A Comparative Electron Microscopic Study on Two Strains of Ascites Tumor Cells and Their Sublines Resistant to Antitumor Agents

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SUMMARY

A fine structural analysis of ascites tumor cells in the original sensitive strain and in the subline resistant to antitumor agents was performed with the Yoshida ascites sarcoma; its subline resistant to methylbis(β-chloroethyl)amine N-oxide (MBAO); ascites hepatoma AH 13; and its sublines resistant to MBAO, mitomycin C, and 6-mercapto purine (6-MP), respectively. In the sublines resistant to MBAO the endoplasmic reticulum was more scarce than in the corresponding original strain. The vertical arrangement of the elongated endoplasmic reticulum and the annulate lamellae, which were encountered frequently in the original Yoshida ascites sarcoma, could not be detected in its MBAO-resistant subline. The Golgi complex was also simpler, and the lamellae were poorly defined in the MBAO-resistant sublines. However, no significant difference was observed between the mitomycin C- or 6-MP-resistant sublines and the original strain. As to the plasma membrane, nuclei, and nucleoli, significant differences could not be detected between the resistant sublines and the corresponding original strain.

Many studies have been made from various points of view on the development of resistance to various antitumor drugs in experimental tumors. However, studies on morphological aspects are few. One of the present authors reported that no significant difference was observed with regard to either ascitic properties or histological features between the original strains and sublines resistant to alkylating agents of the Yoshida ascites sarcoma or the ascites hepatoma AH 13, which are both composed of single, free tumor cells. On the other hand, AH 7974, which is composed of cell aggregates and single isolated tumor cells, showed a significant difference in both ascitic properties and histological features between the original strain and a resistant subline (5, 6). Recent studies with the electron microscope have contributed greatly to our knowledge of the fine structure of cell components and have led to a better understanding of their functional significance. It is of interest to compare the fine structure of both the sensitive and resistant tumor cells in order to define some of the cytological changes which occurred in the development of resistance to antitumor agents.

MATERIALS AND METHODS

The following original sensitive strains and resistant variants of ascites tumors were used in this study: the Yoshida ascites sarcoma; its subline resistant to methylbis(β-chloroethyl)amine N-oxide (MBAO), one of the alkylating agents; and ascites hepatoma AH 13 and its sublines resistant to MBAO, 6-mercapto purine (6-MP), and mitomycin C, respectively. These resistant sublines were established by passing tumor cells through animals treated with the agents. Details concerning the development and biological properties of these resistant sublines can be found in previous papers (5–7). All these sublines were successfully transmitted in the absence of the agents during the course of this study. Each rat received an intraperitoneal inoculation of about 0.1 ml. of undiluted tumor ascites in the state of nearly pure culture. The tumor cells of each original strain

Received for publication July 28, 1961.
or resistant subline were inoculated into five to seven rats weighing about 110 gm. The rats were highly susceptible to these tumors. Approximately 0.2 ml. of tumor ascites was removed by capillary pipette from the peritoneal cavity when a state of nearly pure culture, after inoculation of the tumor, was reached. The fluid was immediately dispersed in 2 ml. of chilled 1 per cent osmium tetroxide, adjusted with veronal-acetate buffer to pH 7.4, and allowed to stand for 30 minutes in a refrigerator. Thereafter, the specimen was dehydrated in a graded series of ethyl alcohols, impregnated with a mixture of methyl and n-butyl (1:4) methacrylate, and finally imbedded in the same resin by polymerization with benzoyl peroxide at 45°C. Thin sections were cut with a Japan Electron Optics Laboratory microtome, model JUM-5. They were mounted on grids coated with collodion film and stained with uranyl acetate according to the method described by Watson (9). Electron micrographs were obtained with a Japan Electron Optics Laboratory electron microscope, model JEM-5G, at original magnifications ranging from 1,400 to 8,500 and subsequently photographically enlarged as required.

RESULTS

Electron microscopy of the original strain and subline of the Yoshida ascites sarcoma resistant to MBAO (Figs. 1–6).—The plasma membrane and microvilli of the original strain and resistant subline did not show any significant difference in appearance. The ground substance of nuclei appeared to be finely granular. In some the granules were evenly distributed, but in others they were more densely congregated near the nuclear membrane. These findings of nuclei also occurred in the original strain and resistant subline. The number and size of the mitochondria varied from one cell to another within the same strain of tumor. It was our general impression that there was no clear difference between these two strains. The greatest difference was found in the endoplasmic reticulum. In tumor cells of the original strain the rough-surfaced endoplasmic reticulum remained almost unchanged both in number and shape (Figs. 9 and 10). The endoplasmic reticulum of the original strain AH 13 was a relatively small vesicle which dispersed diffusely in the cytoplasm (Fig. 7). In the MBAO-resistant subline it was similar in shape to the original strain, but clearly less in number than the original strain (Fig. 8). Degenerated mitochondria were also frequently encountered in the MBAO-resistant subline (Fig. 8). There was no significant difference in the fine structure of the mitochondria between the mitomycin C- and 6-MP-resistant subline and the original strain (Figs. 9 and 10). The Golgi complex in the original strain of AH 13 was relatively well developed. However, it was somewhat simpler in the MBAO-resistant subline (Fig. 8). The mitomycin C- and 6-MP-resistant subline showed well developed Golgi complex as in the original strain (Figs. 9 and 10). Table 1 presents a summary of the differences encountered in the ultrastructural analysis of the original strains and resistant sublines.

DISCUSSION

No significant difference was observed in the plasma membrane, nuclei, and nucleoli between the original strain of the Yoshida ascites sarcoma or ascites hepatoma AH 13 and their sublines resistant to MBAO, mitomycin C, and 6-MP. However, the endoplasmic reticulum of the sublines resistant to MBAO was more scanty compared with that in the corresponding original strain. The vortical arrangement of the elongated endoplasmic reticulum and the annulate lamellae, which were encountered frequently in the original Yoshida ascites sarcoma, could not be detected.
in its MBAO-resistant subline. These differences in the endoplasmic reticulum were so definite that it was easy to distinguish between the resistant sublines and their original strains. Furthermore, the Golgi complex of the MBAO-resistant sublines was simpler in structure than that of the original strains. The cristae of mitochondria also seemed to be less regular in the MBAO-resistant subline of AH 13. Fawcett (3) and Howatson (8) reported that the structure of mitochondria is variable, depending upon the physiological conditions. Yasuzumi and Sugihara (10) also reported that the cristae and limiting membrane of mitochondria are frequently found destroyed in the Yoshida ascites sarcoma. As judged from these facts, it seems to be difficult to conclude that the significant difference between the original strain and MBAO-resistant subline was observed in the mitochondria. That the endoplasmic reticulum is variable in tumor cells was reported by Epstein (1, 2) and Fawcett et al. (4). However, since the difference in the endoplasmic reticulum between the original strain and MBAO-resistant subline was definite and it could not be detected between the mitomycin C- or 6-MP-resistant subline and their original strain, the difference in the endoplasmic reticulum observed in this study is considered to be related to the susceptibility to MBAO. It may be significant to confirm whether these morphological alterations are directly related to the development of resistance to MBAO or are merely secondary changes associated with the development of resistance; but sufficient data to solve this problem have not yet been obtained.

### TABLE 1

**A SUMMARY OF THE DIFFERENCES ENCOUNTERED IN THE ULTRASTRUCTURAL ANALYSIS OF THE ORIGINAL STRAINS AND RESISTANT SUBLINES**

<table>
<thead>
<tr>
<th>STRAIN</th>
<th>MITOCHONDRIA</th>
<th>ENDOPLASMIC RETICULUM</th>
<th>GOLGI COMPLEX</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Yoshida ascites sarcoma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Original strain</td>
<td>Elongated vesicle</td>
<td>Relatively well developed</td>
<td></td>
</tr>
<tr>
<td>MBAO-resistant subline</td>
<td>Annulate lamellae observed frequently</td>
<td>Lamellae poorly defined</td>
<td></td>
</tr>
<tr>
<td><strong>Ascites hepatoma AH 13</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Original strain</td>
<td>Cristae somewhat less regular than in original strain</td>
<td>Relatively well developed</td>
<td></td>
</tr>
<tr>
<td>MBAO-resistant subline</td>
<td>Small vesicle</td>
<td>Somewhat simple</td>
<td></td>
</tr>
<tr>
<td>Mitomycin C-resistant subline</td>
<td>Similar to original strain in shape, but less in number</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-MP-resistant subline</td>
<td>*</td>
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* No clear difference.

**Figs. 1-3.**—Appearance of cell constituents in the original strain of the Yoshida ascites sarcoma.

**Fig. 1.**—Survey picture of the original strain of the Yoshida ascites sarcoma. The tumor cells are surrounded by a dense membrane (CM) and show microvilli (MV). The nucleus (N) and nucleolus (NU) are clearly visible. Most of the cytoplasmic elements appear accumulated in the region of the nuclear hof. The elongated endoplasmic reticula (E) are seen. Some of them form a vortical arrangement (E*). Spherical or elongated mitochondria (M) of various sizes are present. Annulate lamella (AL) is also seen. ×9,800. The inset shows an annulate lamella at higher magnification. ×24,000.

**Fig. 2.**—The elongated endoplasmic reticulum (E) with ribonucleoprotein particles on its limiting membranes is seen in a vortical arrangement. ×29,000.

**Fig. 3.**—Double-layered nuclear membranes (NM) are visible. Mitochondria (M) showing an internal structure consisting of cristae and well developed Golgi complex (G) are seen. ×28,000.
FIGS. 4–6.—Appearance of cell constituents in the MBAO-resistant subline of the Yoshida ascites sarcoma.

Fig. 4.—Survey picture of the MBAO-resistant subline of the Yoshida ascites sarcoma. The endoplasmic reticulum (E) is more scanty, and it is short in length as compared with that of the original strain. X5,000.

Fig. 5.—The endoplasmic reticulum (E) is composed of small round or rodlike vesicles with ribonucleoprotein particles on its limiting membranes. L represents a lipide droplet. X44,000.

Fig. 6.—The lamellae of the Golgi complex (G) are poorly defined as compared with those of the original strain (Fig. 3). X33,000.
Fig. 7.—Tumor cell of the original strain of the ascites hepatoma AH 13. The endoplasmic reticulum (E), most parts of which are relatively small and vesicular, is found scattered throughout the cytoplasm. Mitochondria (M) showing relatively regular cristae are seen. The peripheral portion of the cell is the glycogen area (G.A). X14,800.

Fig. 8.—Tumor cell of the MBAO-resistant subline of the ascites hepatoma AH 13. The endoplasmic reticulum (E) is similar in shape but clearly less in number than that in the original strain. Destroyed mitochondria (M) are prominent. X14,800.
Fig. 9.—Tumor cell of the mitomycin C-resistant subline of the ascites hepatoma AH 13. Numerous small vesicular sections of the endoplasmic reticulum (E) are scattered throughout the cytoplasm. Significant differences in the mitochondria (M) and Golgi complex (G) cannot be detected between the original strain and this subline. X13,000.

Fig. 10.—Tumor cell of the 6-MP-resistant subline of the ascites hepatoma AH 13. Also, in this subline numerous small vesicular parts of endoplasmic reticulum (E) are found dispersed diffusely in the cytoplasm as in the original strain. The Golgi complex (G) is composed of lamellae and small vesicles or vacuoles. X15,000.
ACKNOWLEDGMENTS

The authors are indebted to Dr. M. Hoshino, Department of Pathology, Nagoya University School of Medicine, for his kind help in various phases of this work, and to Mr. Nonoyama and Mr. Maeda, Electron Microscope Section, Nagoya University, for their technical assistance.

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