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

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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

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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,