Metastatic Potential Prediction by a Visual Grading System of Cell Motility: Prospective Validation in the Dunning R-3327 Prostatic Adenocarcinoma Model

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ABSTRACT

A method for accurate prediction of prognosis in individual patients with prostatic carcinoma does not exist. The limitations of pathological grading systems may result from the failure of standard pathological examination of fixed dead tissue to accurately assess the biological and metastatic behavior of live tumor cells. Many of the sublines of the Dunning R-3327 rat prostatic adenocarcinoma are histologically similar yet differ in metastatic potential. Cells from the Dunning model were grown in culture and filmed by time-lapse videomicroscopy. These cells exhibited characteristic membrane ruffling, pseudopodal extension, and cellular translation that could be graded with 80% reproducibility. Individual cells from sublines with high metastatic potential were separated from cells from sublines of low metastatic potential in 96% of cases. We have applied our cell motility grading system to prospectively classify the metastatic potential of neoplastic cells. The mean motility grades of sublines of high and low metastatic potential differed significantly (Mann-Whitney-Wilcoxon, P < 0.0005). Among seven sublines in which the grading system was developed, individual cells were correctly classified as high or low metastatic in 71% of cases by ruffling or pseudopodal extension, 73% of cases by translation, and 75% of cases by motility index, an average of the three parameters of motility. Among four newly tested sublines, cells from a low metastatic and high metastatic subline were perfectly classified. Cells from two other low metastatic sublines were misclassified. When all 88 cells from the 11 sublines were classified, high metastatic cells were detected with 94% sensitivity and 50% specificity. The predictive value of a determination of low metastatic was 93%, whereas the predictive value of an assignment of high metastatic was 52%. The ability to detect and accurately classify most highly metastatic cells were detected with 94% sensitivity and 50% specificity. The predictive value of a determination of low metastatic was 93%, whereas the predictive value of an assignment of high metastatic was 52%. The ability to detect and accurately classify most highly metastatic cells were detected with 94% sensitivity and 50% specificity. The predictive value of a determination of low metastatic was 93%, whereas the predictive value of an assignment of high metastatic was 52%.

INTRODUCTION

In the United States, prostatic carcinoma is the second leading cause of death from cancer in males. Although autopsy studies have revealed prostatic carcinoma in over 30% of men over 50 yr of age (1), only 26,000 American men die of prostatic carcinoma annually (2). The outcome of men with prostatic carcinoma is largely dependent upon the aggressiveness of their disease, outcome is usually determined by the development of metastatic disease. We evaluated a grading system of cancer cell motility for assessment of the metastatic potential of prostatic cancer in an animal model that provided a series of tumors with a wide range of metastatic potential.

At present many grading systems are used to assess the metastatic potential of prostatic carcinoma. Whether these grading systems describe architectural (3), cytological (4), or some combination of both features (5-8), they share the shortcoming of relying upon the examination of dead tissue to predict the biological behavior of a living dynamic tumor system. At present no grading system has utilized living cancer cells that may possess biological properties that are related to the aggressiveness of neoplasms. Cancer cell motility is an obvious example of a property that would be lost with fixation but may prove important as a predictor of patient prognosis.

Cancer cell motility was suggested in 1863 by Rudolph Virchow (9) but was first documented by George Gey with time-lapse cinematography in 1931 (10). Sumner Wood demonstrated that cancer cell motility occurred in vivo (11). Donald Merchant was the first to recognize the motility of human prostatic carcinoma in primary culture. Hosaka and coworkers demonstrated differences in cell motility between various rat hepatoma cell lines that exhibited different propensities for metastasis (12). Haemmerli and Strauli studied the motility of human cells from six squamous carcinoma cell lines and suggested that their motility in vitro reflected their invasive behavior in vivo (13, 14). Time-lapse videomicroscopy has replaced the more cumbersome time-lapse cinematography and many less precise methods for studying cell motility (15-19). Manger and Heckman used time-lapse videomicroscopy to quantify translation by mean angular change of direction in addition to mean cellular velocity (20). Verschueren and Van Larebeke (21) developed a method to measure cellular translation as well as changes in cell contour. Fulton directed attention to three subtypes of cell motion (22). She described swimming due to flagella and cilia, ameboid motion and fibroblastic movement by pseudopodal extension, and bleb formation and ruffling. These studies more precisely described cellular motility and suggested that cancer cell motility may be related to metastatic potential. However, no quantitative system has been used to grade cellular motility and predict metastatic potential, and once developed, any grading system of cancer cell motility must be validated prospectively. We developed a grading system and characterized the in vitro motility of living cancer cells from the Dunning R3327 rat prostatic adenocarcinoma captured by time-lapse videomicroscopy (23). Membrane ruffling, pseudopodal extension, and cellular translation (Fig. 1) described cancer cell motility with reproducibility superior to current histological grading systems. Characteristic cell motility patterns permitted identification of five histologically similar Dunning R-3327 prostatic adenocarcinoma sublines. The grading system of cell motility was then applied to the separation of 7 sublines of the Dunning system by metastatic potential (24). Although no previous biological, biochemical, or morphological test accurately described the metastatic potential of the Dunning sublines (25), we distinguished the sublines and individual cells of high and low metastatic potential. Mean motility grades of...
ruffling, pseudopodal extension, or translation distinguished 3 high metastatic sublines from 4 low metastatic sublines. The combination of translation and pseudopodal extension identified correctly 27 of 28 cells as high or low metastatic potential (24).

The motility grades which separated retrospectively cells of high and low metastatic potential have been used to predict metastatic potential. Cells from 7 sublines in which the grading system was developed were evaluated blindly and prospectively. The motility grading system was tested in 4 sublines that have not been studied previously.

MATERIALS AND METHODS

Eleven sublines with a wide range of metastatic potential from the Dunning R-3327 tumor system of serially transplantable rat prostatic adenocarcinoma were studied. All sublines grew when inoculated into inbred Copenhagen rats (Harlan Sprague Dawley, Indianapolis, IN). The characteristics of 3 high metastatic (AT-3, MAT-Lu, MAT-LyLu) and 5 low metastatic (G, H, H1-F, AT-1, AT-2) sublines are well established and were summarized in Table 1 (25-28). The anaplastic Chilhood Adenocarcinoma (CUB) subline was described in 1983 (29, 30), and was provided by Jun Shimazaki and colleagues of Chiba University, Chiba, Japan. The poorly differentiated PIF-1 and anaplastic PAT-2 were developed at Johns Hopkins from tumors originally obtained from Norman H. Altman of the Papanicolaou Cancer Institute, Miami, FL. For these 2 sublines, the in vivo tumor volume-doubling time and androgen sensitivity were determined as described previously (31). The metastatic ability of the various sublines was determined as described previously (31). This entailed inoculation of at least 10 intact male Copenhagen rats/group s.c. with 1 x 10^6 viable cells from each of the Dunning sublines. All rats were followed until death. Complete autopsies were performed on each rat to determine the number and sites of distant metastases in each rat.

Except for the H tumor, all sublines were maintained as continuous in vitro cell lines as described previously (25). H tumor was harvested from a tumor-bearing animal. The tissue was minced and then digested to single cell suspensions by serial treatment with 0.5% collagenase and 0.1% trypsin solutions. Eight time-lapse videomicroscopic films of isolated cells from each of the 11 sublines were made under similar conditions with a standard technique. Approximately 10^6 cells were plated in T-25 plastic tissue culture flasks (Falcon, Oxnard, CA) and equilibrated at 37°C in 3 ml of Roswell Park Memorial Institute 1640 medium with 2 mM L-glutamine (MA Bioproducts, Walkersville, MD) which contained 10% fetal calf serum (Hyclone Labs, Inc., Logan, UT), 250 nm dexamethasone (Sigma Chemical, St. Louis, MO), 100 units/ml of potassium penicillin G, and 100 units/ml of streptomycin sulfate under an atmosphere of 5% CO2-95% air. Twelve to 24 h later cells were viewed with an inverted microscope (Zeiss IM35, Thornwood, NY) fitted with Hoffman optics. A programmable control box (Red Lion Controls, York, PA) coordinated illumination of the field for manual maintenance of focus and image capture. Images magnified 400 times were recorded every 15 s by a high-resolution black and white video camera (Model MTI 66; Dage, Michigan City, IN) and time-lapse videorecorder (TL AG-6050; Panasonic, Secaucus, NJ) on one-half in vertical helical scan. The 720 frames were played at 24 frames per s which produced a 30-s film at 360 times normal speed.

Eighty-six films of single cells were graded by two observers who had no knowledge of the identity of the specimens. Membrane ruffling, pseudopodal extension, and cellular translation (Fig. 1) were graded from 0 (no motility observed) to 5 (excess motility) by each observer. The grades of both observers were summed to yield final scores from 0 to 10 for each motility parameter. The three motility grades were summed and divided by three to yield a motility index for each cell. In addition, motility parameter grades were averaged to determine whether any two types of motility provided information superior to single grades or the motility index.

We prospectively compared the motility grades of 32 cells from high metastatic sublines and 56 cells from low metastatic sublines. For each group, motility grades were described by mean ± SE and compared by Mann-Whitney-Wilcoxon analysis for the probability that motility grade could distinguish metastatic potential. Next, we determined prospectively whether the analysis of only 8 cells from each of 11 sublines could predict the metastatic potential of the sublines. From each subline the motility grades of 8 cells were averaged to yield subline motility grades. The grades from each subline were compared to previously reported motility grades which distinguished cells of high and low metastatic potential to classify the metastatic potential of these 11 sublines. Finally we prospectively tested the accuracy of this motility grading system to analyze a single cell from a subline to predict the metastatic potential of that subline. The number of individual cells correctly identified as high or low metastatic was tested for statistical difference from chance assignment by x^2 analysis. The sensitivity (true positive divided by true positive + false negative), specificity (true negative divided by true negative + false positive), and predictive values of positive (true positive divided by true positive + false positive) and negative (true negative divided by true negative + false negative) tests were determined for prediction of metastatic potential. All analyses were performed for 56 cells from the 7 sublines in which the grading system was developed as well as 32 cells from 4 sublines which had not been previously evaluated.

### Table 1 Biological characteristics of eleven sublines of the Dunning R3327 rat prostatic adenocarcinoma

Eleven sublines of the Dunning R3327 rat prostatic adenocarcinoma differed in histological differentiation, growth rate, androgen sensitivity, and metastatic potential. The histological differentiation was interpreted from histological sections of in vivo tumors from the 11 sublines. The in vivo tumor doubling time, androgen sensitivity, and metastatic potential were determined as described previously (31).

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Histology</th>
<th>Growth rate (days)*</th>
<th>Androgen sensitivity</th>
<th>Metastatic potential*</th>
<th>Site of metastases</th>
<th>Host survival (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low metastatic potential</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AT-3 tumor</td>
<td>Poorly differentiated</td>
<td>4.0 ± 0.2*</td>
<td>Yes</td>
<td>Low (50)*</td>
<td>Lungs</td>
<td>75 ± 6</td>
</tr>
<tr>
<td>H tumor</td>
<td>Well differentiated</td>
<td>21 ± 6</td>
<td>Yes</td>
<td>Low (50)</td>
<td>Lungs</td>
<td>365 ± 21</td>
</tr>
<tr>
<td>H1-F tumor</td>
<td>Poorly differentiated</td>
<td>4.8 ± 1.8</td>
<td>No</td>
<td>Low (25)</td>
<td>Lungs</td>
<td>120 ± 5</td>
</tr>
<tr>
<td>PIF-1 tumor</td>
<td>Moderately differentiated</td>
<td>3.7 ± 1.1</td>
<td>No</td>
<td>Low (10)</td>
<td>Lungs</td>
<td>87 ± 8</td>
</tr>
<tr>
<td>CUB tumor</td>
<td>Anaplastic</td>
<td>3.4 ± 0.6</td>
<td>No</td>
<td>Low (10)</td>
<td>Lungs</td>
<td>67 ± 4</td>
</tr>
<tr>
<td>AT-1 tumor</td>
<td>Anaplastic</td>
<td>2.5 ± 0.2</td>
<td>No</td>
<td>Low (25)</td>
<td>Lungs</td>
<td>51 ± 7</td>
</tr>
<tr>
<td>AT-2 tumor</td>
<td>Anaplastic</td>
<td>2.5 ± 0.2</td>
<td>No</td>
<td>Low (50)</td>
<td>Lungs</td>
<td>63 ± 8</td>
</tr>
<tr>
<td>High metastatic potential</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAT-Lu tumor</td>
<td>Anaplastic</td>
<td>2.7 ± 0.3</td>
<td>No</td>
<td>High (50)</td>
<td>Lungs [50-350]*</td>
<td>45 ± 3</td>
</tr>
<tr>
<td>MAT-LyLu tumor</td>
<td>Anaplastic</td>
<td>1.7 ± 0.3</td>
<td>No</td>
<td>High (50)</td>
<td>Lymph nodes and lung [25-250]</td>
<td>39 ± 5</td>
</tr>
<tr>
<td>AT-3 tumor</td>
<td>Anaplastic</td>
<td>1.8 ± 0.2</td>
<td>No</td>
<td>High (50)</td>
<td>Lymph nodes and lung [20-200]</td>
<td>40 ± 7</td>
</tr>
<tr>
<td>PAT-2 tumor</td>
<td>Anaplastic</td>
<td>1.8 ± 0.2</td>
<td>No</td>
<td>High (10)</td>
<td>Lymph nodes and lung [40-300]</td>
<td>32 ± 5</td>
</tr>
</tbody>
</table>

* Tumor volume doubling time (days).

* Low metastatic potential, <10% of s.c.-inoculated rats develop distant metastases; high metastatic potential, >90% develop distant metastases.

* Mean ± SD.

* Numbers in parentheses, the number of animals/group upon which these data are based.

* Numbers in brackets, range in the number of lung metastases per rat.

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Fig. 1. Cell motility grading system. Ruffling and pseudopodal extension were demonstrated by differences in cell membrane at Time 1 (left) and Time 2 (right). Ruffling represented rapid rhythmic movement of short segments of cell membrane. Pseudopodial extension was nonrhythmic extension and retraction of short segments of cell contour over comparatively longer distances. Vectoral translation was graded displacement of cell centroid over time. (Reprinted by permission of the Journal of Urology from J. Urol., 138: 168-170, 1987.)

Table 2 Separation of cells of high and low metastatic potential by motility parameters

Thirty-two cells of high metastatic potential and 56 cells of low metastatic potential were filmed in vitro by time-lapse videomicroscopy. Ruffling, pseudopodal extension, and translation were graded from 0 to 10. The motility grades of high and low metastatic cells were compared.

<table>
<thead>
<tr>
<th>Motility parameters</th>
<th>Motility grade of 32 high metastatic cells</th>
<th>Motility grade of 56 low metastatic cells</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ruffling</td>
<td>8.90 ± 0.20</td>
<td>6.46 ± 0.36</td>
<td>0.00000029</td>
</tr>
<tr>
<td>Pseudopodal extension</td>
<td>7.12 ± 0.38</td>
<td>4.22 ± 0.40</td>
<td>0.000015</td>
</tr>
<tr>
<td>Translation</td>
<td>7.04 ± 0.44</td>
<td>3.90 ± 0.44</td>
<td>0.00047</td>
</tr>
<tr>
<td>Ruffling + pseudopodal extension</td>
<td>8.02 ± 0.24</td>
<td>5.34 ± 0.36</td>
<td>0.00000081</td>
</tr>
<tr>
<td>Ruffling + translation</td>
<td>7.96 ± 0.28</td>
<td>5.18 ± 0.38</td>
<td>0.000028</td>
</tr>
<tr>
<td>Translation + pseudopodal extension</td>
<td>7.08 ± 0.38</td>
<td>4.06 ± 0.42</td>
<td>0.000011</td>
</tr>
<tr>
<td>Motility index*</td>
<td>7.68 ± 0.30</td>
<td>4.86 ± 0.38</td>
<td>0.000024</td>
</tr>
</tbody>
</table>

* Probability of separation by Mann-Whitney-Wilcoxon analysis.  
* Mean ± SEM.  
* Motility index = (ruffling + pseudopodal extension + translation) divided by 3.

RESULTS

Eleven sublines of the Dunning R-3327 rat prostatic adenocarcinoma models with low and high metastatic potential were described in Table 1. The 88 individual cells of the 11 Dunning sublines were separated into two statistically distinct populations by single motility grades, paired motility grades, and motility indices (Table 2). The cells of high metastatic potential had higher mean motility grades than the cells of low metastatic potential. Regardless of the motility descriptor(s) examined, the two groups of cells were clearly different (Mann-Whitney-Wilcoxon, P = 0.000047 to 0.00000029).

A motility profile for each subline resulted when the motility grades for 8 cells from each subline were averaged. Ruffling, pseudopodal extension translation, and motility index varied widely between Dunning tumor sublines (Fig. 2). Among sublines of low metastatic potential, G, H, and AT-2 had low ruffling, pseudopodal extension, and translation grades which resulted in low motility indices. These three sublines were separated clearly from the high metastatic MAT-LyLu, AT-3, and PAT-2 sublines. Considerable overlap existed between the low metastatic PIF-1, CUB, and AT-1 and high metastatic MAT-Lu sublines. Within sublines, variation of motility grades averaged 33%. Intraassay reproducibility, (100 - % of coefficient variation) 67%, was similar to the 75% intraassay reproducibility determined previously for the 5 sublines G, AT-1, AT-2, AT-3, and MAT-LyLu (23).

Subline motility grades were compared to the previously established motility grades which distinguished sublines of high and low metastatic potential (24) to test this grading system prospectively. Whether single motility parameters, pairs of parameters, or the motility index was used, classification errors resulted (Figures 3 and 4). Among the 7 sublines in which the classification scheme was generated, ruffling misclassified AT-1 tumor as a high metastatic subline. Pseudopodal extension misclassified HI-F as high metastatic and MAT-Lu as low metastatic. Translation mislabeled 2 sublines of low metastatic potential, HI-F and AT-1, as high metastatic. Motility index also labeled HI-F and AT-1 as high metastatic. Pairs of motility grades did not improve classification accuracy. Four sublines which had not contributed to the generation of classification motility grades also varied widely in their motility profiles. The high metastatic PAT-2 subline and the low metastatic H subline were properly classified by ruffling, pseudopodal extension, translation, and motility index. All 4 scores misclassified the CUB and PIF-1 sublines which had low metastatic potential.
The metastatic potential predicted by a grading system of cell motility for 88 individual cells was compared to the actual metastatic potential of their subline of origin. The sensitivity, specificity, and predictive value of a positive and negative test were calculated for the motility parameters ruffling, pseudopodal extension, and translation as well as motility index.

<table>
<thead>
<tr>
<th>Predicted metastatic potential</th>
<th>Actual metastatic potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>High Low High Low High Low</td>
</tr>
<tr>
<td>Low</td>
<td>29 30 26 22 30 29 30 28</td>
</tr>
</tbody>
</table>

Sensitivity = (true positive) divided by (true positive + false negative).
Specificity = (true negative) divided by (true negative + false positive).
Predictive value + test = (true positive) divided by (true positive + false positive).
Predictive value - test = (true negative) divided by (true negative + false positive).

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The 88 cells were individually classified by comparison of each parameter, pairs of parameters, and motility index with previously established grades which distinguished cells of high and low metastatic potential (24) (Table 3). Fifty-six cells from 7 sublines in which the grading system was developed and 32 cells from 4 previously untested sublines.

<table>
<thead>
<tr>
<th>Motility parameters</th>
<th>Cells from 7 sublines</th>
<th>Cells from 4 previously untested sublines</th>
<th>All 88 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ruffling</td>
<td>40 (71)</td>
<td>15 (47)</td>
<td>55 (63)</td>
</tr>
<tr>
<td>Pseudopodal extension</td>
<td>40 (71)</td>
<td>10 (63)</td>
<td>60 (69)</td>
</tr>
<tr>
<td>Translation</td>
<td>41 (73)</td>
<td>16 (50)</td>
<td>57 (65)</td>
</tr>
<tr>
<td>Motility index</td>
<td>42 (75)</td>
<td>16 (50)</td>
<td>58 (66)</td>
</tr>
</tbody>
</table>

* Statistically different from random assignment by $\chi^2$ analysis ($P < 0.05$).
* Numbers in parentheses, percentage.

The 88 cells were individually classified by comparison of each parameter, pairs of parameters, and motility index with previously established grades which distinguished cells of high and low metastatic potential (24) (Table 3). Fifty-six cells from 7 sublines in which the grading system was developed were correctly classified with regard to metastatic potential with an accuracy of 75% by motility index, 73% by translation grade, and 71% by ruffling or pseudopodal extension grade. When 32 cells from 4 sublines which had not been previously studied were classified, accuracy did not differ from metastatic potential assignment by random chance ($\chi^2, P > 0.5$). When all 88 cells were considered, each parameter and motility index classified cells by metastatic potential with an accuracy statistically greater than by random assignment ($\chi^2, P < 0.05$) (Table 3). Pseudopodal extension was most accurate and classified 68% of 88 cells correctly. In each group of cells tested, when motility parameters were paired, no improvement in classification accuracy resulted.

When the high metastatic potential of a primary tumor was predicted by examination of the motility of a single cell, motility index was the best classifier (Table 4). Highly metastatic cells were detected with 94% sensitivity and 50% specificity. The predictive value of a positive test was 52%, and the predictive value of a negative test was 93%. Thus, if these cells were representative of primary tumors in Copenhagen rats that had not metastasized and were surgically excised, 94% of tumors of high metastatic potential would be identified and removed, whereas 48% of rats would have undergone unnecessary removal of the primary tumor (100 - 52% predictive value of a positive test). An error would have been made in 7% of rats who were spared operation by this test. The high metastatic potential of their tumors would not have been identified.

DISCUSSION

A spontaneous prostatic adenocarcinoma was found in 1961 in the dorsal prostate of the 3327th rat examined by Dr. W. F. Dunning (32). Since its discovery, the transplantable Dunning R-3327 rat prostatic adenocarcinoma line has spontaneously given rise to several distinct variants with differing histology, growth rates, androgen sensitivity, metastatic potential, and lethality (25-30). The usefulness of this model for human prostatic carcinoma has stimulated many investigators to search for a technique which distinguishes sublines of high and low metastatic potential. Failed biological discriminators include in vivo growth rate, androgen sensitivity, in vitro growth rate, culture saturation density, and androgen-dependent and -independent clonogenic potential (25). Lowe and Isaacs (28) derived an index from 3 of 9 measured hydrolytic enzymes which separated 3 sublines of high metastatic potential from 2 sublines of low metastatic potential. Brendler et al. (33, 34) applied this concept to discrimination of normal, benign, and malignant human prostatic tissue and prediction of hormonal responsiveness of prostatic carcinoma with some success. Failed morphological discriminators can be classified into ultrastructural, cytological, and architectural techniques. Electron microscopy of sublines in vivo and in vitro failed to distinguish the 2 groups of sublines. Chromosomal number and DNA content per cell were similar (25). Nuclear size and shape measured by flow cytometric forward and perpendicular laser light scatter identified characteristic indices for 4 Dunning sublines (35). Unfortunately, the index was heavily influenced by growth rate and...
thus failed to recognize metastatic potential independently. Lastly, high and low metastatic sublines cannot be distinguished by their appearance in culture or by their histological appearance in vivo (25).

We combined time-lapse videomicroscopy and a visual grading system to evaluate cell membrane ruffling, pseudopodal extension, and translation in normal and malignant rat prostate cells. The characteristic patterns of motility of normal and neoplastic cells from the Dunning sublines enabled us to identify these cells (23). Furthermore, the characteristic motility of cells of the Dunning tumor sublines enabled us to distinguish cells of high and low metastatic potential. For each motility parameter, a grade was established that most accurately separated cells of high and low metastatic potential (24). We applied this test for metastatic potential in a prospective and blinded fashion to cells from 11 sublines of the Dunning R-3327 prostate adenocarcinoma model. Among the 7 sublines in which the grading system test was developed, both HI-F and AT-1 were usually misclassified as high metastatic, whether subline grades or individual cell grades were considered. Four sublines not previously studied by time-lapse videomicroscopy had metastatic potential predicted. The H tumor which metastasizes rarely and the recently developed and highly metastatic PAT-2 tumor were classified with near-perfect accuracy. CUB and PIF-1 sublines have low metastatic potential and were misclassified by the visual grading system of cancer cell motility as high metastatic sublines. Overall, the metastatic potential of 68% of 88 individual cells was correctly classified by motility index which represented a significant improvement over assignment by chance alone. Although many cells of low metastatic potential were misclassified, this test for high metastatic potential rarely failed to detect a cell of high metastatic potential.

Motility may be required for metastasis but not sufficient. Although motility is required for movement from the primary tumor and through tissue and vascular walls, a cell or cluster of cells must also be able to exist independently of its neighbors, use hydrolytic enzymes to degrade ground substance and interrupt cell-cell contact, exist within the vascular space, and grow at the metastatic site in order to establish a metastasis. In the Dunning model, cells from the highly metastatic sublines MAT-Lu, MAT-LyLu, AT-3, and PAT-2 have extremely high motility. Both individual cell and subline mean motility grades were uniformly high, which permitted the recognition of 94% of highly metastatic cells by our grading system. Among sublines of low metastatic potential, H tumor, G tumor, and AT-1 have very low motility and are rarely misclassified as high metastatic cells or tumors. In contrast, HI-F and AT-2 have intermediate motility and are misclassified as highly metastatic in 50% of cases. CUB and PIF-1 are highly motile and are misclassified as high metastatic in almost all cases. The failure of correct classification could not be explained by heterogeneity of motility within sublines. Homogeneously motile cells lacked metastatic potential; most cells from the AT-2, HI-F, CUB, and PIF-1 tumors possess the capacity for metastasis presumed by the presence of motility. Because these cells metastasized in less than 10% of animals at the time of death from the primary tumor, these cells must have lacked other key characteristics necessary for metastasis.

Recognition of highly motile cancer cells harvested from the primary tumor would suggest presence of metastatic disease. If careful staging failed to reveal presence of metastases, either subclinical micrometastases are present or the cancer cells lack the other requirements for establishment of metastases. In the latter case, removal of the primary would be curative and administration of adjunctive therapy unnecessary. On the other hand, appreciation of micrometastases may enable curative adjunctive therapy. Isaacs (36) demonstrated in the Dunning prostatic carcinoma model that chemotherapeutic and hormonal therapies were much more effective at low tumor volumes. Unfortunately, the tumor volumes used in that animal study were well below the tumor volumes required for detection of metastases in human patients by current methods. In humans, if hormonal or chemotherapy is initiated after metastatic disease is detected by current methods, results are palliative at best without proven improved survival. Therefore, if adjunctive therapy with currently available agents is to have an effective role in prostatic carcinoma, metastatic disease must be detected or its presence predicted at microscopic tumor burdens.

If the absence of motility reliably predicts the inability of a tumor to metastasize, then removal of the primary is required only if death would occur as the result of primary tumor growth or later acquisition of metastatic capability. Unnecessary surgical intervention in patients with incidental prostatic carcinoma could be avoided. However, primary tumors of high metastatic potential must be removed and hence should not be mislabeled. In the Dunning model, when 8 cells were sampled to provide a subline motility grade, highly metastatic sublines were not misclassified. When subjected to a more rigorous analysis where metastatic potential was determined by the examination of a single cell, 6% of cells of high metastatic potential had motility grades insufficient for recognition of their high metastatic potential. Conversely, when a cell was classified as low metastatic, only 7% were from high metastatic sublines. The 24% variability in cancer cell motility grades within cells of the Dunning sublines suggested that assignment of metastatic potential by examination of a single cell from a subline was inadequate. When the Dunning tumor model is subjected to motility analysis by in vivo harvest of single cells, the need for analysis of many cells may increase. In human tumors which have not been subjected to the rigorous selection of maintenance for many years in vitro, the heterogeneity of motility within the primary tumor may be even greater. A more reproducible quantitative grading system of cell motility with a capacity to sample larger populations of cells in an automated fashion may be required to extend this grading system from the in vitro to the in vivo Dunning model and eventually to the human patient. However, we have developed a technique which permitted separation of the Dunning sublines by metastatic potential and tested it successfully in a prospective blinded fashion. Analysis of the motility of live cancer cells to predict the biological behavior of neoplasms is conceptually more attractive than examination of dead fixed histological sections. Grading systems based upon cancer cell motility may offer additional information beyond standard pathological grading systems. Cancer cell motility grading systems deserve further development and testing for the prediction of prognoses of cancer patients.

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REFERENCES


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