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Expression of alpha-synuclein during eye development of mice (*Mus musculus*), chick (*Gallus gallus domisticus*) and fish (*Ctenopharyngodon idella*) in a comparison study

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ABSTRACT

Synucleins are small proteins associated with neurodegenerative diseases, alpha-synuclein is a Parkinson's disease-linked protein of ubiquitous expression in the central nervous system. This study aimed at the localization of alpha-synuclein during eye development of mice (*Mus musculus*), chick (*Gallus gallus domisticus*) and fish (*Ctenopharyngodon idella*) by immunohistochemical staining in a comparison study. The results showed that alpha-synuclein expression increased gradually with the development of ciliary body, iris, retina and cornea of mice at E17, P1, P3, P7 and chick at E5, E10, E15 with unequal appearance of alpha-synuclein expression. Also, it was not detected in iridocorneal angle during eye development of mice and chick. Alpha-synuclein expression during fish eye development at P10, P15, P20 was not detected either in the ciliray body or Iris regions and it was pronounced with sharp signals in the highly specialized tissue of the iridocorneal angle at P20. Also, the expression was gradually increased from P15 to P20 in fish retina and cornea. The pattern of expression and distribution of alpha-synuclein during the development of ciliary body and iris of mice, chick and fish has not been previously characterized, The data concluded that alpha-synuclein has important cellular function during eye development of studied animals.

1. Introduction

Synucleins are small proteins (14.5–20 kDa) with chaperonic properties (Souza et al., 2000; Surgucheva et al., 2005). Alpha-synuclein belongs to the Synuclein family, which comprises alpha-, beta-, and gamma-synuclein (Surguchov et al., 1999; Tofaris and Spillantini, 2007). Alpha-synuclein was initially identified as a component of acetylcholine vesicles in the electric fish Torpedo californica (Maroteaux et al., 1988). It is a neuronal protein expressed in numerous areas throughout the brain (Ueda et al., 1993; Iwai et al., 1995; Irizarry et al., 1996; Bayer et al., 1999; Solano et al., 2000). Alpha-synuclein is abundant in the neuronal cytosol, presynaptic terminals and synaptic plasticity (Maroteaux et al., 1988; Iwai et al., 1995; George et al., 1995; Clayton and George, 1999). High levels of this protein are found in midbrain dopaminergic neurons (Solano et al., 2000; Neystat et al., 1999; Kingsbury et al., 2004), it was shown that alpha-synuclein plays an important pathological and physiological function, alpha-synuclein have been implicated specifically in several diseases, e.g., Alzheimer's disease (Ueda et al., 1993; Mezey et al., 1998; Lippa et al., 1999), Parkinson's disease (Kruger et al., 1998; Iwatsubo, 2003), Lewy body dementia (Trojanowski et al., 1998). Another member of the family, gamma-synucleinis involved in breast cancer (Ji et al., 1997; Jia et al., 1998). ovarian tumors, and other disorders (Ji et al., 1997; Jia et al., 1998; Ninkina et al., 1998). Alpha-synuclein has an important physiological function, in prenatal development and in the formation of the nervous system (Ltic et al., 2004). It is expressed in neurons during human gestation, starts disappearing in early childhood and reappear in the adult neurons (Raghavan et al., 2004). Alpha-synuclein has important role during mouse embryonic development (Zhong et al., 2010), during lens development (Surgucheva et al., 2010) and expressed in the retina of different species of vertebrates (Martinez-Navarrete et al., 2007). The aim of the present study is to understand the normal cellular function of alpha-synuclein during eye development of mice, chick and fish by immunohistochemistry staining in a comparison manner.

2. Material and methods

Mature males and virgin females of mice, *Mus musculus*, weighing approximately 30–35 g were obtained from animal house,

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Faculty of Medicine, Assiut University for Experimentation. Two females were mated with one male and zero day of pregnancy was determined by appearance of vaginal plug. Normal five eyes from Prenatal stage E17 and postnatal stages at P1, P3, P7 were used for study. Fertile chicken eggs of Egyptian Fayoumi (*Gallus gallus domisticus*) were obtained from chicken culture station of species preservation at Sohag, Normal eyes of five chick embryos at E5, E10 and E15 day of incubation at 37 °C were used for study. Newly hatched larvae (one day old) of grass carp, *Ctenopharyngodon idella* were obtained from fish culture station in alahiwa at Sohag, Egypt. Daily renewal of fresh water and fish food supply were carried out with permanent aeration. The use of experimental animals in this study was conducted under the guidance of the basic standards in the care and use of laboratory animals, which has been prepared and published by the institutional animal care committee. Five Normal eyes at P10, P15, and P20 during larval development were used for study. The eyes at different stages of mice, chick and fish were fixated in caryno's fixative, dehydrated in ethyl alcohol, cleared in methyl benzoate and mounting in paraffin wax. The tissue prepared as described were cut in 5 μm sections and stained by hematoxylin and eosin for general histology (Drury and Wallington, 1976).

For immunostaining of alpha-synuclein, paraffin wax sections of Carnoy's fixed tissues (5 μm thick) from eyes of mice, chick and fish at different stages were mounted on Superfrost/Plus glass slides. The slides were deparaffinized in xylene, rehydrated in ethanol and retrieved for re-antigenicity by using 10 mM citrate buffer at pH6 in 100 °C for an hour (Buchlowalow and Bocker, 2010). Then the sections were treated for 10 min with hydrogen peroxide block and sections were blocked in 5% BSA dissolved in 0.1 M phosphate buffer (pH7.4) overnight and then incubated with primary antibody against alpha-synuclein (Rabbit anti-human Synuclein-alpha polyclonal antibody, Spring Bioscience, USA) for three hours at room temperature. Sections were then washed using phosphate buffer and incubated with secondary antibody (FITC, goat anti-Rabbit, Sigma Aldrich, USA) by dilution 1:1000 in phosphate buffer, then washed and mounted by using vectashield (sigma). Sections were analyzed and photographed by using fluorescent microscope (Axio Scope A1, Zeiss, Germany).

3. Results

To evaluate the role of alpha-synuclein during normal eye development of mice, immunohistochemistry of alpha-synuclein was performed during prenatal stage E17 and postnatal stages P1, P3 and P7. The signals of alpha-synuclein increased from E17 to P7 (Fig. 1J–M). At E17 the signals was not detected in ciliary body (Fig. 1J), and the expression start at p1 (Fig. 1K, arrows) with bright signals at p3 (Fig. 1L, arrows) between the inner and outer layers of ciliary body that was accompanied with branching and development of ciliary body. The expression was more clearly in the branching of ciliary body processes (Fig. 1M, arrows) and in the inner layer of iris (Fig. 1E) at P7. The retina showed increase in the signals intensity of alpha-synuclein from low at E17 to high at P7 (Fig. 1R–U), at E17 faint signals were detected in the neural layer (NL) (Fig. 1R, arrows), P1 showed increase in the expression in the ganglion cell layer (GCL) and inner plexiform layer (IPL) (Fig. 1S, arrows). P3 showed more expression than E17 and P1 in the GCL and IPL (Fig. 1T). The retina at P7 showed increase of expression in the GCL, IPL and outer plexiform layer OPL (Fig. 1U). In addition, the signals of expression in cornea was increased from E17 to P7 (Fig. 1V–Y). The normal chick eye development showed increase in expression of alpha-synuclein from E5 to E15 (Fig. 2). Alpha-synuclein signals was not detected in any structures of chick eye at E5 (Fig. 2H). The expression was gradually increased from E10 (Fig. 2I) to E15 (Fig. 2D and J) in the non-pigment epithelium (NPE) of ciliary body and Iris. At the same time, gradually increased in expression from E10 (Fig. 2O) to E15 (Fig. 2P) in different region of retina as GCL (ganglion layer cells), IPL (inner plexiform layer), OPL (outer plexiform layer) and photoreceptor layer (PCL) of outer segments (OS). In addition, the cornea showed increase in expression from E10 (Fig. 2R) to E15 (Fig. 2S). Alpha-synuclein expression during normal fish eye development at P10, P15 and P20 by immunohistochemistry showed increase in the staining from P10 to P20, the expression was not detected at P10 either in ciliary body or cornea (Fig. 3G) and faint signals in photoreceptor layer (PCL) of retina (Fig. 3M, arrows). The retina at P15 showed small signal in photoreceptor layer (PCL) of outer segments (OS) (Fig. 3N, arrows) and weak expression in the mesenchyme of iridocorneal angle (Fig. 3H). The expression was more bright at p20 in the retina structures as GCL, IPL, OPL and photoreceptor layer (PCL) of outer segments (OS) (Fig. 3O). On the other hand, the expression was not detected either in the ciliray body or Iris regions (Fig. 3I) but it was pronounced with sharp signals in the highly specialized tissue of the iridocorneal angle (ICA) (Fig. 3I) that start with weak signals at P15. In addition, gradually increased in expression of cornea (C) from P10 to P20 (Fig. 3H and I). This work showed that alpha-synuclein expression was pronounced in the iridocorneal angle (ICA) of fish that was not detected in mice and chick. From the same view, alpha-synuclein expression was pronounced in the ciliary body and iris of both mice and chick that was not exhibited of expression in the ciliary body and iris of fish. This work is consider, the first report on the presence of alpha-synuclein in ciliary body, iris during eye development of mice, chick and fish.

4. Discussion

It has become interesting to explore the normal cellular and developmental functions of alpha-synuclein during eye development of studied animals. Some of the previous studies showed its role during brain mouse-embryonic development (Hsu et al., 1998), in the midbrain/hindbrain junction at E9.5 (Zhong et al., 2010), in the hippocampus and neocortex on P1 and peaked at P7 (Hong et al., 1998), it was first found at E12.5 in the ventral midbrain (Simon et al., 2001), it is highly expressed in postnatal stages (P0, P3, and P14) (Petersen et al., 1999) and it was maintained to adulthood (Vila et al., 2000; Smidt et al., 2004; Michell et al., 2007). In addition, alpha-synuclein might be involved in the migration of neurons at early stages and in synaptogenesis (Zhong et al., 2010) and it has been found in the neurons of the dorsal root ganglia in adult mice (Giasson et al., 2001). Also it is expressed during embryonic development in other tissues and organs, including the nasal mucosa, the sensory ganglia, and their dominating nerve fibers (Zhong et al., 2010). Alpha-synuclein expression was not restricted during development but it has been found in several studies both in physiological and pathological situations (Goers et al., 2003; Specht et al., 2005; Kontopoulos et al., 2006; Yu et al., 2007). Dysfunction of alpha-synuclein has been linked to the onset and progression of neurodegenerative diseases (Clayton and George, 1999; Di Rosa et al., 2003; George, 2002; Trojanowski and Lee, 2002; Surguchov, 2008a,b) where alpha-synuclein accumulates at presynaptic nerve endings in the brain, where it likely participates in the synaptic-vesicle cycle, including neurotransmitter storage, release and reuptake (Abeliovich et al., 2000; Lee et al., 2001; Chandra et al., 2004). An interesting property of synucleins is their ability to change intracellular localization in response to stress, change conditions, etc. (Surgucheva et al., 2006).

The results of this study demonstrate the important of cellular function of alpha-synuclein during eye development of mice, chick and fish. This paper focus on the gradually increase of alpha-synuclein signals during the development of ciliary body and iris