Contract NAS 9-18189

"Monoclonal Antibody Testing for Cancer
Metastasis"

<u>Final Report</u>

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INTRODUCTION

Malignant cells are characterized by the ability to invade surrounding normal tissues. Tumor invasion is abetted by proteolytic enzymes that have been correlated with recurrent disease and metastasis. These enzymes are involved in a *cascade* of proteolytic interactions with other enzymes and inhibitors which allow cancer cells to dissolve surrounding extracellular matrix, thereby enabling the cells to rapidly invade adjacent tissues and migrate to metastatic sites distant from the primary tumor. Among these proteases are the plasminogen activators (PA), collagenase IV, laminase, and in some cases cathepsin D, which together mediate key steps in the invasion process of metastasis.

Cells which have the selective advantage for invasion and metastasis are those capable of regulating their proteolytic activity and proliferation. Cells in the process of invasion would be probably down-regulated for proliferation, but subsequent to attachment and adhesion at a distant site, would then be in a proliferative mode, up-regulating DNA replication.

Urokinase (uPA) can be present in the tissues in several molecular forms. The inactive proenzyme is a single chain protein (scuPA) that is cleaved at Lys.158 to form the double chain, high molecular weight active form (HMW-uPA) of 54 kD. A low molecular weight form (LMW-uPA) can also be produced by cleavage of the HMW-uPA at Lys.135 - Lys.136 giving a 35 kD active enzyme. Recently, it has been shown 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 [Andreasen *et al*, 1986]. Receptor- (membrane) bound uPA is twice as efficient (catalytically) as free fluid-phase uPA [Hollas *et al*, 1991]. The unbound uPA and the LMW form is not responsible for most of the local dissolution of extracellular matrix in the immediate vicinity of the metastatic tumor cell.

High levels of urokinase (>3.49 ng/mg of total protein) extracted from breast tumor tissues have recently been shown, together with plasminogen activator inhibitor 1 (PAI-1), to be a good prognostic indicator for high risk of recurrence and shorter patient survival times [Janicke *et al*, 1991; Duffy *et al*, 1991; Grøndahl-Hansen *et al*, 1993].

In this project, we have attempted to develop immunocytochemical methodologies for the clinical assessment of the expression of urokinase plasminogen activator, which has been implicated to be important for initial steps in tumor invasion, and to relate it to cell proliferation and DNA replication at the single-cell level.

Flow cytometric studies of urokinase in cultured glioma cells:

In order to establish the parameters for immunofluorometric analysis of urokinase (uPA) in tumor cells, studies were initiated with human glioma cell lines, which have been found to produce high levels of uPA. These studies involve both flow cytometry (FCM) and image analysis; the FCM studies permit the correlation of urokinase expression with the cell cycle, while with image analysis one can localize and quantitate uPA in the cytoplasm and cell membrane. Another advantage of the use of cell lines is the ability to study uPA expression in relation to cell proliferation and DNA replication.

Three cell lines have been employed in the studies thus far, all from Dr. Marylou Ingram, Huntington Research Foundation, Pasadena, CA. The three cell lines, which were cultured from patient surgical biopsy material, have different morphological characteristics and growth rates. While alterations in the cells obviously occur in culture, the consistent morphology of these cell lines during passage in culture encourages us to pursue differences in the cell's characteristics, which will hopefully shed some light on the relationship of uPA to the biological behavior of the original tumors.

The first of these lines, CS, grows very rapidly as polygonal cells in monolayers and in the absence of serum, tends to form spheroid structures. The second cell line, HBr09, has a fibroblastoid morphology but has the characteristic immunological marker associated with gliomas, glial fibrillary acidic protein (GFAP, Patau,1988) This cell line grows at about one quarter the rate of CS. A third line, HBr65, was employed for some studies of uPA expression coupled with DNA replication, as well as uPA localization.

Combined anti-uPA and anti-BrdUrd immunostaining for uPA and DNA replication at the single-cell level:

These studies were performed with the cell line, HBr65, also obtained from glioma patients and initiated in primary culture by Dr. Ingram. Cell monolayers were pulsed with IdUrd and dual immunofluorescence staining performed for detection of both iododeoxyuridine (IdUrd) and urokinase. The protocol, developed under this contract in our laboratories, is described below in the **METHODS** section.

Immunocytochemical Studies of Breast Cancer Biopsies:

In this project, biopsies from these women, who have previously been diagnosed with node negative breast cancer, are being analyzed by immunohistochemical, flow and image cytometry techniques in order to correlate specific tumor markers with clinical outcome for each patient.

METHODS

Cell culture of glioma lines:

Cells were grown in 1:1 DMEM:F12 medium containing 15 % fetal bovine serum. The growth medium was replaced with a "production medium" without serum at the point when the cells appeared to be nearly confluent.

For flow cytometry, cells were scraped from flasks, in lieu of trypsinization, in order to preserve membrane-bound antigen. Cells were then washed in PBS and triturated to disperse the cell clumps. Single-cell suspensions were usually achieved, which were then fixed for 15 min in 0.5% paraformaldehyde at room temperature, followed by one hour permeabilization in 70% methanol at 4° C. The cell suspensions were blocked with 1% bovine serum albumin in PBS for 15 minutes, cells were then washed in PBS and antibody titered by staining with increasing dilutions of the primary, anti-uPA monoclonal antibody (#394, obtained from _American Diagnostica_, Greenwich,CT), or the equivalent concentrations of naive mouse IgG2a, both diluted in 1% BSA in PBS. Staining was for one hour, cells were washed and incubated in second antibody, fluorescein-conjugated goat anti-mouse IgG, for flow and image cytometry studies, or rhodamine-labeled goat anti-mouse IgG (_ICN_, Irvine, CA) for image cytometry, diluted in 4% goat serum for one hour. In later experiments, cells were pre-incubated for one hour in 4% goat serum in PBS prior to addition of the second antibody. The immunostained cells were then incubated in ribonuclease (1 mg/ml in PBS) for 30 min and stained for DNA content with 15 μg/ml propidium iodide.

Flow cytometric analysis :

Flow cytometric analysis was conducted with a EPICS _Profile_ flow cytometer (_Coulter Corporation_, Hialeah FL with the 488 nm line of an argon laser. Green light (from fluorescein emission) passed through a narrow bandpass interference filter (520 +/- 10nm), while red light (from PI) was passed through a 630 long pass filter. Bivariate, 64 x 64 channel histograms were obtained for analysis of mean fluorescence intensity. Data also were normalized for area and fluorescence intensity after the background was subtracted. This allowed comparisons among cells from the same samples and comparisons between cell lines and different samples. Statistical analysis of the data was performed by multivariate analysis using Statview 512 (_Abacus Concepts, Inc._)

Dual, anti-uPA/anti-BrdUrd experiments:

These were performed by pulsing tissue culture chamber slides with 10 μM IdUrd for 60 min, and fixing the cell monolayers with 1% paraformaldehyde/.01% Tween 20. Immunostaining for both uPA and IdUrd incorporation was performed by treating the monolayers with DNase (1 mg/ml) prior to immunostaining for uPA by the indirect technique, and then with direct, anti-BrdUrd fluorescein conjugate (Gratzner HG, 1982).

Digital image analysis :

Image analysis was performed using a fluorescence microscope equipped with a Dage SITS high resolution video camera connected to a *QuickCapture* board (*Data Translation, Inc.*) and *NIH Image* using the *MacIntosh* IIfx. Images were stored as TIFF files and later analyzed using *NIHImage* Version 41.1. Individual cells were scanned for mean optical densities and normalized for area. Relative fluorescence was compared after subtracting any background or autofluorescence measured from control cells on the same slide. Areas of concentrated uPA, within the cell and bound to the cell membrane were further analyzed by density slicing and thresholding followed by particulate analysis of those specific areas.

Confocal microscopy :

Confocal microscopy was performed with a Zeiss laser confocal microscope system in the laboratory of Dr. Michael Andreeff, M.D. Anderson Cancer Center, Department of Hematology. uPA was localized by excitation with a helium/neon laser. Serial sections of 250nm thickness were acquired and assembled into sequential, serial sections by software supplied with the confocal microscope.

Fluorescence in situ hybridization: (FISH)

Human glioma cells, HBr65, were trypsinized from tissue culture flask monolayers, centrifuged onto microscope slides, fixed in methanol acetic acid (3:1) and air dried. FISH was performed by standard methodology: the Internally-labeled alpha satellite probe for human chromosome # 10 purchased from *Imagenetics, Inc.* was denatured and employed according to instructions included with the FISH kit. Fluorescent images were photographed with a *Zeiss* Photomicroscope II using a planoapochomatic 63X objective (NA 1.25) on Kodak High Speed Ektachrome P800/1600 film.

Breast tumor immunohistochemistry:

Paraffin blocks were supplied by the OCOG Node Negative Breast Cancer Group through Dr. Harvey Cramer, London Ontario Regional Cancer Centre. Sections were cut, deparaffinized by usual procedures and stained by immunoperoxidase methodology. Tumor grading was by our pathology consultant , Dr. Ruth L. Katz, M.D. Anderson Cancer Center.

Results and Discussion.

Flow cytometric analysis:

Flow cytometry was performed by two-color analysis, with combined immunofluorescence for uPA and DNA content by PI staining. Two parameter histograms are shown in Figure 1 for both cell lines as studied by FCM. As expected, the majority of the uPA expression is found in the G1 compartment of the cell cycle, since the cells were primarily in stationary phase, having been transferred to serum-free conditions.

Attempts have been made to study the expression of uPA during exponential growth as well as in cultures that have been placed in serum-free medium. We conducted flow cytometric analysis of uPA levels in both CS and HBr09 glioma lines. In Table 1 are the relative levels of the uPA as measured by flow immunofluorescence. Further studies are concerned with measurements of uPA by image cytometry, in an effort to distinguish the membrane-bound (receptor) vs cytoplasmic uPA, since flow cytometry only measures fluorescence at "zero resolution".

Both cell lines produce uPA during growth and also during stationary phase. HBr09 produces significantly higher levels of intracellular and membrane-bound uPA. The CS line reveals two G1 populations, low and high uPA- expressing G1 cells, possibly reflecting residual proliferation in the culture. The HBr09 cell line

in contrast shows only high uPA content G1 cells and with a five-fold greater expression of cell-bound uPA of than the uPA expressing population of the CS line. It is to be noted that HBr09 grows extremely slowly, and also represents a low-passage (passage 13) glioblastoma line, whereas CS is highly proliferating, and has been in culture and while is positive for GFAP antigen, is of unknown origin.

Urokinase Localization by Confocal Microscopy:

Confocal microscopy (CFM) is an invaluable technique for localizing substructures and molecules at the microscopic level, and permits three-dimensional visualization. In Figure 2 are shown serial optical sections of an HBr65 cell monolayer which has been immunofluorescently stained for urokinase andexamined by means of CFM. The cell surface as well as cytoplasmic localization of uPA is apparent by these procedure. Similar confocal microscopic analysis of urokinase *receptor* (uPAR) exhibits the same localization on cell membranes (not shown).

Image Analysis:

Image cytometry provides a method for the spatial quantitation of uPA . The relative amounts of the enzyme in various cell compartments can be determined in a manner obviously not possible by flow cytometry. In Figure 3 is shown a fluorescence micrograph of a CS cell which as been immunostained for uPA. Note that the fluorescence is expressed as a large cap on the cell surface. Figure 4 represents a digitized image of a similar field which dramatically shows the distribution of uPA on the membrane as well as within the cells and demonstrates how uPA levels can be spatially quantified.

In Table 1, the distribution of uPA antigen between these locales is displayed. The levels of uPA determined by FCM for these two cell lines is reflected in this image cytometry data, but now the relative amounts within and on the cell membranes can be measured. Interestingly, the overall magnitude of uPA levels is the same, but now is composed of membrane plus cytoplasmic fluorescence. The uPA is predominantly observed to be on the cell surface.

COMPARISON OF UROKINASE IN GLIOMA CELLS

	CELL LINE	MEAN FLUORESCENCE	S.D.	MEMBRANE-BOUND uPA	S.D.	
FLOW CYTOMETRY	IgG	2.05	0.3	-		
	CS	6.13	0.1	-		
	HBR09	32.25	11.3			
	RATIO HBR09 / CS = 7.4 / 1					
IMAGE ANALYSIS	IgG	1.86	0.1	-		
	CS	11.3	0.76 (8%)	78.3	3.41	(4%)
	HBR09	74.1	6.34 (9%)	821.9	47.8	(6%)
	RATIO HBR09 / CS = 7.56 / 1			HBR09 / CS = 10.5 / 1		

Combined urokinase expression and DNA replication:

In Figure 5 are shown fluorescence micrographs representative of the 2-parameter immunostaining for uPA and DNA replication. While no immediate correlation has been observed between replication and uPA expression, the immunolocalization of uPA can be seen to be, as expected, on cell membranes and in the cytoplasm. The nuclei are fluorescent as expected for immunostaining for IdUrd incorporation that is indicative of DNA synthesis. No correlations between DNA replication and uPA expression, either cytoplasmically or on cell membranes, are apparent from our studies thus far.

Cytogenetic analysis of glioma cells by FISH:

Since we have shown uPA to be over-expressed in HBr65 and the structural gene for uPA is located on chromosome10 (Verde *et al*, 1984), numerical chromosome alteration analysis for chromosome 10 was performed on this cell line. As shown in Figure 6, multiple copies of chromosome 10 appear to be present, as determined by FISH with the metaphase alpha satellite probe which is specific for this chromosome. Controls consisting of normal human lymphocytes placed on the same slides and probed simultaneously are diploid (Figure 6b).

Ontario Clinical Oncology (OCOG) Breast Cancer Study

CTI , Inc./DNA Sciences, Inc. in collaboration with the Ontario Clinical Oncology Group(OCOG) Breast Cancer Study, Ontario, Canada, is conducting a study of approximately 650 patients with breast cancer. The patients in this study were subjected to various combinations of radiation therapy and lumpectomy in an effort to establish the efficacy of radiotherapy and surgery. In this project, biopsies from these women, who have previously been diagnosed with node negative breast cancer, are being analyzed by immunohistochemical, flow and image cytometry techniques in order to correlate specific tumor markers with clinical outcome for each patient. This study represents an invaluable opportunity, for additional research funding in addition to the use of the material for screening of new prognostic probes for cancer. In this NASA-supported study, we employed these biopsies for analyzing markers for metastasis, in order to correlate them with urokinase plasminogen activator, or to ascertain whether urokinase is an independent determinant The latter has been determined recently in other institutions to be a significant predictor of time-to-relapse and disease-free survival in node negative breast cancer [Janicke *et al*, 1991; Duffy *et al*, 1991; Grøndahl-Hansen*et al*, 1993].

In Table 2 are the tumor and proliferation markers we have employed for the OCOG specimens

Tumor Markers Tested for OCOG Breast Cancer Study

Marker	Function	Reference
Urokinase	protease	Grondahl/Hansen et al, 1993; Janicke et al, 1991.
Her 2/ neu (c erb 2)	trans-membrane oncogene	Slamon et al, 1989
p53	tumor suppressor gene	Oren, 1992
PCNA	DNA synthesis accessary protein	McGuire WL, 1990

Evaluations of anti-uPA labeled breast cancer sections reveal that normal breast tissue does not contain uPA except for some endothelial cells lining the arterioles. Intraductal carcinomas, however, do express measurable quantities of uPA using immunoperoxidase detection methods.

Measurements of uPA by absorption of immunologic stains as light passes through tumor cells usually is recorded by the image analysis system as the mean optical density of groups of cells in the field of view. While this is more difficult to quantitate, especially for individual cells, since light absorbed by the histopathology counterstains add to the absorption of the uPA antibody labels, this is the current method employed in pathology laboratories. Measurements of fluorescence is a better quantitative method since the light is emitted only from the uPA molecules and it is emitted at a wavelength different from the incident light. Also fluorescent stains are used at lower concentrations than are absorbing stains and the net background from autofluorescence is less than the contribution of non-specific staining by light absorbing stains [Lockett SJ et al, 1991].

Figure 7a shows an example of a breast tumor section immunostained by the immunoperoxidase technique, illustrating the areas of uPA found in foci of tumor cells. A normal kidney section has been stained for a positive control.The images are a digital representation of the immunostained sections. Distinct areas of concentrated uPA are shown (dark areas). Most of the normal tissue does not produce urokinase. Figure 7 b shows the same section, but subjected to "digital slicing" protocol of the NIH Image software. The red areas are of equivalent mean optical density (MOD), demonstrating the process by which we will conduct quantitative image cytometry for clinical specimens in future studies and for our clinical laboratory breast cancer panel.

These methods can be used in retrospective studies where the time to reoccurrence, degree of metastasis and morbidity are known. Cumulative data then can be used to provide a prognostic indicator for the presence of active metastasis.

In Table 3 are shown a summary of the data obtained from this study, as performed by immunohistological staining.The results of the studies with these patients will be applied to our breast cancer prognostic panel offered to physicians.
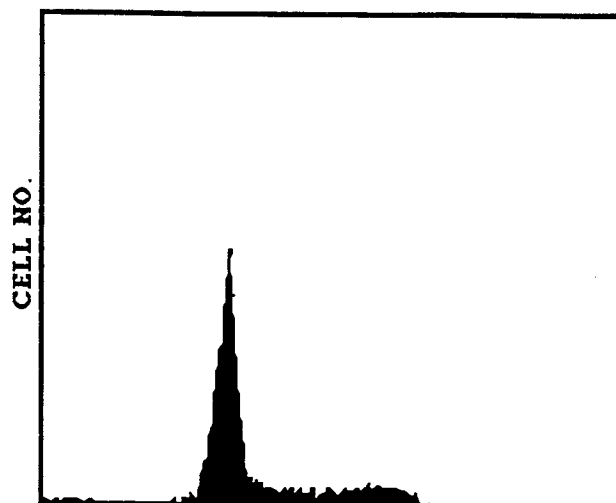
Summary of OCOG Node Negative Breast Cancer Data

BLOCK	UPA	HISTOLOGY	NUCLEAR GRADE	MF/hpf	P53	PCNA	c ERB 2
86-591	1	D	1	2	0	2	0
87-596	3	D	2	2	0		0
86-1798FS1	0	D	1	13	0	25%	0
87-1838	0	DCIS/C+NC	1	2	0	0% ?	
86-1914QS	2	D	1	8	40	18%	
85-2216A	3	L	2	3	0	80%	0
86-3308A	1	L	1	9		24%	0
86-3311 1A	2	D	1	1	0	30%	0
84-3729 1	1	D	1	31	2	31%	3
84-3802 FS	2	D	3	0	1	7%	0
86-3969 QS	2	D	1	15	0		4
86-4220QS	1	D/TUBULAR	3	0	0	1%	0
85-4541QS	2	D	1	2	5	0	
85-4580 B	0	D	1	50	4	75%	0
84-4642 Q3	1	D/TUBULAR	3	0	0	4%	0
85-5307A	1	D	1	2	0	39%	0
86-5301 A	3	D	2	1	5	19%	
87-5475QS	2	LOBULAR	3	0	0	0%	0
85-5535	1	DCIS/C 7 INV	2	1	0	38%	0
86-5905FS	1	D	1	1	0	48%	0
84-6208	0	D/T	3	0	0	55%	0
87-6508 A3	3	LOBULAR	2	2	0	73%	
86-6695 A	2	D	1	5	50+++	23%	0
85-7296FS	3	D/TUBULAR	3	0	0	0	0
874-7467 FS	2	D POOR FIXATION	1	16	70+++		0
87-8159 QS	1	D	3	2	0	2%	0
84-9277	3	D	1	1	0	8%	0
87-9522 QS	3	LOB	2	1	10++	35%	0
85-10589Q	0	D	1	40	0	0%	0
84-10767	2	D	1	11	0	24%	0
85-11331QS	2	D	1	15	1+++	0%	0
84-11944 1	1	D	2	1	0	17%	0
85 239	1				0		
86-591	1				15 +++		
87-596FS	3					6%	
88-1096	1	D	1	11	0	9%	
89-1560	1	no tumour			5	?	0
86-2450 E	2				0		
88-2941QS	1	D	2	12	0	2%	0
88-3000 A	0				MEM	4%	
87-3137 A	0				0		0
88-3692B1	1	D	1	0			
88-3895	3	D	1	1	0		
85 5452 A	2	D	1	3		65%	
88-5768	2	D	1	22	0	61%	0
85 7478 A	1	D	1	16			
89-10179	3	D	1	5	31	42%	0
88-10970 1A	0	D	3	0		0%	0
88-11077	3	D	1	5	0	0%	2
87 1244	2				0		
86 1275 A	2						
88 2321 A	0						
87 2936 A	2				0		
86 3111 A	0						
87 3176	3						
86 3582 A	1						
85 5169	2	2			0		
87 9726	2				0		
395	0	D	1	9	9		
896	1	D	1,2	9	9		
1244	2	D	2	0	0	25	0
1275	2					61	
1596	1	D/scero.	1	12	12	24	0
1717	3	C	2	12	12	89	4
2321	0	D	1				0
2716	3	C	3	5	5		
3137	0		2	1	1		
3160	3	D	3	0	0		0
3506	1	D/Cr	3	0	0	0	0
3786	3	D	1	3	3		
3999	0	D	2	12	12		
4005	1	D	1,2	4	4		
4066	0	D	1	9	9	38	0
?4281	2	D	2	3	3	54	0
5350	1	D	1	9	9	89	4
5452	2					46	
7224	0		1	0	0	0	
7271	0			0	0	0	
7467	2			0	0	28	
7478	1					97	0
7544	0	M/LYMPHS	1	31	31		
8706	2	D	1			2	
10299	3	D	1	4	4		0

FUTURE DIRECTIONS

Future work will involve immunocytochemical studies of plasminogen activator inhibitor (PAI 1) in the same breast tumor sections analyzed for uPA, since other laboratories have implicated an elevation of this factor is highly significant for poor breast cancer prognosis [Janicke *et al*, 1991; Duffy *et al*, 1991; Grøndahl-Hansen *et al*, 1993]. It would be of interest to determine the relative expression of PAI and uPA at the cellular level, not possible by measurements in extracts, as performed by the aforementioned reports. The addition of uPA and PAI 1 assays to panels for other organ sites will be desirable. For example, both prostate and bladder cancer involve metastatic progression and invasion.

The direct correlation between membrane the binding of uPA and invasive activity merits an immunocytochemical study of the uPA receptor (uPAR); it is conceivable that levels of uPAR are an extremely important marker for metastasis.

We would anticipate continuing long term, basic studies of the relationship between DNA replication and uPA expression. The most significant question to be asked is whether there are control mechanisms which down-regulate uPA during cell proliferation, and, reciprocally, up-regulate DNA synthesis and cell proliferation with decreased uPA expression. These types of studies will necessitate *in vitro* experimentation with cell lines, and molecular biological methods to elucidate the complex association between transcription of uPA and the various factors required for cell cycle regulation and DNA proliferation.

Figure 1. Bivariate plots of glioma cells labeled with propidium iodide (PI) for DNA and fluorescein-conjugated antibodies for urokinase (uPA) comparing the uPA and DNA fluorescence for cells in G1 (82% of total cells), S phase (15% of total cells), and G2 + M (22% of total) for two glioma cell lines: CS (upper panels) and HBr09 (lower panels).
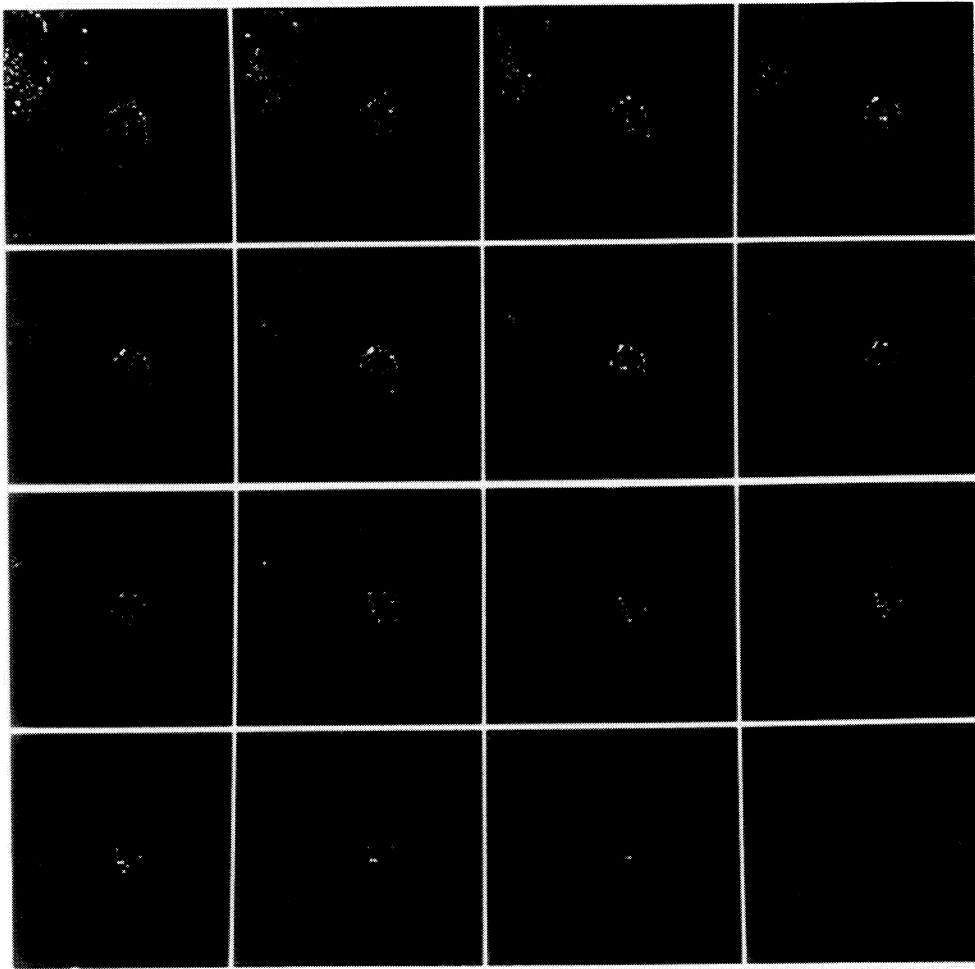
CS #1141 — DNA FLUORESCENCE

	MIN.	MAX.	COUNT	%	MEAN	SD	%HPCY
1	51	77	3458	70.2	69.5	4.1	4.83
2	80	103	541	11.0	90.0	7.1	15.5
3	114	136	515	10.5	125.4	6.4	5.52

CS #1141 — LOG DNA FLUORESCENCE
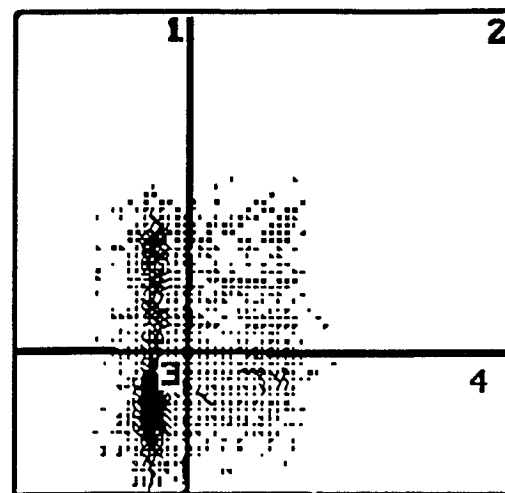
		MIN.	MAX.	COUNT	%	MEAN	SD	%HPCY
1	X	0	21	1098	22.1	17.2	1.5	6.47
	Y	1.58	1023			5.35	0.22	26.8
2	X	22	63	347	7.0	19.1	4.1	9.39
	Y	1.58	1023			4.74	0.24	14.3
3	X	0	21	2720	55.2	17.2	1.4	6.33
	Y	0.10	1.58			0.51	0.18	28.6
4	X	22	63	769	15.6	28.6	4.1	28.6
	Y	0.10	1.58			0.75	0.16	19.2
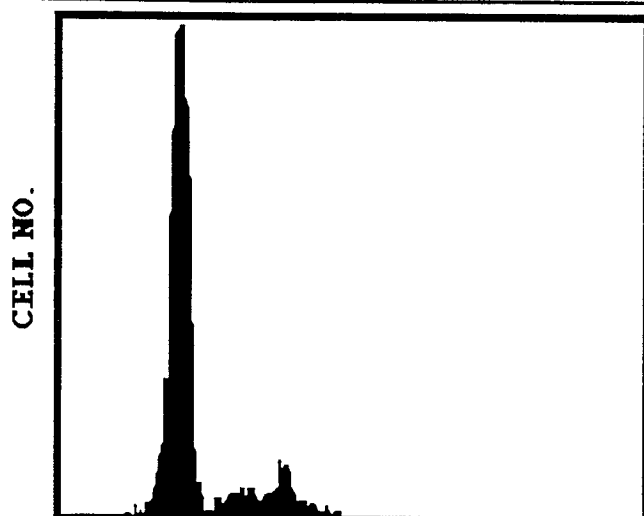
HBR09 #1141 — DNA FLUORESCENCE (PI)

	MIN.	MAX.	COUNT	%	MEAN	SD	%HPCY
1	31	47	1316	81.8	40.9	2.7	7.01
2	46	66	153	9.5	55.3	6.9	11.7
3	68	87	152	9.5	75.8	4.4	3.35

HBR09 #1141 — LOG DNA FLUORESCENCE (PI)
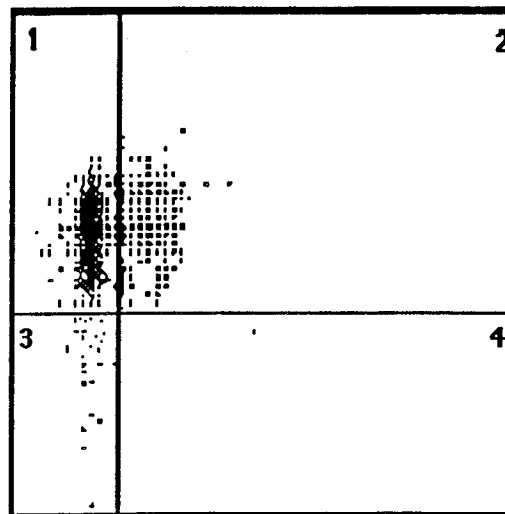
		MIN.	MAX.	COUNT	%	MEAN	SD	%HPCY
1	X	0	13	1329	82.6	9.9	1.0	7.26
	Y	3.24	1023			12.12	0.17	8.62
2	X	14	63	241	15.0	17.4	2.2	13.2
	Y	3.24	1023			15.48	0.18	10.9
3	X	0	13	36	2.2	9.5	1.1	9.59
	Y	0.10	3.26			1.22	0.26	4.08
4	X	14	63	2	0.1	16.1	2.0	6.07
	Y	0.10	3.26			2.43	0.10	1.93

Figure 2. Confocal laser microscope 250nm sections through HBr65 glioma cell.

Figure 3. Immunofluorescence staining of CS glioma cell (scraped from flask) to show "capped" membrane-bound uPA.
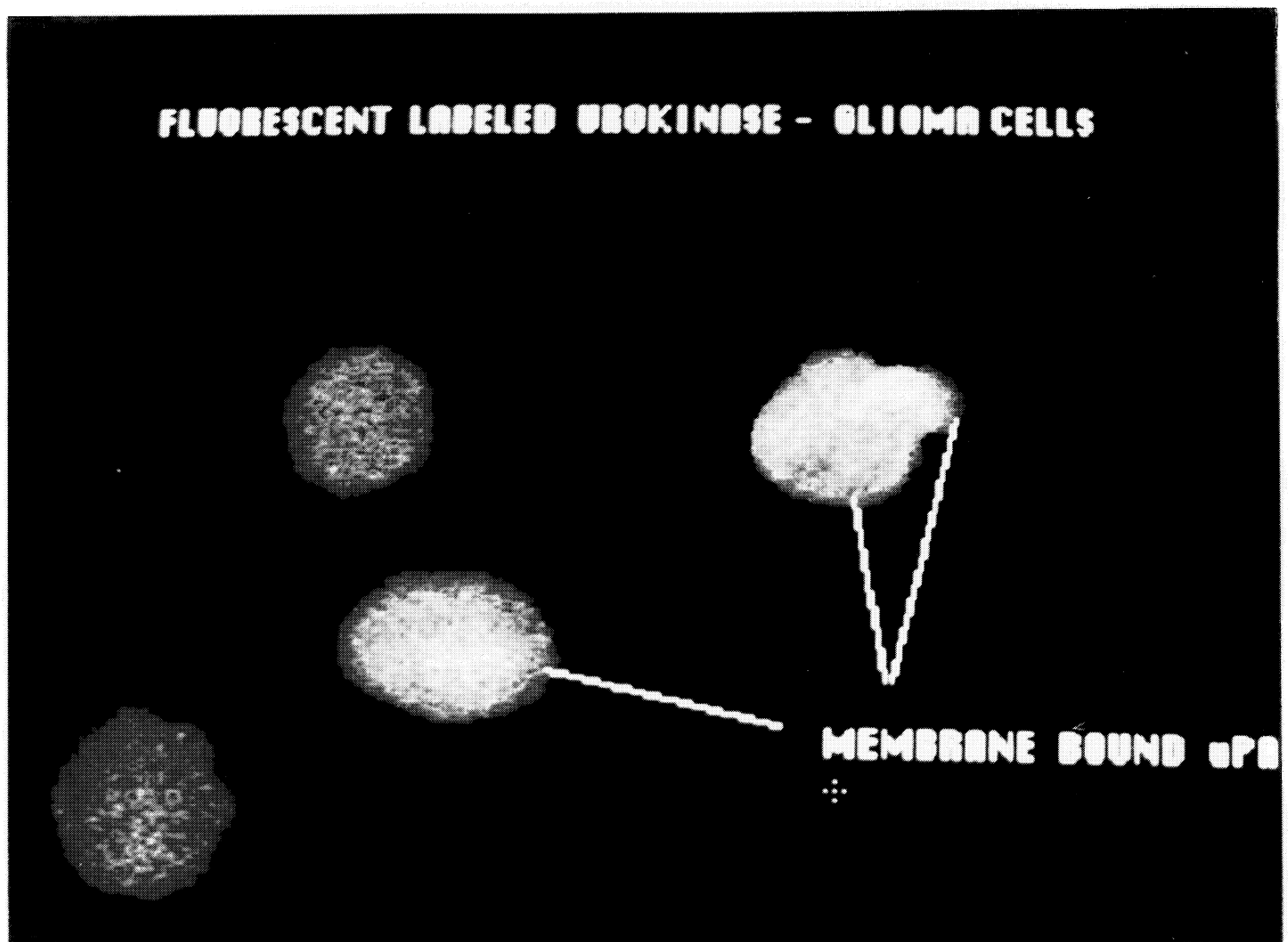

Figure 4. Digitized image of CS glioma cells showing membrane-bound uPA fluorescence.

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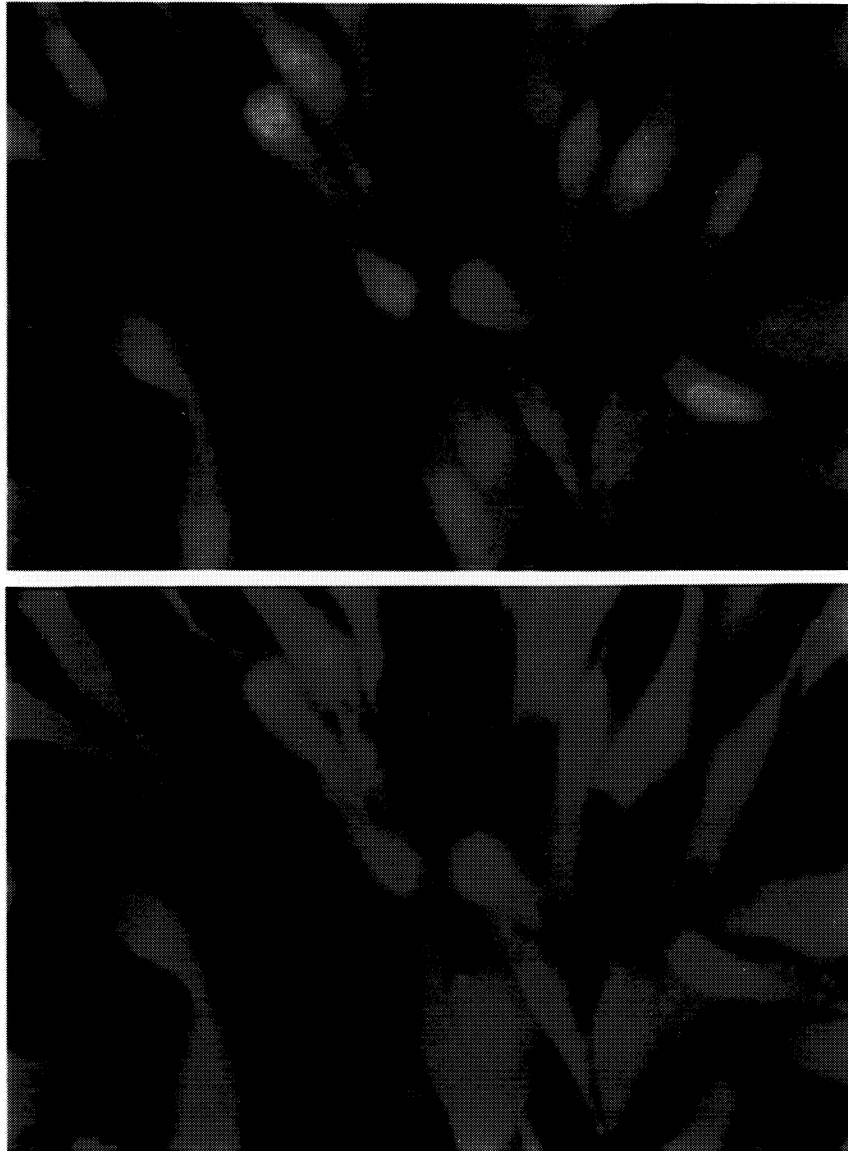
Figure 5. Urokinase immunostaining and DNA replication for human gliomas. Monolayers of cells were pulsed for 1 hr. with 10μM IdUrd, fixed, denatured and immunostained with anti-uPA and anti-BrdUrd Mabs.
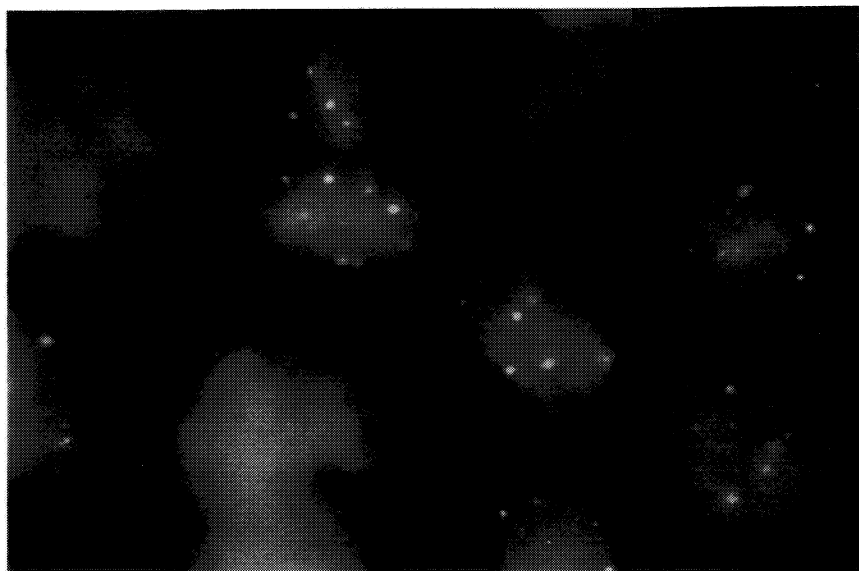
Figure 6a. HBr65 showing multiple copies of chromosome 10 as determined by FISH using an alpha-satellite probe for chromosome 10
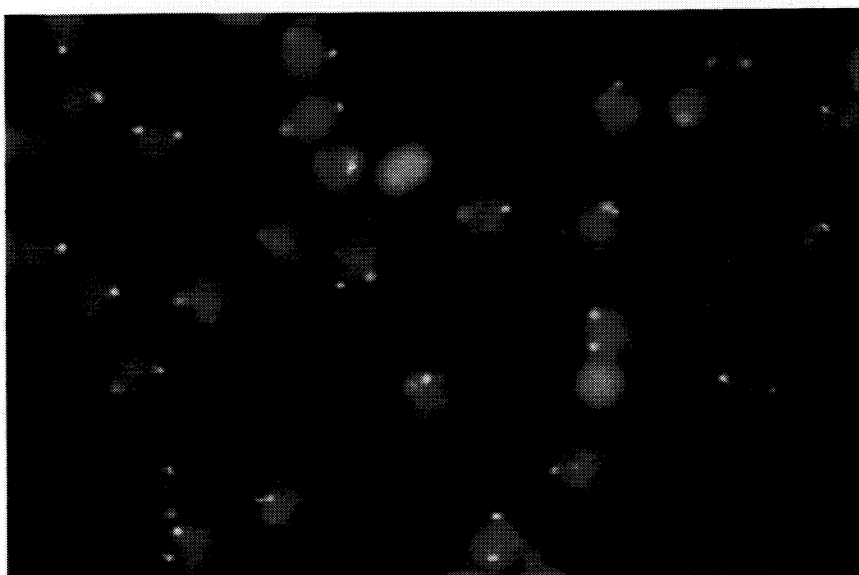

Figure 6b. Human lymphocytes (normal diploid control) probed in the same manner as Figure 6a.
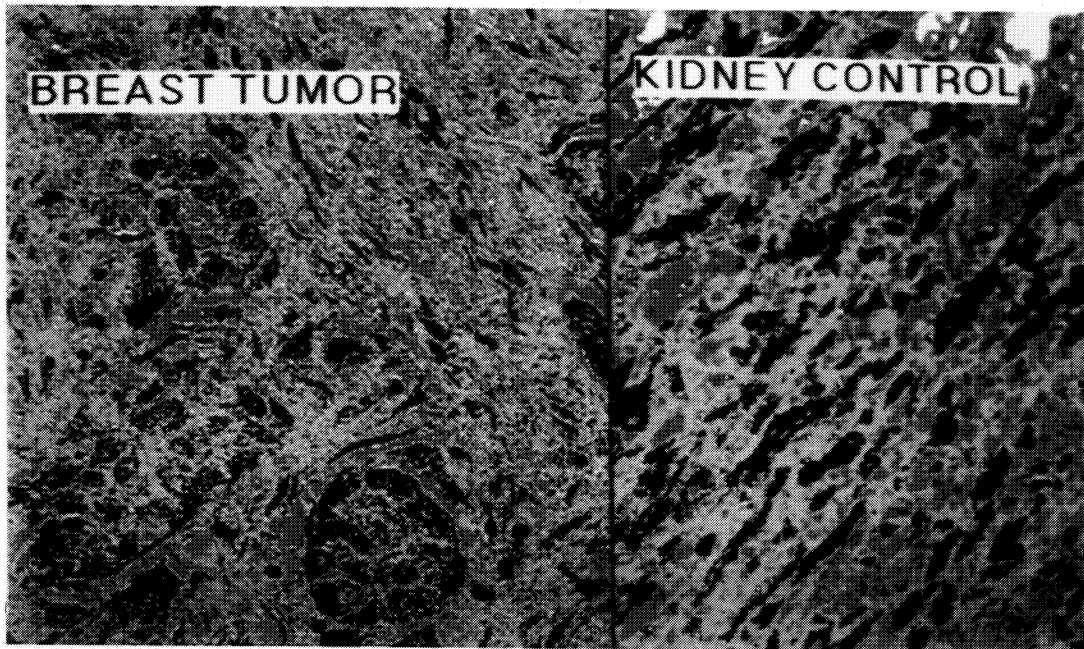
Figure 7a. Breast tumor section and normal kidney (positive control) immunostained and digitized using image cytometry and enhanced with pseudocolor to illustrate the uPA in the foci in the tumor.
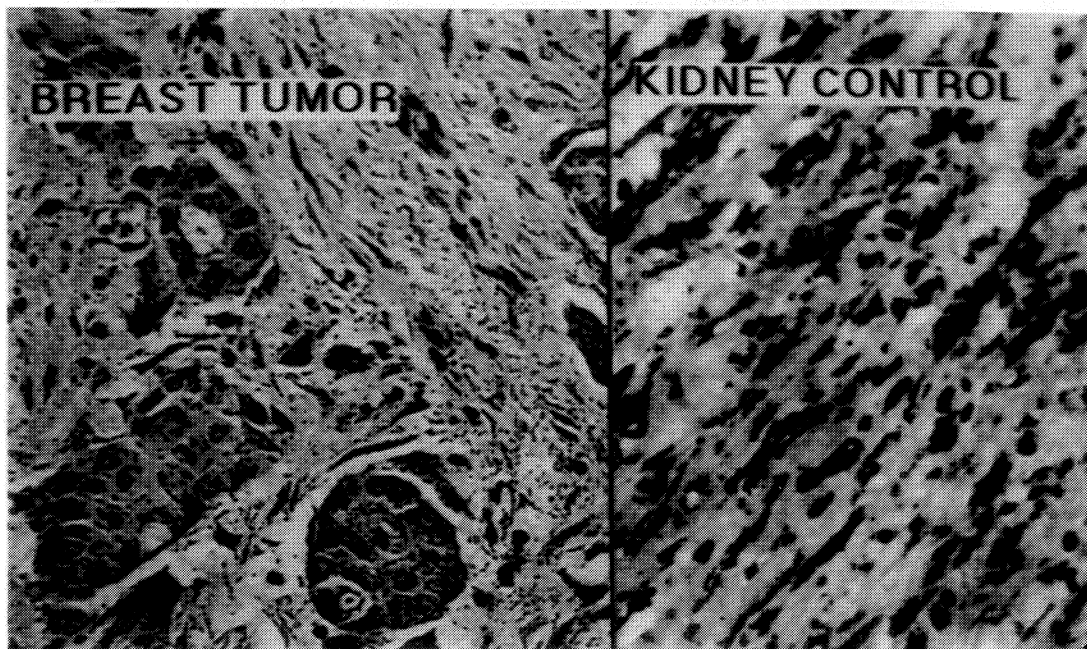

Figure 7b. Same section as 7a showing "digital slicing " to obtain all areas of equivalent MOD (Mean Optical Density) for positive uPA areas.