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Letter to the Editor

THE MOTILE BEHAVIOR OF HUMAN BREAST CANCER CELLS CHARACTERIZED BY TIME-LAPSE VIDEOMICROSCOPY

Dear Editor:

In the development of more effective therapeutic strategies for the prevention and treatment of various cancers, it is important to focus on biological characteristics that differentiate benign and malignant neoplasms. A key difference between benign and malignant tumors appears to be related to cellular motility and invasiveness. Since cancer cell movement is an important component of metastatic potential, various *in vitro* assays have been developed and used to study general cell movement, including chemoinvasion of coated membranes and phagokinetic tracks (1,8). To better define the relationship between cell motility and metastatic potential, the present study used time-lapse videomicroscopy and image analysis to compare and characterize the differences in motile behavior between two human breast cancer cell lines that have been used in numerous *in vitro* studies. These were MDA-MB-231 (MDA) cells, which display a high metastatic potential and MCF-7 cells, which have a low metastatic potential. Similarly, other investigators have used time-lapse videomicroscopy to quantify the motility of other cancer cells (4,6).

MCF-7 human breast cancer cells were provided by Dr. Sam Brooks of the Michigan Cancer Foundation (Detroit, MI), MDA-MB-231 cells were obtained from ATCC (Rockville, MD) and the cells were grown as monolayer cultures in RPMI 1640 media as previously described (3). Cells were plated sparsely in T-25 flasks and their motility was recorded with phase-contrast optics at 400× with a color video camera (Sony, Model CCD-IRIS/RGB). The microscope stage was maintained at a constant temperature (37° C) by a thermostat-controlled stage heater (Fryer, Model A-50-IN). The video signal was fed to a time-lapse videocassette recorder with a time compression ratio of 240:1 (Panasonic, Model AG-6730 S-VHS, Secaucus, NJ). Phase-contrast images of each cell line were recorded for 9–10 h in 3–5 replicate experiments. In each recording the movement of 5–10 cells in a microscopic field was analyzed. At least 30 cells from each cell line were analyzed in this study.

The video images, at different time intervals (10–60 min) over a period of 4–8 h, were captured and digitized with video capture software (Apple Video Player, Videocapture) on a Macintosh computer (Quadra 630-AV). Computer-assisted image analysis was accomplished with the NIH Image 1.59 program. Cell contours of the digital images were manually outlined and their x,y coordinates, area, and perimeter were calculated with the NIH Image program.

Parameters of cell motility and morphology that were calculated for each cell line included rate of movement, total path length, and perimeter index. Rate of cell movement was determined as the distance traveled by the centroid (geometric center) of individual cells divided by time (μm/h) and plotted at the midpoint between each time interval. Total path length was computed as the total path traveled by the cell centroid (in μm) during the experimental period. Perimeter index (PI) was calculated as the perimeter of the cell divided by the perimeter of a perfect circle of equal area. The greater the PI, the less round the cell shape; that is, more elongated or with more cell membrane extensions. Because PI is an index, it can be used to compare cells of different sizes.

Both cell lines, MDA and MCF-7, displayed evidence of cellular motility during time-lapse filming under the culture conditions of RPMI medium supplemented with calf serum but with no additional exogenous growth factors or mitogens. When the videorecordings were viewed at normal playback speed, this motile behavior was clearly visible as undulating, ruffling movements of the cell surface and by actual translocation of the cells. MCF-7 cells typically displayed ruffling activity over most or all of the cell perimeter, whereas MDA cells tended to be more polarized with one or a few distinct areas of the cell perimeter undergoing active ruffling.

Both cell lines displayed active translocation during the recording period (Fig. 1). The rate of movement of MCF-7 cells remained relatively constant at approximately 5 μm/h, whereas the rate of movement of MDA cells varied considerably between 5 and 20 μm/h. This variable movement observed with MDA cells appears to be a common characteristic of motile call behavior, in which there are periods of reduced movement, during which protrusion and withdrawal of lamellipodia occur, followed by bursts of motile activity resulting in cellular translocation. This uneven movement of the MDA cells could be observed readily in the videorecordings. At 2 h into the observation period, the mean cumulative path length of the MDA cell was significantly greater than that of the MCF-7 cells ($P < 0.05$), and this difference in total path length between the cell lines increased with time (Fig. 2). At the end of the 9-h recording period, the MCF-

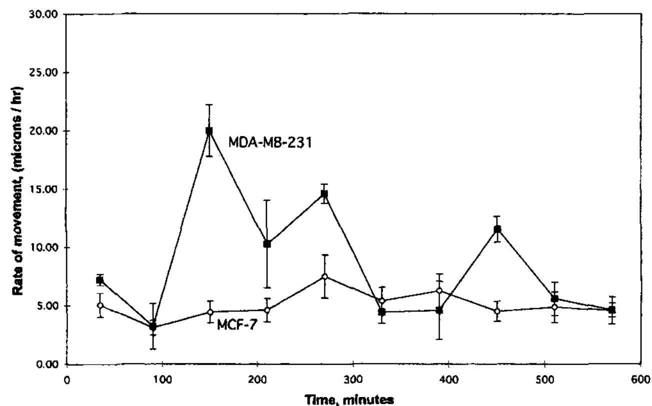

FIG. 1. The rate of cell movement of MCF-7 and MDA-MB-231 cells. *Each point* represents the mean rate of movement of 10 to 15 cells from 2 or 3 recordings ± SEM.

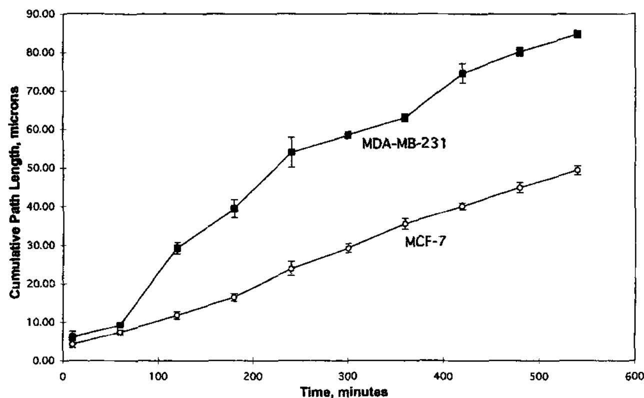

FIG. 2. The cumulative mean path length of MCF-7 and MDA-MB-231 cells. *Each point* represents the mean cumulative path length of 10 to 15 cells from 2 or 3 recordings ± SEM.

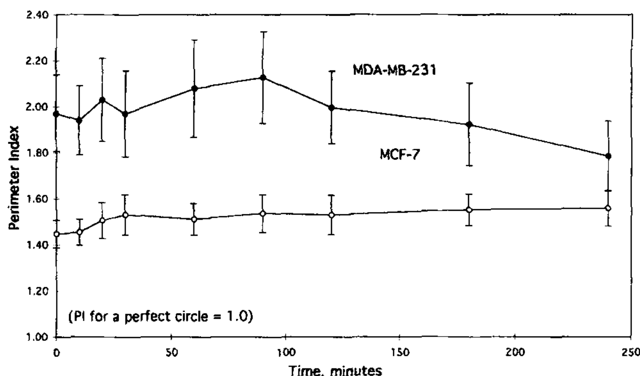

FIG. 3. The perimeter index (PI) of MCF-7 and MDA-MB-231 cells. *Each point* represents the mean PI of 10 to 15 cells from 2 or 3 recordings ± SEM.

7 cells had moved a mean distance of 49.45 μm or 5.4 μm/h while the MDA cell moved 84.79 μm or 9.4 μm/h ($P < 0.01$).

Cell morphology of MDA and MCF-7 cells was quantified by the perimeter index (PI) (Fig. 3). MDA cells had a nearly 50% greater deviation from a circle than MCF-7 cells. These differences were significant for all time points ($P < 0.05$) except for 180 and 240 min. The results quantify the visual observation that MDA cells are generally more elongated than the MCF-7 cells, which tend to be more round or cuboidal. Most analyses of cell morphology have been subjective and descriptive; the use of the perimeter index in this study represents an attempt to express morphology in a quantitative manner. Although the PI is an unambiguous and measurable parameter of cell morphology, it should be noted that a variety of different cell shapes could produce a similar perimeter index. In a separate study, Thurston et al. (9) measured changes in the morphology of 3T3 fibroblasts by measuring cellular "circularity" which was defined as ($\text{perimeter}^2/4 \times \pi \times \text{area}$) and is similar to the perimeter index of the present study. It has been suggested that the process of malignant transformation of epithelial-derived cells involves a change to a more mesenchymal phenotype and morphology (2). This concept seems to be supported by the observation, in the present study, of a more elongated, fibroblastic morphology and greater PI of the invasive MDA cells as compared to the more cuboidal MCF-7 cells with a much lower PI. Similarly, Partin et al. (6) reported that pseudopodial extension showed good correlation with metastatic potential in various sublines of rat prostatic adenocarcinoma.

It is clear that, in addition to motility, there are other important steps in the metastatic process such as tumor initiation, promotion, and progression, protease release, angiogenesis, tumor cell arrest, proliferation, colony formation, and interaction with host defense mechanisms which are not taken into account with the experimental model used in the present study. However, cancer cell motility is a crucial component of the metastatic cascade, enabling neoplastic cells to move through tissues, gain access to the lymphatics and vasculature, and eventually invade and colonize sites distant from the primary tumors (5).

A major advantage of videomicroscopy is that it provides direct observation of cell movement and morphology at every time period during the experiment. The video images can be captured, digitized, enhanced when necessary, and analyzed on the computer. Further, software is available to accurately measure and analyze the kinetic activity of each cell in the microscopic field. Computer analysis can be used to examine directional movement and rate of movement and compare these parameters to cell morphology or changes in morphology. Finally, the videorecording provides an inexpensive and permanent record of the activity of each cell within the microscopic field. The data are readily retrievable for review, modification, or additional analysis.

In summary, the results of the present study demonstrated that MDA cells are inherently more motile than MCF-7 cells *in vitro* and indicate that the time-lapse videomicroscopy method can be used to study parameters of cancer cell motility. These results are supported by previous work that established the invasive and metastatic potential of MDA cells *in vitro* with the Matrigel invasion model (7). Time-lapse videomicroscopy has been used to examine the influence of various factors such as retinoic acid and interleukin-1 on fast plasma membrane movements (10,11) and will be used to study estrogen, antiestrogen and growth factor effects on breast cancer motility and morphology. The results of these studies could provide information that may be useful in predicting the effectiveness of drug and hormonal therapy in the treatment of metastatic breast cancer.

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