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Indirect Response of Early Mouse Embryo to Oxidative Stress Evoked in Apoptosis Model

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Abstract: Quantitative laser microtomography was employed to measure the single cell volume. NMRI mouse zygotes and 2-cell embryos were shown to exhibit the shrinkage at apoptosis induced with ROS (40 min., H_2O_2, 0.2 mM). Unlike early embryos, there was no influence of *in vitro* apoptotic circumstances on mature oocytes, direct precursor of early embryogenesis. Dulbecco's solution prepared from electrochemically reduced bi-distilled water allows the low redox potential in both normal and apoptotic conditions. The data obtained permit us to suggest that ROS sub-milimolar concentrations likely act as the signaling impact. Cys-loo receptors containing thiol group may be considered as the target of hydrogen peroxide molecules.

Key words: Oocyte and early embryo, NMRI mouse, apoptosis, hydrogen peroxide, electrochemically reduced water, quantitative laser microtomography, cellular volume

INTRODUCTION

Electrolysis of water under the action of a constant electric field transforms it into an unstable state. In this case, a fraction of water with alkaline pH and negative Redox Potential (ROP) accumulates in the cathode chamber. This metastable fraction is defined by the term catholyte or ERW (Electrochemically Reduced Water) (Shirahata *et al*., 2012; Henry and Chambron, 2013). The high activity of electrons allows us to consider ERW as an antioxidant solution that compensates for the action of Reactive Oxygen Species (ROS) (Ignacio *et al*., 2012). In other words, it is assumed that a physiological environment based on catholyte will arrest development in a cell of apoptosis caused for example by hydrogen peroxide (Trimarchi *et al*., 2002). Is it so?

This hypothesis was investigated using the experimental model of programmed cell death, induced by oxidative stress in vitro in the zygote of a mouse. As a result, it was shown that with the addition of hydrogen peroxide, apoptosis was observed in the early mouse embryo only in a narrow range of ROP of 120-250 mV which is unexpected in itself. However, ROS does not cause the death of a mature oocyte, a direct precursor of early embryogenesis. The fact confirmed by us that the ERW fraction does not stop the development of apoptosis does not agree with the generally accepted hypothesis. Indeed, the incubation solution on the catholyte shows a lower ROP than the conventional solution. Significant antioxidant activity can compensate for oxidative stress but does not prevent the development of induced apoptosis.

MATERIALS AND METHODS

Conceptual model of cell state change: In the initial stage of induced apoptosis, the cell transition to programmed death occurs through several mechanisms, the transport of hydrogen peroxide to the cell, the oxidation of the receptors on the cytoplasmic membrane, the release of K+ions through the K2p channel into the extracellular space. The listed mechanisms can be in at least two states "On" and "Off". The latter case also corresponds to the complete inactivation of the mentioned mechanism or to its absence. In a symbolic discrete record such a qualitative change in state can be described by a linear classifier-perceptron (McCulloch and Pitts, 1943) (Fig. 1).

RESULTS AND DISCUSSION

Let, X be a set of external stimuli acting on the cell, which generate the input functions of the set $<\varphi_i>$. In the discrete threshold version when $\Psi(X) = 1$, the following condition holds:

$$\sum \alpha_i \varphi_i > \theta$$

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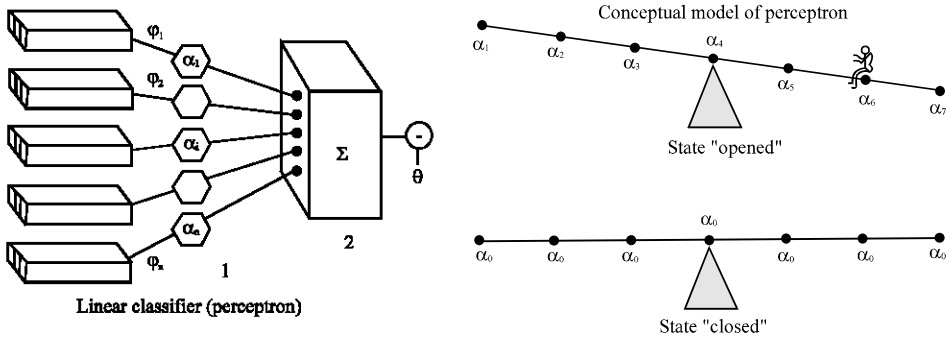

Fig. 1: Scheme of the conceptual threshold model of the change in the state of the cell on which the linear classifier (perceptron) is indicated and also the mechanical analogue explaining the principle of its operation. φ_i are the input functions of the cell receptors that recognize the signals, Φ (φ_i) a family of input functions $\{\alpha_1, \alpha_2, \alpha_3, ..., \alpha_n\}$ is the set of weight coefficients that creates the internal environment of the cell itself, θ-threshold function, Ψ-output function

Each φ_i from the family Φ, contains certain information about whether the output function is true (activation) or false (inhibition) for each external situation X. For example, activation of the receptor will lead to cell death and its inhibition is necessary for the development of the cell.

If Ψ (X) has a positive correlation with the family φ (X) then the weights will be positive whereas for negative correlation the weights will be negative $\{\alpha_i\}$. Swings with distributed load arrangement are a mechanical analogue (Fig. 1).

Suppose that the external situation X is displayed in the form of the location of loads in some zones. Let φ_i (X) = 1 when the load is at the ith point. For the state "open" (Fig. 1), the condition α_i = (i-4), θ = 0 is observed, the result of the calculation will be:

$$\sum_{i}^{n}(i-4),\ \varphi_i\ (X)>\theta = 0$$

and the logical conclusion about the situation-the "swings will tilt to the right", i.e., when recognizing two situations, one will be selected. For example, the receptor is activated if the ligand concentration corresponds to the sensitivity range. In this case, in the presence of an appropriate mediator, the cell will go into the stage of apoptosis. Conversely, the swing is inactive when $\alpha_i \cong 0$ in the "closed" state (Fig. 1). An example can be a "sleeping" state when the mechanisms of initiation of apoptosis by an external factor are disconnected, absent or weakly expressed. In this case, apoptosis can not be induced.

Experimental model of apoptosis: Isolated mature NMRI mouse oocytes and embryos at the zygote and bicellular embryo stage were obtained according to the known method. Apoptosis was induced by incubating (40 min.) oocytes or early embryos in Dulbecco's medium to which hydrogen peroxide was added to a concentration of 0.2 mmol/L (Liu *et al*., 1999). In the experimental group, the incubation solution was prepared on the ERW fractions of the original bidistilled water. The criterions for apoptosis were characteristic morphological features and a decrease in cell volume which were recorded by Quantitative Laser Microtomography (QLSM). The method of preparing the preparation for QLSM and the details of measuring the spatial characteristics of the micro object were discussed earlier (Pogorelov and Pogorelov, 2008).

In the ovule with natural apoptosis state, all the specific features were observed, chromatin fragmentation, cell contraction, appearance of outgrowths on the cytoplasmic membrane. Addition of ROS to the incubation solution did not cause apoptosis in a healthy oocyte. This fact can be explained by the fact that the H_2O_2 molecule is transported to the cell together with water through the AQP3 channel from the aquaporin family (Miller *et al*., 2010) which is not expressed in the mouse oocyte (Meng *et al*., 2008; Jin *et al*., 2011; Xiong *et al*., 2013). This situation corresponds to the state of the "closed" conceptual model (Fig. 1). Induced apoptosis is realized after fertilization already at the stage of a unicellular embryo. Addition of ROS to the usual Dulbecco's solution is accompanied by the appearance of specific changes in the morphology of the zygote against the background of restoration of ROP from 280-250 mV.

Compared with the control (Fig. 2a), the addition of ROS to the incubation solution prepared on the ERW oxidizes the ROP of the medium from 60-120 mV. This level is much lower than the normal Dulbecco's ROP value in which apoptosis is not observed. However, a relatively low ROS value does not abolish induced apoptosis

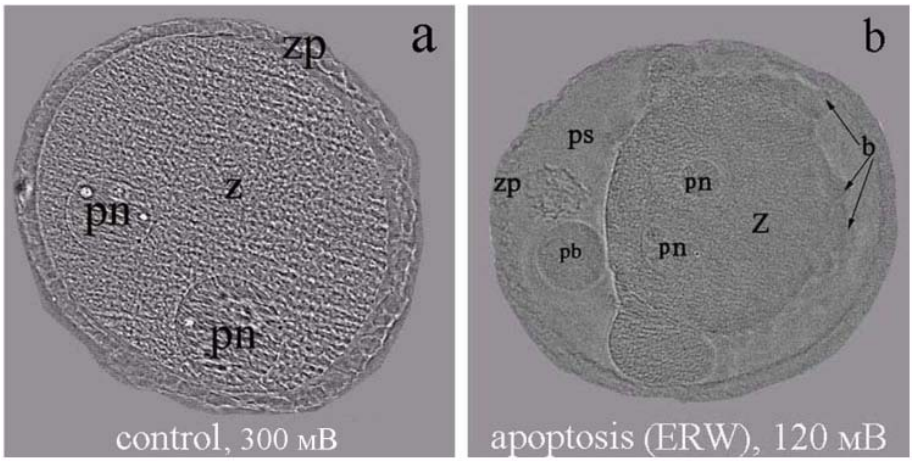

Fig. 2: Image of a unicellular embryo of a NMRI mouse in the equatorial plane on an optical section obtained by QLSM. Control: a) Experimental apoptosis caused by the addition of ROS (40 min, H_2O_2, 0.2 mM) to the Dulbecco incubation solution which is prepared on ERW and b) b-outgrowths, pb-polar body, pn-nucleus of unicellular mouse embryo (zygote), ps-space between shell and cell, Z-zygote, zp-envelope of embryo

(Fig. 2b) in the medium on the catholyte. Thus, there is no direct relationship between the high level of ROS, characteristic of oxidative stress and the transition of the embryonic cell to the state of apoptosis (Fig. 3).

Morphological signs qualitatively illustrate the changes caused by apoptosis. A quantitative criterion for such changes is a decrease in the cell volume at the stage of early apoptosis (Cohen *et al*., 1992). It is shown that the cause of cell contraction can be the yield of the K^+ cation through K_{2p} channel from the family of potassium channels (Hur *et al*., 2012). A statistical comparison of the effect of experimental apoptosis on a cell during the first cell cycle is shown in the diagram (Fig. 3).

Analysis of the obtained data (Fig. 3) in the part of oocytes confirms the conclusion that there is no response of the oocyte to the presence of ROS, regardless of the modification of the incubation medium. The volume of the zygote in the norm is also independent of ERW but it decreases with the development of apoptosis caused by the addition of ROS. The effect of hydrogen peroxide is more pronounced in the incubation medium prepared on the ERW. The main regularities described for the zygote are reproduced in a two-cell embryo (Fig. 3 a, b) but with the difference that the modification of the Dulbecco's ERW environment does not affect the quality of apoptosis.

Concluding the discussion of the results, the following should be emphasized. All data were obtained under conditions of a laboratory model with a relatively short exposure interval. Perhaps the effect of catholyte will be different with prolonged (chronic) using. Under experimental conditions, the mouse oocyte remains resistant to ROS which may be due to the absence on the cytoplasmic membrane of the ovule of this animal species of the AQP3 channel transporting H_2O_2. We use the model of apoptosis induced by exogenous hydrogen peroxide which has long been approved for the early mouse embryo cell (Trimarchi *et al*., 2000; Pogorelov and Pogorelov, 2008). Mature mouse oocyte, the immediate precursor of early embryogenesis has a pronounced potentiation for apoptosis *in vivo* under the influence of endogenous factors.

If fertilization has not occurred then the death of the female sex cell is determined functionally, since, the physiology of the oocyte MII does not imply its development. However, we do not have data on the induction of apoptosis in the mouse oocyte *in vitro* via. exogenous hydrogen peroxide. Apparently, the difficulty of experimental stimulation of programmed cell death in the oocyte was due to the fact that the studies are performed on a unicellular embryo (Liu *et al*., 1999). Although, a mature oocyte is a more promising object from the position of reproducing the results and practical application. In the presence of ROS (H_2O_2, 0.2 mM), apoptosis develops in the early mouse embryo cells against the background of restoration of the Dulbecco's ROP solution to 250 mV. Addition of ROS to Dulbecco's solution prepared on ERW oxidizes the incubation medium to 120 mV. The ROP value remains relatively low, but does not stop induced apoptosis. Thus, the development of apoptosis is observed in a limited range of ROP values (120÷250 mV) which suggests the effect of the exogenous molecule H_2O_2 through the receptor mechanism. In this case, the target of the signal ROS

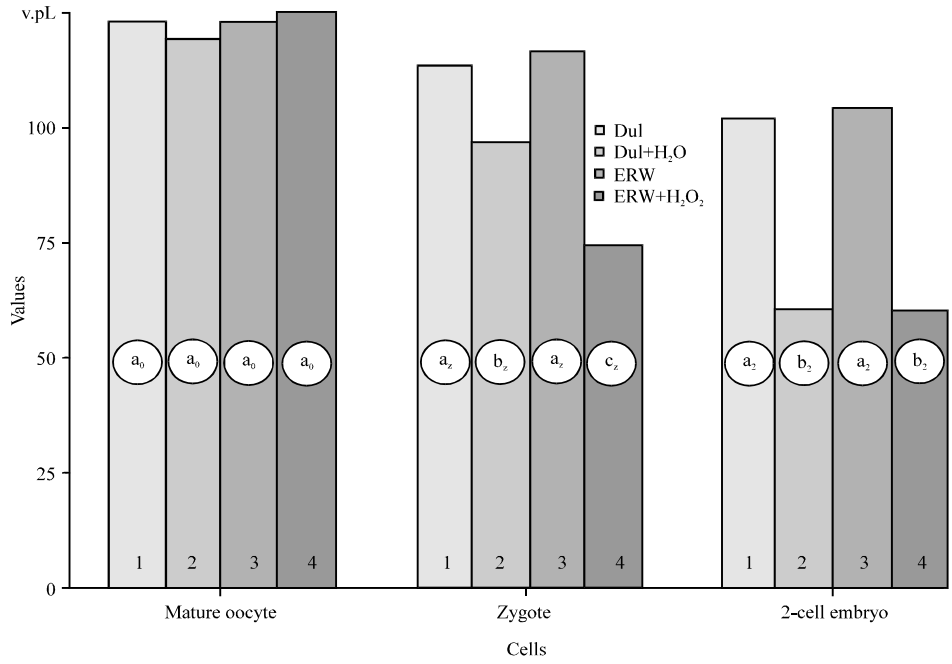

Fig. 3: The change in the volume of the oocyte and early mouse embryo in the experiment with apoptosis induced by the action of ROS. Data received by QLSM. Dul-incubation in Dulbecco's medium (40 min. without ROS), H_2O_2 incubation in Dulbecco's medium with apoptosis induced by ROS (40 min. H_2O_2, 0.2 mM), ERW incubation in Dulbecco's medium (40 min.) ERW, V-the volume of the oocyte or embryo in Picoliter (pL). The data in the columns by sections (oocyte, zygote, 2 embryos), for which the marked letters do not coincide, differ with significance level $p<0.05$. The reliability of the differences was assessed by the student's test with the number of oocytes or embryos not <20 in each group

molecule may be the thiol group (Liu *et al*., 1999) which is part of for example, cis-loop receptors. In terms of the conceptual model, this situation corresponds to the state "openly" (Fig. 1). The cascade of events with apoptosis, their sequence and causes as well as different scenarios of apoptosis activation are the most discussed topics in this field of science. In this part, reference should be made to the following publications (Littler *et al*., 2005; Maeno *et al*., 2000; Shimizu *et al*., 2004).

CONCLUSION

As shown in this reserach, cell contraction precedes the appearance of known morphological and biochemical signs of apoptosis, the development of which was canceled, preventing cell contraction. In the same studies, it was confirmed that at the early stage of apoptosis the decrease in cell volume is due to the release of ions (K^+, Cl^-).

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