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Differential Expression of Metastasis-Associated Cell Surface Glycoproteins and mRNA in a Murine Large Cell Lymphoma

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A metastatic variant cell subline of the Abelson virus-transformed murine large lymphoma/lymphosarcoma RAW117 has been selected in vivo ten times for liver colonization. Highly metastatic subline RAW117-H10 forms greater than 200 times as many gross surface liver tumor nodules as the parental line RAW117-P. Analysis of cellular proteins and glycoproteins indicates reduced expression of murine Moloney leukemia virus-associated p15, p30, and gp70, and increased expression of a sialoglycoprotein, gp150, in the highly metastatic H10 cells. Northern analyses of oncogene expression suggested that mRNA of various oncogenes was expressed equally or not expressed in the RAW117 cells of differing metastatic potential. Differential gene expression was examined using a cDNA library of 17,600 clones established from poly A^+ mRNA isolated from H10 cells. The cDNA library was screened by the colony hybridization technique using probes made from both RAW117-P and -H10 cells. Approximately 99.5% of these cDNA clones were expressed identically in P and H10 cells. Of the few differentially expressed cDNA clones (approx. 150/17,600), one-half of these were identified as Moloney leukemia virus sequences in a separate probing with a radiolabeled Moloney leukemia virus probe. The remainder of the differentially expressed mRNA detected by colony hybridization of the cDNA library were expressed at higher levels (approx. 1/6) or lower levels (approx. 1/3) in the highly metastatic H10 cells.

Key words: tumor metastasis, gene expression, oncogenes, virus antigens, glycoproteins

Highly malignant cells express unique properties that in combination with host environment are important in metastasis formation [1–6]. Some of these unique

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properties have been identified by comparing animal tumor cells of differing metastatic behaviors [1,2,4–6]. One such tumor cell model for large cell lymphoma metastasis has been established from the Abelson murine leukemia virus (AbMLV)-transformed cell line RAW117 [7]. The parental cell line (RAW117-P) of recent origin has a very low potential to metastasize to organs such as lung, liver, spleen, and lymph nodes in BALB/c mice; however, after ten sequential in vivo selections for liver colonization, a variant subline (RAW117-H10) was established that is highly metastatic to liver and rapidly kills its host [8]. This H10 subline forms more than 200 times as many gross surface liver tumor nodules after intravenous or subcutaneous injection than does the parental line [8–10].

Comparison of the biochemical and immunological properties of RAW117-P and -H10 cells indicates that there are specific changes in the highly metastatic cells. For example, differences in the exposures of cell surface proteins [11] and glycoproteins [10,12], amounts of viral antigens [10] and lectin-binding sites [12,13], partitioning behavior in two-phase aqueous solutions [14], sensitivity to host effector systems [15,16], and presence of liver adhesion molecules [17] have been documented in the RAW117 system. We have examined whether these changes are related to the expression of oncogenes or oncogene products [18], and whether the differential expression of RAW117 genes can be used to identify the genes responsible for the malignant behavior of RAW117-H10 cells.

MATERIALS AND METHODS

Cells and Metastasis Assays

RAW117 parental (RAW117-P) cells and a subline selected ten times for liver colonization (RAW117-H10) were established and grown in Dulbecco-modified Eagle's medium (DME) containing 10% fetal bovine serum (FBS) as described previously [8–10]. Cell cultures were used within ten passages from frozen stocks of low-passage cells to eliminate possible drift in metastatic and other properties [9]. Cultures were tested for the presence of *Mycoplasmas* using Hoechst 33258 staining [19] and were found to be negative. RAW117 sublines were assayed for organ colonization by intravenous injection of 5×10^3 viable tumor cells in 0.1 ml phosphate buffered saline (PBS) [5–10]. Mice were killed at specific times after injection, and visible surface tumor nodules were counted in all major organs and were confirmed by histologic examination [12].

Cellular Glycoproteins

Chemical pretreatment of separated RAW117 glycoproteins in polyacrylamide gels and reaction of the glycoproteins with ^{125}I-labeled lectins were performed as described by Irimura and Nicolson [20,21]. The lectins used were wheat germ agglutinin (WGA), *Lens culinaris* hemagglutinin (LCH), and concanavalin A (Con A). All lectins were purified by affinity chromatography and radiolabeled as described previously [20,21]. Standard glycoproteins with known carbohydrate chain structures were separated in adjacent lanes and stained simultaneously with the same ^{125}I-labeled lectins. Binding of ^{125}I-labeled lectins to the RAW117 cellular glycoproteins and to standard glycoproteins was assessed by autoradiography [10–13].

Analysis of mRNA

Total RNA was prepared [22,23] and polyadenylated (poly A^+) mRNA was selected by oligo(dT)-cellulose chromatography [24]. Aliquots were heated at 60°C

for 10 min and were electrophoresed in a 1.1% agarose gel containing 6.5% formaldehyde as described [18,25]. The RNA was transferred onto nitrocellulose paper [26], and hybridized to nick-translated plasmid inserts containing specific sequences [18,25,27].

cDNA Library

Double-stranded complementary DNA (ds-cDNA) made from RAW117-H10 poly A^+ RNA was cloned into pBR322 according to the method of Gubler and Hoffman [28] except that the *Pst* I site was used, and annealing was by homopolymeric tailing (C-tailed insert, G-tailed vector). Colonies were established and replicated by direct contact [29]. Probes were made from RAW117-P and -H10 poly A^+ RNA by preparing ds-cDNA [28], except that ^{32}P-dCTP was added to the second strand synthesis reaction. The filters were hybridized and washed prior to autoradiography [30].

RESULTS

Intravenous (IV) injection of $1\text{–}5 \times 10^3$ RAW117-P cells into groups of BALB/c mice produced few visible lung (median = 0) or liver (median = 0) surface tumor nodules within 2 wk, whereas injection IV of similar members of RAW117-H10 cells produced large numbers of liver tumor nodules (>200 in all animals) but few lung tumor nodules (median = 1). Differences in liver tumor colonies were also found when RAW117-P and -H10 cells were injected subcutaneously (SC) into BALB/c mice [10].

The cell surface glycoproteins of RAW117 cells have been identified by sodium dodecyl sulfate (SDS)-polyacrylamide slab gel electrophoresis and labeling of the gels with ^{125}I-lectins [10,12]. Labeling the separated glycoproteins from RAW117-P and -H10 cells with ^{125}I-Con A revealed a relative decrease in a M_r ~70,000 band and a relative increase in a M_r ~150,000 band in the RAW117-H10 cells (Table I) [10]. When ^{125}I-WGA or ^{125}I-LCH was used to label the separated glycoproteins after electrophoresis, similar increases were found in the amounts of a WGA-binding sialoglycoprotein component of M_r ~130,000–200,000 and a LCH-binding component of M_r ~150,000 (Table I) [13].

The M_r ~70,000 component that is dramatically reduced in expression on RAW117-H10 cells has been identified as gp70, the major Moloney leukemia virus

TABLE I. Expression of Some Viral and Cell Surface Proteins and Glycoproteins in RAW117 Cells

RAW117 subline	Relative amounts of RAW117 component expressed in parallel experiments				
	p15	p30	gp70	gp150	sialo-gp
P	1.00[a]	1.00[a]	1.00[a], 1.00[b]	1.00[b], 1.00[c]	1.00[d]
H5	0.57[a]	0.24[a]	0.26[a], 0.3[b]	1.8[b], ND[e]	ND[e]
H10	0.03[a]	0.03[a]	0.12[a], 0.1[b]	2.5[b], 2.2[c]	2.2[d]

[a]Determined by competition radioimmune assay [10].
[b]Estimated by binding of ^{125}I-Con A to SDS gels [10].
[c]Estimated by binding of ^{125}I-LCH to SDS gels [13].
[d]Estimated by binding of ^{125}I-WGA to SDS gels [13].
[e]ND, not determined

(MoMuLV)-encoded envelope glycoprotein. Competition radioimmune assays on other MoMuLV-encoded components, such as the internal proteins p15 and p30, also revealed lower amounts in the highly metastatic H10 cells (Table I) [10]. Examination of a variety of in vivo and in vitro selected sublines and clones indicated that the loss of gp70 directly correlated ($r = 0.93$) with increased malignancy [9].

RAW117 cells express a variety of transformation-related products. For example, the oncogene p53 was found to be expressed in RAW117 cells [25]. When the expression of p53 was compared in RAW117-P and -H10 cells, however, there was no detectable difference in the expression of the p53 message (Table II) or its encoded product [25]. Examination of the AbMuLV oncogene v-*abl* also revealed its equivalent expression of RAW117 cells of low or high metastatic potential (Table II) [18]. In addition, immunoprecipitation of *abl*-encoded p160 revealed similar amounts of this protein in RAW117-P and -H10 cells [18]. Other oncogenes, such as *fos*, *myc*, and *myb* were either not expressed or equivalently expressed in RAW117 cells of low or high metastatic potential (Table II) [18].

Since several MoMuLV-encoded and other proteins but not oncogenes were found to be expressed differentially in RAW117 cells of low and high malignant potential, we examined differences in gene expression by gene cloning and colony hybridization techniques. A pBR322 cDNA library of 17,600 clones was established from poly A^+ RNA of RAW117-H10 cells [30]. The H10 gene library was screened using the colony hybridization procedure by replicate exposure of the cDNA clones to ^{32}P-cDNA prepared from the poly A^+ RNA of RAW117-P (Fig. 1) and -H10 cells (Fig. 2). Several differences in gene expression were noted in the RAW117 system (Table III) [30]. Most of these differences were attributed to MoMuLV genes, as determined in a separate probing with a ^{32}P-labeled MoMuLV probe (Fig. 3). However, some non-MoMuLV mRNAs were expressed at higher levels in RAW117-P cells, and a few were expressed at higher levels in RAW117-H10 cells (Table III).

DISCUSSION

The numbers and locations of RAW117 metastases in BALB/c mice are determined by a number of tumor cell properties. In our studies, we have found that highly metastatic RAW117 cells progressively lose expression of MoMuLV antigens and glycoproteins [9,10,13] without loss of expression of the AbMuLV oncogene *abl*. These results suggest that host antitumor responses might be involved in selecting RAW117 cells that lose viral antigens and are less sensitive to host effector mechanisms. In an examination of various host antitumor response mechanisms active against RAW117 cells, we found that these cells were equally insensitive to T-cell, NK-cell, or NC-cell-mediated responses [15]. However, when we assayed RAW117

TABLE II. Expression of Oncogenes in RAW117 Cells by Northern Analysis

RAW117 subline	Relative amounts of oncogene mRNA expressed in parallel experiments[a]				
	abl	fos	myc	myb	p53
P	+++	−	+	+	++
H10	+++	−	+	+	++

[a]−, not detectable; +, detectable; ++, expressed in moderate amounts; +++, expressed in high amounts by Northern analysis [18, 25].

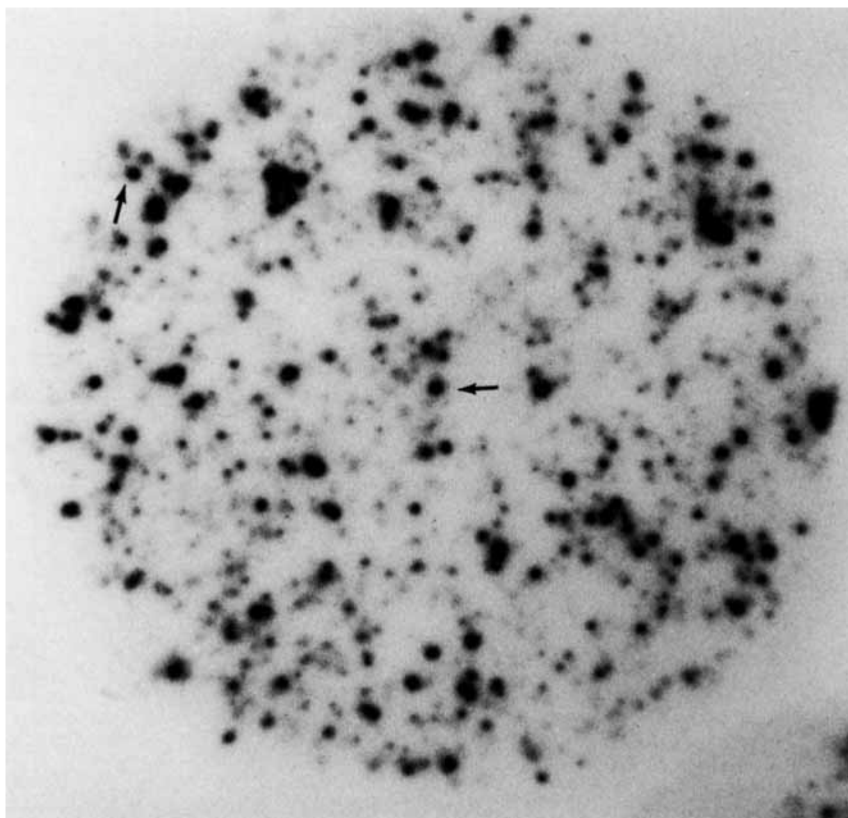

Fig. 1. Colony hybridization of library filter with RAW117-P cDNA (approximately ×1.32 life size). [^{32}P]dCTP-labeled probe derived from RAW117-P poly A$^+$ RNA was incubated with cloned RAW117-H10 cDNA fixed to nylon filters to detect homologous sequences. After subsequent autoradiography, the probe was stripped off to allow reprobing. Arrows indicate differentially hybridizing colonies.

cells for their sensitivities to macrophage-mediated cytolysis and cytostasis, we found that the highly metastatic RAW117-H10 cells were significantly less sensitive to macrophage-mediated responses [16]. Indeed, impairment of macrophage-mediated antitumor responses by administration of chlorine, silica, trypan blue, carrageenan, cyclosphosphamide, or pristane to animals before injection of RAW117 cells increased the malignancies of the low metastatic RAW117-P line [15]. In contrast to our findings, Thorgeirsson et al [31] found that NIH/3T3 cells transfected with T24 c-H-*ras* genes were metastatic in nude mice and were more sensitive to NK- and macrophage-mediated cytotoxicity than control NIH/3T3 cells.

Host antitumor responses may be important in determining the degree of metastasis in some tumor systems, but they are probably not very important in determining the locations of metastases [9,15]. The liver-selected RAW117-H series probably colonies liver, in part, because of an increased expression of liver-binding cell surface receptor(s) [9,17]. Blocking these surface receptors with F(ab')$_2$ antibodies abolishes the ability of RAW117-H10 cells to colonize liver, but not lung [17]. In addition to cell surface receptors involved in organ homing, RAW117 cells selected for liver colonization also show differential growth characteristics in liver-conditioned me-

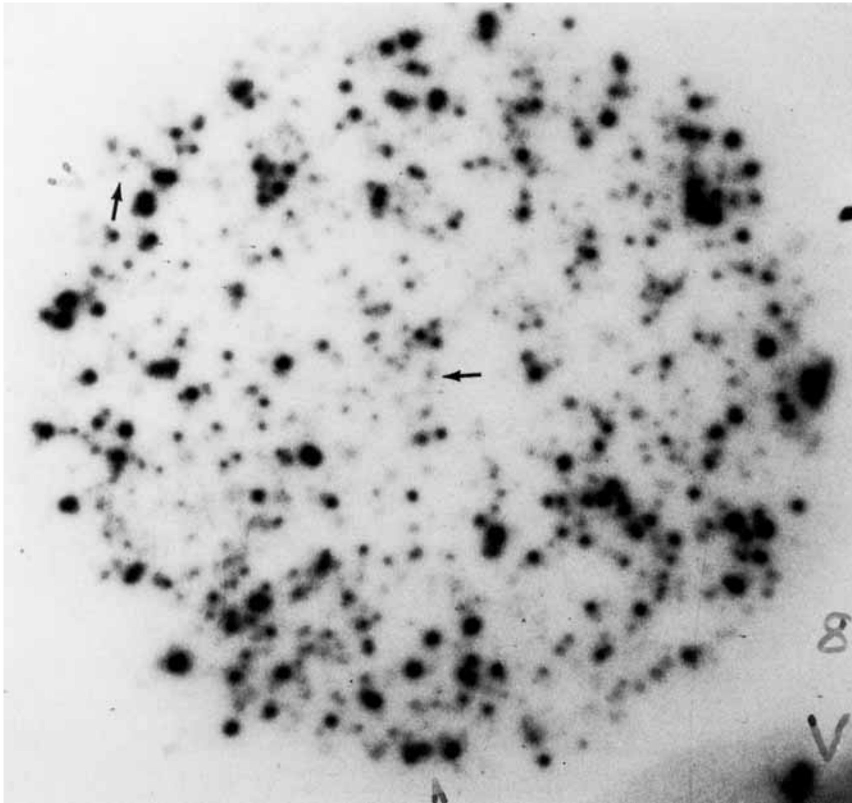

Fig. 2. Colony hybridization of library filter with RAW117-H10 cDNA (approximately ×1.32 life size). Procedure was the same as in Figure 1 except that the probe was derived from RAW117-H10 poly A^+ RNA. Arrows indicate differentially hybridizing colonies.

TABLE III. Differential Gene Expression in RAW117 Cells by Colony Hybridization Analysis

Type	No. colonies	Percentage of abundance
Total library	17,600	100.00
Estimated differentially expressed	~160	~0.9
Estimated total MoMuLV	~75	~0.43
Estimated non-MoMuLV higher in P	~65	~0.36
Estimated non-MoMuLV higher in H10	~20	~0.12

dium, suggesting that the growth of metastatic cells is also regulated, in part, by organ microenvironment [32–34].

We have found that the increased abilities of RAW117 cells to metastasize to liver is not related to oncogene (*abl*, *fos*, *myc*, p53) expression [18,25]. Although examination of advanced neuroblastomas [35] and lung cancers [36] suggested that oncogene expression might be related to malignant properties, we have not found any evidence in the RAW117 system to support this possibility. In other metastatic tumor systems that have been examined for expression of oncogenes, differences were also not found [37], suggesting that overexpression of oncogenes is not a requirement for expression of the highly metastatic phenotype. Once cells are transformed, additional

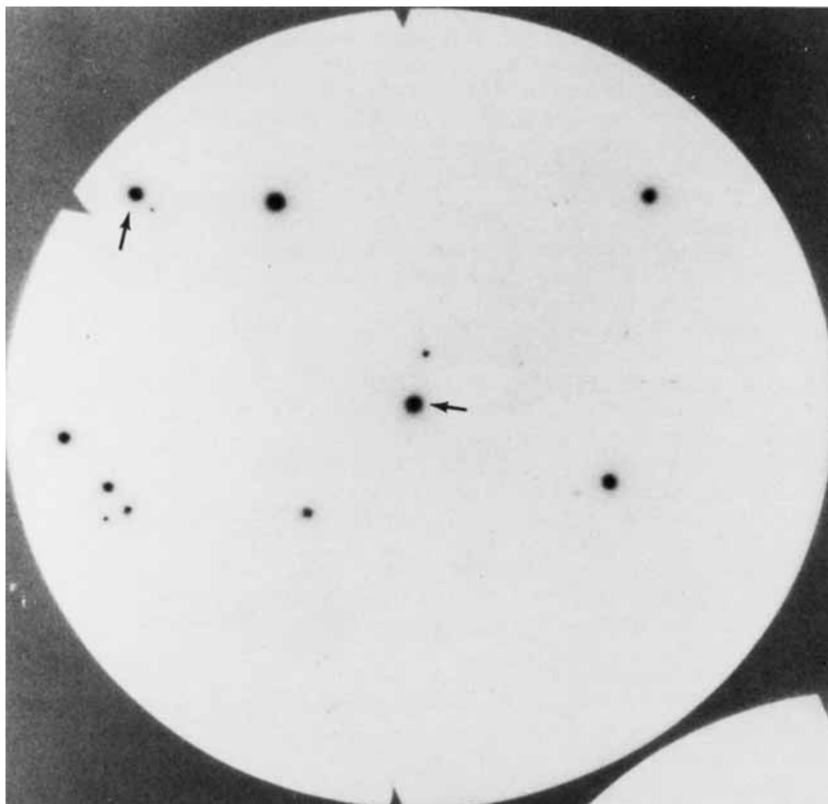

Fig. 3. Colony hybridization of library filter with MoMuLV cDNA (approximately ×1.32 life size). [^{32}P]-labeled probe derived from cloned Moloney leukemia virus cDNA was incubated with cloned RAW117-H10 cDNA fixed to nylon filters to detect homologous sequences. Arrows indicate colonies that exhibited differential hybridization between RAW117-P and RAW117-H10 probes.

expression of oncogenes may not be required to achieve the metastatic phenotype [38,39].

We have identified several non-MoMuLV mRNAs that are differentially expressed in RAW117-P and -H10 cells. Presumably, some of these mRNAs code for proteins and glycoproteins whose expression is required to maintain the highly metastatic phenotype. Determining the identity of the encoded products of these differentially expressed genes could yield additional insights into the components and properties required for a cell to achieve the metastatic state.

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