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Expression of Small Heat Shock Proteins in Tissue Differentiation of Pig Fetuses

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Alpha crystallins are some of the major representatives of the small heat shock proteins' family. At first, they were considered organ-specific, forming the structure of the eye lens, but it is now clear that they are expressed in different tissues and organs, physiologically and under stress. Their expression is also associated with cell proliferation and differentiation.

The aim of the current study is to track out the expression of α-crystallins in some pig fetuses tissues at 20 days, 4, 6 and 8 weeks of pregnancy.

To demonstrate the presence of α-crystallins, indirect immunoperoxidase test was used, with application of obtained by us rabbit anti-alpha-crystallin serum.

The presence of the investigated small stress proteins was ascertained in differentiating tissues – mesenchyme tissue, blood tissue, muscular tissue, cartilaginous tissue, nervous tissue.

The results obtained show that the expression of alpha-crystallins in the investigated pig fetuses is realized early in the prenatal ontogenetic period, at the beginning and during tissue differentiation.

Key words: α-crystallins, cell differentiation, fetus, pig.

Introduction

Under different stress conditions all living organisms react at cellular level by increasing the expression of a little number of specific genes, called heat shock genes. As a result of the activation of these genes cells synthesize stress proteins (heat shock proteins – Hsp), that are able to protect them from the effects of the altered environment conditions. It has been ascertained that stress proteins serve two major functions. First, under physiological conditions, some of them (the so called constitutive Hsp) act the role of molecular chaperones (proteins that maintain the cell homeostasis), by supporting the proper folding of other cellular proteins, protect proteins from aggregation and facilitate the renaturation of partially denaturated proteins, and second, they are synthesized in response to a number of unfavorable for the cell factors, such as high temperature, presence of free radicals, heavy metals, ethanol, ischemia, changes in pH, infectious agents, etc. Their role under such conditions is still unknown, but it is supposed to be similar with that of the constitutive Hsp.

According to their physicochemical characteristics, not by their function, they are classified into several families by their molecular weight – Hsp 110, Hsp 90, Hsp 70,

Hsp 60 and sHsp (small heat shock proteins) with molecular weight of 20-30 кD. Alpha-crystallins are referred to the family of the small heat shock proteins, and they are actually heteropolymers that consist of two types of polypeptide subunits – αA and αB, both having a molecular weight of 20-30 kD. These subunits interact with each other, forming proteins with quaternary structure, whose molecular weight varies between 400 000 and 1 100 000 kD. At first, they were considered organ-specific, as they have been isolated from the eye lens. Recently it was proved that while αA-crystalline is expressed mainly in the eye lens, αB-crystalline is found in other ocular [5] and nonucular tissues in norm [1] and pathology [3], where it probably has a protective function in the presence of stress factors.

There is no data in the specialized literature about the constitutive expression of α-crystallins during the prenatal development of the pig. Researches in that direction would help clear up the relationship between stress proteins and the processes of tissue differentiation, which suggestions are already present in some sources. According to C h a n g et al., 1995 [2], the ceramide that affects cell differentiation induces the expression of α-crystalline in NIH3T3 cells.

The aim of the current study is to trace the expression of α-crystallins in tissues of early-stage pig fetuses, when the processes of tissue differentiation are running.

Materials and Methods

By Sephadex G-200 gel electrophoresis α-crystallins were isolated from pig eye lens extract. The column was equilibrated with the elution buffer (0,05M Tris-HCl with pH 7.5, containing 0,1M KCl, 10 mM β-mercaptoethanol) and a sample of 1 ml pig eye lens extract containing 11,8 mg/ml protein was placed upon the gel. Fractions of 2,5 ml were gathered from the elution. The presence of α-crystallins in the fractions was verified by ELISA using immune anti-alpha-crystallin serum.

Rabbit immune serum against α-crystallins was obtained by subcutaneous immunization. The first immunization was carried out using the fractions containing α-crystallins, emulgated in full adjuvant, and for the second and third – in incomplete adjuvant according to the scheme of Z i g l e r et al., 1980 [7]. Seven days after the last immunization blood sample was taken from the rabbit and the serum tested by ELISA.

The pig fetuses were separated into four groups, according to their stage of development – 20 days, 4 weeks, 6 weeks, 8 weeks. They were taken from a slaughterhouse, fixed in 10 % formaldehyde solution and embedded in paraffin. From each block 7 μm sections were cut and mounted on adhesive coated microscope slides. On each slide two sections were mounted – a control and a test one. For verifying the localization of α-crystallins, avidin-biotin-peroxidase complex method has been used in the following sequence:

– Deparaffination and dehydration of the sections in xylol and passing (them) through successively decreasing solutions of alcohol for 3 min in each, at room temperature.

– Blocking of the endogenous peroxidase with 3% H_2O_2 for 10 min.

– Washing of the sections in PBS and blocking of the nonspecific binding with 2,5% horse serum for 20 min at room temperature in humidity chamber.

– Incubation of test sections with polyclonal rabbit anti-alpha-crystalline immune serum (primary antibody) and the controls with PBS, overnight at 4°C in humidity chamber.

– After washing with PBS three times, the sections were incubated with biotinylated horse anti-rabbit IgG (secondary antibody) for 60 min at room temperature.

– After washing again for three times in PBS, the sections were incubated with streptavidin-HRP for 45 min at room temperature.

– The reaction was visualized using 3,3′-diaminobenzidine tetrahydrochloride (DAB) as chromogen and stopped after 5 min with distilled water.

– The sections were counterstained with hematoxylin, dehydrated and cover-slipped in Canada balsam.

All steps were performed using Universal Elite ABC kit (Vectastain, Burlingame, CA 94010). For locating the presence of α-crystallins, the sections were examined with Olympus BX 40 microscope (Olympus Corp. Japan) and digital pictures were taken.

Results and Discussion

The immunoperoxidase test revealed that α-crystallins are expressed in different tissues of the investigated fetuses.

At the age of 20 days the degree of expression in mesenchyme tissue is imperceptible. The presence of α-crystalins is observed in the developing mesenchyme tissue after the forth week. This temporary embryonic tissue is differentiated very early and consists of actively dividing cells that consequently differentiate in various directions.

The blood tissue, that is derived from the mesenchyme tissue, shows poor expression at the age of 20 days, but in the rest of the fetuses, the presence of α-crystallins in the blood cells, observed in the hemopoetic organs, as well as those in the blood vessels is marked.

In differentiating cartilaginous tissue a strong expression of α-crystallins was found. Until the age of 4 weeks the differentiation of the cartilaginous tissue has not started yet. At the age of 6 weeks expression is observed in developing cartilage, but the reaction is much stronger positive in the differentiating cartilaginous tissue at the age of 8 weeks. The cytoplasm localization of α-crystallins is clearly manifested.

The different types of muscle tissue start developing at different age. Firstly that happens with the cardiac muscle tissue, where the reaction for α-crystallins is positive in all groups. A little fainter reaction is observed at the age of 20 days, when the differentiation of the cardiac muscle cells is just beginning.

Developing skeletal muscle fibers are observed not until the age of 8 weeks, when they show strongly positive reaction. At earlier stages the differentiation of the skeletal muscle tissue has not started yet. P. T a l l o t, J. F. G r o n g n e t и J. C. D a v i d [2] have ascertained a reduction in the expression of α-crystallins in skeletal muscles of piglets over the age of 28 days, when the differentiation of the skeletal muscle fibers has been already completed.

The nervous tissue is derived from the ectodermal cells of the nervous tube, which differentiate in two directions: glioblasts (located close to the lumen of the nervous tube, which consequently differentiate to become glial cells) and neuroblasts (located peripherally, precursors of the neurons). Positive reaction for α-crystallins is observed in the cytoplasm and processes of the neuroblasts in all fetuses.

Conclusion

On the basis of the results obtained we can conclude that the expression of the small heat shock proteins – α-crystallins is carried out early during the prenatal development. The fact that stronger expression is observed in differentiating tissues (mesenchyme, blood, cartilaginous, muscle and nervous tissues) affords an opportunity to assume that α-crystallins are connected with the processes of tissue differentiation.

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