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Cell–cell association in ascites Dalton's lymphoma and the effect of cisplatin *in vivo*

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Scanning electron microscopic studies revealed that ascites Dalton's lymphoma cells are distributed singly (25–30%) or in groups of 2–3 cells (55–60%) and 5 or more cells (10–15%) connected together. The percentage of single cells and groups of 2–3 or more cells changes with tumor growth. The number of single cells is maximal 72 h after tumor transplantation. Control tumor cells revealed the presence of blebs—ruffles all over the cells. Cisplatin treatment of the cells *in vivo* brings about definite changes in the arrangement of blebs—ruffles over the cells. At the later stages of cisplatin treatment disintegration and breaking of the plasma membrane occurs, which ultimately results in the lysis of tumor cells.

Key words: Cell–cell association, cisplatin, lymphoma.

Introduction

Cisplatin is a potent chemotherapeutic agent which has been successfully used against a wide spectrum of malignancies.[1–3] The primary mechanism of the antitumor activity of cisplatin probably resides in its ability to inhibit DNA synthesis.[4,5] It has also been reported that cisplatin has an effect on the surface of tumor and normal cells and brings about definite changes in the lectin agglutinability of the cells.[6] A change in the topographical pattern of lectin binding sites and the removal of cell surface sialic acid–mucopolysaccharides after cisplatin treatment of the cells have been observed.[7]

Many structural and functional properties of malignant cells are related to changes in the cell surface–cell membrane.[8,9] The presence of cell–cell contacts has been described in solid tumors[10] and a few ascitic tumors.[11,12] Cell association in malignant cells has been suggested to regulate the pattern of growth and malignancy in tumors.[13] Cell connections form the channels which probably regulate the hydrophilic pathway between adjacent cells and thus help in transport of ions and small molecules from one cell to another.[14,15]

The pattern of cell distribution, cell–cell association and the effect of cisplatin have not been investigated in ascites Dalton's lymphoma cells. The present scanning electron microscopic (SEM) studies were undertaken to find the pattern of cell surface topography and cell–cell association with reference to the effect of cisplatin on ascites Dalton's lymphoma cells *in vivo*.

Materials and methods

Transplantable ascites Dalton's lymphoma tumor is maintained in C_3H/He male or female mice of 9–10 weeks age, weighing 20–22 g, by serial intraperitoneal transplantation of 1×10^7 viable tumor cells (0.25 ml in phosphate-buffered saline, PBS). Tumor transplanted animals survive for 20–24 days with a mean survival time of 22 days. Cisplatin was a gift from Professor Litterst of the National Institutes of Health, USA. Cisplatin was thoroughly mixed in normal saline (0.89% NaCl) in the dark 10–15 min before use.

On the tenth day of tumor transplantation, mice were intraperitoneally injected with cisplatin (8.0 mg/kg body weight). After 8 h or 1, 2, 4, 6 and 8 days of cisplatin injection, mice were killed by cervical dislocation and ascites tumor was removed, centrifuged and washed once with PBS (0.15 M NaCl, 0.01 M sodium phosphate buffer, pH 7.4). The cell pellet was diluted with PBS (1:3, weight: volume) gently and used for SEM. The cell suspensions were fixed in 2.5% glutaraldehyde prepared in 0.1 M sodium cacodylate buffer for 10 min at 4°C.

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Then the cells were further processed for SEM by the usual drying technique and coated with gold. The cells were observed and photographed under a Jeol scanning electron microscope operated at 15 kV. Control animals were injected with the same amount of normal saline; ascites Dalton's lymphoma tumor was collected and processed for SEM. To study the cell distribution patterns, ascites fluid was collected from the tumor transplanted animals every day up to 10 days. It was diluted with PBS and a minimum of 1000 cells were counted under phase contrast on a Neubauer hemocytometer.

Results

Dalton's lymphoma cells collected on the tenth day of tumor transplantation were observed to be distributed as single cells (25–30%) or in groups of 2–3 cells (55–60%) and 5 or more cells (10–15%) connected together. The cell distribution patterns in ascites fluid collected every day after tumor transplantation showed that the percentage of single cells increases and the percentage of groups of 2–3 cells decreases at the beginning of tumor growth up to 72 h of tumor transplantation. From 96 h onward, an increase in the percentage of 2–3 associated cells was noticed with a concomitant decrease in the percentage of single cells (Table 1).

Tumor cells were noticed to be in various advanced stages of the formation of cellular connections with neighboring cells. More than one cellular process–connection could arise on a cell, each appearing on the side of the cell exactly opposite to the process–connection arising on the adjacent cell (Figure 1 a–e). Cells in advanced joining stages may lead to the fusion of 2–3 cells, resulting in the formation of a multinucleated tumor cell (Figure 1 f–h).

Table 1. Cell distribution during the growth of ascites Dalton's lymphoma

Time after transplantation (h)	Distribution (% of total)		
	Single cells	Groups of 2–3 cells	Groups of 4 or more cells
0	28.2	59.8	12.0
24	41.0	48.2	10.8
48	52.4	37.2	10.4
72	58.2	32.5	9.3
96	43.1	46.4	10.5
120	36.8	51.5	11.7
144	32.3	55.2	12.5
168	27.6	59.3	13.1
192	25.8	60.4	13.8
216	27.4	59.6	13.0
240	28.3	59.4	12.3

Control tumor cells showed the presence of blebs–ruffles all over the cell (Figure 2a). Cisplatin treatment of Dalton's lymphoma *in vivo* does not show significant changes in the pattern of cell–cell association but it brings about definite changes or reorientation in the arrangement of ruffles and blebs over the cells. 8 h of cisplatin treatment resulted in the appearance of thick, pronounced, flat ruffles–blebs (Figure 2b). Treatment for 1–2 days leads to the movement of blebs–ruffles from the top surface of the cells towards the marginal areas (Figure 2c,d). At 4 days of cisplatin treatment many small dense ruffles are seen towards the cell margin and also there is possible disintegration in the cell membrane (Figure 2e). At 6 days of cisplatin treatment more disintegration and breaking of the plasma membrane is noted with the removal of some cellular material suggesting the lysis of tumor cells (Figure 2f). Later, at 8 days of the treatment, most of the plasma membrane is lyzed in small fragments (Figure 2g).

Discussion

Dalton's lymphoma cells in the ascites fluid were observed to be mainly distributed in islands of 2–3 cells (55–60%) or as single cells (25–30%). Few groups (10–15%) were noted to contain 5 or more cells connected together (Figure 1i). However, the percentage of groups of tumor cells or single cells in the ascites fluid was noted to change with the tumor growth in the animal after tumor transplantation. The percentage of single cells increases and groups of 2–3 cells decreases up to 72 h after tumor transplantation (Table 1). This indicates that, in the beginning of the tumor growth, single cells may be multiplying at a preferentially higher rate so that the number of single cells increases. The association of single cells into groups of 2–3 cells may occur with the age of the tumor, resulting in increase in the percentage of groups of 2–3 cells after 3 days of tumor transplantation. This hypothesis is supported by the observation that the cells were noticed to have tendencies to form cellular connections with the neighbouring cells (Figure 1a–e). Cells showed the appearance of one or more cellular connections–processes arising exactly opposite to the cellular processes arising at the adjacent cell

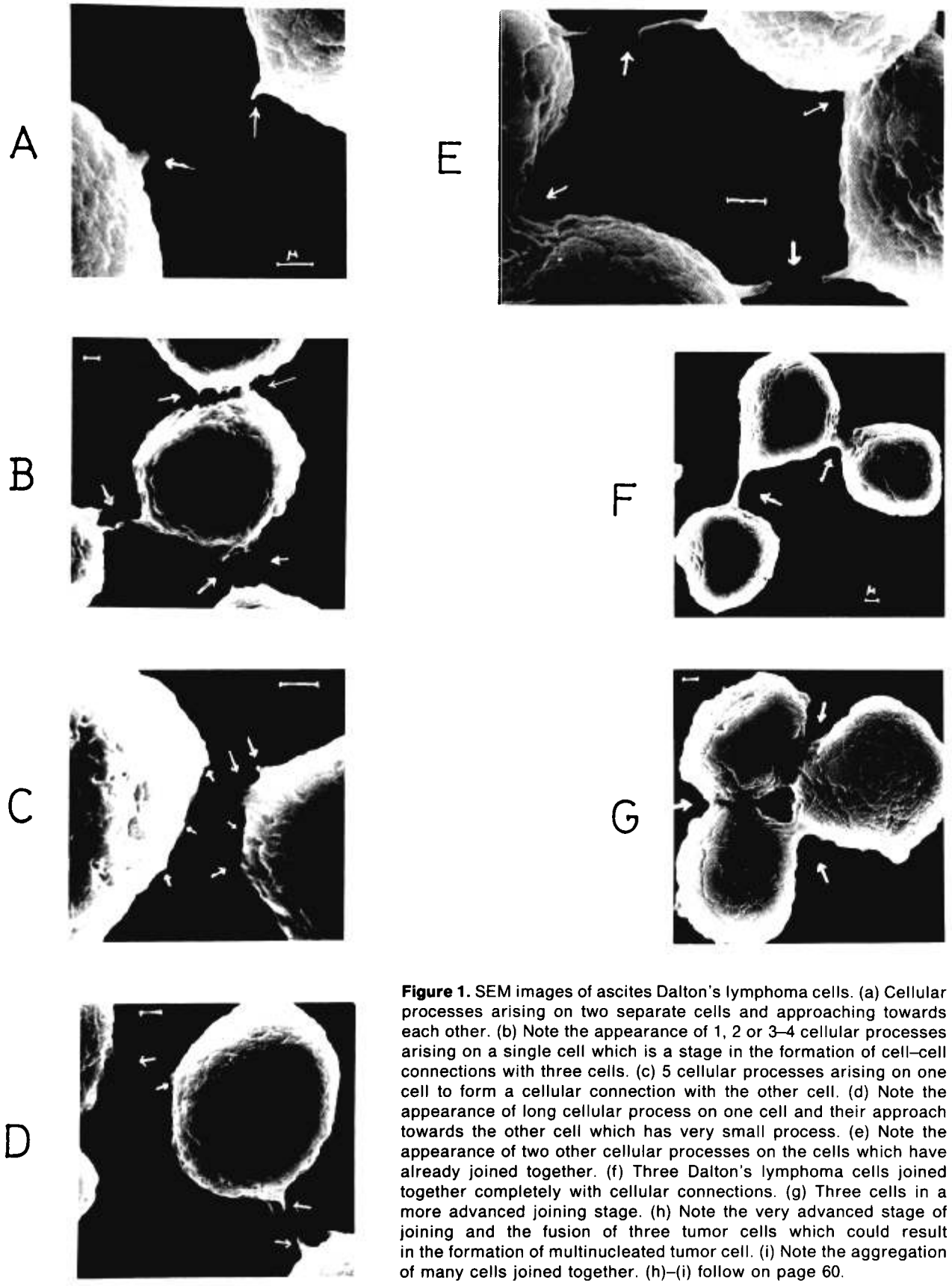

Figure 1. SEM images of ascites Dalton's lymphoma cells. (a) Cellular processes arising on two separate cells and approaching towards each other. (b) Note the appearance of 1, 2 or 3–4 cellular processes arising on a single cell which is a stage in the formation of cell–cell connections with three cells. (c) 5 cellular processes arising on one cell to form a cellular connection with the other cell. (d) Note the appearance of long cellular process on one cell and their approach towards the other cell which has very small process. (e) Note the appearance of two other cellular processes on the cells which have already joined together. (f) Three Dalton's lymphoma cells joined together completely with cellular connections. (g) Three cells in a more advanced joining stage. (h) Note the very advanced stage of joining and the fusion of three tumor cells which could result in the formation of multinucleated tumor cell. (i) Note the aggregation of many cells joined together. (h)–(i) follow on page 60.

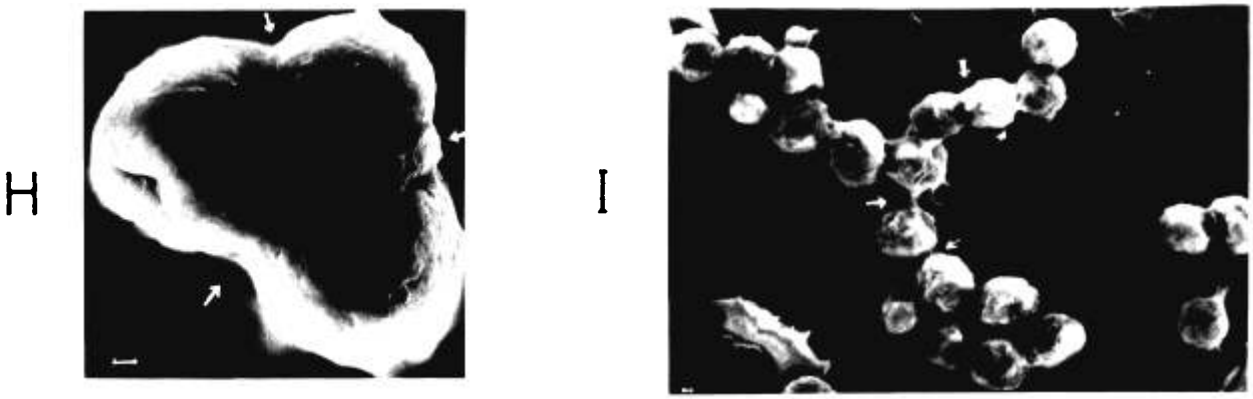

Figure 1. (*continued*)

(Figure 1b–d). An almost similar trend in the distribution of Zajdela ascitic hepatoma cells has been reported up to 5 days.[12] The presence of groups of 2–3 or more cells in ascites fluid may be helpful in the acquisition of the characteristic growth properties of tumor cells in the host. Cell-to-cell adhesion in malignant cells has been suggested to be a multifunctional process and it regulates the pattern of growth and behavior of malignancy in tumors.[13] The cell–cell connections have been reported to contain different types of junctions which are arranged in a manner such that a direct channel is formed through the plasma membrane of both of the apposing cells. These channels probably regulate the hydrophilic pathway between adjacent cells and thus help in transport of ions and small molecules from one cell to another.[14,15] In the present observations also the pattern of tumor growth seems to be regulated through cellular connections, as the percentage of single cells or groups of 2–3 cells changed with the tumor growth. At very advanced joining stages of cell–cell association, fusion of 2–3 cells may take place, resulting in the formation of a multinucleated tumor Dalton's lymphoma cell (Figure 1h) which probably acquires a more stable metabolic existence.

Ascites Dalton's lymphoma cells showed the presence of numerous cytoplasmic blebs and ruffles all over the cells with some infoldings of plasma membrane (Figure 2a). The membrane infoldings have been well marked in ascitic cells.[12] Porter *et al.*[16] reported the presence of unusual ruffles on several virally and spontaneously transformed Balb/C 3T3 cells and showed that ruffles appeared around the cell margin and occupied a significant part of the top surface of the cells, a feature probably related to the known capacity of tumor cells to phagocytose their environment. Other transformed cells that have been reported to have large numbers of ruffles on the surface of interphase cells include mouse sarcoma 180, mouse thymoma cells, rat sarcoma 4337, mouse hepatoma 129 cells,[17] and malignant melanoma A 375 cells.[18] Blebs have also been reported to appear in unusual numbers on many transformed cell lines, e.g. adenovirus-type-5-transformed hamster embryo cells, human carcinoma A 549, rhabdomyosarcoma A 1186, and mouse embryo cells transformed chemically.[18] It is likely that blebs result from alterations of the cortical microfilament network.[19]

Cisplatin treatment of Dalton's lymphoma cells *in vivo* did not show significant changes in the pattern of cell–cell association in groups or as single cells which in turn suggests that it does not change cell–cell connections. However, cisplatin treatment showed significant changes in the arrangement–movement of ruffles and blebs over the cells (Figure 2a–g). Gradual movement and arrangement of ruffles–blebs from the top surface of the cells towards the leading edge of the cells was noticed from 8 h to 4 days of cisplatin treatment *in vivo* (Figure 2a–e). The disintegration of plasma membrane observed at 4 days of the treatment was noted to be more pronounced at 6 and 8 days of cisplatin treatment. The direct disintegration and breaking in the plasma membrane could lead to the lysis of tumor cells after cisplatin treatment. Ribereau-Gayon *et al.*[20] reported that bacterially fermented mistletoe preparation (BFMP) treatment brings significant modifications of cell surface of rat hepatoma cells and disintegration of the plasma membrane takes place in the antitumor effect of BFMP. In the present studies also reorientation in the arrangement of cell surface ruffles–blebs and the

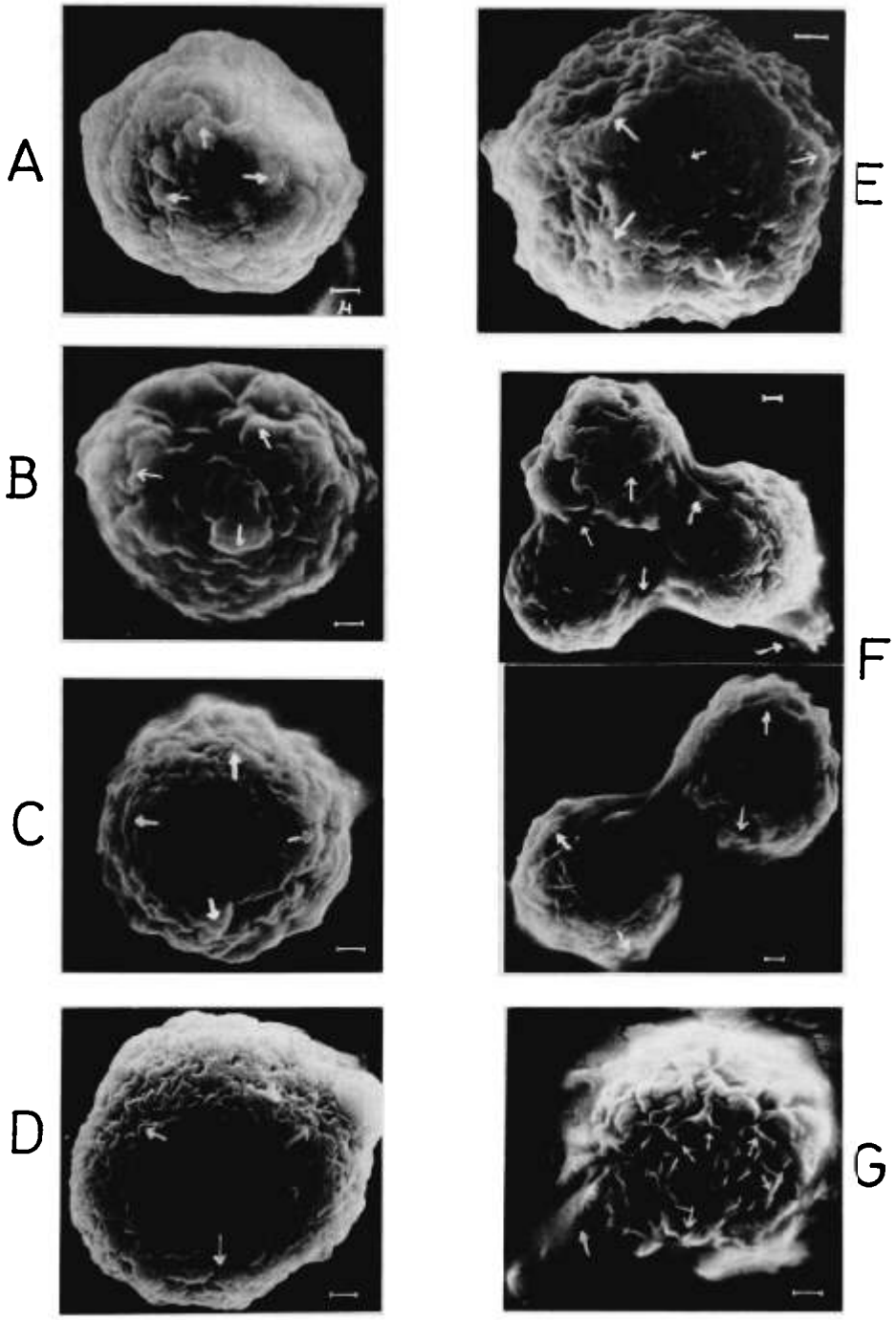

Figure 2. SEM images of Dalton's lymphoma cells treated with or without cisplatin in vivo. (A) Control, showing blebs–ruffles distributed all over the cell. (B) After 8 h of treatment showing thick, more conspicuous blebs–ruffles. (C) After 1 day of the treatment note the movement of blebs–ruffles towards the marginal areas of the cell. (D) After 2 days of treatment accumulation of more ruffles–blebs towards marginal areas of the cell. (E) After 4 days of treatment showing that disintegration in the plasma membrane has started. (F) After 6 days of treatment showing more disintegration in the plasma membrane and release of substances from the cell. (G) After 8 days of cisplatin treatment showing the complete breakdown and disintegration of the plasma membrane. Arrows indicate the movement of cell surface ruffles–blebs and/or disintegration in the plasma membrane.

disintegration in the plasma membrane resulting from cisplatin treatment seem to be the direct cause of tumor cell lysis. This is supported by the observation at 6–8 days of cisplatin treatment when most of the plasma membrane is noted to be disintegrated with the removal of some cellular material (Figure 2f,g).

Conclusion

Ascites Dalton's lymphoma cells are distributed as single cells and in groups of 2–3 or more cells connected together. The joining of 2–3 cells may lead to the fusion of the cells which ultimately could result in the formation of a multinucleated tumor cell. Cell distribution patterns of single cells and islands of 2–3 or more cells change with the age of the tumor after tumor transplantation. Cisplatin treatment of the tumor cells *in vivo* does not affect the pattern of cell–cell association but it brings about significant changes in the arrangement of ruffles–blebs over the cells and causes disintegration in the plasma membrane to lyze the tumor cells.

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