Contact Inhibition of Movement in the Cultures of Transformed Cells

(cell collision/neoplastic fibroblasts/microcinematography/mouse sarcoma virus)

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ABSTRACT Results of cell-cell collisions were studied with the aid of time-lapse microcinematography in primary cultures of normal mouse-embryo fibroblast-like cells and in cultures of transformed mouse cells of two types: (a) primary fibroblast-like cells transformed by Moloney mouse sarcoma virus; (b) neoplastic fibroblasts of the CIM strain.

Collisions of normal fibroblast-like cells and CIM cells in mixed cultures were also analyzed. Classification of the results of collisions was based on observation of the movements of the active cell edge during the first hour after the moment when this edge had contacted another cell. Three types of collision results were detected: halt of the active edge, overlapping, and underlapping.

The relative number of overlappings was not higher and that of halts not lower in the cultures of transformed cells as compared with those of normal cells. Analysis of the collisions of normal fibroblasts with transformed cells gave similar results. Thus, the altered morphology of the cultures of these transformed cells cannot be explained by loss of contact inhibition of movement leading to increased ability of cells to move over the surfaces of other cells after collision.

Contact inhibition of movement was defined by Abercrombie (1) as the prohibition, when contact between cells had occurred, of continued movement such as would carry one cell over the surface of another. Contact inhibition includes the paralysis of movements, adhesion, and retraction of the leading edge at the site of its contact with another cell (1). This set of phenomena prevents the overlapping of one cell by another.

Contact inhibition is a characteristic form of locomotory behavior of normal fibroblast-like cells in culture. Transformation of cultured fibroblasts caused by oncogenic viruses and other carcinogenic agents is believed to be accompanied by the loss or diminishment of contact inhibition of movement. Analysis of the literature shows that this wide-spread opinion is based mainly on the altered morphology of the cultures of transformed cells: these cultures often have multilayered areas with considerable degree of overlappings of the cell nuclei; elongated cells may form criss-cross patterns.

However, as stressed by Abercrombie (1), conclusions about the loss of contact inhibition in culture based on this type of indirect evidence may be erroneous. The only direct way to find out whether contact inhibition is observed in some culture is to analyze the results of individual cell-cell collisions with the aid of microcinematography. This paper describes the results of the analysis of collisions in cultures of normal mouse-embryo fibroblasts and in the cultures of two types of transformed mouse fibroblasts. Relative frequencies of various results of collisions were similar in all types of cultures. Thus, transformation of fibroblasts was not accompanied by decreased contact inhibition of movement.

MATERIALS AND METHODS

Cells. Mouse fibroblast-like cells of three types were used:

(A) Normal embryo cells were obtained by trypsinization of 16- to 18-day embryos of C3HA mice. Cells were grown for 4–5 days in large (100 ml) culture flasks, then seeded into the chambers used for microcinematography.

(B) Embryo cells transformed by mouse sarcoma virus (MSV) of the Moloney type. The extract of pooled neoplasms induced by MSV in C3HA mice was used as infecting material; this extract contained $4 \times 10^4$ focus-forming units of the virus per ml. 2-Day-old cultures of normal embryo cells (2 $\times 10^6$ cells per flask) were washed twice with saline, incubated with 0.5 ml of the virus-containing extract for 1 hr at 37°, washed with saline, trypsinized, and seeded into the large flasks. Simultaneously, the same suspension was seeded into 10-ml culture flasks containing cover slips at the bottom. Cover slips with cultures were fixed at various times and stained with hematoxylin.

Numerous foci of transformed cells were seen in the infected cultures at 3–4 days; these foci became confluent at 6–7 days. 6- to 8-Day-old cultures were seeded again into the chambers for microcinematography and filmed a day later. Morphology of MSV-transformed cultures was similar to that described by various authors (2–4). Numerous cells with two or three very long and narrow cytoplasmic processes were seen. These elongated cells criss-crossed each other under various angles and formed multilayered groups.

(C) Neoplastic fibroblasts of the CIM strain. This strain was obtained by serial cultivation of the cells of primary sarcoma induced by implanted polyion film in a CBA mouse. At the time of the experiments the cells were in culture for about 1 year and were in their 40th–50th passage. Transplantation of 10 cells of this strain was sufficient to produce tumors in 90–100% of syngeneic mice. The elongated cells that had from 2 to 5 long cytoplasmic processes were most characteristic for this strain. Even in cultures of low density (about 1 $\times 10^5$ cells per cm²), these cells criss-crossed each other and formed multilayered groups.

Abbreviation: MSV, mouse sarcoma virus (Moloney).

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Numerous groups of cells piling upon one another were seen in the cultures that were near their saturation density (about $12 \times 10^4$–$14 \times 10^4$ cells per cm²).

**Cinematography.** Cells for cinematography were grown in special chambers with parallel glass walls. Eagle's medium (supplied by the institute of Polyomethylite, Moscow) with 10% bovine serum was used in all the experiments. The filming was started 2 hr after the last change of medium; duration of filming was usually about 24 hr.

The set for time-lapse microcinematography with phase-contrast optics was used. 35-mm film was used; magnification at the film was $\times 50$. Intervals between the frames were 30 sec. The films were analyzed frame by frame on a projection screen.

Two variants of cells were filmed:

(1) Isolated cultures of cells of one of the three types listed above (A, B, or C). Cultures with cell density of about $2 \times 10^4$–$4 \times 10^4$ cells per cm² were used. Cells in these cultures occupied not more than 50% of the whole area of the substrate; this was a necessary condition for analysis of individual collisions.

(2) Mixed cultures of normal embryo cells and CIM cells (A + C). Cultures of normal cells were grown for 5 or 6 days. Then a wound was made in the cell sheet and simultaneously CIM cells ($5 \times 10^4$ cells per ml of the medium) were seeded into the medium. In other experiments, the order of cell seeding was reversed; that is, normal cells were seeded into the dense wounded culture of CIM fibroblasts. The filming was started 2 hr after wounding. The fields of view near the edge of the wound were selected for filming. Cells of one type moved from the edge of the wound and collided with the cells of another type recently attached to the glass.

**CLASSIFICATION OF COLLISIONS IN FILMS**

Active and nonactive parts of the cell edge were easily distinguished in the cells of all of the types studied. Continuous formation of protrusions (lamellipodia) and ruffling were characteristic for the active edge. Outlines of nonactive parts of the edge were smoother and more stable.

The active edge was regarded as translocating upon the substrate if the position of the lateral points and of the center of this edge moved a distance of 20 μm or more in 2 hr. Usually, translocation of the active edge was followed by translocation of the whole cell in the same direction. In a small number of cases, cell translocation during the period of observation was less pronounced than that of the active edge; in other words, the cell became elongated in the direction of the translocation of its active edge.

We registered as cell–cell collisions all the cases in which translocating active edges had first moved on the substrate 20 μm or more in one direction and then contacted the edge of the other cell. By "contact" we mean contact of the images of these two cells in the frame.

A necessary condition for registration of collision was that at least one of the contacting edges has to be active and translocating before making the contact. The edge of the second cell participating in the collision could be active or nonactive. Accordingly, two variants of collisions were distinguished:

(A) Collision of the translocating active edge of one cell with the nonactive edge of the other cell. These collisions will be designated as "head-side collisions."

(B) Collision of translocating active edge of one cell with the active (translocating or nontranslocating) edge of the other cell. These collisions will be designated as "head-on collisions."

Three types of results of collisions were distinguished:

(i) Halt. All collisions that did not result in overlapping or underlapping were regarded as halts. During the head-side collisions of this type, forward movement of an active edge stopped after this edge made contact with another cell. Head-on collisions classified as halts were of two varieties: (a) only one active edge had been translocating before the contact and stopped translocation after the contact; (b) both edges were translocating before the contact; after the contact they both stopped movement in the direction that produced collision.

The halt was usually accompanied by cessation of ruffling and of the formation of lamellipodia ("contact paralysis," see ref. 1). However, the cessation of forward locomotion rather than cessation of ruffling was used as the basis for the diagnosis of halt because classification based on this sign was easier.

**TABLE 1. Results of cell–cell collisions in isolated cultures of normal mouse-embryo fibroblast-like cells**

<table>
<thead>
<tr>
<th>Result of collision</th>
<th>Type of collision</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Halt</td>
<td>Head-side</td>
<td>23</td>
</tr>
<tr>
<td>Underlapping</td>
<td>Head-on</td>
<td>12</td>
</tr>
<tr>
<td>Overlapping</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>37</td>
</tr>
</tbody>
</table>

* All figures in this and other tables show absolute numbers of collisions observed in films.
† In one case, it was not clear whether overlapping or underlapping occurred; this collision was not included in the table.

**TABLE 2. Results of cell–cell collisions in isolated cultures of mouse-embryo fibroblast-like cells transformed by mouse sarcoma virus**

<table>
<thead>
<tr>
<th>Result of collision</th>
<th>Type of collision</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Halt</td>
<td>Head-side</td>
<td>7</td>
</tr>
<tr>
<td>Underlapping</td>
<td>Head-on</td>
<td>12</td>
</tr>
<tr>
<td>Overlapping</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>21</td>
</tr>
</tbody>
</table>

**TABLE 3. Results of cell–cell collisions in isolated cultures of neoplastic mouse fibroblasts of the CIM strain**

<table>
<thead>
<tr>
<th>Result of collision</th>
<th>Type of collision</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Halt</td>
<td>Head-side</td>
<td>5</td>
</tr>
<tr>
<td>Underlapping</td>
<td>Head-on</td>
<td>9</td>
</tr>
<tr>
<td>Overlapping</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>15</td>
</tr>
</tbody>
</table>
Cessation of forward locomotion of the active edge often, but not always, was accompanied by retraction of this edge. We did not try to subdivide the halts into smaller groups on the basis of the degree of retraction. Even if this subdivision could have been done reliably, it was not necessary for the purpose of this work.

(ii) Underlapping. After contact, the translocating edge continued its forward movement upon the substrate so that eventually this edge was under the lower surface of the second cell. The movement of the underlapping edge under the other cell was not seen clearly, especially in head-side collisions. Therefore, it was often impossible to determine the exact distance traveled by this edge. We classified as underlappings all the cases of collisions in which most of the translocating active edge disappeared under the other cell.

Head-on collisions resulting in underlappings were rare. In these cases one active edge stopped or remained nontranslocating while the second active edge moved under the first one.

(iii) Overlapping. After contact, the translocating active edge continued its forward movement not upon the substrate but over the surface of another cell. The collision was classified as overlapping if the active edge traveled not less than 6-7 μm over the surface of the other cell. If the active edge retracted before it passed this minimal distance over the surface of the other cell, collision was classified as halt. In head-on collisions classified as overlappings, one active edge stopped or remained nontranslocating while the second edge moved over the surface of the other cell. We did not see head-on collisions in which both active edges would continue their forward movement after the contact, so that simultaneously the first edge would overlap the second one, while the second edge would underlap the first one.

Classification of the results of collisions described above was based on analysis of the translocation of the active edge during the first hour after the moment when two cells contacted each other. Alteration of the direction of or the rate of translocation at later time intervals was not taken into account, when classification was made. In certain cases, the direction of translocation of the active edge was changed during the first hour after the contact. Usually, these were the collisions in which underlapping or significant (that is, exceeding 6-7 μm) overlapping was then followed by retraction. These collisions were classified, respectively, as underlappings or overlappings but never as halts.

RESULTS

The results of collisions observed in various types of cultures are summarized in the Tables 1–4. The relative frequencies of various results of collisions were similar in all cultures. Overlappings were relatively rare: they were seen in less than 10% of all the collisions. Overlapping observed in all cultures were usually partial, that is, the movement of the active edge over the surface of the other cell was followed by the retraction of this edge. Halt was the most common result of head-on collisions in all types of cultures. In cultures of CIM cells and of MSV-transformed cells, these halts were more often accompanied by the pronounced retraction of active edges than in cultures of normal cells. Underlapping was often observed after head-side collisions in all types of cultures. Underlapping in normal cultures was usually partial: an active edge disappeared under the surface of the other cell, but later, either this edge or the body of the upper cell retracted. In transformed cultures, one could often see complete underlapping of one cell by another: the whole cell passed unimpeded under the central part of the body of the other elongated cell.

DISCUSSION

The behavior of CIM cells and of MSV-transformed cells in cultures is typical for transformed fibroblasts: considerable degree of multilayering and criss-crossing can be seen even in the sparse cultures; eventually, these cells form multilayered cultures with high saturation density. Nevertheless, analysis of cell–cell collisions in the isolated cultures of these cells did not reveal any changes, suggesting the loss of contact inhibition of movement: the relative frequency of halts was not decreased and that of overlappings was not increased in these cultures, as compared with those of normal fibroblasts. Analysis of the collisions between normal cells and neoplastic CIM fibroblasts also revealed efficient contact inhibition of movement. Effective contact inhibition was earlier observed in cultures of neoplastic mouse fibroblasts of the L strain (5). Recently, the absence of overlappings after collisions of 3T3 cells transformed by simian virus 40 had been briefly described (6). Thus, microcinematographic analysis of cell–cell collisions had demonstrated efficient contact inhibition of movement in cultures of several types of transformed fibroblasts. Certain other neoplastic lines may have diminished contact inhibition (7).

However, it is obvious that transformed fibroblasts can form morphologically abnormal cultures without aquired the increased ability to move over the surfaces of other cells after collision, that is, without the loss of contact inhibition of movement. The nature of cellular changes responsible for morphological alteration of cultures is not clear. Deficient attachment to the substrate may be very important (5, 8, 9).

Abnormal attachment to the substrate may alter the cell shape and possibly modify certain characteristics of collisions, such as the degree of underlapping, frequency, and the degree of retraction. Another contributing factor may be the decreased ability of transformed fibroblasts to form stable cell–cell adhesions (10). Further studies are needed to find out which of these cellular changes, if any, is essential for the development of cultures with altered morphology.