan, 410003, China*

[3]*Department of Anatomy & Histology & Embryology, Kunming Medical University, Kunming, Yunnan, 650500, China*

[4]*Department of Clinical Medicine, Kunming Medical University, Kunming, Yunnan, 650500, China*

[5]*Department of Histology & Embryology, Xiangya School of Medicine, Central South University, Changsha, Hunan, 410003, China*

ARTICLE INFO

Article history:
Received 24 Aug 2017
Received in revised form 26 Sep 2017
Accepted 25 Oct 2017
Available online 28 Oct 2017

Keywords:
Development
Vimentin
Glial fibrillary acidic protein
Central nervous system

ABSTRACT

Objective: To investigate the distribution and contents of vimentin (Vim) and glial fibrillary acidic protein (GFAP) immunoreactivities in the central nervous system (CNS) of normal newborn, adult and aged rats.

Methods: In this study, thirty healthy and normal Sprague–Dawley rats were simply classified into three groups: Newborn (7 days aged), adult (5 months aged) and aged (24 months aged) rats. Brains and spinal cord were dissected and cut into frozen sections. The expression of Vim and GFAP in CNS were detected by confocal immunofluorescence.

Results: In each group, Vim was expressed in all the regions of CNS including the hippocampal, cerebral cortex, the third ventricle and spinal cord, and the expression was highest in neuron-like cell of newborn rats, while Vim was mainly expressed in cell bodies in adult and aged rats. GFAP was expressed in all the regions of CNS including the hippocampal, cerebral cortex, the third ventricle and spinal cord, and the expression was in astrocytes of aged rats. In the third ventricle, Vim was detected in all groups, and only observed in neuron-like cells of newborn. Meanwhile, the GFAP expression showed no significant differences between adult and aged rats in this region. The co-localization of Vim and GFAP were mainly observed in hippocampus and cerebral cortex of newborn, but this co-localization was found in the third ventricle of the rats in all groups.

Conclusion: Our data demonstrate for the first time that the expression of Vim and GFAP in the rat's CNS during development. This data may provide a foundation for the further mechanistic studies of these two main intermediate filaments during development of CNS.

1. Introduction

The development of the central nervous system (CNS) is a very complex process. Glial cell as one of the major cellular components of the nervous system, plays a very important role in the development of the CNS. Research has shown that in the early development, radial glial cells can differentiate into astrocytes, neurons and other different types of cells after the completion of the neuronal migration [1]. These processes of

First author: Hua Luo, Xiangtan Medicine & Health Vocational College, Xiangtan, Hunan, 411104, China.
Tel: +86 13873280875.
E-mail: 89433121@qq.com
✉Corresponding author: Mingying Luo, Department of Anatomy & Histology & Embryology, Kunming Medical University, Kunming, Yunnan, 650500, China.
Tel: +86 13668717526
E-mail: luomingying0403@163.com

Peer review under responsibility of Hainan Medical University.
Foundation project: This study was supported by National Natural Science Foundation of China (No: 81500377), the Joint Special Fund between Yunnan Provincial Science and Technology Department and Kunming Medical University (No: 2015FB009, 2015FB153) and Program for Students Innovation in Kunming Medical University.

differentiation are characterized by changes of cytoskeleton composition, which are the standards for neuronal and glial formation during development [2]. Intermediate filaments (IFs) are the most complex set of protein among the cytoskeleton, and their expression has obvious specificity during the developmental stage of the nervous system [3]. The presence of these IFs molecular markers as well as regional distribution in CNS of different vertebrates is very important in ontogenetic and phylogenetic studies. Previous studies have revealed that astrocytes simultaneously express two different types of vimentin (Vim) and glial fibrillary acidic protein (GFAP) at different developmental stages or in some pathological conditions, which showed dynamically altered expression patterns [4]. At early development, radial glia and immature astrocytes express Vim as IFs, while GFAP is mainly expressed in mature astrocytes. With the development of the CNS and the differentiation of glial cells, GFAP become the main IFs protein [5,6]. Although the presence of Vim and GFAP in the nervous system has been reported, as far as we are aware, to date there is no detailed and systematic study of their distribution and development changes in rats. The aim of this work is to analyze comparatively the expression and distribution of these two glial IFs proteins in the CNS during development by confocal microscopy immunofluorescence. The results of this study revealed for the first time a complex developmental pattern of Vim and GFAP in different regions of the CNS.

2. Material and methods

2.1. Animals and sample preparation

The present study was performed with the permission of the local animal ethics committee. All protocols were in conformity with the guidance suggestions for the care and use of laboratory animals issued by the Ministry of Science and Technology of the People's Republic of China. Thirty healthy and normal Sprague–Dawley rats were simply classified into three groups: newborn (7 days aged), adult (5 months aged) and aged (24 months aged) rats which were used in this study. All experimental animals were provided by the Animal Center, Xiangya School of Medicine, Central South University. The animals were deeply anesthesia with 10% chloral hydrate (0.4 mL/100 g) by intraperitoneal injection, and perfused through the heart with 0.9% saline solution, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4 at 4 °C). The brains and spinal cord were rapidly dissected and post-fixed in the same fixative at 4 °C for 2 h, then immersed in 15%, 30% gradient sucrose overnight for cryoprotection. A total of 15 μm thickness coronal sections were prepared for further immunofluorescence.

2.2. Immunofluorescence

In the following treatment, all washing and incubation solutions, with the exception of those containing primary antibodies, were employed at room temperature. After pretreatment with 1% bovine serum albumin for 30 min to reduce nonspecific background staining, the sections were incubated overnight in a moist chamber at 4 °C with mouse anti-vimentin antibody (1:50, Sigma) and rabbit anti-GFAP antiserum (1:1 200, Sigma) overnight, then slides were washed in phosphate-buffered saline (three times, 10 min each) and incubated in the secondary antibodies for 2 h, goat anti-mouse (1:200, Vector) and Cy3-conjugated goat anti-rabbit IgG (1:200, Invitrogen). After rinsing in phosphate-buffered saline, the sections were followed by Cy2-conjugated Streptavidin (1:500, Biotrend) for 1 h. To exclude nonspecific immunostaining, the negative controls were obtained by omission of the primary antibodies, replaced by phosphate-buffered saline.

2.3. Quantitative measurements and statistical analysis

Images were obtained on a Nikon confocal microscope (Nikon, Japan). The quantitation of immunofluorescence intensity of Vim and GFAP was performed with the quantitation software EZ-C1 3.70. Briefly, one channel with format 512 and appropriate filters was used. A full range of gray values from black to peak white (0-pixel to 255-pixel intensity level) was set during the whole process of measurements. The intensity of fluorescence was expressed as arbitrary units (AU)/μm^2.

All data are presented as mean ± SEM. Statistical comparisons between groups were performed with Student's *t*-test. Differences among means at $P < 0.05$ were considered as significant ($P < 0.01$).

3. Results

3.1. Expression of Vim and GFAP in rat hippocampus during development

In newborn rats, Vim with long processes were strongly stained in molecular layer and polymorphic layer and weakly in pyramidal layer of hippocampal CA1–CA3. Compared to newborn, Vim neuron-like cells was significantly decreased while still evident in the pyramidal layer. In aged rats, the profile of Vim was resembled to the adult rats, just immunoreactivity further diminished. In dentate gyrus, Vim were mainly observed in molecular layer and faint in granular and polymorphic layer of the newborn rats. With development, the expression of Vim was gradually diminished. Furthermore, Vim immunoreactivity were rarely seen in aged rats.

In newborn rats, GFAP immunoreactivity was very scarce in molecular layer and polymorphic layer and even devoid in pyramidal layer of hippocampal CA1–CA3. As in adult rats, GFAP mainly expressed strongly in astrocytes and localized in the molecular and polymorphic layer of hippocampal. With development, GFAP astrocytes-like cells were abundant in molecular layer and polymorphic layer of hippocampal. In dentate gyrus, GFAP was observed in molecular layer and weakly in polymorphic and granular layer of newborn rats. As adult rats, GFAP increased obviously. Among aged rats was the most strongest in polymorphic and granular layer. In hippocampus, the co-expression of Vim and GFAP were only observed in newborn rats. The immunofluorescence intensity of Vim and GFAP in hippocampus CA1–CA3 and dentate gyrus of each group is shown in Table 1.

3.2. Expression of Vim and GFAP in rat cerebral cortex during development

In newborn rats, Vim was widely localized in cerebral cortex, which showed long and thin fibers. As compared to newborn,

Table 1
Immunofluorescence intensity (AU/μm^2) of Vim and GFAP in hippocampus.

	Groups	CA1	CA2–CA3	DG
Vim	Newborn rats	155.2 ± 16.1	174.2 ± 18.2	139.2 ± 21.1
	Adult rats	123.3 ± 16.5[a]	112.7 ± 10.3[a]	86.0 ± 8.5[a]
	Aged rats	106.1 ± 11.2[a b]	103.8 ± 14.2[a b]	48.1 ± 6.1[a b]
GFAP	Newborn rats	49.2 ± 7.2	152.8 ± 10.0	89.6 ± 15.2
	Adult rats	187.8 ± 20.5[a]	204.1 ± 19.3[a]	176.8 ± 23.4[a]
	Aged rats	210.7 ± 25.4[a b]	223.4 ± 15.4[a b]	193.2 ± 22.8[a b]

[a] $P < 0.01$ compared with newborn rats. [b] $P < 0.01$ compared with adult rats.

Vim projections of neuron-like cells were observed and only stained in cell bodies of adults rats, while the expression showed no significantly difference between aged rats and adult rats.

In newborn rats, GFAP was weakly. As adult rats, GFAP was increased throughout all regions of the cerebral cortex, the radially fibers were the dominant features in the midmeningeal surface and ependymal area of the lateral ventricle. As compared to adult rats, the GFAP was most abundant in aged rats. Double immunostaining showed Vim and GFAP co-expressed occurred in newborn rats. The immunofluorescence intensity of Vim and GFAP in the cerebral cortex of each group was shown in Table 2.

3.3. Expression of Vim and GFAP in rat third ventricle of cerebrum during development

In all brain ventricles, the expression of Vim and GFAP showed the same pattern between different ages, so the third ventricle was selected for example. In newborn rats, the lumen of the third ventricle was relatively small and exhibited in rhombus in coronal section, Vim neuron-like cells were distributed widely surrounding the third ventricle. As development, the lumen enlargement was significant and positive fibers of Vim fibers were stretched radially from the third ventricle to the outer surface. In aged rats, the lumen was furtherly enlarged and Vim expression was the same as adult rats. Interestingly, there was no significant change in Vim among all groups in the third ventricle.

In newborn rats, GFAP was faint. As adult rats, more dense and frequent GFAP staining was observed close to the lumen of the third ventricle. In aged rats, GFAP expression was also increased. In all groups, the co-expressed Vim and GFAP were arranged closely, and were characterized with long base and narrow shape, which was near the lumen of the third ventricle. The immunofluorescence intensity of Vim and GFAP in the third ventricle of each group is shown in Table 3.

Table 2
Immunofluorescence intensity (AU/μm^2) of Vim and GFAP in cerebral cortex.

Groups	Vim	GFAP
Newborn rats	150.6 ± 18.1	53.2 ± 5.1
Adult rats	125.0 ± 9.4[a]	113.2 ± 13.6[a]
Aged rats	118.2 ± 13.6[a]	197.6 ± 20.2[a b]

[a] $P < 0.01$ compared with newborn rats. [b] $P < 0.01$ compared with adult rats.

Table 3
Immunofluorescence intensity (AU/μm^2) of Vim and GFAP in third ventricle.

Groups	Vim	GFAP
Newborn rats	224.0 ± 29.3	124.1 ± 16.2
Adult rats	223.1 ± 25.9	183.2 ± 20.1[a]
Aged rats	223.9 ± 19.3	179.6 ± 19.2[a]

[a] $P < 0.01$ compared with newborn rats.

Table 4
Immunofluorescence intensity (AU/μm^2) of Vim and GFAP in spinal cord anterior horn of each group.

Groups	Vim	GFAP
Newborn rats	147.2 ± 10.5	58.2 ± 8.5
Adult rats	124.2 ± 15.3[a]	116.2 ± 10.3[a]
Aged rats	106.7 ± 14.1[a b]	212.6 ± 14.3[a b]

[a] $P < 0.01$ compared with newborn rats. [b] $P < 0.01$ compared with adult rats.

3.4. Expression of Vimentin and GFAP in rat spinal cord anterior horn during development

The expression in the posterior horn of the spinal cord displayed the same patterns as the anterior horn, so the anterior horn was taken for example. In newborn rats, Vim was significantly expressed in the anterior horn of spinal cord on the cross-section. In adult rats, a certain number of Vim neuron-like cells were observed and localized in cell bodies. With development, the expression was gradually decreased.

In newborn rats, GFAP was weakly expressed. As development, GFAP was gradually increased and mainly expressed in astrocytes, and the expression was strongest in aged rats. The immunofluorescence intensity of Vim and GFAP in anterior horn of each group is shown in Table 4.

4. Discussion

The main findings of this study are as follows: (1) Expression of Vim and GFAP showed different temporal patterns during development of rat CNS, in the hippocampal, cerebral cortex and spinal cord. Vim expression was decreased with age, whereas GFAP was increased, but there was no obvious change for these proteins in the third ventricle of cerebrum of all groups; (2) Vim was mainly localized in neuron-like cells, but GFAP mainly in astrocytes; (3) The co-localization of Vim and GFAP was observed in cortex and hippocampal of newborn rats, while always be in the third ventricle in all groups.

4.1. The expression of Vim in rat CNS during development

The development is characterized by a change in the composition of IFs. Among IFs, Vim is frequently used as a radial glias marker, and is mainly expressed in undifferentiated neural stem cells, neural progenitor cells and glial precursor cells, which are closely related to the capability of plasticity and regeneration in CNS [7,8]. In immature brain, especially during the very early stages of development, Vim occurs evenly in immature precursors of nerve and most glial cells [9]. In this

study, we also observed that Vim was highly expressed in immature CNS of newborn rats, including the hippocampus (CA1–CA3), cerebral cortex and spinal cord. Furthermore, it was evident that in the newborn rats, Vim was distributed in the cytoplasm and projections of neuron-like cells. These results suggested that Vim was involved in the development of CNS in neonatal period. As compared to the newborn rats, Vim expression was significantly decreased in the CNS of adult and aged rats where Vim only stained the cell bodies, indicating that Vim may be involved in the differentiation and maturation of neuronal and glial cells during development. The mechanism of Vim in the development process is very complicated. Curchoe *et al*. confirmed that early migratory neural crest stem cells can participate in neural crest differentiation into neurons and glial cells by upregulating Vim expression and remodeling of F-actin [10]. Farach *et al*. showed a regulated pattern of O-GlcNAc modification of Vim filaments in the developing chick optic tectum and supposed a role for O-GlcNAc-modified Vim in radial glia, but not in neurons during brain development [11]. Our observation about Vim expression profile in the CNS is in a line with a previous report that showed Vim has a transient expression in immature nerve cells, and decreased in the development of mature nerve cells [12]. A novel finding about Vim expression in the CNS during development was that high level of Vim expression was presented in the third ventricle of different aged rats, indicating that Vim expression in different regions of the CNS is inconsistent during brain development and aging. Alternative explanations for this phenomenon might be closely related to the structure and function of brain tissue in different parts. This is consistent with Lazzari studies on leopard gecko that has shown in different regions of the CNS, the staining intensity appears not to be identical even in the same cellular type [13], and emphasized that different brain regions require different functional neurons and glial cells to adapt to different neuronal needs.

4.2. Expression of GFAP in rat CNS during development

GFAP is one type of III intermediate filament protein and is expressed by radial glia, adult astrocytes and neural stem cells [8,14]. Previously studies reported that even though radial glial cells are considered to be an immature astrocytes. At the early stage of embryonic development, radial glial cells play an indispensable role in the formation of axons during the formation of neural circuits and are temporally and undergo direct transformation into astrocytes shortly after neuronal migration [2]. In present study, we showed that the expression of GFAP in newborn was weak in the hippocampus (CA1–CA3), cerebral cortex and spinal cord, and was increased in adults and was abundant in aged rats. This protein was mainly expressed in astrocytes. Astrocytes have supportive roles for neurons and are very important for proper formation of the CNS during embryonic and fetal development [15]. Astrocytes also play major roles in controlling the ionic environment of neurons. Some astrocytes develop processes with expanded perivascular feet that cover capillary endothelial cells and contribute to the blood–brain barrier. In addition, astrocytic functions are essential for neuronal survival. They regulate constituents of the extracellular environment, absorb local excess of neurotransmitters, and secrete numerous metabolites and factors regulating neuronal activities. Based on theses important functions of astrocytes, it is conceivable for an increased expression of GFAP in adult CNS of rats. Preliminary published data obtained in cytokine-induced activation of astrocytes in the periventricular brain found that the response of astrocytes to inflammation is enhanced in aged brain [16], this is in agreement with our finding that GRAP was highly expressed in aged rat CNS indicating that astrocytes are activated aging brain.

Experiments *in vitro* have shown that the nervous system developed to a certain period of time, Vim and GFAP often co-localize in astrocytes and astrocytes precursor cells in the form of filaments coexistence [12]. The ratio of GFAP to Vim reflects the degree of differentiation and the functional status of these cells. At the early stage of the CNS formation, the IFs of the radial glial cells is predominantly Vim, when they become more mature astrocytes, they began to express GFAP and progressively replaced Vim. Meanwhile, Dahl *et al*. demonstrated that the major cytoskeletal proteins of neuroglia, Vim-GFAP transition in rat brain occurs at the time of early myelination, usually in postnatal period of 12 d–14 d [17]. These views could partially explain the reasons for the GFAP increase and Vim decrease in adult and aged rats in cerebral cortex, hippocampal and anterior horn. In addition, we also found the co-expression of Vim and GFAP located in cortex and hippocampal of newborn, but it was not apparent in adult and aged rats. Mi *et al*. detected only Vim expression in the astrocytes precursor cells from 16 d to 19 d of embryonic stage and 1 d after birth, while Vim and GFAP can be observed in perinatal astrocytes [18]. Based on above-mentioned information, we propose that the Vim and GFAP cells in neonatal rat cerebral cortex and hippocampal could be co-expressed by astrocytes, however, the expression in other cell types remains to be further determined.

Moreover, in present study, we also observed a large number of cells with co-localization of strong Vim and weak GFAP in long coraua and narrow shape which were arranged closely in the third ventricle. According to its location and shape we assume it may be elongated cells, which is a type of incomplete conversion of glial cells between the radial glial cells and astrocytes, and participate in and promote axonal regeneration in mammal [19,20]. But the possible functions need further study.

In conclusion, our study showed a noticeable distribution and development changes of Vim and GFAP immunoreactivity during CNS in rats. This data may serve as a foundation for further study on the role of Vim and GFAP during development and maturation of the nervous system, and for the study on their significance in nerve injury, repair, degenerative disease and regeneration.

Conflicts of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This work was supported by grants from NSFC of Chinese government (NO: 81500377), grants from the Department of Yunnan Science and Technology-Kunming Medical University Joint Special Fund (NO: 2015FB009, 2015FB153), and grants from Kunming Medical University Students Innovation Program.

References

[1] Kaur C, Rathnasamy G, Ling EA. Biology of microglia in the developing brain. *J Neuropathol Exp Neurol* 2017; **76**(9): 736-753.

[2] Nulty J, Alsaffar M, Barry D. Radial glial cells organize the central nervous system via microtubule dependant processes. *Brain Res* 2015; **1625**: 171-179.

[3] Sukhorukova EG, Kruzhevskiĭ DÉ, Alekseeva OS. Glial fibrillary acidic protein: the component of intermediate filaments in the vertebrate brain astrocytes. *Zh Evol Biokhim Fiziol* 2015; **51**(1): 3-10.

[4] Garcia A, Masot J, Franco A, Gazquez A, Redondo E. Immunohistochemical evaluation of the goat forestomach during prenatal development. *J Vet Sci* 2014; **15**(1): 35-43.

[5] Leduc C, Etienne-Manneville S. Intermediate filaments join the action. *Cell Cycle* 2017; **16**(15): 1389-1390.

[6] Hol EM, Pekny M. Glial fibrillary acidic protein (GFAP) and the astrocyte intermediate filament system in diseases of the central nervous system. *Curr Opin Cell Biol* 2015; **32**: 121-130.

[7] Kirkcaldie MTK, Dwyer ST. The third wave: intermediate filaments in the maturing nervous system. *Mol Cell Neurosci* 2017; **17**: 30062-30063.

[8] Parlakian A, Paulin D, Izmiryan A, Xue Z, Li Z. Intermediate filaments in peripheral nervous system: their expression, dysfunction and diseases. *Rev Neurol (Paris)* 2016; **172**(10): 607-613.

[9] Chen P, Cescon M, Megighian A, Bonaldo P. Collagen VI regulates peripheral nerve myelination and function. *FASEB J* 2014; **28**(3): 1145-1156.

[10] Curchoe CL, Maurer J, McKeown SJ, Cattarossi G, Cimadamore F, Nilbratt M, et al. Early acquisition of neural crest competence during hESCs neuralization. *PLoS One* 2010; **5**(11). e13890.

[11] Farach AM, Galileo DS. O-GlcNAc modification of radial glial vimentin filaments in the developing chick brain. *Brain Cell Biol* 2008; **36**(5–6): 191-202.

[12] Vinci L, Ravarino A, Fanos V, Naccarato AG, Senes G, Gerosa C, et al. Immunohistochemical markers of neural progenitor cells in the early embryonic human cerebral cortex. *Eur J Histochem* 2016; **60**(1): 2563.

[13] Lazzari M, Franceschini V. Intermediate filament immunohistochemistry of astroglial cells in the leopard gecko, *Eublepharis macularius*. *Anat Embryol (Berl)* 2005; **210**(4): 275-286.

[14] Deka H, Sarmah R, Sharma A, Biswas S. Modelling and characterization of glial fibrillary acidic protein. *Bioinformation* 2015; **11**(8): 393-400.

[15] Coelho-Aguiar Jde M, Bon-Frauches AC, Gomes AL, Veríssimo CP, Aguiar DP, Matias D, et al. The enteric glia: identity and functions. *Glia* 2015; **63**(6): 921-935.

[16] Sheng W, Zong Y, Mohammad A, Ajit D, Cui J, Han D, et al. Pro-inflammatory cytokines and lipopolysaccharide induce changes in cell morphology, and upregulation of ERK1/2, iNOS and $sPLA_2$-IIA expression in astrocytes and microglia. *J Neuroinflammation* 2011; **8**: 121.

[17] Dahl D. The Vimentin-GFAP protein transition in rat neuroglia cytoskeleton occurs at the time of myelination. *Neurosci Res* 1981; **6**(6): 741-748.

[18] Mi H, Barres BA. Purification and characterization of astrocyte precursor cells in the developing rat optic nerve. *J Neurosci* 1999; **19**(3): 1049-1061.

[19] Liu Z, Li Y, Cui Y, Roberts C, Lu M, Wilhelmsson U, et al. Beneficial effects of gfap/vimentin reactive astrocytes for axonal remodeling and motor behavioral recovery in mice after stroke. *Glia* 2014; **62**(12): 2022-2033.

[20] Chauvet N, Parmentier ML, Alonso G. Transected axons of adult hypothalamo-neurohypophysial neurons regenerate along tanycytic processes. *J Neurosci Res* 1995; **41**(1): 129-144.