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COMPARATIVE AND ONTOGENIC BIOCHEMISTRY

Alpha-Fetoprotein in Retina and Lens of the Human Eye at Early Stages of Prenatal Development

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Abstract—The presence of alpha-fetoprotein (AFP) was shown in the retina and lens of the human fetal eye at different stages of prenatal development. PCR analysis revealed AFP mRNA neither in the retina nor in the lens, whereas in the fetal liver (control) AFP mRNA was found to be expressed. The data obtained indicate that AFP is not synthesized in retinal and lens cells of the human fetal eye but is imported from elsewhere to be taken up by these cells. The presence of AFP in the retina and lens implies its involvement in early morphogenesis and differentiation of these ocular tissues during prenatal human development.

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Key words: alpha-fetoprotein, human fetus, retina, lens, vitreous body.

INTRODUCTION

Alpha-fetoprotein (AFP) is an embryo-specific glycoprotein, a major component of blood serum in vertebrates during embryonic and fetal development. At early embryonic stages, AFP is synthesized by cells of the visceral yolk sac and hepatic endoderm as well as (to a lesser extent) the gastrointestinal tract, and after 8-week gestation—mainly by hepatic cells [1–4]. From the liver, AFP is released into the blood circulatory system and transported to target tissues of developing human embryos and fetuses. Apart from blood serum, AFP was also found in other human fetal tissues such as the cerebrospinal fluid, bile, meconium [5], amniotic fluid [6], vitreous body [7, 8].

AFP plays a crucial role in human prenatal development. The AFP level in blood serum of pregnant women is used for diagnostics of some disturbances in fetal development [9]. The elevated AFP level in the maternal blood indicates neural tube defects while its low level is intrinsic to Down's syndrome [10].

The detection of AFP in the cerebrovascular system and the presence of its specific receptors in neurons of some vertebrate's embryos implies an appreciable role of this protein in the CNS development. Using immunoperoxidase labeling, it was shown that AFP is present in neurons of different regions of the developing brain in mouse, rat and chicken embryos. It was also localized to the inner nuclear layer of the retina in a 9-week fetus of the

AFP concentration (IU/mL) in the vitreous body, retina and lens during prenatal development of the human eye ("–" means no measurements)

Age, weeks (number of fetuses)	Vitreous body	Retina	Lens
14 (1)	2030600	–	–
16 (3)	1060600	–	2599
	–	10078	–
	186900	41730	52218
18/19 (1)	498499	10825	3164
22 (1)	111700	–	2387
24 (2)	349200	30516	16892
	920600	54402	15018
31 (1)	5328	–	346

baboon [11]. When cultivating retinal neurons of chicken embryos, it was demonstrated by the addition of AFP into the culture medium that differentiating neurons and ganglionic cells take up AFP [12]. These observations made on animals indicate that the intracellular presence of AFP results not from its synthesis *in situ* but its import via neuronal uptake [11].

The vitreous body, being an internal ocular medium, plays a considerable role in the differentiation of the adjacent retina and lens [13–15]. During the human prenatal development, the vitreous body was found to contain AFP [7, 8]. In other tissues of the developing human eye, AFP was not studied. The knowledge of the AFP distribution across ocular tissues is important for the evaluation of its physiological role in the development of ocular structures.

In this work, along with the vitreous body, we studied the AFP level in the lens of the human fetal eye.

MATERIALS AND METHODS

The study was carried out on the human eye's retina, lens and vitreous body obtained from the abortive material. Fetuses were supplied to the Kulakov Research Center of Obstetrics, Gynecology and Perinatology within the framework of legislation of the Russian Federation on health protection of citizens and according to the approved list of medical indications. The age of fetuses corresponded to terms determined by an obstetrician.

Under a binocular microscope MBS-9, the retina was cut off along the limb border allowing the vitreous body to be extracted together with the lens, with the latter separated carefully thereafter. The retina was separated from the pigment epithelium, washed (together with the lens) in 0.9% NaCl, weighed, diluted 2–3 times by distilled water, and homogenized. The vitreous body and retinal homogenates were centrifuged at 12 000 rev/min on the Eppendorf centrifuge 5417R at 4°C for 30 min. Then, supernatants were collected to be used further for measuring the AFP concentration with the use of the Elecsys AFP assay (cat. No. 04481798190) on the immunochemical cobas e 411 analyzer (F. Hoffman–La Roche Ltd., Switzerland). The ages of fetuses used for assaying the AFP concentration in the retina, lens and vitreous body are show in Table 1.

To analyze AFP gene expression in tested tissues, the retina and lens from 12-week fetuses were used along with the 16-week fetal liver taken as a control. Total RNA isolation from tested tissues was carried out using the TRI Reagent mixture (Sigma). cDNA libraries were synthesized on total RNA using the Sileks kit (Russia). PCR was conducted in 25 μL of the reaction mixture (0.625 units of Color Taq DNA Polymerase, 1x buffer, 250 mM dNTP, 400 mM specific primers, 0.5 μL cDNA) using the Eppendorf Mastercycler amplificator (Germany). The reaction conditions were the following: the primer annealing temperature—58°C, the number of cycles—30. PCR results were analyzed using horizontal gel electrophoresis in 2% agarose gel on 1x TAE buffer. Ethidium bromide was used as an intercalating dye. Electrophoresis was carried out in the Horizon58 chamber (Life Technologies Inc., USA). Gel was photographed on the GelDoc XR transilluminator (Bio-Rad Laboratories, USA) using the Quantity One software (Bio-Rad Laboratories, USA). cDNA libraries were pre-normalized by the housekeeping gene RPL18s (Fig. 1).

RESULTS AND DISCUSSION

The AFP concentration in the vitreous body, retina and lens of the human fetal eyes is presented

in Table. Between 14th and 24th weeks of gestation, the vitreous body displays a high AFP level. In the retina and lens, the AFP level is 1 or 2 orders of magnitude lower than in the vitreous body but, at the same time, shows high values until the 24th week. At the 31st week, there is a sharp drop in the AFP level both in the vitreous body and the lens (the retina was not measured), as consistent with the well-known tendency toward decreasing concentration of this protein in the fetal blood circulation [16].

RCR analysis (Fig. 1) shows that mRNA to AFP are detected only in the liver (control, whereas in the retina and the lens it is lacking. This indicated that AFP is not synthesized during development in retinal and lens cells themselves but is taken up from outside.

A high AFP concentration that we found in the vitreous body, retina and lens in human fetuses in the 2nd trimester of gestation is well in line with the idea of the AFP involvement in the differentiation of retinal neurons and lens fibers, as shown in the vertebrate CNS and retina [11]. In mice and rats, it was demonstrated that AFP binds with a high degree of affinity to estrogens and thus participates in the regulation of sexual differentiation of the brain [17, 18]. The ability of AFP to bind estrogens suggests that this protein protects the developing fetus from the influence of maternal estrogens [19].

AFP shows a high affinity to polyunsaturated fatty acids (PUFA) [20]. During the prenatal development of human fetuses, PUFA are not synthesized, and major functions of AFP in embryonic and fetal periods include transport of PUFA from the maternal blood through the placenta to fetal tissues [21]. Apparently, AFP supplies PUFA, which are essential for the assembly cell membranes and growth of retinal neurons and lens fibers [22, 23]. In many embryonic tissues of vertebrates there have been found AFP receptors which are involved in PUFA transport to cells. It has been shown that the developing retina and the brain in mammals and birds display a strong immunoreactivity for AFP [11], and these tissues are exactly those that have a high PUFA level [24].

By now, there is ample evidence that AFP and polypeptide growth factors share some common properties. It has been demonstrated that AFP is able to modulate activity of growth factors, and this ability can be realized through its effect at different stages of the cascade mechanism of signal transmission by binding of growth factors to membrane receptors [19]. During the 2nd trimester of gestation, there occur most critical events in the development and differentiation of the human eye. Maximum AFP concentration during this period indicates a meaningful involvement of AFP in growth and differentiation of the retina and lens.

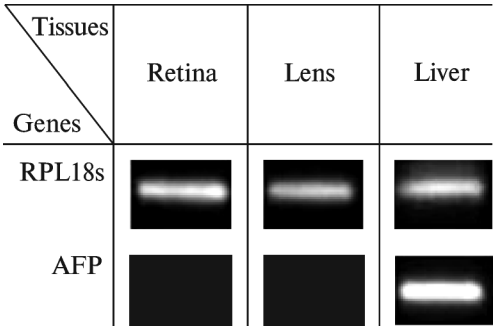

Fig. 1. PCR analysis of alpha-fetoprotein (AFP) gene expression in the retina and lens of 12-week human fetuses and 16-week human fetal liver (control).

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