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Morphological and Immunocytochemical Characteristics of Human Tumor Cell Lines for Use in a Disease-Oriented Anticancer Drug Screen

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Abstract. *A panel of 60 human tumor cell lines is currently being used in the U.S. National Cancer Institute's* in vitro *anticancer drug screen. The panel is organized into 7 subpanels; 6 leukemia/lymphoma lines comprise one subpanel, and 54 other lines are organized into subpanels representing solid tumors of the central nervous system (CNS), colon, lung, ovaries, kidneys and melanomas. In the present study, the leukemia and lymphoma cell lines were analyzed by flow cytometry for appropriate CD antigens; all but 1 line showed patterns of expression consistent with their reported derivations. The solid tumor lines were characterized individually using morphological and immunocytochemical techniques to determine their relative degrees of representativity for the subpanels within which they are currently grouped. Histological, histochemical and ultrastructural examinations were performed on cell lines grown under identical conventional culture conditions and as xenografts in nude mice. Immunocytochemistry using panels of antibodies raised against 6 types of intermediate filaments, 7 adenocarcinoma-associated antigens, 7 melanoma/neuro-ectodermal-associated antigens, 3 neuroendocrine-associated antigens, 9 urinary tract associated antigens, and 4 markers of muscle differentiation was done on cells grown in monolayer culture. Central nervous system (CNS) cell lines lacked expression of glial fibrillary acidic protein, but all had other features consistent with derivation from glioblastoma. Lines derived from adenocarcinomas of the colon, lung and ovary, for the most part, expressed adenocarcinoma-associated antigens and showed histological and/or ultrastructural evidence of gland formation and other adenomatous features. Most of these lines were poorly differentiated. Lines derived from large-cell and squamous-cell cancers also showed some characteristics consistent with their reported origins, except for one line which showed immunocytochemical and morphologic characteristics consistent with rhabdomyosarcoma. The 2 lines derived from small cell lung cancer (SCLC) lacked neurosecretory granules and 3 other SCLC markers but showed morhologic features consistent with SCLC. Most melanoma cell lines strongly expressed melanoma-associated antigens and were morphologically similar to human melanoma. Five lines produced premelanosomes, melanosomes or melanin. Most of the renal cancer cell lines showed morphologic or immunocytochemical features consistent with renal clear cell carcinoma. Collectively, these morphological and immunocytochemical analyses provide information concerning tissue of origin, tumor type, degree of differentiation and other biologic features essential to the use of these lines in a disease-oriented* in vitro *antitumor drug screen and to the interpretation of data derived therefrom.*

The National Cancer Institute is implementing a new investigational primary *in vitro* antitumor drug screen in which all new agents are tested initially against a broad panel of human tumor cell lines, arranged in a series of subpanels representing various major categories of human cancer. An important design goal of the screen is to facilitate the detection and selection of line-specific or subpanel-specific antitumor leads for follow-up *in vivo* evaluation in xenograft models employing the sensitive lines identified in the primary screen. The rationale, evolution and current status of this experimental screening approach are described elsewhere (1-3).

An element critical to the design of this «disease-oriented» (2) screening model is the composition of the cell line panels. Tumor types from which the lines were derived should be representative of well-defined human tumor classifications, and individual cell lines must faithfully reflect appropriate biological properties of the tumor of origin. To determine if

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these minimal criteria were met among lines initially available to us, and to aid in the selection of the most appropriate lines for interim use in a pilot screening panel, in-depth characterization of candidate lines was conducted to define various biologic and pharmacologic traits, and to determine their suitability for use under assay conditions (4-5).

As a part of the above essential cell line characterization, detailed morphological and immunocytochemical evaluations also were performed. There were two major objectives of these evaluations. The first was to define the baseline characteristics of the individual cell lines, to confirm their tissues of origin, tumor type and degree of differentiation, and to relate certain biological properties to those attributed to tumors of similar histologic origin. These are described in the present manuscript. The second was to define features of the cell lines of importance in interpreting drug responses. Some of these features being analyzed include multidrug resistance as well as other factors related to drug resistance (e.g., topoisomerases, protein kinase C, tubulin, glutathione-S-transferase, etc.), growth factor receptor expression, and oncogene expression. These studies are in progress and will be reported in a later publication. The use of the current panel of 60 lines in a pilot-scale antitumor screen is described in detail in a separate paper (5). The panel, characterized in detail herein, was adopted by NCI for routine screening operations starting in 1990 (2-3).

Materials and Methods

Cell lines and cell line cultivation. The sources of the majority of the cell lines used in this study have been previously reported (4). Sources of the remaining lines are given in Table I. Cultivation of the lines, cryopreservation and quality assurance procedures are described in detail elsewhere (4,5). All cell lines were adapted to RPMI 1640 cultufe medium supplemented with 5% fetal bovine serum (heat-inactivated) and 2mM L-glutamine. The cultivation of human tumor cell lines under standardized culture conditions permits comparison of morphological and immunocytochemical features, as well as growth and drug sensitivities among tumor cell types.

When possible, cell lines were also established and grown as xenografts in nude mice, by conventional techniques (15). Xenografts were derived by implanation of freshly thawed cell stocks, or in some cases, by injection of cells harvested from logarithmic growth phase cultures into athymic NCr-nu mice (Animal Production Area, National Cancer Institute, FCRDC, Frederick, MD or Taconic, Inc, Germantown, NY). Xenografts of cell lines were grown subcutaneously, with the exception of OVCAR-3 which was cultivated intraperitoneally.

Immunocytochemical assays of solid tumor cell lines. The anti-human antibodies used to characterized solid tumor cell lines, together with their reported antigenic specificities, working dilutions, and sources, are summarized in Table II. Optimal dilutions of antibodies were determined by titration assay against known positive and negative control samples.

When flask cultures were 70-90% confluent, cells were harvested with a cell scraper (Costar, Cambridge, MA). Cytology specimens were prepared on glass slides using a Cytospin 2 cytocentrifuge (Shandon, Pittsburg, PA) operated at 750 rpm for three minutes, or by application to Teflon coated slides containing multiple uncoated assay wells (Cel-line Assoc., Inc., Newfield, NJ). Immunostaining was performed on air-dried, unfixed specimens (for cell surface antigens) or on specimens fixed in acetone at -70°C for 1 minute (for intracellular antigens), with commer-

Table I. *Sources of selected screening panel cell lines.*

Histology cell line	Reference	Contributor (Original source)
CNS		
SF-268	6	P.L. Kornblith
SNB-78	7	M.L. Rosenblum, Univ. of California
XF-498L	8	NCI/DCT/DTP[a]
Colon adenoca.		
HCT-15	9	ATCC (D.L. Dexter, Roger Williams General Hospital)
KM-12	10	I.J. Fidler, Univ. of Texas M.D. Anderson Hospital and Tumor Institute
KM-20L2	10	I.J. Fidler, Univ. of Texas M.D. Anderson Hospital and Tumor Institute
Lung adenoca.		
HOP-18	—	M. Liu, Johns Hopkins Univ. School of Medicine
HOP-62	—	M. Liu, Johns Hopkins Univ. School of Medicine
Lung-Lg. cell ca.		
HOP-92	—	M. Liu, Johns Hopkins Univ. School of Medicine
LXFL529L	—	H.-H. Fiebig, Univ. of Freiburg, West Germany
Melanoma		
M-14	11	D.H. Kern, John Wayne Cancer Clinic, UCLA School of Meidicine
M19-MEL	11	D.H. Kern, John Wayne Cancer Clinic, UCLA School of Meidicine
UACC-62	—	A. Leibovitz, University of Arizona
UACC-257	—	A. Leibovitz, University of Arizona
Renal carcinoma		
ACHN	12	S.M. Schmid, Southern Research Inst. (T.F. Hogan, Middleton Memorial VA Hospital
RXF-393L	8	NCI/DCT/DTP[a]
RXF-631L	8	NCI/DCT/DTP[a]
TK-10	13	R.V. Clayman, Washington Univ. School of Medicine
786-0	14	R.D. Williams, University of Iowa
Lymphoma		
SR	—	W.J. Urba, Program Resources, Inc.

[a]Developed under contract by Program Resources, Inc., from human tumor xenografts contributed by H.-H. Fiebig, University of Freiburg, West Germany

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Table II. *Antibodies used for solid tumor cell line characterization.*

Clone/ Designation	Immunizing agent/ Specificity	Ig Subclass	Derivation[a]	Working dilution	Source
Intermediate filaments					
AE-1	Type I acidic keratins	IgG_1	Mouse-asc	1:10	CRL (Cambridge, MA)
AE-3	Type II basic keratins	IgG_1	Mouse-asc	1:10	CRL (Cambridge, MA)
Vimentin	Vimentin 57 Kd	IgG_1	Mouse-sup	1:10	CRL (Cambridge, MA)
GFAP[b]	GFAP 56 Kd	IgG_1	Mouse-sup	1:10	CRL (Cambridge, MA)
Neurofilament	Neurofilament 70 & 200 Kd	IgG_1	Mouse-sup	1:10	CRL (Cambridge, MA)
Desmin	Desmin 56 Kd	IgG_1	Mouse-sup	1:10	CRL (Cambridge, MA)
Adenocarcinoma associated surface antigens					
CEA	Carcinoembryonic Antigen	Ig	Rabbit-ser.		
8H12	Cell line MCF7	IgG_{1k}	Mouse-asc.	1:50	DuPont (Boston, MA)
IBD12	Cell line MCF7	IgM_1	Mouse-asc.	1:50	DuPont (Boston, MA)
47D10	Cell line A-549 (GP 67-98)	IgG_{1k}	Mouse-asc.	1:50	DuPont (Boston, MA)
B72.3	Mammary Carcinoma (TAG-72)	IgG_1	Mouse-asc.	1:50	DuPont (Boston, MA)
OC125	Cell line OVCAR-3	IgG_1	Mouse-asc.	1:40	Centocor (Malvern, PA)
19-9	Cell line SW1116	IgG_1	Mouse-asc.	1:200	Centocor (Malvern, PA)
Melanoma associated antigens					
S-100	S-100 protein	IgG	Rabbit-ser.		Signet (Dedham, MA)
R24	Cell line SK-MEL-28	IgG_3	Mouse-asc.	1:10	Signet (Dedham, MA)
B5.2	Cell line SK-MEL-93	IgG_{2a}	Mouse-asc.	1:10	Signet (Dedham, MA)
BD18	Cell line SK-MEL-37	IgG_1	Mouse-asc.	1:10	Signet (Dedham, MA)
L101	Cell line SK-MEL-33	IgG_{2a}	Mouse-asc.	1:10	Signet (Dedham, MA)
Ta99	Cell line SK-MEL-23	IgG_{2a}	Mouse-asc.	1:10	Signet (Dedham, MA)
Neuroendocrine associated antigens					
MOC 1	SCLC[e] cells	IgG_1	Mouse-sup.	1:10	Sanbio bv (Uden, Neth)
123 C3	SCLC membrane prep.	IgG_1	Mouse-asc.	1:20	Sanbio bv (Uden, Neth)
NSE[f]	Bovine brain g/g homodimer (90 kd)	Ig	Rabbit-ser.	1:150	ICN (Lisle, IL)
Urinary tract associated antigens					
J143	Cell line 253-J	IgG_1	Mouse-asc.	1:100	CRL (Cambridge, MA)
S4	Cell line SK-RC-7	IgG_{2a}	Mouse-asc.	1:100	CRL (Cambridge, MA)
S22	Cell line SK-RC-7	IgG_1	Mouse-asc.	1:100	CRL (Cambridge, MA)
S27	Cell line SK-RC-7	IgG_1	Mouse-asc.	1:100	CRL (Cambridge, MA)
F23	Kidney epithelium (GP 140)	IgG_{2a}	Mouse-asc.	1:100	CRL (Cambridge, MA)
F32	Cell line SK-RC-1	IgM	Mouse-asc.	1:10	CRL (Cambridge, MA)
Om5	Bladder TCC[g]	IgG_1	Mouse-asc.	1:10	CRL (Cambridge, MA)
T16	Cell line T-43	IgG_{2b}	Mouse-asc.	1:100	CRL (Cambridge, MA)
T43	Cell line T-24	IgG_1	Mouse-asc.	1:10	CRL (Cambridge, MA)
Muscle associated antigens					
DE-U-10	Desmin (porcine stomach)	IgG_1	Mouse-asc.	1:200	ICN (Lisle, IL)
MG-1	Human skeletal myoglobin	IgG_1	Mouse-asc.	1:400	ICN (Lisle, IL)
MY-32	Skeletal muscle myosin	IgG_1	Mouse-asc.	1:600	ICN (Lisle, IL)
B4	Actin (chicken gizzard)	IgG_1	Mouse-asc.	1:500	ICN (Lisle, IL)

[a]Abbreviations: asc. = ascites fluid; sup. = hybridoma supernatant; ser. = whole serum [b]Glial fibrillary acidic protein; [c]Epidermal growth factor receptor; [d]Parentheses enclose antigen characterization information, e.g. glycoprotein, 170 kd; [e]small cell lung cancer; [f]Neuron specific enolase; [g]Transitional cell carcinoma.

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Table III. *Expression of intermediate filaments.*

Panel	Cell line	AE-1	AE-3	Vimentin	GFAP	Neurofilament	Desmin
CNS	SF-268	0	0	100++	0	0	0
	SF-295	0	0	50+	0	0	0
	SF-539	0	0	75++	0	0	0
	SNB-19	0	0	100++	0	0	0
	SNB-75	0	0	100++	0	0	0
	SNB-78	0	0	100++	0	0	0
	U-251	0	0	100++	0	0	0
	XF-498	0	0	100++	0	0	0
Colon	COLO-205	50++	50++	0	0	0	0
	DLD-1	95++	100++	0	0	0	0
	HCC-2998	100++	0	0	0	0	0
	HCT-15	100++	95++	0	0	0	0
	HCT-116	95++	100++	0	0	0	0
	HT-29	100++	50++	0	0	0	0
	KM-12	100+	100++	0	0	0	0
	KM-20L2	100++	100+	0	0	0	0
	SW-620	75++	50++	75++	0	0	0
Lung-Ad Ca	A549	50+	100++	100+	0	0	0
	EKVX	10++	75++	75++	0	0	0
	HOP-18	100++	100++	25++	0	0	0
	HOP-62	100++	100++	90++	0	0	0
	NCI-H23	1++	1++	100++	0	0	0
	NCI-H322	40+	30+	0	0	0	0
	NCI-H522	1++	0	75++	0	0	0
Lung-Lg cell	HOP-92	50+	0	100++	0	0	0
	LXFL529L	0	0	100+	0	0	100++
	NCI-H460	20+	40++	100++	0	0	0
Lung-Squamous	NCI-H226	60+	100++	100+	0	0	0
Lung-Sm. cell	DMS 114	0	0	70+	0	0	0
	DMS 273	0	0	90+	0	0	0
Melanoma	LOX-IMVI	0	0	100+	0	0	0
	MALME-3M	0	0	100++	0	0	0
	M14	0	0	100++	0	0	0
	M19-MEL	0	0	100++	0	0	0
	SK-MEL-2	0	0	100++	0	0	0
	SK-MEL-5	0	0	100+++	0	0	0
	SK-MEL-28	0	0	100++	0	0	0
	UACC-62	0	0	100++	0	0	0
	UACC-257	0	0	100++	100	0	0
Ovary	IGROV-1	40+	60+	70++	0	0	0
	OVCAR-3	90+	70+	0	0	0	0
	OVCAR-4	80+	90+	0	0	0	0
	OVCAR-5	80+	100+	0	0	0	0
	OVCAR-8	0	0	100+	0	0	0
	SK-OV-3	40+	15+	100+	0	0	0
Kidney	ACHN	95++	50++	100+++	0	0	0
	A498	75+	0	100++	0	0	0
	CAKI-1	25+	0	100++	0	0	0
	RXF-393L	0	0	100++	0	0	0
	RXF-631L	0	0	100+++	0	0	0
	SN12-C	75++	100++	100++	0	0	0
	TK-10	95++	0	95++	0	0	0
	UO-31	90++	0	100++	0	0	0
	786-0	50++	0	80+	0	0	0

cially available kits using the peroxidase anti-peroxidase technique (Signet Laboratories, Inc., Dedham, MA) the avidin-biotin complex method (Vector Laboratories, Inc., Burlingame, CA), or alkaline phosphatase methods (BIO/CAN Scientific, Portland, ME). The three techniques gave comparable results. Negative controls consisting of isotypic immunoglobulin or ascitic fluid at a similar protein concentration to the primary antibody were run for each sample. Slides were prepared from a minimum of 2 separate cultures for each cell line. Microscopically, three separate fields, each consisting of approximately 100 cells, were selected randomly from each slide, and the percentage of positively-stained cells was counted. In addition, the relative staining intensity was qualitatively assessed on a scale of + to +++, with + representing detectable staining,

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Table IV. *Expression of adenocarcinoma associated antigents.*

Panel	Cell line	CEA	8H12	IBD12	47D10	B72.3	OC125	19-9
CNS	SF-268	0	0	0	0	0	0	0
	SF-295	0	0	0	0	0	0	0
	SF-539	0	0	0	0	0	0	0
	SNB-19	0	0	0	0	0	0	0
	SNB-75	0	0	0	0	0	0	0
	SNB-78	0	0	0	0	0	0	0
	U-251	0	0	0	0	0	0	0
	XF-498	0	0	0	0	0	0	0
Colon	COLO-205	100++	100++	100+	100+	5+	10+	100+++
	DLD-1	25++	70++	80++	5++	5+	0	80+++
	HCC-2998	0	25++	95+	5+	10+	75++	35++
	HCT-15	0	0	100++	0	0	0	0
	HCT-116	0	0	0	0	0	0	0
	HT-29	100++	100++	100++	100+	0	0	60+++
	KM-12	100++	0	80++	50++	20+	0	0
	KM-20L2	100++	50++	0	100++	5+	0	45+++
	SW-620	1++	10++	20++	50+	0	0	20++
Lung-Ad Ca	A549	10++	0	0	5++	5+	0	0
	EKVX	2++	0	0	10++	15+	95++	0
	HOP-18	100++	10++	0	100++	5+	35+	50+++
	HOP-62	0	0	0	0	0	0	0
	NCI-H23	0	0	0	0	0	0	0
	NCI-H322	0	0	0	10++	5+	60+	0
	NCI-H522	1++	0	0	5++	0	0	0
Lung-Lg cell	HOP-92	0	0	0	100+	0	0	0
	LXFL529L	0	0	0	0	0	0	0
	NCI-H460	0	0	0	0	0	0	0
Lung-Squamous	NCI-H226	0	0	0	0	0	0	0
Lung-Sm. cell	DMS 114	0	0	0	0	0	0	0
	DMS 273	0	0	0	0	0	0	
Melanoma	LOX-IMVI	0	0	0	0	0	0	0
	MALME-3M	0	0	0	0	0	0	5+
	M14	0	0	0	0	0	0	0
	M19-MEL	0	0	0	0	0	0	0
	SK-MEL-2	0	0	0	0	0	0	5+
	SK-MEL-5	0	0	0	0	0	0	0
	SK-MEL-28	0	0	0	0	0	0	0
	UACC-62	0	0	0	0	0	0	0
	UACC-257	0	0	0	100+	0	0	0
Ovary	IGROV-1	0	0	0	0	0	5+	0
	OVCAR-3	0	0	100++	0	20+	65++	25++
	OVCAR-4	1++	0	100+	0	75+	35+	0
	OVCAR-5	100++	20++	0	100++	25+	45+	95+++
	OVCAR-8	0	0	0	0	0	0	0
	SK-OV-3	0	0	0	0	25+	5+	10+
Kidney	ACHN	0	0	0	0	0	0	0
	A498	0	0	0	0	0	0	5+
	CAKI-1	0	0	0	0	0	0	30+
	RXF-393L	0	0	0	0	0	0	0
	RXF-631L	0	0	0	0	0	0	0
	SN12-C	0	0	0	0	0	0	0
	TK-10	0	0	0	0	0	0	0
	UO-31	0	0	0	0	0	0	15+
	786-0	0	0	0	0	0	0	0

and ++ strong and +++ very strong staining respectively. Results from all fields and slides for each cell line were averaged and rounded to the nearest 5%.

Histology and histochemistry. Histology and histochemistry were performed on xenografts of the cell lines. Tumors (0.5-1.0 cm) were removed from animals, fixed in 10% neutral buffered formalin and embedded in paraffin. Five µm thick sections were stained with hematoxylin-eosin, alcian blue-PAS (for differentiation of mucopolysaccharides), Kreyberg stain (for keratin), Lilie stain (for melanin), and Bodian stain (for argyrophilic substances).

Electron microscopy. Ultrastructural examination was performed on solid tumor cell lines grown in monolayer culture and on xenografts of cell

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Table V. *Expression of melanoma associated antigens.*

Panel	Cell line	S-100	R24	B5.2	BD18	L101	Ta99
CNS	SF-268	0	0	50++	0	45+	0
	SF-295	0	0	0	5+	60+	0
	SF-539	0	0	10+	0	10+	0
	SNB-19	0	0	0	0	60+	0
	SNB-75	65++	0	65+	70+++	80+	0
	SNB-78	0	0	80+	0	65+	0
	U-251	0	0	0	0	40+++	0
	XF-498	100+++	70+	85++	75++	100+++	0
Colon	COLO-205	0	0	0	0	75+	0
	DLD-1	0	0	0	0	25+	0
	HCC-2998	0	0	25+	0	70+	0
	HCT-15	0	0	0	0	0	0
	HCT-116	0	0	0	0	0	0
	HT-29	0	0	0	0	0	0
	KM-12	0	0	0	0	50+	0
	KM-20L2	0	0	0	0	0	0
	SW-620					85+	0
Lung-Ad Ca	A549	0	0	0	0	50+	0
	EKVX	0	0	0	0	85++	0
	HOP-18	0	40+	30+	10+	0	0
	HOP-62	0	0	0	0	85++	0
	NCI-H23	0	0	0	0	0	0
	NCI-H322	0	0	0	0	0	0
	NCI-H522	0	0	0	0	0	0
Lung-Lg cell	HOP-92	0	0	0	25+	50++	0
	LXFL529L	0	0	0	0	10+	0
	NCI-H460	0	0	0	0	55++	0
Lung-Squamous	NCI-H226	0	0	0	0	5+	0
Lung-Sm. cell	DMS 114	0	0	0	0	0	0
	DMS 273	0	0	0	0	25+	0
Melanoma	LOX-IMVI	0	5+	10+	0	0	0
	MALME-3M	70+++	90++	95++	50+	100+++	50+
	M14	50+	45+	90++	85++	5++	0
	M19-MEL	60+	45+	75+++	10++	75+++	35+
	SK-MEL-2	45+	45+	40++	60++	55+++	0
	SK-MEL-5	0	60+	0	25+	75+++	40+++
	SK-MEL-28	0	80+	30+	65+	90+++	10+
	UACC-62	0	60+	80+	45+	90+	0
	UACC-257	5+	30+	55++	15+++	60+++	90+++
Ovary	IGROV-1	0	0	0	0	0	0
	OVCAR-3	0	0	35+	0	70+	0
	OVCAR-4	0	0	0	0	70+	0
	OVCAR-5	0	0	0	25+	35+	0
	OVCAR-8	0	0	90+	0	100++	0
	SK-OV-3	35+	0	0	0	35+	0
Kidney	ACHN	0	0	0	0	95++	0
	A498	0	0	0	0	5+	0
	CAKI-1	0	0	0	0	80+++	0
	RXF-393L	0	0	0	0	85++	0
	RXF-631L	0	0	0	0	20+	0
	SN12-C	0	0	50+	0	40+	0
	TK-10	0	0	0	0	80+	0
	UO-31	0	0	0	0	95+++	0
	786-0	0	0	0	0	95+	0

lines. For *in vitro* lines, cells were scraped from culture vessels after the monolayers had reached 70-90% confluency. The cells were sedimented into pellets by centrifugation at approximately 200 × g for 10 minutes, and fixed in 1,25% glutaraldehyde in 0.1 M cacodylate buffer (pH=7.2) for 1 hour. For xenografts, several pieces of tissue ranging in diameter from 0.5 to 1mm were cut from non-necrotic areas and fixed for 24 hours in 2.5% glutaraldehyde in 0.1 M cacodylate buffer. Cell pellets and tissue pieces were post-fixed in osmium tetroxide, dehydrated in graded solutions of ethanol and embedded in Epon 812 resin (Fluka AG, Bucks, Switzerland). Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a JEOL JEM-100CX electron microscope using an accelerating voltage of 60 KV.

Flow cytometry of leukemia and lymphoma cell lines. Phenotypic characterization of the cell lines K-562, MOLT-4, HL-60, CCRF-CEM, RPMI 8226 and SR were conducted by flow cytometry. Cells were suspended in

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Table VI. *Expression of neuroendocrine associated antigens.*

Panel	Cell line	NSE	MOC 1	123C3
	SF-268	5+	25+	30+
	SF-295	0	0	0
	SF-539	0	0	0
	SNB-19	0	25+	50+
	SNB-75	0	40+++	75+++
	SNB-78	30+	35+	50+++
	U-251	70+	80++	90++
	XF-498L	0	50+	95+++
Colon	COLO-205	0	0	0
	DLD-1	0	0	0
	HCC-2998	0	0	0
	HCT-15	0	0	0
	HCT-116	0	0	0
	HT-29	0	0	0
	KM-12	0	0	0
	KM-20L2	0	0	0
	SW-620	0	0	0
Lung-Ad Ca	A549	0	0	0
	EKVX	65+	0	0
	HOP-18	0	0	0
	HOP-62	0	0	0
	NCI-H23	0	0	0
	NCI-H322	0	0	0
	NCI-H522	0	0	0
Lung-Lg cell	HOP-92	0	0	0
	LXFL529L	0	85++	95+++
	NCI-H460	0	55++	25++
Lung-Squamous	NCI-H226	0	0	0
Lung-Sm. cell	DMS 114	0	0	0
	DMS 273	0	5+	5+
Melanoma	LOX-IMVI	0	0	0
	MALME-3M	70+	0	0
	M14	0	5+	5+
	M19-MEL	0	45+	80+++
	SK-MEL-2	0	0	5++
	SK-MEL-5	20+	0	10+
	SK-MEL-28	0	0	0
	UACC-62	0	60+	50++
	UACC-257	45++	40+	50++
Ovary	IGROV-1	0	75+++	75++
	OVCAR-3	0	45++	75++
	OVCAR-4	0	65++	75++
	OVCAR-5	0	0	0
	OVCAR-8	0	0	0
	SK-OV-3	0	0	25+
Kidney	ACHN	0	0	50++
	A498	0	0	0
	CAKI-1	0	0	0
	RXF-393L	0	55++	75++
	RXF-631L	0	0	0
	SN12-C	40+	0	0
	TK-10	0	0	0
	UO-31	0	55++	50+++
	786-0	0	10+	50+++

phosphate buffered saline (PBS) containing 2% heat-inactivated pooled human AB serum for 5 minutes to block Fc receptors or in PBS-2% BSA for CD16 (Fc receptor III) staining. Fluorescein (FITC)-conjugated antibodies to CD2, CD3, CD4, CD5, CD10, CD16, CD19, CD25, CD45, CD57, and CD71; phycoerythrin (PE)-conjugated antibodies to CD8, CD14, CD23, CD33, CD38, CD56 and HLA-DR; and unconjugated antibodies to human IgG, IgM, kappa and lambda light chains were obtained from Becton-Dickinson Immunocytometry Systems (Mountain View, CA). FITC-conjugated anti-CD7, CD116, CD29 and CD45RA were obtained from Coulter Corporation (Hialeah, FL). FITC-conjugated antiglycophorin and unconjugated anti-CD18 were obtained from GenTrak, Inc. (Plymouth Meeting, PA). Cells incubated with unconjugated primary antibodies were washed and incubated with fluorescein-conjugated goat anti-mouse IgG (Tago, Inc., Burlingame, CA). Stained

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Table VII. *Expression of urinary tract associated antigens.*

Panel	Cell line	J143	S4	S22	S27	F23	F31	Om5	T16	T43
CNS	SF-268	70++	0	0	0	0	5+	0	0	55++
	SF-295	95++	0	0	0	0	55++	50++	0	70+++
	SF-539	55++	0	0	0	25++	0	0	0	20++
	SNB-19	95++	0	0	0	0	0	0	0	70+++
	SNB-75	85+++	30+	60+++	0	25+	85+	65+	0	85+++
	SNB-78	40++	0	0	0	15+	0	0	0	90++
	U-251	95+++	0	0	0	0	30+	0	0	50+++
	XF-498L	95+++	0	0	0	0	0	0	0	75+
Colon	COLO-205	100+	0	0	70+	0	90+	0	50+	100+++
	DLD-1	100++	10+	0	10+	0	25+	0	80++	70+++
	HCC-2998	75++	0	0	35++	0	0	0	0	75+++
	HCT-15	85++	0	0	0	0	95+	0	45++	75+++
	HCT-116	0	0	0	0	0	80+	0	0	90+++
	HT-29	95++	0	0	0	0	35+	0	95++	80+++
	KM-12	0	0	0	85+++	0	60++	0	0	90+++
	KM-20L2	95+++	5+	0	5+	0	0	0	65++	40+++
	SW-620	10+	0	0	0	0	70+	5+	0	100+++
Lung-Ad Ca	A549	100+++	0	0	0	10+	95+	0	0	100+++
	EKVX	85++	0	0	0	5+	0	5+	0	95+++
	HOP-18	80+++	0	0	0	10+	0	80+	0	80++
	HOP-62	90+++	0	50++	0	30+	80+	0	0	85+++
	NCI-H23	20+	0	0	0	0	50+	0	0	75++
	NCI-H322	85++	0	0	0	0	65+	0	85+++	95+++
	NCI-H522	0	0	0	0	0	0	0	0	40+
Lung-Lg Cell	HOP-92	60+++	0	70++	10+	20+	25+	0	32++	55++
	LXFL529L	0	0	0	0	0	0	0	0	60+
	NCI-H460	40++	0	0	0	0	40+	0	0	95++
Lung-Squamous	NCI-H226	100+++	0	0	70+	0	100+	0	0	95++
Lung-Sm. Cell	DMS 114	0	0	0	0	0	0	0	0	45+
	DMS 273	85++	0	0	0	5+	20+	0	0	25++
Melanoma	LOX-IMVI	100+++	0	0	0	50++	0	0	0	90+
	MALME-3M	90+++	0	0	0	0	0	0	0	95++
	M14	85++	0	0	0	0	0	0	0	95++
	M19-MEL	80++	0	0	0	0	65++	0	0	70+++
	SK-MEL-2	40++	0	0	0	0	40+	0	0	70++
	SK-MEL-5	15++	0	0	0	0	75++	0	0	65+++
	SK-MEL-28	55++	0	0	0	0	60+	60++	0	85+++
	UACC-62	25++	0	0	0	0	0	0	0	95++
	UACC-257	55++	0	0	0	0	65+	0	0	70+++
Ovary	IGROV-1	80+++	0	0	70+++	0	50+	0	0	55++
	OVCAR-3	55+++	0	0	5+	5+	0	0	25++	75+++
	OVCAR-4	90++	0	0	0	0	0	0	85++	75+++
	OVCAR-5	95++	0	0	0	0	80+	45++	95++	90+++
	OVCAR-8	90++	0	0	0	55+	100+	0	0	95++
	SK-OV-3	90+++	20+	0	25++	80++	60++	0	80++	60++
Kidney	A498	45++	0	0	25++	10+	95++	0	0	100+
	ACHN	100+++	0	5+	90++	0	10+	0	0	50+++
	CAKI-1	85+++	20+	95+++	15+	70+++	85+	0	15+	75+++
	RXF-393L	50++	20++	35++	50++	65++	35+	0	0	80+++
	RXF-631L	80++	0	0	5+	5+	0	0	0	95+++
	SN12-C	85+++	0	0	0	80++	0	0	0	55+
	TK-10	85+++	60+	80+++	75++	0	0	5+	0	95++
	UO-31	85+++	0	50+	0	45+	80+	0	0	85++
	786-0	85+++	90+++	55++	95++	85++	95++	0	0	100+++

cells were washed in protein-free phosphate buffered saline, fixed with buffered 1% paraformaldehyde and stored in the dark at 4°C overnight prior to analysis. Flow cytometric analysis of cells was performed on a Coulter Profile flow cytometer (Hialeah, FL) with 4 decade log amplification.

Viable cells were bitmap gated based on light scatter for analysis. Two color immunofluorescent samples were corrected for spectral overlap using color compensation. The photomultiplier tube (PMT) voltage used to analyze fluorescence signals was adjusted for each cell line bases on the intensity of autofluorescence obtained on fluorescently labelled subclass

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Table VIII. *Selected ultrastructural features of cell lines cultivated in monolayer culture and as xenografts in nude mice.*

Cell line	Cultivation method	RER[a]	Golgi	Inclusions	Surface features	Junctions	Cell/Tissue organizations
CNS							
SF-268	in vitro	+[b]	-	++fp[c]	-	groups	
	in vivo[d]						
SF-295	in vitro	+	-	+++fp	+dls	-	
	in vivo	+	-	-	-	-	
SF-539	in vitro	+	-	++fp	+dls	groups	
	in vivo[d]						
SNB-19	in vitro	+	-	+++fp	-	-	
	in vivo	+++	-	+fp	+dls	-	
SNB-75	in vitro	++	-	+++fp	-	groups	
	in vivo[d]						
SNb-78	in vitro	++	-	++fp	+dls	groups	
	in vivo[d]						
U-251	in vitro	+	-	++fp	-	-	
	in vivo	+++	-	-	+dls	chords	
XF-498	in vitro	++	-	+++fp	+dls	chords	
	in vivo	++	-	++fp	+dls	chords	
COLON							
COLO-205	in vitro	+	+	-	+++mv	+ds,za	sheets/glands
	in vivo	+	-	-	-	++ds	sheets/glands
DLD-1	in vitro	+	+	-	+mv	++ds	sheets
	in vivo	+	-	mucous	+mv	++ds	sheets
HHC-2998	in vitro	++	+	-	++mv	++ds	chords/sheets
	in vivo	+	+	-	-	++ds	chords/sheets
HCT-15	in vitro	+	+	-	+mv	++ds	sheets
	in vivo	+	-	-	-	+ds	sheets
HCT-116	in vitro	+	+	mucous	++fp	++ds	chords/sheets/glands
	in vivo	++	-	-	++mv	+++ds	chords/sheets/glands
HT-29	in vitro	+	+	mucous	++mv	++ds,za	chords/sheets/glands
	in vivo	+	++	mucous	++mv	++ds,za	chords/sheets/glands
KM-12	in vitro	+	+	-	++fp	+ds	groups
	in vivo	+	+	-	-	-	groups
KM-20L2	in vitro	+	+	mucous	++mv	++ds,za	chords/sheets/glands
	in vivo	+	-	mucous	+mv	+ds,za	chords/sheets/glands
SW-620	in vitro	+	+	-	+fp	+ds	groups
	in vivo	++	-	mucous	+mv	+ds	sheets/glands
LUNG-AD CA							
A549	in vitro	+	++	-	+fp	+ds	chords
	in vivo	+++	-	mucous	++mv	++ds	chords/sheets/glands
EKVX	in vitro	+	+	-	++mv	++ds,za	chords/sheets/glands
	in vivo	+	+	mucous	+mv	++ds	chords/sheets/glands
HOP-18	in vitro	+	++	mucous	++fp	++ds	chords/sheets
	in vivo[d]						
HOP-62	in vitro	+	++	mucous	++fp	++ds	chords/sheets
	in vivo[d]						
NCI-H23	in vitro	+	+	-	++mv	+tj	groups
	in vivo[d]						
NCI-H322	in vitro	+	-	-	++mv	+ds,za	chords/sheets/glands
	in vivo	++	-	mucous	+mv	+++ds	chords/sheets
vsMCI-H522	in vitro	+	-		+fp	++ds	sheets/glands
	in vivo	++	-		+mv	++ds	chords/sheets/glands
LUNG-LG CELL							
HOP-92	in vitro	++	+	-	+fp	-	-
	in vivo	+	+	-	-	-	-
LXFL529L	in vitro	+	++	-	+fp,mv	-	-
	in vivo	+	++	mf	+fp,mv	+dls	-
NCI-H460	in vitro	-	+++	-	++mv	+tj	groups
	in vivo	+	+	-	+mv	-	groups

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Table VIII *continued*

LUNG-SQUAMOUS							
NCI-H226	in vitro	+	+	tf	-	+tj	groups
	in vivo	+	+	tf	-	-	groups
LUNG-SM. CELL							
DMS 114	in vitro	+	-	-	-	-	-
DMS 273	in vivo[d]						
	in vitro	+	-	-	-	-	-
	in vivo	+	+	-	-	-	-
MELANOMA							
LOX-IMVI	in vitro	++	-	-	-	+ds	groups
	in vivo	-	-	-	-	++tj	chords
MALME-3M	in vitro	+	-	ms,pms	+fp	-	-
	in vivo	-	+	ms,pms	+fp	+tj	groups
M14	in vitro	+	+	pms	+fp,mv	-	-
	in vivo	+	+	pms	+fp	+tj	chords
M19-MEL	in vitro	+	++	-	+fp	-	-
	in vivo	+	+	pms	-	-	-
SK-MEL-2	in vitro	+	+	-	+fp	-	-
	in vivo	+	+	-	-	-	groups
SK-MEL-5	in vitro	+	+	ms	-	-	-
	in vivo	++	-	-	-	+tj	groups
SK-MEL-28	in vitro	+	+	-	+fp	+tj	groups
	in vivo	++	-	-	-	+tj	groups
UACC-62	in vitro	+	+	-	++fp	-	-
	in vivo	+	-	-	+fp	+tj	groups
UACC-257	in vitro	+	+	ms,pms	+fp	-	-
	in vivo	+	+	pms	-	+tj	groups
OVARY							
IGROV-1	in vitro	+	-	-	+mv	++ds	chords/sheets/glands
	in vivo	+	-	-	++mv	++ds	chords/sheets
OVCAR-3	in vitro	+	-	-	+mv	++ds,za	chords/sheets/glands
	in vivo	+	-	-	++mv	+++ds	chords/sheets/glands
OVCAR-4	in vitro	+	-	-	+mv	++ds	chords/sheets
	in vivo	+++	-	mucous	+mv	++ds,za	chords/sheets/glands
OVCAR-5	in vitro	+	++	-	+mv	++ds,za	chords/sheets/glands
	in vivo	++	-	mucous	+++mv	+++ds,za	chords/sheets/glands
OVCAR-8	in vitro	+	-	-	+mv	++ds	chords/sheets/glands
	in vivo	+	-	-	++fp	+ds	sheets
SK-OV-3	in vitro	+	+	-	+mv	++ds	chords/sheets/glands
	in vivo	+	-	-	-	+ds	chords/sheets
KIDNEY							
ACHN	in vitro	+	-	-	+fp,mv	++ds	groups/small glands
	in vivo	+	-	-	+fp,mv	+++ds	chords
A-498	in vitro	++	-	-	+++mv	+ds	chords/sheets/glands
	in vivo	+++	-	-	+mv	++ds	chords/sheets/glands
CAKI-I	in vitro	++	-	-	++mv	++ds	chords/sheets/glands
	in vivo	+++	-	-	+mv	++ds	chords/sheets/glands
RXF-393L	in vitro	+	-	-	+++mv	+ds	groups
	in vivo	++	+	-	-	+ds	chords/sheets
RXF-631L	in vitro	+	-	-	+fp	++ds	groops/chords
	in vivo	+	-	-	+fp	+++ds	chords
SN12-C	in vitro	+	-	-	+mv	+ds	groups
	in vivo	+	-	-	-	+ds	sheets/glands
TK-10	in vitro	+	-	-	++fp,+mv	+ds	-
	in vivo[d]						
UO-31	in vitro	+	-	-	++mv	++ds	chords/sheets/glands
	in vivo	+	-	-	-	-	groups
786-0	in vitro	+	-	-	+fp,mv	+ds	groups
	in vivo[d]						

[a] Rough endoplasmic reticulum
[b] + = small amount, ++ moderate amount, +++ large amount, - = absent
[c] Abbreviations: Surface features: fp = filopodia, mv = microvilli; junctions: tj = tight junctions, dls = desmosome like structure, ds = desmosomes, za = zonula adherens; Inclusions: mf = myofilaments, tf = tonofilaments, dcsg = dense cored secretory granules, ms = melanosomes, pms = premelanosomes
[d] Suitable xenograft not available

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Table IX. *Histologic features of human tumor cell lines implanted in nude mice.*

Panel	Cell line	Passage in vivo	Host connective tissue	Special stains	Degree of differentiation	Tissue organization consistent with
CNS	SF-268	1	+[a]	–	undifferentiated	glioblastoma
	SF-295	4	+	–	undifferentiated	glioblastoma
	SF-539	na[b]				
	SNB-19	2	±	–	undifferentiated	glioblastoma
	SNB-75	na				
	SNB-78	na				
	U-251	4	±	–	undifferentiated	glioblastoma
	XF-498	2	+	–	undifferentiated	glioblastoma
Colon	COLO-205	2	++	±mucin	poor	AdCa[c]
	DLD-1	9	+++	+mucin	undiff.-poor	undiffer. Ca.
	HCC-2998	5	++	+mucin	moderate-poor	AdCa
	HCT-15	2	+++	+mucin	poor-moderate	AdCa
	HCT-116	1	+	–	undifferentiated	undiffer. Ca.
	HT-29	1	+++	++mucin	moderate	AdCa
	KM-12	3	+	±mucin	poor	AdCa
	KM-20L2	2	++	+mucin	poor-moderate	AdCa
	SW-620	2	++	–	undifferentiated	undiffer. Ca.
Lung-Ad Ca	A549	8	+	±mucin	poor-moderate	AdCa
	EKVX	4	++	++mucin	moderate	AdCa
	HOP-18	1	+++	+mucin	moderate-poor	AdCa
	HOP-62	2	±	–	undifferentiated	undiffer. Ca.
	NCI-H23	6	±	–	undifferentiated	undiffer. Ca.
	NCI-H322	8	++	+mucin	moderate	AdCa
	NCI-H522	7	+	–	undifferentiated	undiffer. Ca.
Lung-Lg Cell	HOP-92	1	±	–	undifferentiated	undiffer. Ca.
	LXFL529L	1	++	–	undifferentiated	undiffer. Ca.
	NCI-H460	2	++	–	undifferentiated	undiffer. Ca.
Lung-Squamous	NCI-H226	2	+	–	undifferentiated	undiffer. Ca.
Lung-Sm. Cell	DMS 114	na				
	DMS 273	1	+	–	undifferentiated	SCLC
Melanoma	LOX-IMVI	1	–	–	undifferentiated	undiffer. Ca.
	MALME-3M	2	+	+melanin	moderate-poor	melanoma
	M14	2	+	+melanin	moderate	melanoma
	M19-MEL	2	–	–	moderate	melanoma
	SK-MEL-2	2	±	–	poor-moderate	melanoma
	SK-MEL-5	5	+++	–	moderate	melanoma
	SK-MEL-28	2	±	–	undifferentiated	melanoma
	UACC-62	2	+	–	poor	melanoma
	UACC-257	4	+	–	poor-moderate	melanoma
Ovary	IGROV-1	1	+++	+mucin	poor	AdCa
	OVCAR-3	8	++	±mucin	poor-undiffer.	AdCa
	OVCAR-4	1	++	+mucin	moderate	AdCa
	OVCAR-5	8	+++	+++mucin	well	AdCa
	OVCAR-8	1	+	–	undifferentiated	undiffer. Ca.
	SK-OV-3	2	++	–	undifferentiated	undiffer. Ca.
Kidney	ACHN	1	±	–	poor-moderate	RCCa
	A498	2	+	–	poor	tubular AdCa
	CAKI-1	2	+	–	poor	RCCa
	RXF-393L	1	+	–	poor	RCCa
	RXF-631L	1	±	–	moderate-poor	RCCa
	SN12-C	1	+	–	poor	RCCa
	TK-10	na				
	UO-31	1	+	–	undifferentiated	undiffer. Ca.
	786-0	na				

Footnotes:
[a]± very little; + small; ++ moderate; +++ large amount; – absent
[b]na: xenograft not available
[c]AdCa: adenocarcinoma; SCLC: small cell lung carcinoma; RCCa: renal cell carcinoma; Ca: carcinoma

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control samples and these controls were used to determine the percentage of positive cells.

Results

Immunocytochemical assay. Reaction of the cell lines with antibodies against various intermediate filaments is shown in Table III. Cell lines of neuroectodermal origin (central nervous system [CNS], melanoma, lung small cell) were uniformly negative for cytokeratins (AE-1 and AE-3), while lines of epithelial origin, with few exceptions, showed strong expression. Vimentin was strongly expressed by lines of neuroectodermal origin as well as most lines derived from carcinomas of the lung and kidney. Eight of 9 colon and half of the ovarian lines did not stain for vimentin. The cell lines were all negative for glial fibrillary acidic protein (GFAP), neurofilament and desmin, except for LXFL529L, which stained heavily with both anti-desmin antibodies used. LXFL529L also reacted with antimyoglobin (11+), and anti-myosin (9++) but not with anti-actin. No other cell line reacted with any of these antibodies.

Cell lines derived from CNS, lung (squamous, large or small cell) and renal tumors, and from melanomas, did not react with the antibodies against adenocarcinoma associated antigens, with few exceptions (Table IV). Most colon carcinoma lines reacted with the majority of these antibodies except for OC125. All ovarian carcinoma lines except for OVCAR-8 reacted with OC125, and showed sporadic staining with the other antibodies.

Two CNS and 5 melanoma lines were positive for S-100 protein (Table V), while all other lines were negative except for SK-OV-3. Four of the 5 antibodies developed against human melanoma cell lines were relatively specific for the melanoma and CNS lines tested, while the fifth (L101) showed broad reactivity with all cell line panels. LOX-IMVI demonstrated little reactivity with any of these antibodies.

NSE was demonstrated in 3 of the CNS and 3 of the melanoma lines but was not expressed by either of the small cell lung cancer lines or by any cell line of epithelial origin except for EKVX and SN12-C (Table VI). MOC1 and 123C3 showed similar patterns of reaction, staining a high percentage of the CNS, lung large-cell, melanoma, ovarian, and a lower proportion of the renal cell lines. Colon and lung adenocarcinoma lines did not stain. Only one of the small-cell lung cancer lines (DMS 273) reacted with these antibodies at a very low rate.

Three of the 9 antibodies (J143, F31, T43) recognizing urinary tract associated antigens, showed broad reactivity with most cell line panels, and little specificity for the renal cancer cell lines (Table VII). Reactivity of S4, S22, S27 and F23 was most concentrated in the renal panel, although S27 reacted with several of the colon and F23 with a number of the CNS and lung adenocarcinoma cell lines. T16 stained most of the colon and ovarian lines, but few from the other panels.

Ultrastructural characteristics. Selected ultrastructural features of the cell lines cultivated *in vitro* in monolayer culture and *in vivo* as xenografts in nude mice are summarized in Table VIII. For the most part, ultrastructural characteristics were comparable for a given cell line cultivated *in vitro* or *in vivo*.

The CNS lines, as a group, were typified by large oval to elongated, irregularly shaped cells containing large nuclei with peripherally clumped chromatin and prominent, often multiple nucleoli. A moderate amount of rough endoplasmic reticulum and abundant polyribosomes were usually present, and numerous filopodia were observed emanating from the surfaces. Cell-to-cell junctions were uncommon, and there was little tendency toward tissue organization. Extracellular collagen fibrils were observed in xenografts of SF-295, SNB-19 and U-251, but not in any samples from *in vitro* cultures.

Lines derived from adenocarcinomas of the colon, lung and ovaries generally showed similar ultrastructural features, although certain individual lines within each of the panels showed distinctive variations. In general, cells were moderate to large insize and oval to columnar in shape. Nuclei were relatively large with prominent nucleoli, usually singular. The cytoplasm contained small to moderate amounts of rough endoplasmic reticulum. There were distinct Golgi complexes in most lines, except in the ovarian lines in which they were mostly lacking. Several of the lines contained subpopulations with intracytoplasmic mucous granules. Microvilli were common, sometimes relatively dense, on cell surfaces. There was a strong tendency toward organization into epithelial-like tissues composed of chords and sheets of cells. Desmosomes were frequently apparent. Several of the lines demonstrated formation of multicellular glands with numerous microvilli lining the acinar surfaces of cells, and well-formed intercellular junctional complexes composed of desmosomes and zonula adherens near the luminal borders. Evidence of mucous secretion into glands was occasionally present.

Two of the cell lines derived from large-cell undifferentiated carcinoma of the lung (HOP-92, NCI-H460) were composed of large round to oval cells with large nuclei containing prominent, usually multiple, nucleoli. Small to moderate amounts of rough endoplasmic reticulum were observed in both lines. Tight-junctions or desmosomes were rare and there was little organization into multicellular structures. NCI-H226 was similar in appearance to these large cell lines with the exception that distinct intracytoplasmic tonofilament bundles were occasionally found. Cells of LXFL529L were pleomorphic, especially when grown *in vitro*. Most were large, round to oval with large irregular nuclei and multiple promiment nucleoli. A smaller percentage were extremely large and fusiform; some were biopolar or tripolar with large centrally located nucleus or nuclei, and others were monopolar with the nucleus at one end. In addition, multinucleated giant cells were frequently observed. Cytoplasmic organelles included rough endoplasmic reticulum, numerous mitochondria, prominent Golgi complexes and smooth endoplasmic reticulum. In a few cells, bundles of parallel thick and thin filaments consistent with myofilaments were observed. Desmosomes were not found, but in xenografts, desmosome-

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Table X. *Flow cytometric evaluation of leukemia and lymphoma.*

Antibody	(description)	Percent positive					
		PRMI-8226	K562	HL-60	CCRF-CEM	MOLT 4	SR
CD2	Pan T	0	0	2	58	87	4
CD3	Pan T	2	0	0	35	2	0
CD5	T Subset	ND[b]	ND	ND	68	36	ND
CD5	Pan T/B Cell Subset	0	0	0	100	100	0
CD7	T Cell/NK	ND	ND	ND	100	64	ND
CD8	T Subset	ND	ND	ND	57	59	ND
CD45	Pan Leukocyte	1	85	99	100	100	88
CD14	Pan Monocyte	0	0	3	0	0	0
CD16	FcR II, Granulocyte/NK	2	2	0	2	3	36
CD56	NK/Adhesion	97	46	0	0	3	0
CD71	Transferrin Receptor	83	80	77	99	97	15
CD33	Pan Myeloid	98	79	99	2	2	24
CD38	Broad/Plasma Cell	100	49	0	100	100	0
CD25	Activation	0	0	0	0	0	10
HLA-DR	B Cell/Activation	0	0	0	0	0	99
CD45RA	Restricted	2	56	2	3	18	48
CD29	VLA-chain/Broad	84	95	98	99	99	92
CD18	LFA B-chain	4	ND	98	ND	ND	0
CD11B	CR3/adhesion	ND	3	8	ND	ND	ND
CD23	Fc- Receptor	ND	60	ND	ND	ND	ND
CD32	IgG FcR II	ND	90	ND	ND	ND	ND
Glycophorin		ND	85	ND	ND	ND	ND
CD19	Pan B	0	0	0	0	0	0
CD10	Pre-B/Granulocytes	2	ND	ND	ND	ND	ND
PCA-1	Plasma Cell	100	ND	ND	ND	ND	ND
IgG		1	ND	ND	ND	ND	ND
IgM		0	ND	ND	ND	ND	ND
Kappa		0	ND	ND	ND	ND	ND
Lambda		28	ND	ND	ND	ND	ND

[a]Data are reported as the percentage of positive cells as determined by comparison to cells stained with fluorscently labelled subclass controls.
[b]ND=not done

like structures (opposing membrane densities without characteristic converging tonofilaments, attachment plaque and intermediate line) were occasionally encountered.

The cells of the lines derived from small cell lung carcinomas were round to oval and small to moderate in size. Nuclei were irregular to round, contained multiple nucleoli, and filled a major portion of the cells. Cytoplasmic organelles included small amounts of rough endoplasmic reticulum. Characteristic dense-cored secretory granules were not observed in either DMS 114 or DMS 273.

Melanoma lines were composed of moderately-sized round to oval cells with large nuclei often with prominent multiple nucleoli. Cytoplasmic organelles included small to moderate amounts of rough endoplasmic reticulum, Golgi complexes, numerous mitochondria, and in 5 of the lines melanosomes and/or premelanosomes. Most cell lines demonstrated the presence of tight-junctions, although desmosomes were very infrequent, and there was some organization of cells into groups, chords or sheets.

Cell lines derived from renal carcinoma contained moderate to large cells with large irregular nuclei, prominent nucleoli, abundant rough endoplasmic reticulum and mitochondria and surfaces expressing microvilli or filopodia. Cells were organized into chords and sheets with numerous desmosomes. Gland-like structures were present with 3 of the renal lines, but these lacked the junctional complexes found with the adenocarcinoma lines previously described.

Histological characteristics of xenografts. A summary of selected histological features of xenografts derived from cell lines is given in Table IX. Xenografts of CNS cell lines were heterogenous in their morphologic appearance, but all showed features consistent with glioblastoma.

Xenografts of cell lines derived from adenocarcinomas of the colon, lung and ovaries varied widely in their degrees of differentiation from well-differentiated to undifferentiated. Several lines showed organization into tubular and/or acinar-glandular arrangements, which, in more differentiated tumors, comprised the major part of the neoplasms. Evidence of intracellular and extracellular mucin was demonstrated by most of the tumors. Mitotic figures, sometimes bizarre, were common, being more frequent in the colon than in the ovarian or lung tumors.

HOP-92, NCI-H460 and NCI-H226 produced xenografts consisting of relatively large undifferentiated cells with large nucleoli showing no obvious organization of cells. Multi-

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nucleated giant cells were occasionally present. No staining for mucin or keratin was apparent, nor were there any other morphologic indicators of adenomatous or squamous differentiation. Xenografts of LXFL529L were similar in appearance to those described above, but fusiform cells were present, giant cells were more frequent and pseudo-rosette formations around capillaries were often encountered.

The xenograft of DMS 273 was composed of small round to oval cells with relatively large hyperchromatic nuclei and little cytoplasm. In less densely staining nuclei, prominent nucleoli were frequently apparent. No particular tissue organization was apparent.

Melanoma cell line xenografts consisted of moderately-sized round to oval cells containing relatively large nuclei and very prominent, often eosinophilic nucleoli. With most lines, cells were organized into chords and sheets, and M19-MEL showed some formation of alveolar structures. MALME-3M and M14 contained subpopulations staining positively for melanin.

The renal cell lines grew as poorly differentiated tumors with round to oval to spindle-shaped cells with large nuclei and prominent nucleoli. Where tissue organization was present, it was in the form of chords and tubules with occasional acinar formations. In addition, ACHN, CAKI-1, RXF-393, RXF-631L and SN12-C showed areas with some clear cell differentiation.

Flow cytometry of leukemia and lymphoma cell lines. The results of flow cytometric analysis of leukemic cell line phenotypes are summarized in Table X. The RPMI-8226 cell line was <99% positive for intense expression of the antigens PCA-1 and CD38, a pattern which is consistent with that of a plasma cell. These cells did not express the B cell antigen CD19 or HLA-DR both of which are lost from B cells upon differentiation to plasma cells. These cells were also positive for the myeloid marker CD33 and the NK cell antigen CD56, but lacked the Fc receptor CD16 which is also usually found on NK cells. The RPMI-8226 cell line did not express surface IgM, IgG or kappa light chain, but did weakly express surface lambda chain.

The K-562 myelogenous leukemia cell line expressed both the pan-leukocyte antigen CD45 and the red blood cell associated antigen glycophorin. No reactivity was observed with T or B markers or with the pan-monocyte marker CD14. Antibodies to the myeloid antigen CD33, Fc receptors CD23 and CD32, and the antigens CD56 and CD38 showed complex staining patterns with subpopulations of cells showing clear reactivity with these antibodies.

The HL-60 promyelocytic line expressed the pan-leukocyte marker CD45 and was <95% positive for the CD33 myeloid marker. Minor subsets of HL-60 cells shosed clear reactivity with the monocyte marker CD14 and with CD11B. These cells were negative for T and B cell antigen expression.

The CCRF-CEM and MOLT-4 cell lines were analyzed at the same fluorescence PMT voltages to allow direct comparison of both phenotype and fluorescence intensity since both are of T cell lineage. While both lines were CD45 positive, the CCRF-CEM line had 2-3 fold higher expression based on mean fluorescence intensity of positives. While both cell lines were 100% positive for the T cell-associated antigen CD5, expression of the CD2 and CD3 antigens differed between the two cell lines. The CCRF-CEM line contained clear subsets of CD2 positive and negative while the pattern of CD2 expression was identified as a shift in the fluorescence intensity of the entire population with no clear distinction between positives and negatives. Both lines had complex staining patterns following incubation with CD4-FITC and CD8-PE, with cells present in all quadrants of a quadstat analysis. In the CCRF-CEM line 40% of cells were identified as CD4+, CD8+ while in the MOLT-4 line the dominant subset was CD4-CD8+.

The SR cell line proved difficult to characterize by surface marker phenotype. It weakly expressed CD45 but was negative for T cell, B cell and monocyte markers. There was weak staining with the NK, granulocyte marker CD16 but the cells did not express the NK antigen CD56 and had intense expression of HLA-DR which distinguishes them from granulocytes. In contrast to the other lines tested, the SR cell line had low reactivity with anti-transferrin receptor, CD71.

Discussion

Antibody specificities. Intermediate filament expression is widely used as a reliable method of tissue identification and tumor classification (16-18). In general, cytokeratins are expressed by most neoplasms of epithelial origin, vimentin primarily by mesenchymal and some neuroectodermal tumors, GFAP by astrocytic malignancies, neurofilament triplet proteins by neural cancers, and desmin by striated, cardiac and many smooth muscle sarcomas. Antibodies AE-1 and AE-3 were selected to detect cytokeratins due to their combined broad reactivity with virtually all acidic and basic cytokeratin proteins (16). As expected, they reacted exclusively with cell lines of epithelial origin and were not detected in CNS, small-cell lung or melanoma cell lines, and were absent, or only weakly detected in some of the more undifferentiated carcinoma lines. Vimentin was strongly expressed by CNS and melanoma cell lines, and also by most members of the carcinoma panels except for the colon and the more well-differentiated ovarian lines. The expression of vimentin by cultured epithelial cells of different types is a common finding (18). GFAP was not detected in any of the cell lines tested. Decreased or total loss of GFAP expression is observed in some poorly differentiated astrocytic tumors and is a frequent finding in cell lines derived from astrocytin neoplasms. All cell lines examined were negative for neurofilament and desmin except for LXFL529L which was strongly positive for the muscle-differentiation marker desmin.

Several antibodies with specificities for adenocarcinoma-associated antigens were chosen for cell line characterizations. Carcinoembryonic antigen (CEA) is a high molecular weight surface glycoprotein expressed by the fetal gstrointes-

Table

Panel

CNS

Colon

Lung

Lung

Lung

Lung

Mela

Ovar

Kidr

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Table XI. *Comparison of original tumor diagnosis with cell line phenotypic characteristics.*

Panel	Cell line	Clinical information[a]				Cell line phenotype consistent with
		Age	Sex	Prior treatment	Tumor histology	
CNS	SF-268[b]	24	F	Rad	Astrocytoma-anaplastic	Glioblastoma
	SF-295[b]	67	F	Rad	Glioblastoma-multiforme	Glioblastoma
	SF-539[b]	34	F	Rad/BCNU/5FU/HU/6MP	Glioblastoma-multiforme	Glial cell neoplasm
	SNF-19[b]	47	M	None	Glioblastoma-multiforme	Glial cell neoplasm
	SNB-75	?	F	None	Glioblastoma-multiforme	Glial cell neoplasm
	SNB-78	?	?	?	Glioblastoma-multiforme	Glial cell neoplasm
	U-251	75	M	?	Glioblastoma-multiforme	Glioblastoma
	XF-498[b]	63	M	None	Glioblastoma-multiforme	Glioblastoma
Colon						
	Colo-205[b]	70	M	5FU	Adenocarcinoma-anaplastic	Adenocarcinoma-pd
	DLD-1	?	M	None	Adenocarcinoma-md[c]	Adenocarcinoma-vpd
	HCC-2998	?	?	?	Adenocarcinoma	Adenocarcinoma-p/md
	HCT-15	?	?	None	Adenocarcinoma-md	Adenocarcinoma-p/md
	HCT-116	?	M	?	Carcinoma	Adenocarcinoma-vpd
	HT-29[b]	44	F	None	Adenocarcinoma-md	Adenocarcinoma-md
	KM-12	?	?	None	Carcinoma	Adenocarcinoma-pd
	KM-20L2	?	?	?	Carcinoma	Adenocarcinoma-p/md
	SW-620[b]	51	M	?	Adenocarcinoma	Carcinoma-ud
Lung-Ad Ca						
	A549	58	M	?	Adenocarcinoma	Adenocarcinoma-p/md
	EKVX[b]	?	M	?	Adenocarcinoma	Adenocarcinoma-md
	HOP-18[b]	57	F	None	Adenocarcinoma-md	Adenocarcinoma-p/md
	HOP-62[b]	60	F	None	Adenocarcinoma-pd	Carcinoma-ud
	NCI-H23	?	M	None	Adenocarcinoma	Carcinoma-ud
	NCI-H322	52	M	None	Adenocarcinoma-ba	Adenocarcinoma-md
	NCI-H522	?	M	None	Adenocarcinoma	Adenocarcinoma-vpd
Lung-Lg Cell						
	HOP-92[b]	62	M	None	Large cell, ud carcinoma	Large cell ud carcinoma
	LXFL529L[b]	34	F	None	Large cell, pd, anaplastic	Rhabdomyosarcoma
	NCI-H460	?	M	?	Large cell ud carcinoma	Large cell ud carcinoma
Lung-Squamous						
	NCI-H226	?	M	None	Squamous cell carcinoma	Squamous cell carcinoma-vpd
Lung-Sm. Cell						
	DMS 114	?	M	None	Small cell lung carcinoma	Small cell lung carcinoma
	DMS 273	?	F	Rad/CCNU	Small cell lung carcinoma	Small cell lung carcinoma
Melanoma						
	LOX-IMVI	58	M	None	Melanoma-amelanotic	Carcinoma-ud
	MALME-3M[b]	43	M	None	Melanoma	Melanoma-melanotic
	M14[b]	?	?	?	Melanoma	Melanoma-melanotic
	M19-MEL	?	?	?	Melanoma-amelanotic	Melanoma-premelanotic
	SK-MEL-2[b]	60	M	None	Melanoma	Melanoma
	SK-MEL-5[b]	24	F	None	Melanoma	Melanoma-premelanotic
	SK-MEL-28[b]	51	M	None	Melanoma	Melanoma
	UACC-62	?	?	?	Melanoma	Melanoma
	UACC-257	?	?	?	Melanoma	Melanoma-melanotic
Ovary						
	IGROV-1	47	F	Rad	Cystadenocarcinoma	Adenocarcinoma-pd
	OVCAR-3[b]	60	F	CyPh/CsPt/Adr	Papillary adenocarcinoma-pd	Adenocarcinoma-md
	OVCAR-4[b]	42	F	CyPh/CsPt/Adr	Papillary adenocarcinoma-pd	Adenocarcinoma-md
	OVCAR-5[b]	67	F	None	Adenocarcinoma	Adenocarcinoma-wd
	OVCAR-8[b]	64	F	Ctx/Adr/CsPt/CyPh	Adenocarcinoma-pd	Carcinoma-ud
	SK-OV-3[b]	64	F	Thiotepa	Adenocarcinoma-papillary	Adenocarcinoma-vpd
Kidney	ACHN[b]	22	M	Rad/VB/CCNU/Mto/Pred	Renal cell carcinoma	Renal cell carcinoma, p/md
	A498	52	M	?	Renal cell carcinoma	Renal cell carcinoma-pd
	CAKI-1[b]	49	M	Rad/HU/5FU/Mtx/Ctx	Renal clear cell carcinoma	Renal cell carcinoma-pd
	RXF-393L[b]	54	M	None	Hypernephroma-pd	Renal cell carcinoma-pd
	RXF-631L[b]	54	M	None	Hypernephroma-pd	Renal cell carcinoma, m/pd
	SN12-C	43	M	None	Renal cell carcinoma	Renal cell carcinoma-pd
	TK-10[b]	43	M	None	Renal spindle cell carcinoma	Renal cell carcinoma
	UO-31	?	F	None	Renal cell carcinoma	Renal cell carcinoma-vpd
	786-O[b]	58	M	None	Renal clear cell carcinoma	Renal cell carcinoma

continued

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Table XI *continued*

Leukemia and Lymphoma						
	CCRF-CEM	4	F	Rad/Mtx/Cyx	Acute lymphoblastic leukemia	Acute lymphoblastic leukemia
	K-562	53	F	Bisulfan/PiBr	Chronic myelogenous leukemia	Chronic myelogenous leukemia
	MOLT-4	19	M	VB/6MP/Pred	Acute lymphoblastic leukemia	Acute lymphoblastic leukemia
	HL-60 TB	36	M	None	Promyelocytic leukemia	Promyelocytic leukemia
	RPMI-8226	61	M	None	Multiple myeloma	Multiple myeloma
	SR	11	M	None	Lg cell immunoblastic leukemia	Leukocytic neoplasma/pd

[a]Clinical information as reported in the literature, clinical history, or correspondence with cell line originator? Abbreviations: ?=information not available, adr=adriamycin, BCNU=Bis (chloroethyl) nitrosourea, CCNU=Chloroethylecyclohexylnitrosourea, CyPh=Cyclophosphamide, CsPt=Cisplatin, Ctx=Cytoxan, 5FU=5-fluorouracil, Hu=Hydroxyurea, Mto=Mitotone, Mtx=Methotrexate, PiBr=Piprobroma, Pred=Prednisone, Rad=Radiation, VB=Vinblastine, 6MP=6-Mercaptopurine; [b]Clinical history and/or donor correspondence available; [c]wd=well differ., md=moderately diff.? pd=poorly differ.? vpd=very poorly differ.? ud=undifferentiated.

tinal tract, normal colonic epithelium and a majority of mucus-producing adenocarcinomas of the gastrointestinal tract, lung, breast, ovary and endometrium (for review, see 19). Antibodies 8H12 (20) and IBD12, (21) raised against the human breast tumor line MCF-7, react with human tumors, or cell lines of the colon, lung, prostate and breast. IBD-12 recognizes blood group substance H, and reportedly is expressed inversely in relation to degree of differentiation in colon tumors, although this phenomenon was not observed in our study. 47D10 was developed against the bronchioalveolar adenocarcinoma cell line A549, and recognizes members of a group of surface-associated glycoproteins of 67-98 Kd, which are found in a subset of adenocarcinomas of the pancreas, colon, lung and breast (21, 22). B72.3 was raised against membrane-enriched fractions of a human mammary carcinoma metastasis (23), OC125 against human ovarian carcinoma cell line OVCAR-3 (24), and 19-9 against human colon carcinoma cell line SW-1116 (25). They have been shown to recognize distinct epitopes on human adenocarcinoma-associated mucin molecules (26). Our investigations show specificities consistent with those reported. These antibodies reacted almost exclusively with cell lines derived from adenocarcinomas. Anti-CEA, 8H12, IBD12, 47D10 and 19-9 reacted with most colon lines and some of the more differentiated ovarian lines. A few of the more differentiated lung lines also reacted with these except for 47D10. OC125 reacted with most ovarian lines and a few of those of the colon and lung.

S-100 protein (a thermolabile acidic protein of MW 20-25,000) is present in astrocytes, oligodendrocytes, ependymal cells, Schwann cells, melanocytes, many tumors arising from these cell types, as well as occasionally in carcinomas of non-neural origin (for review, see 27). This is consistent with our results. S-100 was detected in some CNS and melanoma lines, but was lacking in all others except for SK-OV-3. The other melanoma-associated antibodies were raised against various melanoma cell lines. R24 identifies G_{D3} a prominent ganglioside of neuroectodermal tissues, expressed by melanomas, but not most carcinomas (27, 28). B5.2 and L101 detect antigens expressed by melanomas and a subset of astrocytomas, but L101 reoprtedly also reacts with some human cancer cell lines of neural, renal, bladder, lung, and breast origin (27). BD18 identifies the Class II histocompatibility antigens expressed on melanoma cells but not normal melanocytes (28). Ta99 detects a pigmentation-associated glycoprotein expressed by pigmented melanocytes and melanomas, but not by unpigmented tissues (29). In the present study, only cell lines showing ultrastructural and/or histochemical evidence of melanin production, melanosomes or premelansomes reacted appreciably with Ta99. Melanoma lines, except for LOX, each reacted strongly with most or all of the other melanoma-derived antibodies. Most CNS lines reacted with B5.2. L101 reacted with most of the cell lines tested.

NSE (gamma-gamma enolase) catalyzes the interconversion of 2-phospho-D-glycerate and phosphoenolpyruvate and is found in neurons, neuroendocrine cells, and tumors of these cell types, including small-cell lung carcinomas (for reviews, see 17, 20). While this marker is very specific for these cell types, cross-reactivity of available antibodies with the heterodimer, alpha-gamma endase, is unavoidable, and NSE-like immunoreactivity has been reported in melanomas and some other tumors of non-neuroendocrine origin (17). In the present study, NSE was expressed by some CNS and melanoma cell lines, but not by any of the small-cell lung cancer lines. MOC1 (31) and 123C3 (32) were raised against a low passage small-cell line and a membrane preparation from a small-cell lung cancer respectively. In normal tissues these antibodies stain most endocrine cells and a variety of other cells including glial cells, some stromal cells and muscle (123C3 only). Both the reported to react strongly with all small-cell lung cancers and more weakly and focally with a minority of adenocarcinomas of the lung and some other organs. Staining of melanomas was not reported with MOC1. In the present study, MOC1 and 123C3 reacted with only a small percentage of the cells from one of the small-cell lung

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cancer lines. Definitive staining was observed with most of the CNS lines and some of the ovarian, renal and large-cell lung carcinoma lines, but not with lines derived from adenocarcinomas of the colon or lung. Contrary to reported specificities in human tumor specimens, the antibodies reacted strongly with several melanoma cell lines.

The panel of antibodies detecting urinary tract antigens was developed against a variety of human renal and bladder carcinoma cell lines, normal kidney epithelium and a bladder transitional cell carcinoma (33-35). Testing of these antibodies against normal human tissues and cancers of various types (33-37) showed S4, F23 and S27 to react with proximal tubular epithelium and subsets of renal cancer. T43 also reacted with proximal tubular cells and aggressive bladder cancers. J143, T16 and Om5 reacted with urothelium and subsets of bladder cancer. J143 was also positive with most other epithelial tumors. S22 was unreactive with normal tissues, but reacted with some renal carcinomas. F31 reacted with tubular epithelium and some renal cancers. Testing of the antibodies with a broad spectrum of human tumor cell lines (33-35) showed J143 an T43 to react with most cancer lines. The other antibodies while more specific for urinary cancers, also demonstrated variable reactivity with non-urinary tumor lines. Our results are consistent with these. Where common cell lines existed between these prior investigations and the present study (6 common lines), our results, for the most part, agree. Cell lines which were reported negative for specific antibodies but found to be positive in our hands include HT29 with T16, SK-OV-3 with S4, and CAKI-1 with S4. HT29 was reported positive with S27 and U251 positive with F23, but we found then both negative. These few inconsistencies might be attributable either to differences in assay techniques, or to changes in phenotypic traits of the cell lines with serial passage and adaptation to new culture conditions, or both.

Several of the cell lines lacked expression of markers which are usually associated with the tumor type from which they were derived. This is most notably exemplified by the lack of GFAP expression and only limited S-100 protein production by the CNS lines, and the absence of NSE and neurosecretory granules in the 2 small-cell lung cancer lines. Three of the CNS lines used in this study (SF-268, 295 and 539) were derived from human glioblastomas expressing GFAP, but this marker was lost during early serial passage of these and 3 other glioblastoma cell lines (7, 38). In this, and another study, however, U-251 was shown to express GFAP (39), a feature not found under our culture and assay conditions. In addition, 13 other glioblastoma lines which were investigated did not express GFAP, and none of the lines (including U-251) showed production of S-100 protein. Neurosecretory granules were reported in the small-cell lung cancer line DMS 114 (40), but these were not observed in the present study. Loss of original tumor markers by derived cell lines, and failure of cell lines to retain markers throuth serial passage may be explained on the basis of culture conditions selectively favoring growth of tumor or cell line subpopulations which do not express the specific markers, or *in vitro* conditions which do not support differentiation of characteristics found in parent cells. In any event, the importance of detailed characterization of cell lines to define their properties under existing culture conditions is apparent.

Classification of individual cell lines based on their morphologic and immunocytochemical characteristics. All of the cell lines in the CNS panel were derived from malignant glioblastomas, an anaplastic form of astrocytoma often with sarcomatous elements. While none of the lines expressed GFAP, all showed other evidence consistent with an origin from glioblastoma. SNB-75 and XF-498L produced S100 protein and reacted with all antibodies (except GFAP) recognizing glial-associated markers (B5.2, MOC1, 123C3). SF-268 and SNB-75 did not produce S100 but reacted with the other 3 antibodies, and SNB-19 and U-251 reacted only with MOC1 and 123C3. Of the lines for which xenografts were available, (SF-268, SF-295, SNB-19, U-251 and XF-498L) all revealed histologic features consistent with glioblastoma.

Adenocarcinomas of the colon, lung and ovary exhibited diverse degrees of differentiation from well-differentiated to undifferentiated. A well-differentiated cell line (OVCAR-5), and to a lesser extent moderately differentiated cell lines (HT-29, EKVX, NCI-H322, OVCAR-4), showed distinctive histological and ultrastructural evidence of gland formation in tubular or glandular patterns with abundant mucus production. There was variable, but detectable levels of CEA and most of the other adenocarcinoma-associated antigens. Poorly differentiated lines (COLO-205, KM-12, NCI-H322, IGROV-1) showed some evidence of gland formation, usually substantiated only at the ultrastructural level, and little mucus production. CEA and many of the other adenocarcinoma-associated antigens were strongly detected in poorly-differentiated colon lines but not in lung and ovarian lines. The majority of the other lines appeared poorly-differentiated to undifferentiated at the histological level, but showed convincing ultrastructural evidence of gland formation and other adenomatous features and/or definitive expression of some adenocarcinoma-associated antigens. For these reasons they were classified as very poorly differentiated adenocarcinomas. SW-620, HOP-62, NCI-H23 and OVCAR-8 were composed of undifferentiated cells with little or no tendency toward tissue organization and no other convincing evidence of adenomatous origin, and were classified as undifferentiated carcinomas.

HOP-92 and NCI-H460 showed epithelial character, but little other morphologic or immunocytochemical evidence of differentiation, features consistent with a diagnosis of large-cell undifferentiated carcinoma. NCI-H226, while otherwise similar to these lines, was characterized by well-formed tonofilament bundles in some cells, thus demonstrating squamous differentiation, and is considered to be a very poorly differentiated squamous cell carcinoma. LXFL529L was derived from a lung tumor originally diagnosed as an anaplastic large cell undifferentiated carcinoma. However,

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the cell line strongly expressed desmin, and a small but significant percentage of the cells also produced myoglobin and myosin. These characteristics, coupled with the *in vitro* morphology, the ultrastructural demonstration of myofilaments, and the lack of any markers of epithelial differentiation, provide strong evidence for a classification of rhabdomyosarcoma.

The two lines derived from small-cell lung cancers did not express cytokeratins or antigens associated with adenocarcinomas, melanomas or astrocytomas. While they did not show characteristic secretory granules or immunocytochemical markers of small-cell lung cancer, their morphological appearance was consistent with small-cell lung cancer and they were classified as such.

All melanoma cell lines (except for LOX) exhibited immunocytochemical and morphologic characteristics diagnostic of human melanoma. MALME-3M and M-14 were histochemically positive for melanin, and melanosomes or premelanosomes were ultrastructurally demonstrated in MALME-3M, M14, M19-MEL, SK-MEL-5 and UACC-257. In comparison with these other melanoma lines, LOX showed little definitive morphological or immunocytochemical evidence of melanoma origin, and has the appearance of an undifferentiated carcinoma.

Histologically, most of the renal cell lines were poorly differentiated to undifferentiated. However, acinar gland-like formations were demonstrated ultrastructurally in cell culture preparations of ACHN, CAKI-1, RXF-393L, RXF-631L and SN12-C showed localized areas demonstrating clear-cell differentiation compatible with renal origin. For two of the renal lines (TK-10, 786-0) xenografts were unobtainable, and no available histological or ultrastructural evidence clearly supported their renal origin. These were classified as renal cell carcinomas primarily based on their strong reaction with S4, S22 and S27.

All of the leukemia and lymphoma cell lines, except for SR, showed patterns of antigen expression consistent with their reported derivations. SR was untypeable with the methods used, and was classified as a poorly differentiated leukocytic neoplasm.

A comparison of the tumor diagnosis from which each cell line was derived with a phenotypic classification of individual cell lines based on their characteristics observed under the conditions of this study in shown in Table XI. For the most part, cell line characteristics were consistent with original tumor diagnoses, but substantial new information was derived concerning degree of differentiation and certain biological traits, especially in those cell lines for which little information was previously available.

In summary, this investigation has shown that among these lines, which are currently being used in a disease-oriented antitumor screen (2, 5), a majority have well-defined characteristics that appear to suitably justify their interim inclusion into designated «disease-specific» subpanels as selected representatives of major categories of human cancers of the central nervous system, colon, lung, ovary, kidney, breast and melanomas. However, there clearly were some exceptions and, further, most of the cell lines were poorly-differentiated to undifferentiated. A concentrated effort is currently underway to obtain a greater diversity of well-characterized lines more representative of the spectrum of histologic types found in the respective human carcinoma categories. Acquisition or development of such lines will be an essential prerequisite to the further refinement or expansion of the cell line panel which provides to key part of the conceptual basis for the NCI's current experiment in disease-oriented antitumor drug screening.

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