UNITED STATES PATENT

Morrison

[54] QUANTITATIVE METHOD OF MEASURING METASTATIC ACTIVITY

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[51] Int. Cl. 6 ............................... C12Q 1/68; G01N 33/74; G01N 33/48

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[58] Field of Search .......................... 435/7.23, 6, 15, 435/973, 968; 436/800, 805, 64

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ABSTRACT

The metastatic potential of tumors can be evaluated by the quantitative detection of urokinase and DNA. The cell sample selected for examination is analyzed for the presence of high levels of urokinase and abnormal DNA using analytical flow cytometry and digital image analysis. Other factors such as membrane associated urokinase, increased DNA synthesis rates and certain receptors can be used in the method for detection of potentially invasive tumors.

21 Claims, 2 Drawing Sheets
CS GLIOMA CELLS

DNA FLOURESCENCE (P1)

FIG. 1A

LOG uPA FLOURESCENCE

DNA FLOURESCENCE (P1)

FIG. 1B
FIG. 2A

HBR09 GLIOMA CELLS

DNA FLUORESCENCE (PI)

FIG. 2B

LOG 
JPA FLUORESCENCE (FITC)

DNA FLUORESCENCE (PI)
QUANTITATIVE METHOD OF MEASURING METASTATIC ACTIVITY

This application is a continuation of application Ser. No. 08/097,186, filed Jul. 27, 1993, now abandoned.

ORIGIN OF THE INVENTION

The invention described herein was made by an employee of the United States Government and may be manufactured and used by or for the Government of the United States of America for governmental purposes without the payment of any royalties thereof or therefor.

BACKGROUND OF THE INVENTION

Tumor cells of some cancers secrete enzymes that break down the intracellular matrix and invade adjacent tissues. The production of serine protease enzymes such as plasminogen activator enzymes have been observed in connection with the metastasis of tumor cells into healthy tissues. Plasminogen activator enzymes have been linked to cancer detection, using assays of enzymes extracted from the tumor cells (Camiolo, S. M., Markus, G. and Piver, M. S. “Plasminogen activator content of gynecological tumors and their metastases.” Gynecological Oncology 26: 364–373, 1987) or assays of supernatants from tissue culture of the tumor cells. (Hasui, Y. et al., “Comparative study of plasminogen activators in cancers and normal mucosa of human urinary bladder.” Cancer Research 49: 1067–1070, 1989. Harvey, S. R., et al., “Secretion of plasminogen activators by human colorectal and gastric tumor explants.” Clin. Exp. Metastasis 6: 431–450, 1988.)

Urokinase-type plasminogen activator (urokinase or uPA), a serine protease, is not produced in most normal cells, except at low levels in certain types of normal kidney cells, colon and gastric mucosa, and endothelial cells lining small arteries. Urokinase (uPA) can be present in the tissues in several molecular forms. The inactive proenzyme (scuPA) is composed of 411 amino acids. ScuPA is converted to the active enzyme by cleavage at Lys.158–Lys.139, Lys.158 and formation of the double chain, high molecular weight form (HMW-uPA) at Lys.135–Lys.136 giving a 33 KDa active enzyme.

The active urokinase enzymes convert plasminogen into plasmin, which in turn, dissolves intravascular fibrin blood clots and intracellular fibrin matrix components as well as activating collagenases, laminases, and other related enzymes which are important to the anchorage and growth regulation of cells. Urokinase is produced in a number of tumors such as lung, colon, gastric, uterine, breast, brain cancer and malignant melanoma. High levels of urokinase (>3.5 ng/mg of total protein) extracted from breast tumor tissues is an indicator for high risk of recurrence and shorter patient survival times. Janicke, et al. Fibrinolysis 4:69–78, 1990. Data from these clinical studies showing that a) measurements of uPA in plasma are of no value and that b) measurements of uPA in cytosol fractions give some prognostic value (0.12 benign vs. 1.65 ng/ml in metastatic breast cancer) and c) measurements of the uPA extracted from all of the tumor cells by Triton-X 100 treatment have even more significance (0.23 vs. 3.21 ng/mg of metastatic breast cancer). However, the standard deviation (S.D.) of these measurements represents ±65% for benign breast cancer and ±75% for metastatic tissues. Clearly, cell extractions cannot make the precise distinctions that are necessary as to the presence or absence of uPA, since the extractions are from tissues containing some normal cells, some cancer cells that may have no (or very little) uPA and those that may have significant amounts of uPA. (Schmidt, M. et al., “Tumour-associated fibrinolysis: the prognostic relevance of plasminogen activators uPA and tPA in human breast cancer.” Blood Coagulation and Fibrinolysis 1: 695–672, 1990). Also cells from primary lung and colon tumor produce more uPA than cells from metastatic tumors, but different methods of extraction and assays give widely variable results (Markus, G. et al., “Plasminogen activator secretion of human tumors in short-term organ culture, including a comparison of primary and metastatic colon tumors.” Cancer Research 43: 55–75–5525, 1983.).

It recently has been shown in cancer cells that the HMW active form of urokinase, bound to the tumor cell membrane, is responsible for the local lysis of the extracellular matrix, hence the tissue invasion mechanism for metastasis. (Hollas, W., Blasi, F., and Boyd, D. “Role of urokinase receptor in facilitating extracellular matrix invasion by cultured colon cancer.” Cancer Research 51: 3690–3695, 1991.) The unbound uPA and the LMW form is not responsible for local dissolution of extracellular matrix in the immediate vicinity of the metastatic tumor cell. (Cubellis, M. V., Wun, T. and Blasi, F. “Receptor-mediated internalization and degradation of urokinase is caused by its specific inhibitor PAI-1.” EMBO Journal 9: 1079–1085, 1990.) Thus the direct measurement of membrane-bound urokinase is more important to the prognostic accuracy than is measurement of all urokinase (membrane-bound and free uPA) present in cells or in the tissue specimen.

Total urokinase has been measured from tumor tissue and secretion by cultured explants. These are difficult to quantitate from biopsy to biopsy, especially if the measurements are made on a large group of cells. The data obtained is an average value of all normal and cancer cells, rather than a measurement of each individual cell. However, direct measurements of intracellular and extracellular urokinase have not been made previously.

A DNA content histogram of normal cells shows a single diploid peak (at G1 phase) and a tetraploid peak (at G2+M phase). In most tumors, abnormal DNA content of tumor cells is detected as a second G1 peak or multiple peaks. Abnormal DNA (DNA aneuploidy) is considered as an independent indicator of tumor aggressiveness and poor prognosis that is used to supplement cytopathology grading. Flow cytometry can be used to measure total DNA content as shown in U.S. Pat. No. 4,780,406, Doblere et al. entitled Flow Cytometric Measurement of Total DNA and Incorporated Halodeoxyuridine issued Oct. 25, 1988, which is incorporated herein by reference. Cells dissociated and prepared for flow cytometry can be analyzed for cell cycle stage as well as DNA content simultaneously. Flow cytometry measurement of the percentage of proliferating tumor synthesizing DNA (S-phase cells) is an independent indicator of malignancy. High percentages (15–20%) of S-phase tumor cells usually indicates an aggressive malignancy and usually correlates well with abnormally high DNA content. The labeling index (LI) obtained by pulse-labeling cells with DNA precursors represents the rate that DNA is being synthesized in tumor cells. Usually, a LI=4% is associated with a higher probability of recurrent malignancy. (Merkel, D. E. Dressler, L. G. and McGuire, W. L., “Flow cytometry, cellular DNA content and prognosis in human malignancy.” J. Clin. Oncology 5: 1690–1703, 1987.)

SUMMARY OF THE INVENTION

A method has been developed for quantitative detection of urokinase and correlating the urokinase levels to the DNA
content and additionally to the DNA synthesis rate for evaluation of metastatic potential of tumor cells. First, the cells to be examined and evaluated are selected. The cells can be obtained from known tumor cell lines cultured for research purposes, from tumor biopsies or cytological samples from patients or any other source of tissue to be examined for metastatic activity of tumor cells. The cell sample preparations are incubated with antibodies specific to urokinase in order to directly measure its presence in the isolated cells on a cell by cell basis. The sample of cells may be prepared in suspension for analysis by analytical cytometry techniques such as flow cytometry, digital image analysis or sectioned and prepared as histology slides for digital image analysis. The intranuclear DNA is also labeled or stained (using labels or staining including propidium iodide, Hoechst 33258 and others known to those skilled in the art) to allow quantitative measurements of DNA content. Viable cells also can be pulse-labeled with DNA precursors, BrdU, or IdU for a specific uptake period, then incubated with fluorescent-labeled antibodies against the precursor to quantitatively measure the DNA synthesis rates in those cells. Measurements of DNA and urokinase may also be made on the same cells. DNA and urokinase measurements, may be made essentially simultaneously if flow cytometry is used, or sequentially from the same source cell population.

The cells or histology sections are incubated with antibodies or anti-antibodies specific for urokinase. Antibodies or anti-antibodies to specific forms of urokinase may also be used. The antibodies can be monoclonal or polyclonal antibodies that bind specifically to urokinase. Further, intracellular urokinase is measured as well as extracellular urokinase which is outside or associated with the cell membrane outer surface. The antibodies specific to urokinase may be labeled with a fluorescent marker detectable by analytical cytometry techniques. Also DNA content and synthesis rate based on DNA stains or pulse-labeled uptake of DNA precursors is measured by image analysis and flow cytometry. The same cell sample can be measured for DNA content and urokinase by vital staining the DNA and labeling the urokinase with a marker so that both the DNA and urokinase can be measured simultaneously using two-color image analysis or flow cytometry.

The presence of urokinase, particularly the inactive proenzyme scuPA and the high molecular weight form II, is correlated to DNA content to determine if the profile of the urokinase to DNA ratio is within a range indicating metastatic activity. The cells are measured individually on a cell by cell basis so that the population examined can be ranked to determine whether a high percentage of the cells have high urokinase and abnormal DNA which indicates immediate concerns about the spread of the cancer from the primary tumor and therefore more aggressive post-operative treatments. No previous method has been developed to accurately measure the intracellular urokinase content, membrane-bound urokinase and cellular secretion levels and then correlate those urokinase levels with DNA content, DNA synthesis, and other markers of aggressive tumor growth to determine the metastatic potential in an individual patient.

**BRIEF DESCRIPTION OF THE DRAWINGS**

**FIG. 1A** is a graphic representation of DNA found in CS glioma cells, measured by fluorescent flow cytometry, which shows the DNA content relative to the stage of the cell cycle.

**FIG. 1B** is a two dimensional plot of the log fluorescence from urokinase (ordinate) vs. the log fluorescence from the DNA contained in the CS glioma cells, as measured by flow cytometry.

**FIG. 2A** is a graphic representation of DNA found in HBR09 glioma cells, measured by fluorescent flow cytometry, which shows the DNA content relative to the stage of the cell cycle.

**FIG. 2B** is a two dimensional plot of the log fluorescence from urokinase (ordinate) vs. the log fluorescence from the DNA contained in the HBR09 glioma cells, as measured by flow cytometry.

**DETAILED DESCRIPTION OF THE METHOD**

The method for analysis of the metastatic potential of cells can be used for diagnostic, research, or any other purpose. It is not the intent to limit the type of cell that can be isolated and evaluated nor to limit the type of histological tissue that can be labeled with the fluorescent anti-urokinase antibodies for measuring the urokinase in situ in the tissue.
The following is a general description of the method including the preferred method. It is not intended to disclose every mode to practice the invention, and substitutions and modifications to the steps described herein may be made without departing from the scope of the invention.

Antibodies can be formed against the two major molecular forms of uPA using established methods of isolating the HMW-uPA or LMW-uPA urokinase protein then immunizing animals to produce the antibodies. Typically, the uPA antigen is isolated from human urine or cell culture medium by absorption chromatography. Most commercial anti-uPA antibodies are murine antibodies made against human urokinase. The commercial antibodies (such as AD#394 and AD#3689 from American Diagnostic, Inc.) can detect the 54 kDa HMW-uPA or the 33 kDa LMW-uPA forms of uPA, but they cannot distinguish between the 54 kDa HMW-uPA active and proenzyme uPA molecular forms (also 54 kDa). The HMW-uPA is used by the tumor cells to rapidly invade adjacent tissue. Therefore, it is important to distinguish between HMW-uPA with its proenzyme scuPA and LMW-uPA. The commercial anti-uPA antibodies can inhibit the normal enzymatic activation of plasminogen to plasmin.

Presently, none of the commercial anti-uPA antibodies are specific for detection of the actual cleavage sites where activation of the proenzyme (scuPA) occurs. Plasmin converts scuPA to HMW-uPA. Measurements of scuPA may be performed by precubation of duplicate samples with and without plasmin and measuring the presence of HMW-uPA in both samples and quantifying the difference attributable to scuPA. Anti-uPA antibodies can be used as the primary antibody in sandwich assays or in permeabilized cytology preparations, wherein either the anti-uPA antibodies can be fluorescently labeled or a secondary, fluorescent labeled, antibody can be attached to the anti-uPA primary antibody; thus permitting fluorescence image analysis or laser activated flow cytometry analysis to determine the amount of the intracellular and membrane bound urokinase in each cell. Also the anti-uPA antibodies themselves can be conjugated with markers having detectable physical or chemical properties. The markers are well known in the field of immunohistochemistry. The preferred method utilizes markers with detectable light emissions by fluorescence, phosphorescence or luminescence, however, markers that absorb light, such as peroxidase, and radioactive labels can also be used. Primary and secondary detection of the antibodies may be useful in the invention with different types of cell or tissue samples.

The cells to be examined are first isolated. The cells may be from a tumor, fine-needle biopsy or cytological sample. The anti-uPA antibodies are incubated with the cells. In a preferred method for examination of intracellular and membrane-bound extracellular urokinase, the cells are labeled with fluorescent markers for digital image analysis. Using digital image analysis the anti-uPA antibodies can be located and quantitatively measured in both the cytoplasm and where the urokinase is bound to the cell membrane. These measurements are used statistically to give the relative distribution and absolute concentrations of membrane-bound and cytoplasmic urokinase in biopsy cells. This data can then be used to statistically compare the urokinase levels and distribution in those cells with tumor cells from other patients, thus giving a quantitative benchmark as to the metastatic state of those tumors in each individual patient. This data also can be used in retrospective studies where the time to reoccurrence, degree of metastasis and morbidity are correlated. DNA content, DNA synthesis rate or hormone receptors. For example, fluorescent antibodies to both the DNA or hormone receptors can be used with different
emission characteristics, thus the flow cytometer can simultaneously measure two or more color fluorescence of the two or more antibodies. Digital image capture also can be performed at multiple wavelengths thus enabling measurements of more than one intracellular protein or membrane receptor and DNA in the same tissue specimens.

Digital analysis measurement of the amount of urokinase, in the cytoplasm or membrane-bound, also can be used with the measurement of DNA content by flow cytometry. The identical cell population is not necessarily used for each analysis, however, since aliquot samples are used from the same cells that were isolated from the surgical biopsy.

Another alternative method also measures the synthesis rate of DNA in the cells by pulse labeling with DNA precursor amino acids (BdUR or IdUR) and then labeling with fluorescent antibodies against the DNA precursor to determine the amount of incorporation per unit time. (Gratzner, H. G. and Leif, R. C. “An immunofluorescence method for monitoring DNA synthesis by flow cytometry.”* Cytometry* 6: 385–389, 1983.). The DNA synthesis rate can either be measured by flow cytometry or fluorescence image analysis and thus compared directly to the levels of urokinase in the tumor cells.

Another alternative method is measurement of urokinase using fluorescence image analysis of de-paraffinized or frozen histology sections from biopsy tissues. DNA can be measured simultaneously using a vital fluorescent stain (PI or DAPI) or fluorescent labeled antibodies against BdUR or IdUR that emits fluorescence at a different wavelength than does the labeled anti-uPA antibodies. Supporting flow cytometry data can be obtained from thick sections from the tumor specimens, whereupon the cells are dissociated, treated with RNAse, the nuclei freed from the cytoplasm and flow cytometry performed on the nuclei. This data is then compared to the urokinase levels or image analysis data on cells taken from the same biopsy tissue.

To illustrate the method of this invention the correlation of urokinase on a cell by cell basis and DNA content have been measured on a cell by cell basis and DNA content have been measured in HBM-UPA (with commercial anti-uPA antibodies HMW-uPA that cannot distinguish between scuPA and active HMW-uPA). FIG. 1B illustrates the correlation of the uPA content vs. the DNA content in the CS cells, while FIG. 2B illustrates a different pattern of uPA content vs. the DNA content in the more invasive HBR09 cells. Table 1 summarizes the presence of DNA and uPA in the CS and HBR09 tumor cell lines. It should be noted that the difference in uPA content between the CS and HBR09 cell types is more pronounced when the cells are in the resting cells (G1/G0) compared to the those cells that are actively synthesizing DNA and dividing (S & G2M). In other tumor cells the difference may be greater in S and G2M phases.

**Table 1**

<table>
<thead>
<tr>
<th>PHASE (CELL CYCLE)</th>
<th>G1/G0</th>
<th>S + G2M</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CELL I.D.</strong></td>
<td><strong>% Cells</strong></td>
<td><strong>DNA S.D.</strong></td>
</tr>
<tr>
<td>IgG (Control)</td>
<td>78.8</td>
<td>16.7</td>
</tr>
<tr>
<td>CS</td>
<td>77.3</td>
<td>17.2</td>
</tr>
<tr>
<td>HBR09</td>
<td>82.6</td>
<td>9.8</td>
</tr>
<tr>
<td>RATIO of uPA HBR09/CS = 6.43</td>
<td>RATIO of uPA HBR09/CS = 3.27</td>
<td></td>
</tr>
</tbody>
</table>

5,869,238
/autofluorescence levels below the gating value selected from identical runs with negative controls. In general, the DNA fluorescence for the HBR09 cells in the S and G_{2M} phases (FIG. 2B, zone 2) is higher than the CS cells (FIG. 1B, zone 2). Furthermore, the distribution of HBR09 cells containing high levels of uPA in the resting phase (FIG. 1B, zone 1) is more focused than the CS cells (FIG. 2B, zone 1). Eighty-eight percent of the HBR09 cells in the resting phase and 96% of the cells in S and G_{2M} (zone 2) have high levels of urokinase in contrast to the CS cells where 42% in the resting phase and only 54% of the cells in S and G_{2M} have significant levels of urokinase. Also the mean content of uPA per HBR09 cell is greater than the CS cells in all phases of the cell cycle.

Flow cytometry can be used for disassociated cell samples to initially screen for the abnormal DNA content and uPA profile indicating aggressive tumors. Cells with abnormal DNA and high levels of uPA particularly in the S phase merit attention for potentially aggressive tumor tissue and further examination by digital image analysis. However, the analysis by flow cytometry using this method may be sufficient with existing pathological procedures and tests to make a determination on treatment of a dangerously malignant tumor.

Image analysis was performed on fluorescent uPA antibodies in CS and HBR09 cells. Image analysis can quantify the presence of uPA by measuring the fluorescence emitted by labeled antibodies that are bound directly to uPA. Table 2 illustrates the comparison of flow cytometry analysis and digital image analysis of fluorescent-labeled uPA antibodies, wherein each sample of glioma cells were split into two groups for parallel assays of uPA. Table 2 shows the high levels of uPA in CS and HBR09. However, in-comparison HBR09 has much higher levels of uPA than CS as confirmed by both flow cytometry and image analysis. Note that the image analysis method show a greater ratio of UPA in the HBR09 cells as compared to the CS cells and that the standard deviation of the mean fluorescence is smaller (8-9%) compared to the flow cytometry data (10-35%) on the same cells.

<table>
<thead>
<tr>
<th>TABLE 2</th>
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<tbody>
<tr>
<td>COMPARISON OF UROKINASE IN CONTROL AND BRAIN CANCER CELLS USING FLUORESCENT-LABELED uPA ANTIBODIES</td>
</tr>
<tr>
<td>CELL LINE</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>FLOW IgG (Control)</td>
</tr>
<tr>
<td>CYTO- CS</td>
</tr>
<tr>
<td>METRY HBR09</td>
</tr>
<tr>
<td>RATIO HBR09/CS = 5.261*</td>
</tr>
<tr>
<td>IMAGE IgG (Control)</td>
</tr>
<tr>
<td>ANAL- CS</td>
</tr>
<tr>
<td>YNOS HBR09</td>
</tr>
<tr>
<td>RATIO HBR09/CS = 7.56/1</td>
</tr>
</tbody>
</table>

*RATIO HBR09/CS = 7.4 after autofluorescence is subtracted

Image analysis is vastly important in detecting metastatic activity by direct measurement of urokinase and DNA for use in the evaluation of the presence of tumor cells with invasive characteristics comprising the steps of:

(a) selecting the cells to be examined;

(b) incubating a sample of the cells with antibodies specific to urokinase and a label specific to DNA;

(c) measuring directly the antibodies specific for the amounts of urokinase present in the sample on a cell by cell basis;

(d) measuring the DNA content simultaneously as step (c) in the same sample of the cells isolated in step (a) on a cell by cell basis; and

(e) identifying the cell populations in the sample on a cell by cell basis for DNA aneuploidy and high urokinase amounts; and

(f) further evaluating the cell populations identified in step (e) for DNA content for resting cells (G1/G0) and synthesizing and dividing cells (S and G2+M); and

(g) correlating DNA content for resting cells (G1/G0) and synthesizing and dividing cells (S and G2+M) with high urokinase levels to identify tumor cells with more...
performed by analytical cytometry and the antibodies specific to urokinase.

wherein the label specific to urokinase is a detectable light emitting marker.

wherein steps (c) and (d) the simultaneous measurements are performed by analytical cytometry and the antibodies specific to urokinase and the label specific to DNA have a marker detectable by analytical cytometry techniques.

wherein the antibodies specific to urokinase are monoclonal antibodies.

wherein the antibodies specific to urokinase are polyclonal antibodies.

wherein the urokinase antibodies are specific to the group of claim 1.

wherein the antibodies specific to urokinase have a fluorescent marker.

wherein the antibodies specific to urokinase have comparably low levels of plasminogen activator inhibitor content and high levels of urokinase content.

wherein the antibodies specific to urokinase identifying the more metastatic cells have lower DNA content as compared to the high DNA content of tumor cells with less invasive characteristics which have high urokinase content and low plasminogen activator inhibitor content.

wherein the antibodies specific to urokinase have a detectable light emitting marker.

wherein the antibodies specific to urokinase are monoclonal antibodies.

wherein the antibodies specific to urokinase are polyclonal antibodies.

wherein steps (c) and (d) the simultaneous measurements are performed by analytical cytometry and the antibodies specific to urokinase and the label specific to DNA have a marker detectable by analytical cytometry techniques.

wherein the antibodies specific to urokinase have a fluorescent marker.

wherein the antibodies specific to urokinase have comparably low levels of plasminogen activator inhibitor content and high levels of urokinase content.

wherein the antibodies specific to urokinase identifying the more metastatic cells have lower DNA content as compared to the high DNA content of tumor cells with less invasive characteristics which have high urokinase content and low plasminogen activator inhibitor content.

wherein the antibodies specific to urokinase identifying the more metastatic cells have lower DNA content as compared to the high DNA content of tumor cells.

wherein the antibodies specific to urokinase have higher DNA content.

wherein the antibodies specific to urokinase identifying the more metastatic cells have lower DNA content as compared to the high DNA content of tumor cells.

wherein the antibodies specific to urokinase have their DNA content identified and isolated in step (e).

wherein the antibodies specific to urokinase identifying the more metastatic cells have lower DNA content as compared to the high DNA content of tumor cells.

wherein the antibodies specific to urokinase identifying the more metastatic cells have lower DNA content as compared to the high DNA content of tumor cells.

wherein the antibodies specific to urokinase identifying the more metastatic cells have lower DNA content as compared to the high DNA content of tumor cells.

wherein the antibodies specific to urokinase identifying the more metastatic cells have lower DNA content as compared to the high DNA content of tumor cells.
19. A method of measuring metastatic activity by direct quantitative detection of urokinase and DNA of claim 12 comprising the additional steps of incubating a sample of the cells with antibodies against plasminogen activator inhibitors and measuring quantitatively the presence of plasminogen activator inhibitor bound antibodies by flow cytometry and correlating the plasminogen activator inhibitor content to the urokinase content identifying the more metastatic cells that have comparably low levels of plasminogen activator inhibitor content and high levels of urokinase content.

20. A method of measuring metastatic activity by direct quantitative detection of urokinase and DNA of claim 12 comprising the additional steps of incubating a sample of the cells with urokinase receptor markers and measuring quantitatively the urokinase receptor density and further correlating those cells with high urokinase receptor density with those cells with high urokinase content and low plasminogen activator inhibitor content which are characteristic of high invasiveness.

21. The method of measuring metastatic activity by direct quantitative detection of urokinase and DNA of claim 12 wherein the urokinase antibodies are specific to the group consisting of scuPA, LMW-UPA and HMW-UPA or mixtures thereof.

* * * * *