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Contact stimulation of cell migration

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Summary

Mass migrations of dense cell populations occur periodically during embryonic development. It is known that extracellular matrices, through which the cells migrate, facilitate locomotion. However, this does not explain how cells, such as neural crest, can migrate as a dense cohort of cells in essentially continuous contact with one another. We report here that unique behavioral characteristics of the migrating cells may contribute to cohesive migration. We used time-lapse video microscopy to analyze the migration of quail neural crest cells and of two crest derivatives, human melanoma cells and melanocytes. These cells migrated poorly, if at all, when isolated, but could be stimulated up to 200-fold to travel following contact with migrating cells. This phenomenon, which we have termed "contact-stimulated migration," appeared to activate and sustain migration of the mass of cells. Cells that became dissociated from the others ceased directional migration, thereby limiting aberrant cell dispersion. Fibroblasts were minimally responsive to this novel phenomenon, which may be crucial for major, mass cell migrations.

Key words: cell migration, neural crest, melanoma.

Introduction

During embryogenesis there are many instances of mass cell migrations in which cells travel in dense cohorts. Probably, the most extensively studied of these is the exodus of neural crest cells from the region of the dorsal neural tube. Until now, "contact inhibition of locomotion" has been the most plausible explanation for directional migration by cohorts of neural crest cells (Abercrombie, 1970; Abercrombie and Heaysman, 1953, 1954; Heaysman, 1978; Erickson, 1985, 1988; Newgreen and Erickson, 1986). According to the concept of contact inhibition of locomotion, when two cells meet while migrating toward one another, they are inhibited from continuing forward locomotion if it would cause one cell to pass over the other (Abercrombie, 1970; Abercrombie and Heaysman, 1953, 1954; Heaysman, 1978). However, as noted by Abercrombie and others, this model does not adequately explain the driving force behind the mass migration of a population of actively motile cells (Abercrombie, 1970).

In addition to contact inhibition of locomotion, other events of cell-cell contact alter the behavior of migrating cells. Curtis found that independently migrating slime mold amoebae, upon contacting a passing stream of cells, would bind to the trailing end of one of the migrating cells, and fall into line following the cell to which it had attached (Curtis, 1967). Middleton discovered that embryonic chicken pigmented retinal epithelial (PRE) cells spread and migrated poorly as single cells, frequently altering their course and making little progress in any one direction. When, however, they contacted other PRE cells, complex cell-cell adhesions formed, the cells polarized and the peripheral cells formed broad lamellapodia. These outer cells seemed to be attempting to migrate (Middleton, 1976, 1977; Middleton and Pegrum, 1976). This response may be similar to epithelial wound healing, in which cells at the edges of the wound migrate inward to close it.

Cell-cell contacts can have diverse effects on migrating cells. We examined the effects of cell-cell contacts on migrating and non-migrating primary neural crest cells, as well as neural-crest-derived melanoma cells and melanocytes. When migrating cells contacted non-migrating cells, the migrating cells underwent classic contact-inhibition of locomotion. The non-migratory cell was also affected by the encounter. Following a quiescent period of 1-4 h after initial contact, the non-migrating cell also began to translocate. As noted by others, isolated cells were poor migrators, but our key observation is that contact with migrating cells can induce isolated cells to resume motility. It is possible that the aberrant dispersion of migrating cells is prevented by this behavior, in which isolated cells cease persistant migration and migrating cells in contact with one another continue effecient progression.

Materials and methods

Cell culture

Primary *Coturnix coturnix japonica* neural crest cells were isolated from 36-h fertile eggs (Truslow Farms, Inc., Chestertown, MD, 21520, USA). Embryos were dissected in serum-free Dulbecco's modified Eagle's medium (4.5 g/l glucose), and neural tubes from approximately somite 5-to-16 were transferred to fibronectin-coated (10 μg/ml human plasma fibronectin) culture dishes containing the same medium, supplemented with 10% heat inactivated

fetal calf serum plus 2 mM glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin (Duband and Thiery, 1987). For some experiments neural crest cells were allowed to migrate out on the dish overnight before the neural tubes were removed. These cells remained in the dishes until migration had stopped and were then harvested with trypsin and replated as scattered cells in another dish. At other times, intact neural tubes were added to experimental dishes and neural crest cells were recorded as they migrated outward in cohorts, sometimes encountering previously-plated scattered neural crest cells.

Sk-Mel-24 human melanoma cells (a gift from Dr. Anthony Albino, Memorial Sloan-Kettering Cancer Center, New York, NY 10002, USA) were maintained in the same supplemented Dulbecco's modified Eagle's medium as the primary neural crest cells. Primary human foreskin melanocytes were purchased and grown in serum-free defined Melanocyte Growth Medium (MGM), then harvested for experiments with trypsin as recommended by the supplier (Clonetics Corp., San Diego, CA 92123, USA).

All cells were maintained at 37°C with 7.4% CO_2. During time-lapse recordings, cells were in normal growth medium kept at 37°C and supplemented with CO_2.

Cell migration measurements

Migration rates for scattered cells (1.5×10^3 cells/ml) were determined using cells suspended with 0.25% trypsin and plated at low cell density in 35 mm plastic Petri dishes (Falcon, Becton Dickinson Labware, Lincoln Park, NJ 07035, USA) coated with 10 μg/ml human plasma fibronectin. For pellet outgrowth assays, cells (3.5×10^4 cells/pellet) were centrifuged for 10 min (1000 revs/min in a Beckman TJ-6 centrifuge with an H-4 rotor; Beckman Instruments, Inc., Fullerton, CA 92634, USA) in V-well, 96-well plates (Linbro/Titertek, Flow Laboratories, Inc., McLean, VA 22102, USA) blocked with 1% BSA. After centrifugation the resultant pellets were incubated in a tissue-culture incubator for 2 h before transfer to fibronectin-coated Petri dishes. Migration was recorded at 1 frame/6 min with an inverted microscope (Model ICM 405, Carl Zeiss, Oberkochen, Germany) and video camera (Newvicon Model C2400, Hamamatsu Photonics, Hamamatsu City, Japan). Images were computer enhanced with Image 1 software (Universal Imaging Corp., West Chester, PA 19380, USA) and stored on optical discs (Panasonic Model TQ 2028F, Matsushita Electric Corp., Secaucus, NJ 07094, USA) for later analysis.

Video recordings of individual cells and the leading edge of pellet outgrowths were traced onto acetate sheets in direct contact with the video monitor. For scattered cells, all cells which remained in the field of view for four hours were traced. The tracings were digitized with SigmaScan software (Jandel Scientific, Corte Madera, CA 94925, USA) to obtain migration rates per unit of time. Tracings of a stage micrometer were made with the same microscope and magnification, recording equipment and monitor to standardize the scanning software to the scale of experimental cell tracings. The rates of individual cells in a single experiment were averaged and expressed as μm/h. Tracings of neural tube and cell pellet outgrowths were measured at 12 mm intervals, perpendicular to the leading front. The outgrowth measurements for 4 h intervals were averaged and expressed as μm/h. Two-tailed Student *t*-tests were used to determine significant differences in migration rates. Because the nature of the method used to generate quantitative data can affect results, it is important to emphasize that the process of contact stimulation was strikingly apparent by visual observation to both naive and experienced observers of the time-lapse recordings.

Results

We first used quail (*Coturnix coturnix japonica*) neural crest cells migrating in vitro from explanted neural tubes to examine the question of how cells migrate while in contact with surrounding cells. It has been reported that when neural crest cells become isolated from a migrating cohort in vitro, they lose directionality and velocity (Newgreen et al., 1979). We confirmed this loss of locomotion in isolated cells. However, we discovered that *Coturnix* neural crest cells that cease migration can be induced to resume rapid locomotion by contact with other cells migrating from explanted neural tubes (Fig. 1). Neural crest cells harvested from outgrowths of explanted neural tubes were cultured as scattered cells in fibronectin-coated culture dishes. Single cells were essentially non-migratory, locomoting in no consistent direction at only 2.8 μm/h. However, when contacted by other neural crest cells migrating from explanted neural tubes, the quiescent cells were stimulated to migrate directionally at 26.6 μm/h (Fig. 1; Table 1). There was a lag of 1-2 hours between physical contact with a translocating cell and initiation of contact-stimulated migration.

We analyzed this phenomenon further using the neural-crest-derived human melanoma cell line SK-Mel-24. When SK-Mel-24 cells were dispersed in culture, they barely moved (1.7 μm/h), even when in limited contact with other cells through cell processes. However, they could be activated to migrate by high cell density: when concentrated by centrifugation and plated as pellets, the cells migrated out much like crest cells exiting a neural tube at 13.2 μm/h. As with primary neural crest cells, contact between migrating SK-Mel-24 cells and dispersed static cells induced active migration in the immobile cells (Fig. 2). Time-lapse recordings showed a requirement for cell-cell contact and adhesion. Cell-cell attachments were evidenced by the stretching of adherent cell processes and the snapping back of those processes as cells parted. Forty eight-hour conditioned medium from migrating cells could not stimulate migration by isolated cells (data not shown). To confirm that the migratory behavior of SK-Mel-24 cells was not

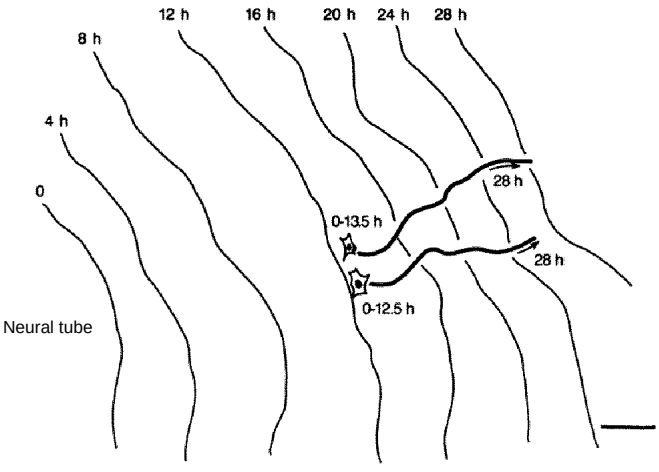

Fig. 1. Tracings of video images of *Coturnix* neural crest cells migrating from an explanted neural tube plated on fibronectin. The position of the leading edge of outgrowth is indicated at 4-h intervals. Also shown are the individual paths of initially static cells that were stimulated to migrate by contact with cells of the leading edge. Bar, 100 μm.

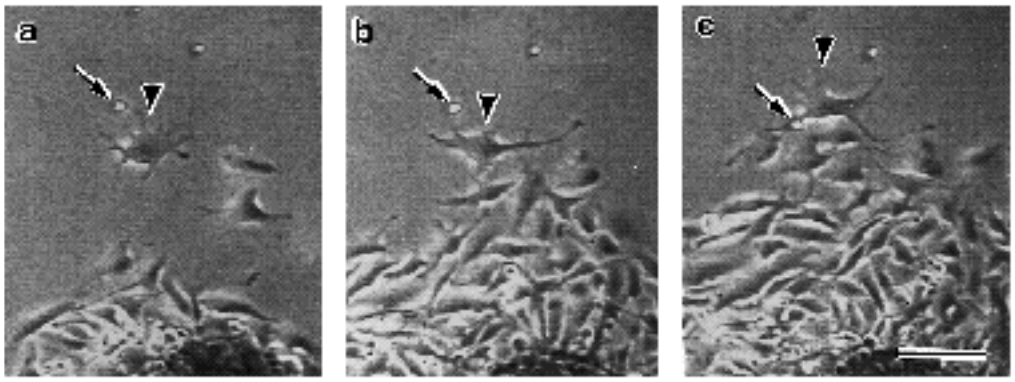

Fig. 2. SK-Mel-24 cells on fibronectin migrate from a pellet, contact a static cell and stimulate it to migrate. (a) Static cell (arrowhead) and leading edge of pellet outgrowth. Note the piece of cellular debris (arrow) as location markers. (b) Cells at the front contact the static cell. (c) The static cell begins to migrate and is now between the two location markers. Digitized tracings from the video recording indicated that the leading edge was migrating at 13.0 μm/h, and the contact-stimulated cell migrated at <0.04 μm/h prior to contact and at 11.4 μm/h after initiating migration. Bar, 100 μm.

Table 1. *Video recordings of migrating cells*

Cell type	Migration (μm/h)		Fold stimulation	*P* value
	Unstimulated	Stimulated		
Neural crest	2.8 ± 1.8 (*n*=36)	26.6 ± 2.8 (*n*=26)	9.5	0.0005
SK-Mel-24	1.7 ± 2.0 (*n*=27)	13.2 ± 4.6 (*n*=23)	7.8	0.0001
Melanocytes	⩽0.04 (*n*=9)	7.7 ± 3.6 (*n*=9)	200	0.0001
WI-38	13.0 ± 7.0 (*n*=23)	15.6 ± 6.8 (*n*=13)	1.2	0.2747
Dermal fibroblasts	9.4 ± 5.3 (*n*=26)	13.7 ± 4.1 (*n*=21)	1.5	0.0021

Video recordings of isolated unstimulated cells and of stimulated cells migrating from pellets or explanted neural tubes were traced and digitized to obtain migration rates. Results were compared with two-tailed Student's *t*-tests.

related to malignant transformation, we compared them with primary newborn human melanocytes. Scattered melanocytes were virtually non-migratory, even when in contact via cell processes. However when plated as high density pellets, the cells migrated outward and could accelerate the migratory speed of scattered cells at least 200-fold (Table 1).

SK-Mel-24 cells began stimulated migration after a lag of 2-4 hours. Contact-inhibition responses occurred in the migrating cell during the lag time before a stationary cell began to move. The migratory stimulator cell typically passed around the static cell and resumed its original direction of migration. When the stimulated cell began to move, it was incorporated into the cohort of migrating cells approaching from behind. When the lag period was briefer, the stimulated cell could begin migrating at the leading edge of the outgrowth. This process was much slower than the phenomenon of contact-induced blebbing described by Tickle and Trinkaus (1976) for *Fundulus* cells, which occurs in seconds.

Not all migratory cells travel in coherent masses. When most fibroblasts are cultured in vitro they migrate independently as single cells. We therefore examined the capacity of two human fibroblastic cell types to undergo contact stimulation (Table 1). The migration rate for scattered WI-38 embryonic lung fibroblasts was 13.0 μm/h, and merely increased to 15.6 μm/h following contact. Dermal foreskin fibroblasts migrated separately at 9.4 μm/h and their rate increased to only 13.7 μm/h after contact. In striking contrast to neural crest derivatives, therefore, contact stimulation does not appear to affect the migratory behavior of fibroblasts.

Discussion

Contacts between migrating cells can elicit varied responses. Chick heart fibroblasts alter their migratory behavior in response to cell-cell contacts (Abercrombie, 1961). Leading cells, migrating outward from tissue explants, are polarized in that they extend lamellapodia only along the free leading edge. This form of contact inhibition of locomotion constrains the formation of lamellapodia and is believed to give direction to the migratory path. When a chick heart fibroblast separates from the cohort of cells, it produces multiple lamellapodia, which seem to compete with each other for dominance. The result is that the cell makes frequent changes in direction and little migratory progress until it rejoins the outward migrating mass of cells and again becomes stably polarized. It was further found that while cells that had no contacts with other cells migrated more slowly than those with contacts, the number of contacts did not have significant effect on the rate of migration (Abercrombie and Heaysman, 1966).

Some transformed cells do not display contact inhibition upon collision with other cells, and even display what has been called a reversed contact-inhibition response (Curtis, 1967). In heterologous collisions between normal chick heart fibroblasts and fibrosarcoma cells, only the fibroblasts showed contact inhibition. In contrast, after collisions the fibrosarcoma cells travelled further forward than they would have if the collision had not occurred (contact progression). They also showed a decreased tendency to change direction and even tended to turn toward marginal contact sites (Paddock and Dunn, 1986). Experiments are underway to

determine if contact-stimulated migration can occur between different cell types.

Chicken pigmented retinal epithelial (RPE) cells attached poorly and were minimally motile as isolated cells. However, when they contacted one another they too became polarized and extended lamellapodia along the free edge. Complex cell adhesions formed between cells of these multiple-cell islands and the peripheral cells appeared to be trying to migrate (Middleton, 1976, 1977). In these experiments neither the scattered cells nor the island cells exhibited persistant translocation. The only manner in which scattered RPE cells contacted cell islands was via the very limited movements of nearby isolated cells or via the gradual expansion of cell islands. Therefore, while Middleton's experiments demonstrated contact-stimulated cell spreading, they did not show stimulated migration. In our experiments small groups of spread cells also failed to migrate, until contacted by migrating cells. However, both SK-Mel-24 cells migrating from pellets and neural crest cells exiting neural tubes exhibited persistant migration, as did contact-stimulated cells. Thus, to turn on contact-stimulated migration in initially non-migratory cell masses it may be necessary for cells to have more extensive cell-cell contacts than exist in spread-cell islands, which have only two-dimensional peripheral contacts. However, isolated cells can be induced to migrate by substantial cell-body contact with only a single migrating cell on a two-dimensional substratum.

Earlier studies have demonstrated that cell-cell contact can alter the migratory behavior of already-translocating cells and that contact can also induce spreading in isolated, minimally motile cells. In this study we demonstrate that contact between migrating and non-migrating cells can induce de novo migration. Our findings suggest that while "contact inhibition of locomotion" may influence directionality and provide a mechanism with which cells can bypass obstacles, it is the novel complementary phenomenon of "contact-stimulated migration" that can initially switch on and maintain the capacity of cells to travel as dense populations. We suggest that under the combined influences of contact-stimulated migration and contact-inhibition of locomotion, cells that have ceased migration can be stimulated to translocate while cells that remain with the cohort translocate swiftly and in a persistent direction.

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