Properties of the Ehrlich Ascites Tumor Cell as Determined by Electron Microscopy, Ultracentrifugation, and Hydrostatic Pressure

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SUMMARY

Ehrlich ascites tumor cells were ultracentrifuged in a gum arabic solution, or as stacks of cells in 0.9% sodium chloride solution for periods ranging from 20 to 30 min. Those suspended in gum arabic become greatly stretched and are sometimes pulled into two parts. The nuclei are displaced centrifugally and the relatively dense nucleolus (sometimes there are more than one) is forced against the nuclear envelope and adjacent plasma membrane causing them to become greatly stretched. Sometimes the nucleolus is thrown through both the nuclear envelope and plasma membrane becoming free of the cell. The interphase chromosomes are more dense than the nucleoplasm, and they are displaced centrifugally. However, when this occurs, it is revealed that the chromosomes adhere to the nuclear envelope. The forces holding them to the inner membrane of the nuclear envelope are relatively strong, thus causing the displaced chromosomes to become greatly stretched, or even broken, before they are detached. When subjected to a comparable centrifugal force, the stratification in the ascites cells is not so complete as has been reported for certain nonmalignant somatic cells.

INTRODUCTION

The study of the cancer cell has been approached from practically every point of view in an effort to understand the transformation from the normal to the malignant state. Notwithstanding the enormous literature on this subject, "Classical and modern investigations have failed to reveal a single morphological sign which is truly specific of cancer cells; however, it is possible to enumerate certain phenomena which, when taken together, characterize the tumor cells" (31).

The centrifuge technic has been widely used in a study of the physical properties of noncancerous cells, particularly large cells such as oocytes, eggs, and protozoa (e.g., 8, 15-17, 30). However, because of their relatively small size and high consistency, somatic cells from which most malignant cells are derived have been little studied by this method (cf. 25, 26).

In fact, it was not until the development of the air-turbine ultracentrifuge (5) that an instrument capable of producing stratification in normal somatic and neoplastic cells became available.

Guyer and Claus (12-14) were the first to study the physical nature of neoplastic cells by use of the air-turbine ultracentrifuge. They observed a difference between the displacement of their components, both of the nucleus and cytoplasm, than did the normal cells, and from this evidence they concluded that neoplastic cells possess "a decidedly greater viscosity than that of normal tissue cells."

On the other hand, Cowdry and Paletta (10), using the same methods as Guyer and Claus (12), but studying the effects on the nuclei only in several different types of malignant cells, observed them to stratify more readily than do the nuclei in their nonmalignant counterparts, and they concluded that the nuclei of malignant cells possess a lower viscosity than do those of nonmalignant cells. Mateyko and Kopeć (28) found that neoplastic cells of the frog kidney stratified more readily and their contents redistributed more rapidly than they did in centrifuged normal cells.

Evidence cited above is conflicting concerning consistency of normal and malignant cells. It is known that certain cell types seem to require more centrifugal force than others to effect a stratification of their components; this may be due in part to differences in density of the cellular components and to the difference in size of the cells (cf. 3). We have not attempted to make a direct comparison of the relative consistency of the ascites cells with that of their progenitors; instead, a comparison of the stratification occurring in the ascites cells with that reported in published accounts of certain other ultracentrifuged somatic cells is discussed.

MATERIALS AND METHODS

A strain of Ehrlich ascites tumor cells was kindly supplied to us by Dr. T. C. Evans of the Radiation Research Laboratory, University of Iowa. The tumor was propagated in young, adult female Swiss mice. Five to seven days after inoculation, the cells were removed from the body cavity of the mouse and placed in a test tube, where they were washed three times in...
0.9% sodium chloride solution. Some of the cells were placed in small plastic tubes containing 20 to 30% gum arabic dissolved in 0.9% sodium chloride solution, and others were packed several layers deep within small tubes containing 0.9% sodium chloride solution. The tubes were then placed in an air-turbine ultracentrifuge (5) and centrifuged at approximately 300,000 × g for periods of 20 to 30 min. Upon completion of the ultracentrifugation some of the cells were immediately examined under the phase-contrast microscope and photographed. Others were fixed in Carnoy’s, Bouin’s, and Champy’s solutions and stained in Heidenhain’s hematoxylin or the Feulgen method. For electron microscope studies, the cells were fixed in cold, 3% phosphate-buffered glutaraldehyde solution (pH 7.3) for 1 to 2 hr and postfixed in 1% buffered (pH 7.3) osmium tetroxide (33). The cells were dehydrated rapidly in a series of cold ethanols and embedded in Epon 812 (22). Sections were stained in uranyl acetate (35), lead citrate (32), or in both uranyl acetate and lead citrate. The sections were studied in an RCA EMU 3D or 3G electron microscope.

Ascites cells were also exposed in 0.9% sodium chloride solution to a hydrostatic pressure of 9,000 lb/sq inch for periods of 30 to 60 min. The pressure was generated by a jack (model P76, Blackhawk Mfg. Co., Milwaukee, Wisconsin) and delivered through a pressure hose to a specially constructed cylinder fitted with an automobile hydraulic brake wheel sleeve, which functioned both to separate the oil from the pressure chamber and to transmit the pressure from the jack to the experimental pressure chamber. Experiments were run at room temperature (22°C), and while a continuous record of the temperature within the pressure chamber during an experiment was not made, it was determined that the temperature within the chamber immediately upon its decompression did not vary over ±1°C from that at the beginning of the experiment.

RESULTS

The structure of the Ehrlich ascites cell is so well known that a detailed description of it here seems superfluous. It will suffice to point out that this cell possesses a relatively large nucleus, one or more nucleoli, and a prominent porous nuclear envelope (Fig. 1). A well-developed Golgi apparatus consisting of lamellae and vesicles (GA), scattered filamentous mitochondria (M), and one or more lipid bodies (L) are usually present (Fig. 1). Numerous ribosomes, both free and attached to membranes of the endoplasmic reticulum, occur in the cytoplasm (Fig. 1, ER). A well developed centriole, bodies consisting of granules and vacuoles (lysosomes), and bodies described as virus are often seen in electron micrographs of ascites cells (cf. 1). Projecting from the cortex or ectoplasmic region of these cells are numerous microvilli. Cells exposed to a hydrostatic pressure of 9,000 lb/sq inch for 30 min often show fibrous rootlets extending from the base of the microvilli to a position relatively deep within the cortex (Figs. 2, 3).

Effects of Ultracentrifugation

When washed ascites tumor cells are suspended in a medium of the same relative density or piled one upon the other in a centrifuge tube, subjected to a centrifugal force of approximately 300,000 × g for 20 to 30 min, and examined immediately under the phase-contrast microscope, marked effects on the cells are observed, some of which are illustrated in Figs. 4 to 8. They are often greatly stretched, even to the point of separating into two parts (Figs. 7, 8). The nucleus is relatively dense and displaced centrifugally; the lipid (L) and vacuoles (V) are less dense and are displaced centripetally (Figs. 4–8). The lack of a sharp stratification of the mitochondria in many of these cells (Fig. 4, M) suggests, but does not prove, that they possess a relatively high density as compared to certain normal somatic cells in which the mitochondria are sharply stratified (cf. 3, 4, 6, 11).

Electron micrographs also reveal in some of the ultracentrifuged cells that the mitochondria are not completely displaced from all parts of the cells, but they appear a little more concentrated in the centrifugal and centripetal halves than elsewhere (Figs. 9, 10). It may be that the mitochondria in the ascites cell, like those in the Arbacia egg (19), differ slightly in density, a condition which would account for their concentration in the two ends of the ultracentrifuged cells. The Golgi complex in ultracentrifuged cells is usually found in the centripetal half of the cell (Fig. 9, GA).

The Nucleus

A marked effect of the high centrifugal force is noted on the nucleus. Generally, it is displaced centrifugally and becomes greatly stretched (Figs. 4–10). The densest component in the nucleus is the nucleolus (there are sometimes more than one); it is always forced to the centrifugal end of the nucleus, where it may cause the nuclear envelope and adjacent plasma membrane to become greatly stretched, even to the point of breaking, thus allowing the nucleolus to become detached from the cell (Figs. 5, 6).

Of special interest is the effect of the centrifugal force on the interphase chromosomes. The chromatin is more dense than the nucleoplasm and is displaced centrifugally (Figs. 6–10, DCH). When this occurs, it is observed that some, if not all, of the interphase chromosomes are “attached” to the nuclear envelope, and they adhere so tightly to it that they become greatly stretched (Figs. 4, 6–8, SCH). There seems little doubt that the filaments adhering to the nuclear envelope represent chromosomes, as they readily stain with the Feulgen method. The buoyancy of the centripetally displaced nucleoplasm prevents the collapse of the nuclear envelope at the centripetal end of the cell (Figs. 9, 10 NE). Although not clearly illustrated in the figures, the nuclear envelope at the centripetal end of the nucleus often shows small indentations, presumably caused by the displacement pull of the centrifugal force on the “attached” chromosomes.

The adherence of the interphase chromosomes to the inner membrane of the nuclear envelope is not a special characteristic of neoplastic cells, as it has been observed in certain other types of nonmalignant cells. It has been suggested that the chromosomes may be anchored to the nuclear envelope at chromocentres (29); however, we were unable to obtain evidence in the ascites cells for a specialized structure or body in the chromosome where it is attached to the nuclear envelope.

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Ascites cells are not killed by ultracentrifugation, as evidenced by the fact that they seem to grow as rapidly as noncentrifuged ascites cells when transferred to the body cavity of mice (cf. 11, 12). Mateyko and Kopac (26) report that frog and rat liver cells exposed to a 40% gum arabic solution for 2.5 hr show slight shrinkage. No morphologic evidence was revealed in this study, even in the electron micrographs, to suggest that the gum arabic had produced a marked distortion of the ascites cells, but admittedly it is not an ideal medium on which to cushion cells in the ultracentrifuge.

Hydrostatic Pressure

As shown here, the Ehrlich ascites cells seem to be more resistant to the action of hydrostatic pressure than are certain normal cells (23). Exposure to a hydrostatic pressure of 9,000 lb/sq inch for a period of 30 min produced little effect on these cells. Longer exposure (1 hr) showed effects illustrated in Fig. 11. Here the mitochondria are vacuolated and appear dense, and the endoplasmic reticulum is less definite, but still recognizable. Most, but not all, of the microvilli have disappeared from the surface.

DISCUSSION

Investigators who have studied the biochemistry of the Ehrlich ascites tumor cells are in agreement that they are much more difficult to break than most nonmalignant somatic cells. In fact, it has been necessary to devise special methods to disrupt these cells, such as grinding with sand (21), because "... homogenization in sucrose solution as commonly employed for normal tissue, gave completely unsatisfactory results" (18, cf. 36). The fact that these cells are so difficult to homogenize can be interpreted to mean that the plasma membrane and/or the cytoplasm is more firm and substantial than it is in many other types of nonmalignant cells. As reported here, ultracentrifuged ascites cells are readily stretched, but less sharply stratified than many other types of somatic cells such as certain leukocytes (6). This condition may be interpreted to mean that the cytoplasmic hyaloplasm has a relatively higher consistency or that the cytoplasmic components are nearly of the same density as the hyaloplasm. However, it should be emphasized that from our studies there is no direct evidence that this condition is associated with the state of malignancy, nor does it suggest that all types of malignant cells would react to ultracentrifugation in a similar way. In fact, the results of Guery and Claus (13, 14) and Cowdry and Paletta (10) suggest that different types of neoplastic cells may react to ultracentrifugation in different ways, some showing little stratification, presumably because of their high consistency (viscosity); others, especially their nuclei, stratify readily, indicating a relatively low consistency. The most extensive study on the physical properties of tumor cells is that of Mateyko and Kopac (24-26). They have used both the technics of microdissection and high speed centrifugation to investigate human ascites cells (24), human gynecologic tumors, both benign and malignant (27), and frog adenocarcinoma (28). Some variation in consistency was found to exist among different types of tumor cells, but more often than not they seemed to be of a lower consistency than most nontumor cells.

Other evidence bearing on the consistency of tumor cells has been noted by Lewis (20). After comparing several different types of sarcoma cells in tissue culture with their normal prototype cells, he concluded that the characteristic differences between them was that the cytoplasm of the malignant cells is more dense, suggesting a relatively high viscosity. Physiologic studies seem to indicate that the surface properties of tumor cells differ from those of normal cells and that they are more easily separated from one another than are normal cells (e.g., Ref. 2). However, a study involving the use of the microdissection technic revealed no difference in consistency and structure between the normal and malignant cell (7). Good general discussion of the cytology of the cancer cell may be found in articles by Cowdry (9) and Oberling and Bernhard (31).

A condition which has been observed by a few investigators but which is not of general knowledge is the fact that in certain types of cells the interphase chromosomes are "attached" or adhere securely at one or more positions on their surface to the inner membrane of the nuclear envelope. The nature and significance of this association is not clear. Are the chromosomes always "attached" to the inner nuclear membrane in a given position on their surface, such as the chromocenter (29), or may they adhere to it at any position? In any case, the chromosomes are so tightly bound to the membrane that they are often broken by the centrifugal force before becoming detached (4). Obviously, as the cell enters prophase, the chromosomes automatically shorten, thicken, become detached from the inner nuclear membrane and write about "like cells in a box" (34).

REFERENCES


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1950 CANCER RESEARCH VOL. 28

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