The Occurrence of a Cyclosis-like Phenomenon in Human Lung Cancer Cells in Vitro

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Summary

An undifferentiated human lung carcinoma was resected and explanted in vitro; its primary origin has been subsequently confirmed by autopsy study. Film recordings were made of 45 cancer cells exhibiting a peculiar type of cytoplasmic streaming, designated "pericyclosis." The movement involved the peripheral portion of the cytoplasm and resulted in a continual, circling motion around a central cell mass. Special aspects of the streaming were the relatively constant cycling time of 1 min, the gel-sol interphase appearance, reversal of direction, the occurrence of exactly proportioned biphasic forms, and the invariably presence of cohesion loss and cell rounding. The initiation of the movement, the general association with "death agony," and the relationship to cyclosis of plants are discussed.

Introduction

Although cyclosis is a well-known phenomenon in plants and various protozoa (3, 14), its counterpart in mammalian cells, i.e., cytoplasmic streaming characterized by rotation and circulation, has not been reported. Except for a "searlike" circular motion of the cytoplasm in leukocytes (1), cytoplasmic movement in mammalian cells has been restricted to fairly localized travel of intracytoplasmic organelles (3, 7) and to such phenomena as zeiosis (2, 10) and "death agony" (1).

During an in vitro investigation of human lung cancer, a cyclosis-like activity of documented lung cancer cells was observed with one of the explanted cancers. The movement was circumferential, continuous, and constant in speed and as such had not been previously described. Significantly, the phenomenon occurred only in the cancer cells, despite the presence of numerous other cells of various types in the same preparations. The several dynamic and structural aspects associated with the phenomenon offer potential insight into the membrane nature of this particular cancer cell and also that of nonneoplastic cells in general.

Materials and Methods

Forty-six surgically resected human lung cancers have been explanted in vitro and the outgrowths observed and photographed. Details of the methods used have been previously reported (8). In brief, 1- to 2-mm explants of the lung cancer were cultured in Rose chambers, using a cellophane strip technic, and in Leighton tubes with tube slips. A total of 72 explants were employed, and the cultures were maintained on Eagle's double strength amino medium enriched with 20% calf serum and 5% beef embryo extract ultrafilter. Incorporated in the medium were 100 units/ml of penicillin, 100 μg/ml of streptomycin, glucose (in a final concentration of 500 mg %), L-glutamine (0.25 mg/ml), and phenol indicator in a 0.0025 final concentration. Cultures were incubated at 37°C ± 0.5°C and treated according to changes in pH. A correlative study was made of the cancer cells in the monolayers and explants (12, 13). Cellular activities throughout the study were recorded on 16-mm film with phase contrast microscopy, using scanning speeds (16 frames/sec) and time lapse intervals. In addition, 35-mm photomicrographs were obtained.

Results

Numerous instances of pericyclosis, i.e., peripheral en masse rotation of the cytoplasm (Figs. 5, 6, 10-14) were observed, including 36 examples recorded on 35-mm film and 9 recorded on 16-mm film (time lapse and scanning speed). The phenomenon occurred in 3 of the 4 Rose chamber preparations; the 4th culture degenerated prior to the time pericyclosis was first observed in the others. An appearance consistent with an arrested state of pericyclosis was found in one of the stained Leighton tube preparations. An in vitro search for pericyclosis in the Leighton tubes was not done. Pericyclosis was first noted at 13 days of in vitro life and was seen as late as 30 days in the Rose chambers and 35 days in the stained tube slip from 1 Leighton tube. After 30 days most of the cancer cells had degenerated. Pericyclosis was seen only in cells which had separated from the monolayer, and the time of the 1st observation corresponded with the onset of marked cohesion loss of the monolayer sheets. During pericyclosis all of the isolated cancer cells were in a retracted or "rounded" form. The phenomenon generally began with the appearance of a single cytoplasmic bleb or pseudopod at 1 point along the periphery of the rounded cell. This was shortly followed either by a succession of similar pseudopods at this focal point or herniation of the cytoplasmic membrane next to a pseudopod (Fig. 4). The cytoplasm and the immediately adjacent intracytoplasmic organelles then proceeded to flow through the weakened area and produced a bulge which then became the "head" of the encircling cytoplasmic mass. Once started, the encircling movement continued for a variable length of time (a few min to 2 or 3 days) at a speed...
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which was fairly constant. Cessation of pericyclosis was almost always followed by marked blistering and death of the cell. Exceptions, however, did occur, and the cessation of pericyclosis was followed by recovery of the cell, i.e., a change from a rounded to a spread form (Figs. 13–18).

The shortest time for a complete cycle was 53 sec and the longest was 1 min and 30 sec (2 min and 50 sec for one of the biphasic forms, Fig. 9), and in several instances a 1-min cycle was maintained for a few hours with essentially no change in speed, as shown dramatically in one of the time lapse records. There was no apparent relationship of cell diameter to the cycling time. As a rule the cessation of the pericyclosis movement was a sudden one, but several instances were noted where progressive slowing occurred. In several instances the movement stopped abruptly and then reversed immediately, continuing at approximately the same speed as noted when moving in the opposite direction. Several reversals in direction were noted in the same cell (Figs. 11, 12). Cessation and reversal of the movement were often noted at the point of initiation, and this was generally accompanied by the formation of a blister or bleb. Sometimes the formation of bleb or pseudopod stopped the advancing head, but more often there was a temporary retardation or no effect (Fig. 7). Cellular debris in the path of the advancing head was vigorously pushed aside. On the other hand, debris adjacent to the periphery of the moving mass but away from the advancing head itself moved very little. In a few instances pericyclosis began simultaneously at points on the cell periphery diametrically opposite to each other, thus producing 2 advancing heads of cytoplasmic movement (Figs. 8, 9). The cycling time for the 2 heads was invariably the same in all cases, and each arc of moving cytoplasm occupied exactly half of the cell circumference. Reversal of direction of 1 or both advancing heads of the biphasic forms was not observed. With both the single and the biphasic forms the movement of direction was more often counterclockwise, but the findings are inconclusive. Fusiform cells, macrophages, and other cells persisted for several days after the loss of all cancer cells, and their subsequent degeneration was not associated with cytoplasmic movement other than the usual forms of zeiosis.

Discussion

The type of movement found, namely, the circling of the central portion of a cell by a peripheral en masse movement of the cytoplasm with its intracytoplasmic organelles, is a unique phenomenon. A presumably similar phenomenon has been noted by Bessis with human leukocytes and some other cells in tissue culture; it has been described as a circular movement of “scarf-like” pseudopods (1). There has been no previous report of this type of movement in tissue cultures of human cancers, in particular, primary lung cancer. Other types of cytoplasmic movements may bear a remote relationship, such as the cytoplasmic “streaming” of polymorphonuclear leukocytes (9) and also fibroblasts cultured under anaerobic conditions (4). Movement of this type within plant cells has been described as cyclosis but differs from the phenomenon noted in that it occurs within the interior of the plant cell and is a normal property. There is, however, some resemblance of the rotational form of cyclosis or so-called umlaufbewegung (4) to the present phenomenon.

The restriction of pericyclosis to the cancer cells throughout the entire in vitro study, despite an equal amount of degenerative change of fusiform cells, macrophages, and other cells, indicates an extraordinary peculiarity of the cancer cell. Although all of the cancer cells in the cultures eventually died, relatively few showed this phenomenon. There were no overt morphologic differences between the cancer cells (Figs. 1–3) to indicate impending pericyclosis or to account for the phenomenon in certain cells. None of 44 other lung cancers explanted in vitro exhibited pericyclosis.

The mechanism of the movement has not been established, but there is a consistent relationship to a small focus of weakening of the cytoplasmic membrane with herniation of freely flowing cytoplasm and its organelles in the form of a blister or pseudopod (Fig. 4). The sudden and relatively rapid cytoplasmic flow implies the presence of a gel-sol interphase, and this may be responsible for the force that moves the sol (5). A significant aspect appears to be localized nature of the herniation, and it is intriguing to note that 2 can occur simultaneously at diametrically opposite poles, thus producing a biphasic pericyclosis (Figs. 8, 9). The phenomenon is best understood as an “unwinding” of cytoplasmic membrane with internal streaming only. Although the streaming is triggered by a degenerative type of change, it is important to note that pericyclosis is reversible, with complete recovery of the cell.

An extraordinary finding was the uniform speed of the movement and the generally constant cycling time. Each cycle usually required 1 min regardless of cell size, and the biphasic advancing heads proceeded at identical speeds. The speed at the beginning of the movement was usually the fastest observed and after several hours or days showed a very gradual decrease. Reversals of direction did not result in a change of speed. It is pertinent to note that cyclosis in plants tends to maintain constant speeds, mostly averaging 5–15 μm/sec (3), and that the reversal of cytoplasmic movement in slime molds is generally at 1-min intervals (11).

No attempt was made during the study to influence the cytoplasmic movement, either by adding cysteine to inhibit pericyclosis (6) or by adding sulfhydryl-group-blocking agents to initiate it. Nutrient media withdrawn from the chambers at the time of pericyclosis have been frozen and stored, pending analysis for a possible peculiarity in amino acid consumption by the in vitro cells. In this respect it may be significant that the cancer exhibited marked central necrosis and cavitation in vivo.

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Fig. 1. Cancer cell monolayer (L-68), 7 days in vitro. The identity of this undifferentiated primary lung cancer has been documented by the demonstration of continuity between the in vitro cancer cell monolayer and cancer tissue in the explant itself. The giant sections of the lung cancer showed a very similar and homogeneous cell type. A primary origin elsewhere was excluded by a postmortem examination. Although there is good cellular cohesion, the cell borders frequently overlap (contact inhibition loss) or there is overt layering, 2-3 cells in depth. Phase contrast photomicrograph, × 520 (× 160; 35-mm film).

Fig. 2. Edge of cancer cell monolayer, 2 days in vitro. The 2 cancer cells at the lower margin are partially rounded, and the spread forms above show prominent vacuoles (pinocytosis). The feathery appearance of the cytoplasmic edges is membrane activity. Time phase cinemicrograph, R sec exposure time, abstracted and enlarged from × 240 on 16-mm film, × 2400.

Fig. 3. Cancer cell, 19 days in vitro. This isolated cancer cell has an unusual, almost rectangular, shape and limited membrane activity (upper and lower corners on the right). Two small and 1 very large nucleus, all with prominent nucleoli, are present. Phase contrast micrograph, × 520 (× 160; 35-mm film).

Fig. 4. Cancer cell, 15 days in vitro. At the lower pole of the cytoplasmic periphery of the cell, several blebs have formed and a pseudopod from the bleb has begun to encircle the cell periphery. This phenomenon very often preceded the development of pericyclosis. Shortly after this sequence was taken, the clockwise-moving pseudopod enlarged, lost its segmented appearance, and became a typical example of pericyclosis. The cell had exhibited pericyclosis several min earlier. Note the 2 nuclei with their prominent nucleoli at the extreme margins of the central portion of the cell. Phase contrast photomicrograph, × 520 (× 160; 35-mm film).

Fig. 5. Cancer cell in pericyclosis, 16 days in vitro. The movement is characterized by an advancing head and a main body of cytoplasm, the latter gradually tapering to a very thin band. Two nuclei are present just beneath the advancing head. The granules within the inner half of the cytoplasmic band appear elongated and blurred because of the rapidity of the movement, whereas those which are stationary at the extreme periphery are round and sharply defined. A refractile lymphocyte is seen at the upper pole of the cell periphery; it had been attached to the cytoplasmic membrane at that point for several revolutions. Shortly after this photo was taken the lymphocyte released its hold and was pushed forward by the advancing head of the cytoplasm. A complete cycle required exactly 1 min. Note the cancer cell at the left margin; it is partially rounded and shows 2 prominent membrane projections. Phase contrast photomicrograph, × 520 (× 160; 35-mm film); R sec exposure time.

Fig. 6. Elliptical cancer cell in pericyclosis, 17 days in vitro. Within a short period of time, several reversals of the movements occurred. The advancing head shown here represents a reversal of direction which was preceded by bleb formation. Phase contrast photomicrograph, × 520 (× 160; 35-mm film).
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FIG. 7. Two cancer cells, both with bleb formation and 1 (upper) with pericyelosis, 17 days in vitro. The large bleb just in front of the advancing cytoplasmic head (cell above) slowed the movement of the cytoplasm slightly, but pericyelosis continued essentially unaffected. The cell below did not undergo pericyelosis. Phase contrast photomicrograph, × 812 (× 250; 35-mm film).

FIG. 8. Cancer cell with biphasic pericyelosis, 15 days in vitro. The advancing heads of the moving cytoplasmic bands are at diametrically opposite poles of the cell and each head overlaps approximately equal portions of the tail of its counterpart. A large, eccentrically placed nucleus is present (upper margin). Relatively few granules are present in the cytoplasmic bands. Phase contrast photomicrograph, × 812 (× 250; 35-mm film), 1 sec exposure.

FIG. 9. Cancer cell with biphasic pericyelosis, 17 days in vitro. Very few granules are present in the cytoplasmic bands, and the cycling time was exceptionally long, 2 min and 50 sec. Note the symmetry of the 2 bands. Phase contrast photomicrograph, × 520 (× 160; 35-mm film), 4 sec exposure.

FIG. 10. Cancer cell in pericyelosis, 24 days in vitro. Four nuclei can be seen within the rounded central portion of the cell, and there is a cap-like mass of cytoplasm occupying 1/2 of the cell periphery with a slender tail-like extension around the remainder of the cell. Cytoplasmic streaming within the inner half of the cap is evident as a blurring of the granules, whereas those at the extreme periphery are stationary and thus are sharply demarcated. Time phase cinemicrograph, 1 sec exposure time, 0.0 min, abstracted and enlarged from × 96 on 16-mm film, × 900.

FIG. 11. Cancer cell in pericyelosis, same as that of Fig. 10. The cytoplasmic cap has made several revolutions, whereas the central cell mass has changed relatively little (note the position of the 4 nuclei). Time phase cinemicrograph, 1 sec exposure time, 7 min, 0.0 sec.

FIG. 12. Cancer cell in pericyelosis, same as that of Fig. 11. There has been slight rotation of the central portion of the cell as evident from the change in position of the nuclei. Time phase cinemicrograph, 1 sec exposure time, 24 min, 30 sec.

FIG. 13. Cancer cell in pericyelosis, same as that of Fig. 12. The direction of movement of the advancing head of the cancer cell has suddenly reversed. Time phase cinemicrograph, 1 sec exposure time, 14 hr, 35 min.

FIG. 14. Cancer cell in pericyelosis, same as that of Fig. 13. There has been some rotation of the nuclei at the periphery of the central cell mass, but the nucleus in the center has maintained its position throughout the sequences. Time phase cinemicrograph, 1 sec exposure time, 21 hr, 59 min, 30 sec.

FIG. 15. Cancer cell in pericyelosis, same as that of Fig. 14. The cytoplasmic movement has suddenly stopped, but the outline of the cap is still present. The distinction between the central portion of the cell and the cap is now less apparent. Time phase cinemicrograph, 1 sec exposure time, 22 hr, 33 min, 45 sec.

FIG. 16. Cancer cell after pericyelosis has ceased, same cell as that of Fig. 15. At one pole of the cell a tangential pseudopod has formed (left margin). The cell is now roughly oval, and the cytoplasmic cap is no longer apparent. Time phase cinemicrograph, 1 sec exposure time, 22 hr, 33 min, 45 sec.

FIG. 17. Cancer cell after pericyelosis has ceased, same cell as that of Fig. 16. The rounded cell has now partially spread, and membrane activity can be seen along the left lower margin of the cell. Membrane activity is also present elsewhere, and one focus is evident at the upper left margin. Near this focal membrane activity a small blister has appeared (arrow). Although only 1 nucleus is obvious, the presence of 2 others is indicated by the prominent aggregates of dark granules (a, b). Time phase cinemicrograph, 1 sec exposure time, 29 hr, 18 min, 45 sec.

FIG. 18. Cancer cell after pericyelosis has ceased, same cell as that of Fig. 17. The cancer cell has undergone further spreading, and extensions of the cytoplasm characteristic for these cancer cells have appeared. Membrane activity is present at the margin of such extension (below and center). Time phase cinemicrograph, 1 sec exposure time, 36 hr, 15 min, 15 sec.
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