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*Advances in Brief*

# Migration of Coordinated Cell Clusters in Mesenchymal and Epithelial Cancer Explants *in Vitro*[1]

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## Abstract

**The invasion and migration occurring in primary neoplastic tissue explants were studied by using a three-dimensional collagen matrix model, subsequent time-lapse videomicroscopy, and computer-assisted cell tracking. We show that not only single cells but groups of clustered cells comprising 5 to more than 100 cells detach from the primary tumor lesion and migrate within the adjacent extracellular matrix. These clusters were highly polarized, resulting in a high directional persistence of migration. Locomoting cell clusters were observed in primary cultures from invasive oral squamous cell carcinomas (6 of 9), ductal breast carcinomas (2 of 3), and rhabdomyosarcoma (1 of 1), whereas normal oral mucosa (0 of 4) was cell cluster negative. Thus, locomoting cell clusters could be a novel and potentially important mechanism of cancer cell invasion and metastasis.**

## Introduction

On tissue invasion, tumor cells acquire the capacity to detach from the primary tumor site and penetrate the adjacent tissue (1–4). The current model of cancer cell invasion and motility strongly focuses on individual cells that lose homophilic cell-cell contacts and migrate from the primary tumor to invade adjacent tissue (5–7). In embryonic morphogenesis, the motility of entire cell clusters, besides single locomoting cells, are considered as an important motility mechanism in organ development (8). In histopathological tumor sections, a substantial proportion of invading cancer cells is present in solid tumor masses scattered within the tissue matrix (9). During the metastatic process *in vivo*, clumps of tumor cells can be detected in lymphatic vessels or in the blood stream (10, 11). These and other *in vitro* and *in vivo* observations of scattered cell clumps in cancer (6, 12, 13) have raised the question whether groups or sheets of cancer cells might be able to dissociate from the primary site and migrate within a 3-D[3] tissue environment as coherent aggregates (8, 12, 13).

In the present study, the mechanisms underlying cancer cell invasion were studied by using primary human tumor cultures established within a 3-D collagen matrix model. Time-lapse videorecording and computer-assisted cell tracking methods were used to provide quantitative data analysis of locomotor parameters.

## Materials and Methods

**Tumor Specimens.** Primary tumor specimens were obtained from the Department of Surgery, Montreal General Hospital and the Department of Otolaryngology, Jewish General Hospital (both in Montreal, Canada). Frozen sections and the final pathological diagnosis from paraffin sections were performed in the Departments of Pathology at Montreal General Hospital and at the Jewish General Hospital.

**Incorporation of Primary Tumor Samples into 3-D Collagen Lattices.** Freshly isolated tissue samples were incorporated within 3-D collagen lattices containing 1.5 mg/ml purified native type I dermal bovine collagen (99.9% pure collagen; Vitrogen 100; Collagen Corp., Palo Alto, CA) in Eagle's MEM (Flow) and 5% fetal bovine serum adjusted to pH 7.4, as was described previously for lymphocytes (14, 15). The malignant area of the tumor was detected by frozen section, and an immediately adjacent piece (3–20 $mm^3$) was obtained for culture. Each tumor sample was cut into 20–30 pieces (maximal dimensions, 1 × 1 × 1 mm) and added to 100 μl of the liquid collagen solution in 96-well microtiter plates. This suspension was allowed to polymerize (37°C for 20–30 min; 5% $CO_2$). The depth of the resulting collagen lattices was 3–4 mm.

**Cell Cultivation in Liquid Culture.** Simultaneously, 3–10 tumor pieces were long term cultivated in RPMI supplemented with 2 mM L-glutamine, 10% heat-inactivated FCS (Boehringer Mannheim, Mannheim, Germany), 50 units/ml penicillin (GIBCO, Eggenstein-Leopoldshafen, Germany) and 50 μg/ml streptomycin (GIBCO).

**Time-Lapse Videomicroscopy and Cell Tracking.** After polymerization of the lattices, the interphase from the tissue sample to the adjacent collagen matrix was monitored by bright-field time-lapse videomicroscopy. Each of the 20–30 independent cultures was monitored every 2–3 days over a time period of 3–8 weeks. The magnification was ×64. In control experiments, tumor samples cultivated in liquid culture medium were monitored. The following growth and locomotor characteristics were assessed and quantified: single locomoting cells were characterized as large nonlymphoid and nonmonocytic cells (>20 μm in diameter) with irregular cell shape locomoting within the collagen matrix after detachment from the tissue sample. Outgrowths were defined as organized tissue protrusions into the adjacent collagen matrix without loss of connection to the primary tissue sample. Locomoting cell clusters were defined as detached clustered cells (>3 cells coherent as a group) migrating within the collagen lattice. For the discrimination of passively scattered nonmobile tissue material, the locomotion of detached cell clusters was confirmed by time-lapse videomicroscopy and subsequent cell tracking. The malignant phenotype was confirmed by light microscopy after fixation of the lattices in 10% formalin and staining with hematoxilin and eosin. Cell clusters were examined for atypical cytological features consistent with malignancy, such as nuclear hyperchromasia, prominent and/or multiple nucleoli, and a high nucleus: cytoplasmic ratio.

For cell tracking, the paths of individual cells or locomoting cell clusters were digitized from the video screen as *X/Y* coordinates by using a digitizing tablet (SummaSketch II; Summagraphics, Seymour, CT) and analyzed, as was, described previously (14). The sample period was 3–24 h; the time interval from step to step was 60 or 120 s.

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[1] Supported by Fritz-Bender-Foundation, Munich, Germany. Because the dynamics of the behavior of locomotiong cell clusters described in this manuscript are insufficiently documented by static images alone, essential key sequences of the source tapes have been accepted by Spektrum der Wissenschaft, the German edition of Scientific American, for publication as video documentation (ISBN 3–86025–801-X). An English version of this video production is in preparation.

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[3] The abbreviation used is: 3-D, three-dimensional.

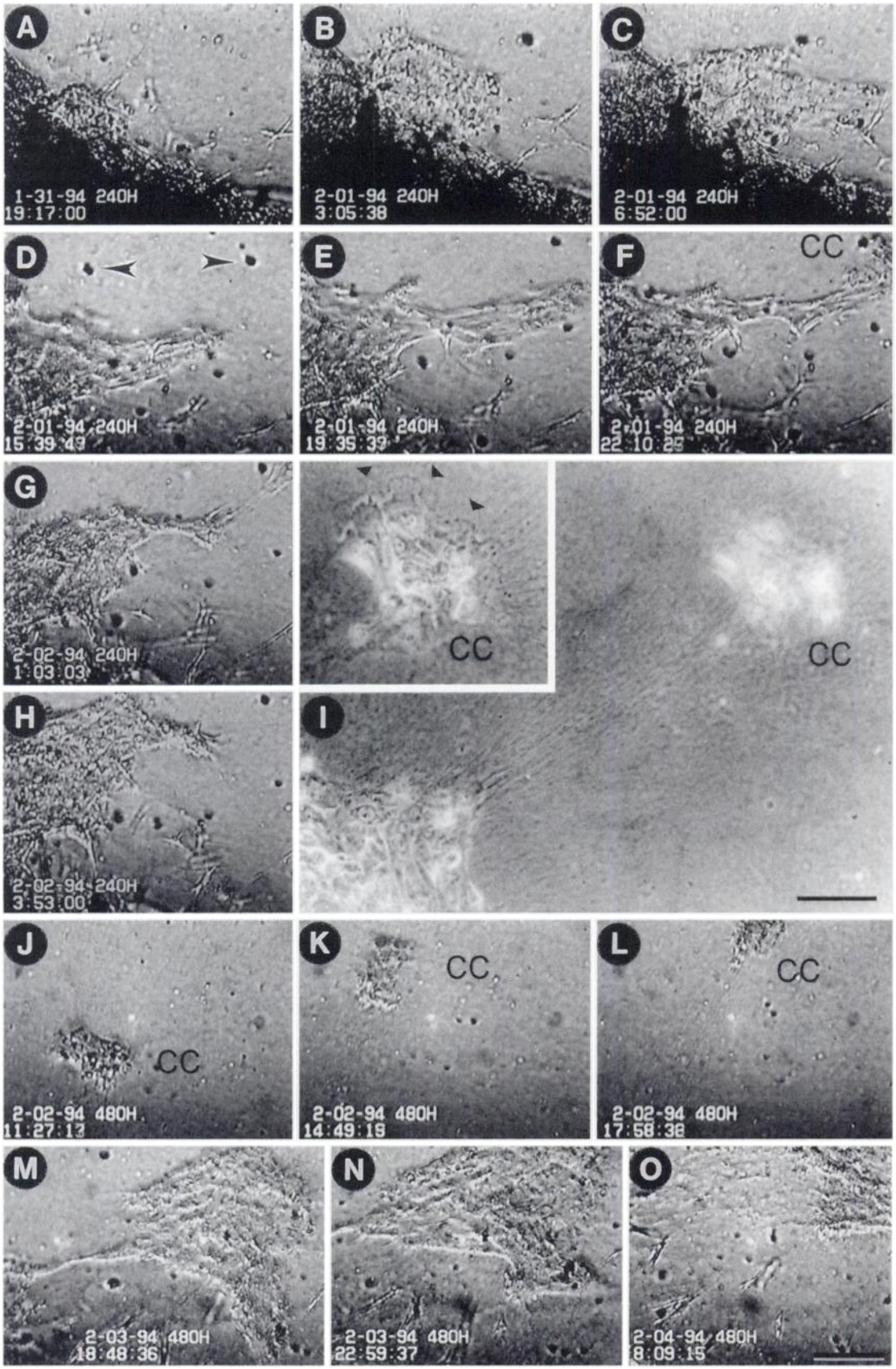


Fig. 1. Growth and local invasion followed by detachment and locomotion of clustered cells in a rhabdomyosarcoma sample. *In vitro* growth (*A-E*) and local invasion of the surrounding 3-D collagen matrix were obtained after 3 days of culture. A small cluster of cells (*CC*; 15–30 cells) detached from the tumor (*F-H*) and migrated within the collagen matrix (*I-L*). The cell cluster shows the formation of filopodia at the leading edge (*I*, arrowheads inset), resulting in coordinated migration of the whole group (*J-L*). The subsequent detachment and migration of a larger cell cluster (>100 cells) is depicted in *M-O*. *I* (inset), locomoting cell cluster migrating at a different level of depth in the 3-D collagen lattice. *D*, single locomoting cells (*arrowheads*). The images were digitized from videorecordings except (*I*), which is a phase contrast image, and represent a field of 600 × 430 μm, the bar representing 120 μm (except *I*, bar = 60 μm). Very similar results were obtained from 4 of 6 independent primary cultures from the same tumor.

Table 1 *Locomotor characteristics of locomoting cell clusters as compared to single locomoting cells from primary rhabdomyosarcoma and squamous cell carcinoma specimens cultivated in a 3-D collagen matrix*[a]

| | Rhabdomyosarcoma | | | Squamous cell carcinoma | | |
|---|---|---|---|---|---|---|
| | Single cells ($n$ = 15) | Cluster ($n$ = 1) | Cluster ($n$ = 1) | Single cells ($n$ = 9) | Cluster ($n$ = 1) | Cluster ($n$ = 1) |
| No. of clustered cells | | 15–30 | >100 | | 20 | 30–50 |
| Observation period (h) | 6.3 | 6.7 | 17 | 3.8 | 7.5 | 7.1 |
| Speed (μm/h) | 193 ± 79 | 66 ± 8 | 32 ± 10 | 160 ± 48 | 78 ± 14 | 70 ± 12 |
| Time migrated (min/h) | 56 ± 3 | 57 ± 0.5 | 50 ± 2 | 49 ± 6 | 55 ± 3 | 54 ± 3 |
| Persistence | 0.08 ± 0.04 | 0.66 ± 0.03 | 0.73 ± 0.06 | 0.17 ± 0.07 | 0.8 ± 0.1 | 0.74 ± 0.11 |

[a] The data were obtained from individually selected explant cultures obtained within a series of 4–30 parallel explants from a given specimen. The locomotor parameters were assessed from the time-lapse videorecordings by using computer-assisted cell tracking, as was described previously (14). The paths were obtained as X and Y coordinates from randomly selected single cells or cells within clusters. The longest possible time interval for cell tracking was defined by the time frame individual or clustered cells migrated within the microscopic field; therefore, the observation period of individually digitized paths varied from 3 to 24 h and is indicated as the average observation period for each sample. The number of cells and clusters investigated is indicated as $n$. For locomoting cell clusters, the data were derived for one individual cluster of each size range as the X and Y coordinates of 5–6 individual cells at different positions within the cluster for a step interval of 2 min; the step interval of single cells was 1 min. The time migrated was calculated as the cumulative amount of steps a cell or a cluster moved at least 1 μm/min. The persistence (directionality) was calculated as the distance migrated from the starting to the end point divided by the length of the total path (1.0 for migration in a straight line, 0.0 for no directionality). For the rhabdomyosarcoma, the smaller cell cluster refers to Fig. 1, *D–L*, the larger cluster to Fig. 1, *M–O*. The clusters from squamous cell carcinomas are derived from 2 different specimens. The data represent mean values ± SD for each group of cells.

## Results

The sequential detachment and migration of the ubiquitous fibroblast, followed by single tumor cells and locomoting cell clusters, were recorded and analyzed for a mesenchymal tumor and epithelial carcinomas of different origins.

**Locomoting Cell Clusters in a Primary Rhabdomyosarcoma.** On cultivation in a 3-D collagen lattice, a primary rhabdomyosarcoma explant developed local invasion after 3 days of culture (Fig. 1, *A-E*). The invasion of the collagen matrix was rapidly followed by the detachment of a small cell cluster (Fig. 1, *F-H*) that migrated within the collagen matrix (Fig. 1, *J-L*). This cell cluster comprised approximately 15–30 cells and measured 70–100 μm in the *X-Y* plane (diameter; Fig. 1*I*, labeled "CC"). Subsequently, the detachment and migration of a large sheet-like cell cluster (200–400 μm in diameter, 100 or more cells) was documented (Fig. 1, *M-O*). No cell divisions were observed within these locomoting clusters.

On migration within the collagen, these clusters maintained an asymetric yet organized morphology. The dynamic formation of ruffling filopodia occurred exclusively at the leading edge of the clusters (Fig. 1*I*, triangles/CC inset). This high degree of polarization was concordant with a persistent directional migration (Fig. 1, *J-L*; Table 1) at speeds ranging from approximately 15–70 μm/h (Table 1), whereas single locomoting tumor cells from the same lesion exhibited random migration (Fig. 1*D*, arrows; Table 1). Thus, the principal motility mechanisms described previously for ameboid migration of polarized individual cells (16) may also apply to more complex yet polarized multicellular locomoting clusters.

**Frequency of Locomoting Cell Clusters in Epithelial Cancer.** Of the 9 squamous cell carcinomas from the oral cavity and 3 ductal carcinomas of the breast, more than one-half of the tumor samples exhibited locomoting cell clusters after 4–12 days of cultivation in collagen lattices (Table 2). The morphology (Fig. 2) and the locomotor characteristics, including speed and directionality (Table 1), of locomoting cell clusters obtained from squamous cell (Fig. 2) and mammary carcinoma explants closely resembled the features described for the rhabdomyosarcoma (Fig. 1). Control cultures of nonneoplastic oral mucosal tissue did not develop locomoting cell clusters (Table 2), suggesting that the collagen matrix invasion model may confine the development of outgrowth and locomoting cell clusters to neoplastic propensities.

Furthermore, in specimens that did produce invasion zones, only 21 ± 13% (± SD) of the individually established explant cultures exhibited outgrowths or locomoting cell clusters (total range, 1 of 27 to 9 of 20), which may be an indicator of significant biological heterogeneity within tumors. Focal degradation of the collagen matrix was seen as areas of matrix lysis in only 3 of 12 tissue samples and was not correlated with the location or the onset of emerging locomoting cell clusters. In no case did locomoting cell clusters result from individual detached and dividing cells. Most interestingly, however, none of the control cultures in liquid culture on two-dimensional plastic substrata established from each specimen in parallel experiments developed the described outgrowth and motility characteristics. Thus, the development of locomoting cell clusters in malignant tumor explants of both mesenchymal and epithelial origin was dependent for expression on the presence of a 3-D extracellular matrix network.

Table 2 *Frequency of single locomoting cells, outgrowths, and locomoting cell clusters in primary epithelial cancer samples and control tissue cultivated in 3-D collagen lattices*

| | Single locomoting cells[a] | Outgrowths | Locomoting cell clusters |
|---|---|---|---|
| Squamous cell carcinoma | 8/9 | 6/9 | 6/9 |
| Ductal carcinoma of breast | 2/3 | 1/3 | 2/3 |
| Normal oral mucosal tissue | 3/4 | 0/4 | 0/4 |

[a] Tumor specimens exhibiting at least 1 positive of 20–30 independent cultures on long-term cultivation/total number of specimens. For each specimen, 20–30 independent cultures were established and monitored every 2–3 days over a time period of 3–8 weeks by videomicroscopy.

## Discussion

These observations support the concept that malignant tissues can develop patterns in cell arrangement and function occurring in embryological morphogenesis (8). The capacity of clustered cancer cells to migrate through the extracellular matrix in a highly coordinated manner might supplement the current concept of primarily individual cells involved in invasion and metastasis, *i.e.*, the concept that individual cells must lose homophilic cell-cell interactions before they can detach from a primary tumor. In contrast, the detachment and locomotion of clustered cells would essentially require a substantial proportion of homophilic cell-cell interactions.

In the past, 3-D collagen matrices have been frequently used for cell motility studies (5, 13–15). In this study using primary tumor samples, the collagen matrix explant model lacking an equivalent of a basement membrane may appropriately represent the cell biology of mesenchymal cancer that initiates within stromal tissues. In a more limited way, the collagen matrix model may reflect the natural history of epithelial cancer after the transition from *in situ* to invasive carcinoma (2). From this stage on, the basement membrane is often focally defective or completely absent, thereby, enabling the carcinoma to directly interact with the adjacent submucosal tissue matrix (2).

It remains to be determined to what extent the locomotion of cell clusters follows biological and biochemical mechanisms established for single cells (1, 2, 16). Locomoting cell clusters may combine a variety of motility mechanisms, including membrane ruffling at the

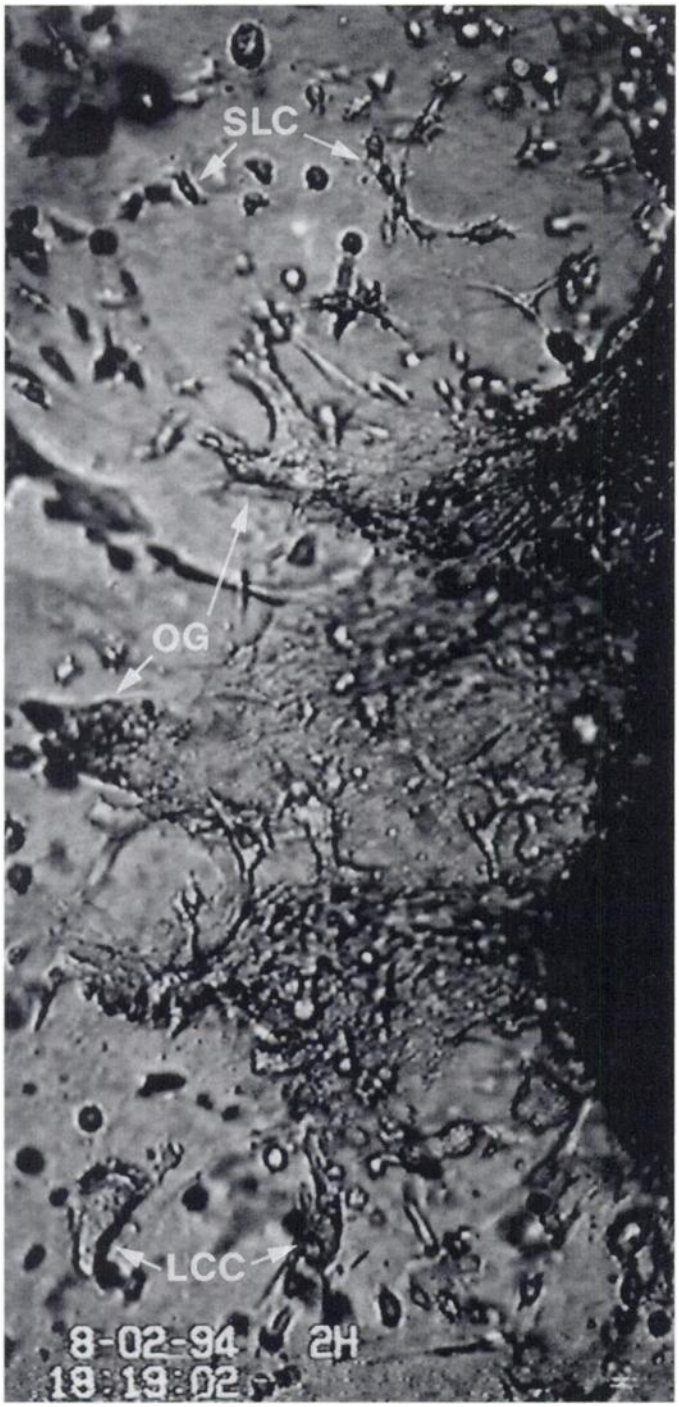


Fig. 2. Outgrowth behavior, single-cell detachment, and locomoting cell clusters of invasive squamous cell carcinoma. The development of detaching single locomoting cells (*SLC*), outgrowths (*OG*), and locomoting cell clusters (*LCC*) was obtained from a primary squamous cell carcinoma of the mouth after 9 days of culture within a 3-D collagen lattice. Single cells may locomote as individual cells, doublets or form chain-like mobile aggregates (*SLC*). The image depicts an area of 600 × 1300 μm.

leading edge (7), adhesion and deadhesion via integrins (15, 17–19), and homophilic adhesion providing the coherence and integrity of the group. Furthermore, contact inhibition of locomotion (8) may maintain a high degree of polarity in locomoting cell clusters contributing to the directional peristence of motility despite the absence of a chemotactic gradient in the collagen lattice.

Although the development of locomoting cell clusters in 3-D collagen matrices potentially adds another dimension to our understanding of the cell biology in invasion and metastasis, it remains to be established how this model relates to *in vivo* reality. However, it is reasonable to speculate that the dissemination of cell clusters may represent an efficient mechanism of cancer spread in the tissue, relative resistance to immunological assault, and cancer cell survival in the circulation (4, 10, 20, 21).

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