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Loosening of the cytomembrane of Ehrlich ascites tumor cell by unsaturated fatty acid

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Abstract

Ehrlich ascites tumor cells affected by oleic and linoleic acids lose their cytomembrane followed by the leak out of ribosomes. Some cells survived through this treatment when they were transplanted into mouse peritoneal cavity, but they changed their characteristics showing wider and less basophilic cytoplasm and smaller nuclei with dense nuclear chromatin and ambiguous nucleoli. In spite of many attempts, no qualitative changes have been found between normal and cancer cells. Recently, Ishikawa found the specific antigenicity of cancer cell membrane which was common to several strains of cancer cells. Grobstein and coworkers have clarified that pancreatic cells can differentiate in association with neighboring mesenchymal cells, probably getting some information. Their works suggest that the cell differentiation will be induced by mutual association of cells by which the cell will receive some substance acting as the information for differentiation. Taking the works of Ishikawa and his collaborators into consideration, it seems that cancer cells may be unable to differentiate by their defective or incomplete cell membrane through which they cannot associate with neighboring cells and fail to get the information. Almost all of the biological characteristics of cancer cells, immaturity, autonomic growth, invasive and metastatic properties independent from the neighboring cell groups, are well explained or consistent with this view. Recently, we found that the cell membrane can be loosened by some unsaturated fatty acids resulting in the leak-out of ribosomes. In this paper it is demonstrated how the Ehrlich ascites tumor cell affected by fatty acids lose their cytomembrane and the ribosomes and how the cells survived through this treatment show different characteristics from the original ones, taking the appearance more matured cells.

BRIEF NOTE

LOOSENING OF THE CYTOMEMBRANE OF EHRlich ASCITES TUMOR CELL BY UNSATURATED FATTY ACID*

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ABSTRACT

Ehrlich ascites tumor cells affected by oleic and linoleic acids lose their cytomembrane followed by the leak out of ribosomes. Some cells survived through this treatment when they were transplanted into mouse peritoneal cavity, but they changed their characteristics showing wider and less basophilic cytoplasm and smaller nuclei with dense nuclear chromatin and ambiguous nucleoli.

In spite of many attempts, no qualitative changes have been found between normal and cancer cells. Recently, Ishikawa found the specific antigenicity of cancer cell membrane which was common to several strains of cancer cells¹. Grobstein and coworkers have clarified that pancreatic cells can differentiate in association with neighboring mesenchymal cells, probably getting some information^{2,3,4}. Their works suggest that the cell differentiation will be induced by mutual association of cells by which the cell will receive some substance acting as the information for differentiation. Taking the works of Ishikawa and his collaborators into consideration, it seems that cancer cells may be unable to differentiate by their defective or incomplete cell membrane through which they cannot associate with neighboring cells and fail to get the information. Almost all of the biological characteristics of cancer cells, immaturity, autonomic growth, invasive and metastatic properties independent from the neighboring cell groups, are well explained or consistent with this view. Recently, we found that the cell membrane can be loosened by some unsaturated fatty acids resulting in the leak-out of ribosomes. In this paper it is demonstrated how the Ehrlich ascites tumor cell affected by fatty acids lose their cytomembrane and the ribosomes

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and how the cells survived through this treatment show different characteristics from the original ones, taking the appearance more matured cells.

MATERIALS AND METHODS

Ehrlich ascites tumor cells grown in the peritoneal cavity of ddN mice were used. They were harvested five to seven days after inoculation of 1 cc of cell suspension, 6,000 to 10,000 cells per cu mm. 1 to 3 cc of the tumor ascites was mixed with 1 to 1/3 volumes of 1 per cent fatty acid emulsion in bovine serum, or of the protein layer obtained by centrifugation of the emulsion, and incubated for 5 to 15 minutes at 37°C. After the incubation the cells were observed under the light and electron microscopes, and some cells were suspended in Hanks' solution and transplanted into the peritoneal cavity of ddN mice, 1 cc of cell suspension and 5,000 to 10,000 cells. For the light microscopy the cells were smeared, fixed with methanol, and stained with Giemsa for general observation, and with Nile blue for fatty acids after formol gas fixation by the method of Cain⁵. Cells were also observed in wet staining with eosin by the method of Eaton⁶. For electron microscopy the cells were fixed with osmic acid or acrolein followed by osmic fixation, embedded in Epon 812 and stained with uranium acetate. The fatty acids used were oleic and linoleic acids for Ishizu Co. Each of these was mixed with bovine serum and emulsified with glass homogenizer and used immediately, or after centrifugation the protein layer was used for the experiment.

RESULTS

The cells incubated with the fatty acid emulsion for 15 minutes showed a scarcity of cytoplasmic basophilia (Fig. 2). Nile blue staining of the cells incubated with fatty acid emulsion revealed the adsorption of fatty acid droplets all over the cells surface (Fig. 3). The protein layer separated from the fatty acid emulsion also showed a similar effect, and the cells lost their basophilic substance with 5 to 15 minutes' incubation. In the earlier stage of incubation less than 5 minutes the leaked-out cytoplasmic basophilia are seen to be adhered to the outside of cells. The cytoplasm looked somewhat reduced in volume but the cells appeared to be the ones having cytoplasm in normal state. By staining with eosin in wet the nuclei were stained red. Under electron microscope the cells in the earlier stage of damage showed the formation of the tongue-like processes in several places of cell surface (Fig. 5), which contained a mass of ribosomes but hardly any other cytoplasmic organellae. The processes were enveloped with the enormously extended cytoplasmic membrane. Some of these envelopes detached from the cell surface with the ribosomes and finally were decomposed

erupting out the ribosomes. The cell membrane of the mother cell was perforated in many places or decomposed completely, but the mitochondria and endoplasmic reticulum with some viruses were found in the original cytoplasm, though some structures were swollen (Fig. 6). The fine structures of the nucleus remained almost unchanged. Some of the cells with damaged cell membrane and having lost ribosomes survived through the treatment when they were transplanted into the peritoneal cavity of mice. One half of the case under observation cells began to proliferate, but the rate of proliferation was rather low, rarely showing mitotic picture on the smeared ascites. On the sixth day the ascites developed slightly, while the control animal had quite well developed ascites. The morphologic picture of the cells was largely different from the original one, having a wide cytoplasm but much less basophilicity. Nuclei were round or kidney-shaped with dense chromatin and ambiguous nucleoli (Fig. 4). They look to be rather matured cells comparing to the original ones. After 15 to 20 days ascites developed well and the tumor cells proliferated actively restoring a strong basophilicity of cytoplasm.

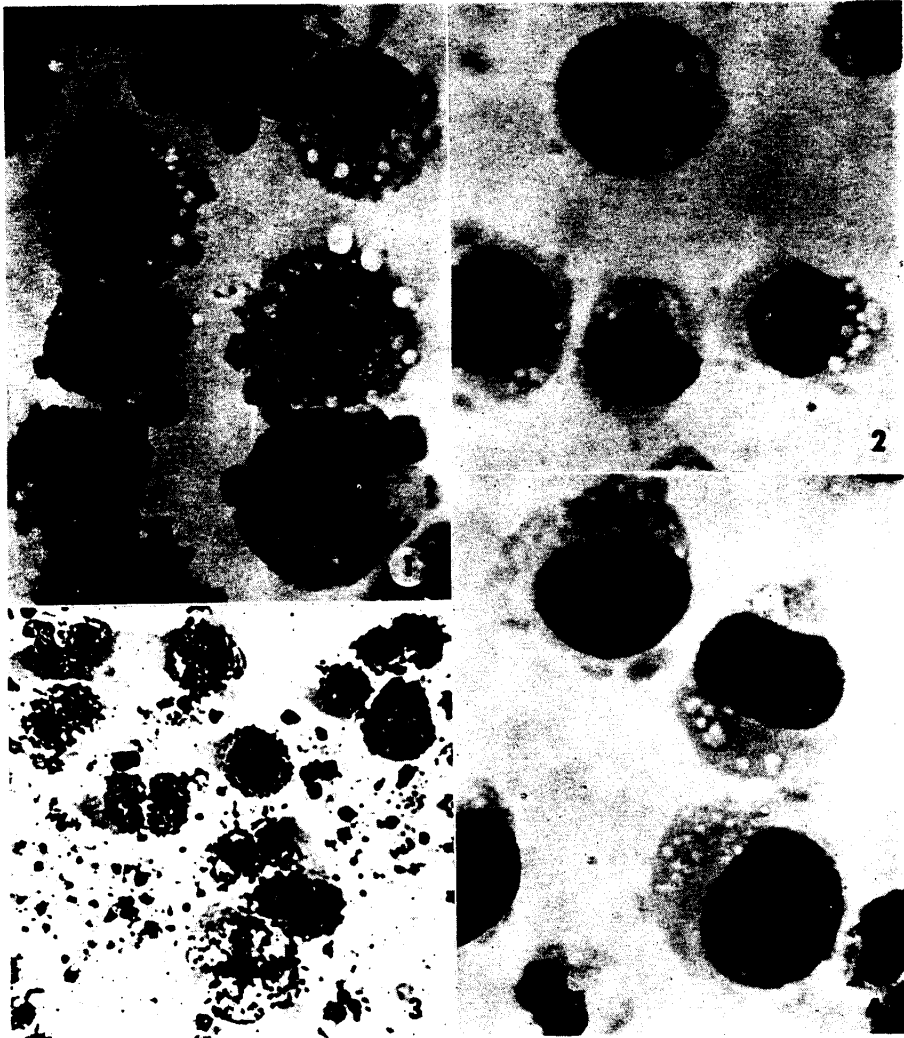
COMMENT

As demonstrated here, Ehrlich ascites tumor cells loosen their cytoplasmic membrane by the action of oleic and linoleic acids followed by the loss of the cytoplasmic basophilia or ribosomes. The mechanism seems to be due to the surface active characteristics of the fatty acids and their specific affinity to cell membrane. The peculiar thing is that the cells lose their ribosomes but not the mitochondria, endoplasmic reticulum or any other organellae, even if the cell membrane is loosened. The point to be asserted is that the cells survive through the treatment in spite of the loosening of cytomembrane and most of ribosomes being lost, and the invasion of the environmental fluid into cytoplasm and even into the nucleus as suggested by the supravital staining test with eosin. Finally they alter the original characteristics. This may be induced by some unknown substance having invaded into the cells, but the most reasonable mechanism in this case will be the loss of ribosomes and RNA responsible for the active synthesis of protein. Another important point is that the cells show an appearance of more matured or differentiated ones with less basophilicity, dense chromatin in the nucleus and ambiguous nucleoli. Later they restored the cytoplasmic basophilicity and showed the characteristics of malignant cells. Therefore, the change should be a temporary one. However, this experiment seems to indicate a possibility that malignant tumor cells will be able to change their characteristics to more mature cells by losing RNA or getting some information from the environment if their cell membrane is loosened.

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EXPLANATION to FIGURES

- Fig. 1 Ehrlich ascites tumor cells on the fifth day of proliferation in mouse peritoneal cavity. Smear and stained with Giemsa. $\times 1,000$
- Fig. 2 The tumor cells incubated with oleic acid-bovine serum mixture for 10 minutes at 37°C . Smear and stained with Giemsa. $\times 1,000$
- Fig. 3 The cells treated similarly as those in Fig. 2 and stained with Nile blue. $\times 400$
- Fig. 4 The tumor cells on the eighth day after transplantation, which were previously treated with the oleic acid-serum mixture for 10 minutes and transplanted. The tumor ascites developed moderately. $\times 1,000$
- Fig. 5 The tumor cells incubated for 5 minutes with the oleic acid-serum mixture, and show the process formation which includes a mass of ribosomes. On the right hand below the detached process is seen. $\times 40,000$
- Fig. 6 A cell incubated with the oleic acid-serum mixture for 10 minutes. Perforated cell membrane and the cytoplasm having mitochondria and endoplasmic reticulum but poor in ribosomes can be seen. $\times 7,500$

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