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Effect of retinoic acid on human neuroblastoma:

Correlation between morphological differentiation and changes in plasminogen activator and inhibitor activity

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Summary. The relationship between plasminogen activator (PA)/plasminogen activator inhibitor (PAI) activity and morphological differentiation was investigated in human neuroblastoma (NB) cells treated with retinoic acid (RA). Conditioned medium from nine NB cell lines and one closely related neuroepithelioma line was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and zymography. All NB cell lines were shown to secrete urokinase (UK)-type PA (mol. wt., 52 kDa), and all except two produced tissue PA (mol. wt., 65 kDa). Identification of the PAs was made based on molecular weight and sensitivity to inhibition by anti-UK and anti-tPA antibodies. Several cell lines expressed PA inhibitory molecules; two molecular-weight forms were observed (35 and 40 kDa) in different cell lines. Complex formation with $^{[125]}$I-labelled proteases revealed specific binding with UK and trypsin but not thrombin, plasmin, or kallikrein. After treatment for 6 days with 1 μ*M* RA, six of the cell lines exhibited an increase in cell-associated and/or secreted tPA activity, corresponding to morphological differentiation of the cells as manifested by extensive neurite outgrowth. A decrease in UK and UK-complex secretion was observed in several of these cell lines. Three cell lines exhibiting no detectable morphological alterations with RA treatment also showed no dramatic changes in PA/PAI activity. These results suggest that morphological differentiation of NB cells may be associated with alterations in the regulation of PA activity.

Introduction

Neuroblastoma (NB) is a common solid tumor of childhood, arising from the sympathetic part of the autonomic nervous system [3]. One of the interesting features of this tumor is its high rate of spontaneous regression and/or ability to mature spontaneously to benign ganglioneuroma [1]. The in vitro treatment of NB cell lines with various agents such as retinoic acid (RA) and dibutyryl cAMP induces cell differentiation typically characterized by extensive neurite outgrowth and cell-growth inhibition. The morphological changes induced by maturational agents in cultured cells appear to parallel the clinical observations, thus enabling the investigation of naturally occurring features of the tumor as well as the effects of potential therapeutic agents.

Many tumor cells secrete high levels of plasminogen activators (PA) compared with their untransformed counterparts [8, 16]. Two main types of biochemically and immunologically distinct PAs have been described: urokinase-type (UK) and tissue-type (tPA). UK is important in cell migration and tissue remodelling, whereas tPA is a fibrin-dependent enzyme that is critical to vascular homeostasis. The regulation of fibrinolytic enzymes occurs through specific protease inhibitors acting on plasmin and PAs. Plasminogen activator inhibitors (PAIs) can be classified into four immunologically distinct types: the endothelial cell type (PAI-1), the placental type (PAI-2), the urinary type (PAI-3) and protease nexin (PN) (reviewed in [16]).

Alterations in PA and PAI activity have been reported to occur during differentiation in a number of diverse, malignant cell types, including melanoma [17], bladder carcinoma [22], HeLa cells [33] and others [16]. When human glioma cells were treated with various maturational agents, decreased PA expression was observed [14]. In general, inhibition of PA was correlated with the induction of a more benign phenotype (i.e., reduced clonogenicity of glioma cells in agar). In contrast, differentiation of NB cell lines has been associated with increased PA expression [2, 10, 20, 29]. This report describes a correlation between neurite outgrowth and PA/PAI regulation and presents preliminary characterization of the fibrinolytic enzymes produced by a number of NB cell lines.

Materials and methods

Reagents. All trans-RA, thrombin, trypsin, plasmin and pancreatic kallikrein were obtained from Sigma Chemical Co. (St. Louis, Mo). UK and anti-human UK IgG were purchased from Alpha Therapeutics (Los Angeles, Calif). Human tPA and anti-human tPA IgG were obtained from American Diagnostics (Greenwich, Conn). All reagents for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were obtained from Bio-Rad (Mississauga,

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Abbreviations: NB, neuroblastoma; RA, retinoic acid; PA, plasminogen activator; PAI, plasminogen activator inhibitor; SDS, sodium dodecyl sulfate; UK, urokinase; tPA, tissue plasminogen activator

Ontario). Heparin-Sepharose CL-6B was obtained from Pharmacia (Dorval, Quebec). [^{3}H]-thymidine (42 Ci/m*M*) and Na[^{125}I] were purchased from Amersham (Arlington Heights, Ill). All reagents for zymography were obtained from sources described elsewhere [7]. Diisopropylfluorophosphate (DFP) and 3-[(3-cholamidopropyl)dimethylammonia] 1-propanesulfonate (CHAPS) were obtained from Sigma Chemical Co. (St. Louis, Mo).

Cell culture. Cell lines used in this study included SMS-KAN and SMS-KCNR (courtesy of Dr. C. P. Reynolds, Bethesda Naval Research Hospital, Bethesda, Md); IMR-5 and NMB/N7 (both courtesy of Dr. F. Gilbert, Mt. Sinai Hospital, New York, NY); La-N-1 and LA-N-2 (courtesy of Dr. R. Seeger, UCLA, California); CHP-126 (courtesy of Dr. J. Roder, Mt.Sinai Research Institute, Toronto, Ontario); and IMR-32, SK-N-SH and SK-N-MC (American Type Culture Collection). All cell lines used in these experiments were mycoplasma-free. All NB cell lines were grown in RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (Hyclone Laboratories, Logan, Utah). The cells were maintained in a humidified atmosphere of 95% air-5% CO_2 at 37° C.

Preparation of cells for PA assays. The NB cells were grown as adherent monolayers in 75-cm^2 tissue-culture flasks (Corning). To prepare conditioned medium (CM), 60%–70% confluent monolayers were washed twice with serum-free medium and placed in culture with RPMI 1640. After incubation for 24 h, the medium was removed and concentrated tenfold by Amicon ultrafiltration (UM10). Lysates were prepared by treating adherent cells with 20 m*M* CHAPS dissolved in phosphate-buffered saline (PBS; pH 7.4). Preliminary experiments were carried out to determine the concentration of RA yielding optimal morphological differentiation. The concentration that induced maximal increase in the formation of neurites in these cell lines amounted to 1 μ*M* RA. Parallel cultures of each cell line were prepared and treated with either 0.1% ethanol (control) or 1 μ*M* RA (dissolved in ethanol) for 3 and 6 days, respectively. This concentration of ethanol had no detectable effect on cell growth, differentiation or fibrinolytic enzyme activity. The medium was removed after 3 days and replaced with fresh medium (RA or ethanol control) and CM, and lysates were prepared as described above after 6 days.

Assay for inhibition of DNA synthesis. To determine the effects of RA on DNA synthesis, 10^4 cells/well were plated in quadruplicate in 96-well tissue-culture plates (Falcon). Cells were treated with RA or ethanol and pulsed with 1 μCi [^{3}H]-thymidine/well after various intervals. After 18 h incubation at 37° C, the cells were harvested and incorporated [^{3}H] was determined with a scintillation counter (Beckman).

PA and PAI analysis. PAs and PAIs were detected by SDS-PAGE followed by zymography [13]. Human UK standards and/or human urine samples were included in all assays as controls and reference points. Human urine was prepared as previously described [18]. Human plasminogen was purified from DFP-treated plasma according to the method of Deutsch and Mertz [9]. In specific experiments anti-PA antibodies were included in the zymography plates as described previously [7, 18, 22]. A total of 200 ml/CM from each of the SK-N-SH and SMS-KAN cell lines was tested for binding to heparin-Sepharose. The column (20 × 2.5 cm) was washed with 0.3 *M* NaCl in 20 m*M* sodium phosphate buffer (pH 7.4) before and after CM was loaded. Elution was carried out with 1.0 *M* NaCl in the same buffer and protein-containing fractions (measured by absorbance at 280 nm) were collected.

Iodination of proteases and autoradiography. UK, thrombin, trypsin, plasmin and kallikrein were labelled with [^{125}I] as previously described [7]. [^{125}I]-labelled proteases were incubated with CM prior to SDS-PAGE for 30 min at 37° C and analyzed alongside Bio-Rad low-molecular-weight protein standards. After SDS-PAGE, gels were dried and placed under Kodak XO Mat film for 48–72 h. Alternatively, [^{125}I]-labelled proteases were added to cell cultures concomitantly with serum-free medium, and 24-h CM was analyzed by SDS-PAGE and autoradiography.

Results

Cell culture

Eight human neuroblastoma cell lines derived from different patients were used in this study. IMR-5 is a subclone of IMR-32. SK-N-MC has recently been reclassified as a neuroepithelioma cell line based on morphology, HL-A expression and cytogenetic rearrangements [25]. Table 1 outlines several features of the cell lines with respect to the

Table 1. General characteristics of human NB cell lines

Cell line	Site of origin	Adr/Chol	Morphological features
LA-N-1	Bone marrow	Adr	Mixture of neuronal and substrate-adherent phenotypes [27]
LA-N-2	Bone marrow	Mixed	Predominantly neuronal, teardrop-shaped cells [26, 27]
SMS-KAN	Ovarian tumor	Adr	Small, polygonal cells; grow in clumps [24]
SMS-KCNR	Bone marrow after chemotherapy	Adr	Similar to SMS-KAN, except cells are smaller [24]
IMR-32	Abdominal mass	Adr	Single cells; neuronal with long processes [31]
IMR-5	Abdominal mass	Adr	Small, neuronal-shaped cells in large clumps (subclone of IMR-32)
NMB/N7	?	?	Identical to IMR-32, but derived from a different patient [6]
SK-N-SH	Bone marrow	Adr	Mixture of 50% flat, fibroblast-like cells/50% neuronal [4]
SK-N-MC	Supra-orbital	Chol	Predominantly small, round, neuroblastic cells with 20% flat cells [5]
CHP-126	Bone marrow	?	Identical to IMR-5, but derived from a different patient [26]

Adr, adrenergic; Chol, cholinergic; ?, no data available

site of origin of the tumor, adrenergic/cholinergic activity and their morphological characteristics in vitro. In addition, these cell lines varied dramatically in growth rate, with the most strongly substrate-adherent cells (IMR-32, SK-N-SH, NMB/N7, IMR-5, CHP-126) reaching confluence most quickly.

PA and PAI activity

Serum-free CM from the human NB cell lines was analyzed by SDS-PAGE followed by zymography (Fig. 1). As summarized in Table 2, all cell lines secreted a 52-kDa protein that comigrated with human high-molecular-weight UK (HMW-UK) and was inhibited by the inclusion of rabbit anti-human UK antibodies into the indicator gel. CM from eight of the cell lines contained a 65-kDa Pa that comigrated with human tPA and was inhibited by rabbit anti-human tPA antibodies (data not shown). Additional experiments revealed that the enzymatic activities in the CM were plasminogen-dependent, further supporting the identification of these bands as UK and tPA (data not shown).

The presence of PAI in the CM was detected by preincubating 100 µl CM with 1 unit low-molecular-weight UK (LMW-UK; mol. wt., 33 kDa), followed by SDS-PAGE and zymography. In five of the cell lines (SMS-KAN, IMR-5, LA-N-1, LA-N-2, SK-N-MC), preincubation with LMW-UK yielded a fibrinolytically active band at 68 kDa, suggesting the presence of a 35-kDa UK-binding protein in the CM. An LMW-UK complex of slightly higher molecular weight (75 kDa) was detected in CM from SK-N-SH, NMB/N7 and IMR-32; the addition of anti-UK antibodies to the indicator gels inhibited the detection of these complexes. Higher-molecular-weight bands (approximately 95 and 105 kDa) were detected in CM and lysates from several cell lines as well; these bands probably represent complexes of PAI with endogenous HMW-UK and tPA, respectively (reviewed in [16]).

Specificity of the NB-derived PAI(s)

To determine the specificity of the NB-derived PAI(s), radioactively labelled proteases were prepared (UK, throm-

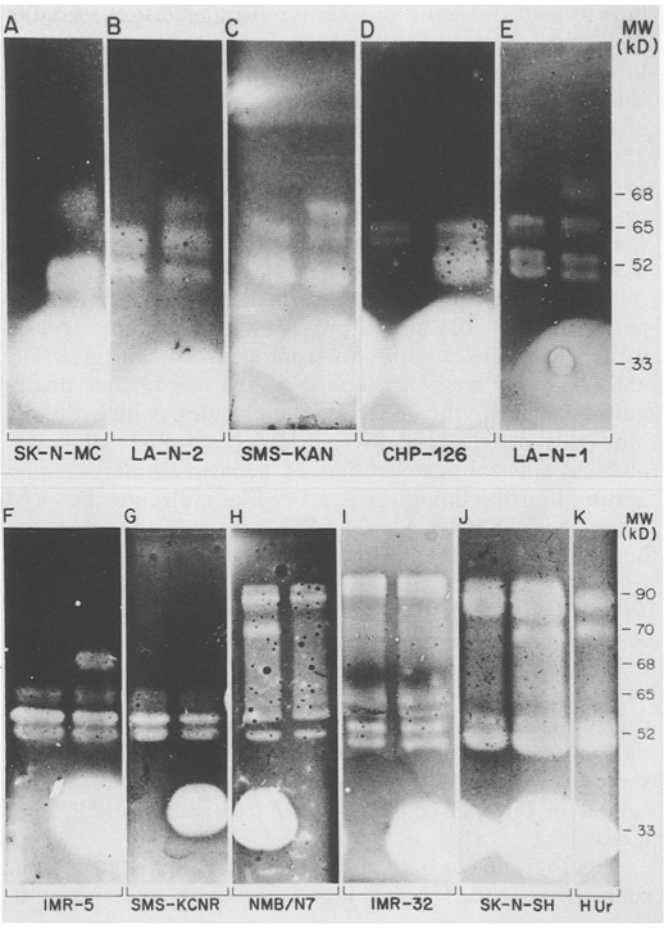

Fig. 1. Zymographic analysis of conditioned medium from ten human NB cell lines. Cells were incubated in serum-free medium and 100 µl concentrated CM was analyzed by SDS-PAGE and zymography. For each cell line, CM was assayed without (*left lane*) or with (*right lane*) prior incubation with low-molecular-weight urokinase (LMW-UK: 33 kDa) for detection of UK-binding proteins. *A*, SK-N-MC; *B*, LA-N-2; *C*, SMS-KAN; *D*, CHP-126; *E*, LA-N-1; *F*, IMR-5; *G*, SMS-KCNR; *H*, NMB/N7; *I*, IMR-32; *J*, SK-N-SH; *K*, dialyzed human urine (H Ur)

Table 2. Plasminogen activator and inhibitor activity in conditioned medium from human NB cell lines

Cell line	HMW-UK (52 kDa)	tPA (65 kDa)	PAI-complex (68 kDa)[a]	PAI-complex (75 kDa)[a]	PAI-complex (90 kDa)[b]
LA-N-1	+[c]	+	+	−	−
LA-N-2	+	+	+	−	−
CHP 126	+	+	−	−	−
SMS-KAN	+	+	+	−	+/−
SMS-KCNR	+	+	−	−	−
NMB/N7	+	+	−	+	+
IMR-5	+	+	+	−	+/−
IMR-32	+	+	−	+	+
SK-N-SH	+	−	−	+	+
SK-N-MC	+	−	+	−	−

[a] PAI + exogenous LMW-UK
[b] PAI + endogenous HMW-UK
[c] Conditioned medium from each cell line was prepared and assayed as described in *Materials and methods*. The presence (+) or absence (−) of PA and PAI-complex activity is indicated

bin, trypsin, plasmin and kallikrein) and tested for complex-forming ability with CM from several of the NB cell lines. When iodinated LMW-UK was added directly to the cells or CM, a shift in radioactivity from 33 to 68 kDa was observed with SMS-KAN and IMR-5, supporting the above-described indication that these cells secrete a 35-kDa UK-binding protein (data not shown). The inhibitor secreted by SK-N-SH formed a complex with UK that was observed zymographically to run slightly higher than that of the previous two cell lines (PAI mol. wt., 40 kDa); this molecular weight was confirmed by the iodination experiments. Of the other proteases tested, only trypsin formed complexes with CM from all three cell lines. The ability of PN to bind heparin-Sepharose further distinguishes it from the other PAI molecules, which do not bind this matrix [16]. SK-N-SH-derived PAI (mol. wt., 40 kDa) was found to adhere to heparin-Sepharose, suggesting that this inhibitor is a PN-like molecule. The PAI produced by SMS-KAN (35 kDa) did not bind to heparin-Sepharose (data not shown).

Effect of RA on NB cell lines

RA induces differentiation of NB cell lines to several different phenotypes [23]. Typically, differentiation is indicated by extensive neurite outgrowth and an inhibition of cell proliferation [28], although in some cell lines differentiation to a Schwannian/melanocytic "flat cell" is observed. Table 3 outlines the changes in morphology observed in the NB cell lines after treatment with RA for 6 days.

Photographs of both control and RA-treated cells (four representative cell lines) taken at 6 days are shown in Fig. 2. Although several cell lines were shown to be resistant to morphological differentiation induced by RA, axonal outgrowth was evident in the majority of lines tested. SK-N-SH ws an exception in that the cells changed from a mixed neuronal/flat-cell morphology to a predominately flat-cell phenotype with RA treatment.

Table 3. Morphological changes observed in NB cell lines after treatment with RA for 6 days

Cell line	Neurite outgrowth	Morphological alterations
LA-N-1	1+ → 3+[a]	Extensive neurite outgrowth involving most cells
LA-N-2	1+ → 2+	Limited neurite outgrowth
SMS-KAN	0 → 4+	Cells spread and flatten Numerous short neurite extensions from individual cells
SMS-KCNR	0 → 3+	Same as SMS-KAN
IMR-5	1+ → 3+	Axonal extensions from cells at edges of large clumps
IMR-32	ND	Neuronal morphology No detectable changes
SK-N-SH	ND	Mixed neuronal/flat morphology to 100% flat cells
SK-N-MC	ND	Neuroblastic, round cells No detectable changes
NMB/N7	ND	Same as IMR-32
CHP-126	1+ → 3+	Same as IMR-5

[a] Extent of morphological differentiation: control cultures→ RA-treated cultures. Cells were scored according to the degree of neurite outgrowth (processes at least twice as long as the soma diameter) and reported as the percentage of differentiated cells: 4+, >76%; 3+, 51%–75%; 2+, 26%–50%; 1+, 5%–25%; 0, <5% ND, none detected

The effect of RA on the inhibition of DNA synthesis was analyzed in four representative cell lines by the incorporation of [^{3}H]-thymidine after 1, 3 and 6 days. As shown in Fig. 3, SMS-KAN was most sensitive to growth inhibition by RA, whereas IMR-5 showed a slight reduction in DNA synthesis. SK-N-MC was completely resistant to this agent, and SK-N-SH cells actually showed slightly higher proliferation in cells treated for 6 days.

Modulation of fibrinolytic activity of NB cells with RA

In light of the variability in morphological response to RA of the different NB cell lines, it was of interest to compare these results to RA-induced changes in PA and PAI activity of the same cell lines. Table 4 presents the results of zymographic analysis of the CM and cell lysates, indicating shifts in PA/PAI activity observed after 6 days of RA treatment. After treatment for 6 days with the differentiating agent, six of the NB cell lines exhibited an apparent increase in secreted and cell-associated tPA activity, corresponding with morphological differentiation of the cells. In several of these cell lines, a decrease in UK secretion and UK-inhibitor-complex formation accompanied the up-regulation of tPA (LA-N-1, IMR-5, SMS-KAN). In addition, these cell lines were generally most susceptible to growth inhibition by RA, suggesting that PA activity on a per-flask basis would represent a lower number of cells. In one cell line (SK-N-SH), conversion to the flat-cell phenotype correlated with an increase in cell-associated and secreted UK as well as UK-inhibitor-complex activity. In cell lines exhibiting no detectable morphological alterations on exposure to RA, no changes in fibrinolytic enzyme activity were observed (SK-N-MC, IMR-32, NMB/N7).

Discussion

Cultured NB cells provide an excellent model with which to study aspects of both tumor and neuronal cell behavior. NB cell lines derived from rat, mouse and human sources have been reported to secrete high levels of PA in vitro [2, 11]. Enhanced levels of PA have been associated with tumor invasion and metastasis [8] and may thus be an important aspect of the malignant behavior of NB.

In this study, we analyzed a panel of human NB cell lines and one neuroepithelioma cell line for PA and PAI activity. All cell lines secreted UK and all except two produced tPA. However, considerable variability in PA activity, both quantitative and qualitative, was observed between the cell lines. This finding is not surprising, in light of the well-established heterogeneity of NB cell lines with respect to morphology, growth characteristics and many other biochemical parameters. No correlation between PA or PAI expression and the cholinergic/adrenergic nature of the cell line could be established. PA inhibitory activity has been described for brain tumors such as rat C6 glioma [12] and human glioma cell lines [9, 14]. Here, we provide evidence for PAI activity in the neural crest-derived tumor, neuroblastoma. Preliminary characterization of the

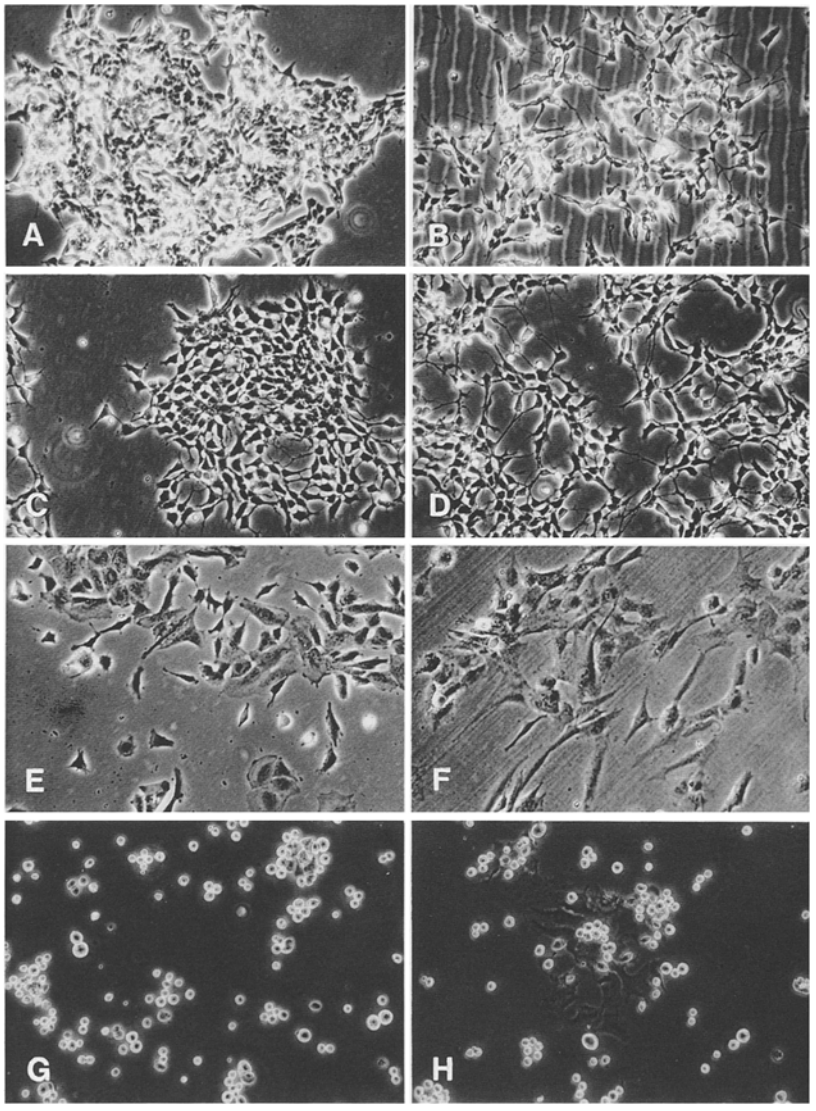

Fig. 2. Alterations in morphology observed in four NB cell lines treated with RA. *A*, SMS-KAN control; *B*, SMS-KAN, RA-treated; *C*, IMR-5 control; *D*, IMR-5, RA-treated; *E*, SK-N-SH control; *F*, SK-N-SH, RA-treated; *G*, *H*, SK-N-MC cells. The morphology of SK-N-MC cells after treatment was essentially identical to that of control cells; the two different morphologies observed in these cultures are demonstrated (phase contrast, × 100)

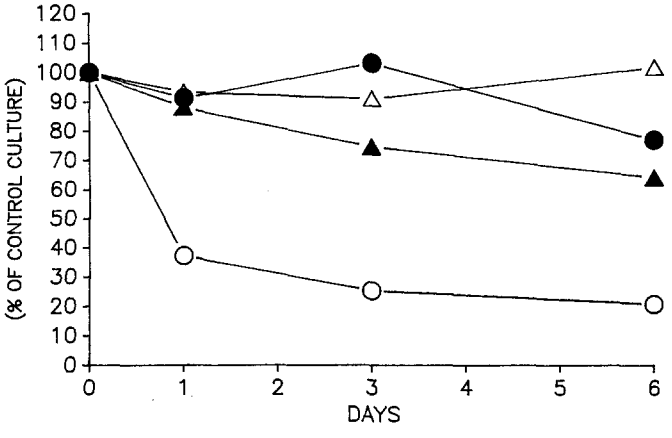

Fig. 3. Time course of [^{3}H]-thymidine incorporation in four NB cell lines treated with RA. Time-course experiment showing the effect of RA on DNA synthesis in four NB cell lines. [^{3}H]-Thymidine incorporation was measured after 1, 3 and 6 days of treatment in ●, SK-N-MC; △, SK-N-SH; ○, SMS-KAN; and ▲, IMR-5 cells. Values represent the average of quadruplicate cultures (SE < 10%)

NB-derived PAI(s) has revealed two different molecular-weight forms secreted by individual cell lines. The 40-kDa PAI secreted by SK-N-SH cells is likely to be a protease nexin, as it has a high affinity for heparin-Sepharose. The SMS-KAN-derived PAI has a lower molecular weight (35 kDa) and does not bind heparin-Sepharose. Preliminary experiments with mRNA from this cell line have indicated that the PA inhibitor produced by these cells is cross-reactive with a PAI-2-specific probe (unpublished observations). However, the specificity of both inhibitors for UK and trypsin but not plasmin, thrombin or kallikrein distinguishes them from other reported PAI molecules.

The mechanism by which chemical agents induce differentiation is not fully understood. One possibility that cannot be excluded from this study is that agents such as RA promote selective outgrowth of subpopulations of RA-resistant cells, resulting in the observed changes. However, it is noteworthy that at least one of our cell lines (IMR-5) is a clone of the line IMR-32 and thus not likely to be a mixture of cell types. Differentiation induction in this line produced changes in tPA levels similar to those in other

Table 4. Changes in PA/PAI activity of NB cell lines treated with RA for 6 days

Cell line	Secreted:				Cell-associated:			
	HMW-UK	tPA	LC[a]	HC[b]	HMW-UK	tPA	LC	HC
LA-N-1	↓↓[c]	↑	↓	ND	NC	↑↑	NC	ND
LA-N-2	NC	NC	NC	ND	ND	↑↑	ND	ND
SMS-KAN	↓↓	↑↑	↓↓	ND	NC	↑↑	NC	ND
SMS-KCNR	↓↓	NC	ND	ND	NC	↑↑	ND	ND
IMR-5	NC	↑	NC	NC	NC	↑↑	ND	ND
IMR-32	NC	↑	NC	NC	NC	NC	ND	ND
SK-N-SH	↑↑	ND	↑↑	↑↑	↑↑	ND	ND	↑↑
SK-N-MC	↑	ND	↑	ND	NC	ND	NC	ND
NMB/N7	↓	NC	NC	↓	↓	ND	ND	ND
CHP-126	↓	↑	ND	ND	NC	↑↑	ND	ND

[a] LC, low-molecular-weight complex (68–75 kDa)
[b] HC, high-molecular-weight complex (90 kDa)
[c] *Arrows* indicate changes in levels of activity according to the size of the zone of lysis detected by zymography
ND, none detected; NC, no change

cells under study; thus, these changes cannot be explained by selective outgrowth of subpopulations.

Treatment of tumor cells with differentiating agents has been shown to modulate fibrinolytic enzyme activity in a number of cell types. In mouse NB cells induced to differentiate morphologically with dibutyryl cAMP, a dramatic increase in cell-associated and secreted PA was observed [19, 29]. Human NB cells exposed to a plasminogen-deficient medium exhibited extensive neurite outgrowth and a five-fold increase in PA activity [2]. Furthermore, Gibson et al. [10] have reported that antipain, leupeptin and DFP reversibly inhibited the morphological differentiation induced by cAMP effectors and serum-free medium. Conversely, the protease nexin-type inhibitor secreted by rat glioma cells has been shown to cause neurite outgrowth in mouse NB cells [15]. Several factors may account for these discrepancies, including (a) the use of different NB cell lines for the detection of morphological changes which, as shown, have very different responses to the same stimulus; (b) variations in the specificity of the protease inhibitors and relative levels of PA and PAI; and (c) intracellular vs extracellular activity. In the latter case, a differentiating agent acting intracellularly may induce changes in addition to the up-regulation of PA, such as the production of other proteases and changes in the secretion of extracellular matrix (ECM) proteins. In fact, Tsokos et al. [30] have shown that the pattern of ECM components secreted by NB cells changes dramatically when the cells are treated with RA. In the present study, it is noteworthy that the four cell lines that did not develop extensive neurites showed no detectable changes in tPA expression following RA treatment.

On treatment with RA, several of the NB cell lines exhibited extensive axonal outgrowth corresponding to a dramatic increase in cell-associated and secreted tPA activity. It is interesting to note that UK activity was not similarly enhanced; in fact, in some cell lines a reduction in UK and UK-complex activity was observed. PA release by differentiating NB cells has been demonstrated to occur predominantly at the growth cones of extending neurites [19]. Recently, Verrall and Seeds [32] have reported specific binding sites for tPA on cultured cerebellar neurons, suggesting that proteolytic activity may be localized to the cell surface. These results suggest that the balance between PA and PAI activity in NB cells undergoing differentiation is directly linked to morphology.

The relationship between PAs and the metastatic potential of tumor cells is still controversial (reviewed in [21]). Since RA is currently being used in clinical trials to assess its efficacy in the treatment of NB (Dr. C. P. Reynolds, personal communication), the responsiveness or unresponsiveness of the primary tumor to alterations in PA/PAI expression and/or neurite outgrowth could have predictive value in identifying patients in whom RA would be effective. In acute myeloid leukemia (AML), a response to chemotherapy has been demonstrated in patients whose AML cells secrete UK either alone or with tPA but not in those whose cells produce only tPA [34]. The ability of other differentiating agents to alter fibrinolytic activity and induce morphological changes in various NB cell lines is presently under investigation in our laboratory.

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