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Stimulation of Human Metastatic Melanoma Colony-forming Cells by an Acid-sensitive Factor in Human Platelet Sonicate[1]

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ABSTRACT

A human platelet sonicate was evaluated for its effects on the growth of human metastatic melanoma colony-forming cells in soft agar from cells in culture and from biopsies. The addition of platelet sonicate increased both cloning efficiency and proliferative capacity in that more and larger colonies were formed. In more detailed studies under growth-limiting conditions, melanoma cellular responses to known growth factors were compared to the activity found in the platelet sonicate. None of the growth factors tested either alone or in combination, including platelet-derived growth factor, epidermal growth factor, α-type transforming growth factor, and β-type transforming growth factor, were capable of inducing melanoma colony formation to the 12-fold stimulation observed with the platelet sonicate. Treatment of platelet sonicate with dithiothreitol, trypsin, or acid resulted in loss of activity for human melanoma. Our results suggest that human platelets contain an acid-sensitive protein which can support the expression of the transformed phenotype of human melanoma, and this factor is distinct from acid-stable activities previously characterized from human platelets.

INTRODUCTION

The ability to grow malignant cells in soft agar allows investigation of subpopulations of tumor cells, specifically the clonogenic cells (11). These cells express their proliferative potential in soft agar by forming colonies under conditions which do not favor colony formation of normal cells. The cloning of tumor cells has been used to evaluate responses of tumor cells to chemotherapeutic drugs (1, 13, 16, 17, 24) as well as biological modifiers (5, 12).

Colony formation in soft agar can also be used to investigate factors which regulate expression of the transformed phenotype. The demonstration by Todaro *et al.* (28) that some tumor cells release TGFs[3] which support reversible anchorage-independent growth of nontransformed cells suggests that these proteins may be important for the maintenance of the transformed phenotype. The subsequent identification by Roberts *et al.* (20) of TGF activity in nonneoplastic tissues suggested that these compounds may play a role in normal cell proliferation as well. Serum contains a platelet-derived TGF-like activity (6); thus in responsive cells serum may provide a stimulus for the expression of the transformed phenotype in soft agar. Colony formation in soft agar may represent the ability of the tumor cell to respond to growth-regulatory molecules provided by serum in the plating medium; therefore low cloning efficiency may reflect, in part, a decreased cellular responsiveness or an increased requirement for such factors.

Tumor cells can proliferate in response to factors contained in human platelets. Such growth enhancement has been observed in monolayer culture with cells from various species including murine and human cell lines (8, 10). Cowan and Graham (7) examined human platelet effects on soft agar growth of a variety of human tumor samples, including melanoma. They observed enhanced colony formation in 47% of their melanoma samples.

We examined in detail the effect of sonicated platelets on cell cloning of melanoma in soft agar. The biological activity of our platelet preparation was demonstrated by colony formation of NRK fibroblasts which were used as nontransformed phenotypic indicators of TGF activity (3). Cells from four human metastatic malignant melanoma cell strains, one long-term melanoma cell line, and four of ten melanoma patient samples responded to platelet sonicate by forming more and larger colonies in soft agar. The quantitation of this proliferative response showed substantial increases in total cell numbers within colonies for cells from cell strains and biopsies. The results demonstrate the ability of an acid-labile platelet sonicate activity to support the expression of the transformed phenotype of human metastatic melanoma.

MATERIALS AND METHODS

Maintenance of NRK Fibroblasts. The NRK clone 49F cells were generously provided by Dr. Harold Moses of the Mayo Clinic. These cells were maintained in DMEM (GIBCO, Santa Clara, CA) supplemented with 10% heat-inactivated FBS (GIBCO), glutamine (0.8 μg/ml; GIBCO), and gentamicin 10 μg/ml; Irvine Scientific, Santa Ana, CA).

Establishment and Culture of Melanoma Cell Strains and Line. Cell strains and the one cell line from human metastatic malignant melanoma were established from patient tumor biopsy samples that had been successfully cloned in agar, as described previously (5). The cells were maintained in monolayer in RPMI 1640 (GIBCO) supplemented with 10% FBS, glutamine (0.8 μg/ml), and gentamicin (10 μg/ml). All experiments that assessed colony formation in soft agar from cell strains and the cell line were done on cells from passages 1 to 7.

Soft Agar Assay. Growth of human malignant melanoma in soft agar has been described (14). The procedure used in this study involved these modifications. Cells were plated in RPMI 1640 supplemented with 10% FBS, glutamine (0.8 μg/ml), and gentamicin (10 μg/ml). In experiments utilizing suboptimal growth conditions for M1RW5 cells, the plating medium was either RPMI plus 1% FBS or DMEM (GIBCO) plus 1% BSA (Sigma). Dose-range curves for optimal cell concentration and colony formation were done for all cell cultures and biopsies (15). Cell concentrations chosen for plating were those in the midrange of concentrations showing a linear relationship between number of cells plated and number of colonies formed. Confluent flasks of melanoma cells were harvested

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[3] The abbreviations used are: TGF, transforming growth factor; NRK, normal rat kidney; FBS, fetal bovine serum; BSA, bovine serum albumin; PBS, phosphate-buffered saline; DTT, dithiothreitol; EGF, epidermal growth factor; PDGF, platelet-derived growth factor; FGF, fibroblast growth factor; NGF, nerve growth factor; GIBCO, Grand Island Biological Co.; DMEM, Dulbecco's minimal essential medium.

using 0.25% trypsin (GIBCO). Melanoma cell strains C822, C8161, C832C, and C8157 were plated in the overlayer at cell concentration of 10,000 cells/plate. The cell line M1RW5 was plated at 5,000 cells/plate. Cells from cryopreserved or fresh patient biopsy samples were plated at 50,000 to 300,000 cells/plate in Ham's F-10 medium (GIBCO). No other additives were used in the patient sample plating medium. The NRK 49F cells were plated at 5,000 cells/plate in DMEM with 10% FBS. The plates were incubated in a sealed, humidified modular incubator chamber (Phillips-Rothenberg, Inc., Del Mar, CA) gassed with a mixture of 95% air and 5% CO_2, and placed in a 37°C incubator. The colonies from short-term melanoma cell strains and melanoma biopsy cells were counted on day 14, while the M1RW5 and NRK colonies were counted on days 7 to 9. The colonies were counted and grouped into size classes based on colony diameter, using an optical image analyzer (Omnicon FAS II; Bausch and Lomb, New York, NY) (25). As a control for cellular aggregation, day 0 counts of M1RW5 and biopsy 8442 cells were performed in the presence or absence of platelet sonicate. We routinely measured both the number of colonies (cloning efficiency) and the number of cells in colonies (proliferative capacity).

Quantitation of Cell Numbers within Colonies. Colonies were sized and counted on the Omnicon FAS II, and cell diameters of individual cells were measured by inspection under an inverted microscope. These values were used in the following formula to calculate the number of cells within colonies of specific size classes as described previously (17).

$$\text{No. of cells/colony} = \frac{2.40\ (\text{colony diameter})^{2.378}}{\text{Cell diameter}^{2.804}}$$

See Table 1 for the number of cells contained within colonies of a particular diameter. The number of cells within each colony size class was multiplied by the number of colonies in that size class to generate the total number of cells per size class. Adding all these numbers results in the total number of cells in colonies per plate.

Platelet Sonication. An outdated unit of human platelets was obtained from the University of Arizona Health Sciences Center Hospital Blood Bank. The platelets were diluted 1:2 in double-distilled water and sonicated at low amplitude for 30 s using an Ultratip Microprobe Sonicator (New Brunswick Scientific, New Brunswick, NJ). Lysate was centrifuged at 5000 × *g* for 15 min and the supernatant was aliquoted into 1-ml samples and lyophilized. Samples were rehydrated with 1 ml of PBS and sterilized by passage through a Millipore 0.22-μm filter prior to use in the colony-forming assay. Platelet sonicate was added to the soft agar overlayers over a concentration range of total protein.

Protein Determination. Protein concentrations were determined by using Bio-Rad Protein Assay (Bio-Rad Laboratories, Richmond, CA). This assay is based on the Bradford method utilizing Coomassie Brilliant Blue G-250 to assess protein binding.

Physical Treatment of Platelet Sonicate. A lyophilized sample of platelet sonicate was hydrated with 0.1 M NH_4HCO_3 in PBS containing 0.065 M DTT (18). The sample was lyophilized in 4 ml of PBS after a 1-h incubation at room temperature. Another sample of sonicate was also subjected to 5% trypsin digestion for 24 h at 37°C. After each treatment, the sonicate was evaluated for the ability to stimulate soft agar growth of M1RW5 cells in 1% FBS-RPMI or 1% BSA-DMEM. Acid stability of the activity was assessed by treatment of the platelet sonicate with 4 mM HCl at 4°C for 24 h. Acid-treated and nontreated samples were assayed in 1% BSA-DMEM with M1RW5 cells. NRK cells were assayed in 10% BSA-DMEM, and they served as a positive control for acid-stable TGF activity.

Table 1

Number of melanoma cells contained within specific sized colonies

Cell strain or line	No. of cells in colony with following diameter				
	42–50 μm	51–60 μm	61–72 μm	73–86 μm	86–104 μm
M1RW5	17	26	39	60	94
C8161	12	19	29	45	70
C8157	43	65	101	155	240
C832C	24	36	56	85	132
C822	13	20	31	47	73

Effects of Other Growth Factors. In addition to platelet sonicate, the M1RW5 cells were assayed for growth factor requirements in soft agar. The following growth factors were added individually to 1% FBS-RPMI: EGF; FGF; NGF; PDGF (Collaborative Research, Inc., Lexington, MA); transferrin (Sigma Chemical Co., St. Louis, MO); or insulin (Eli Lilly and Co., Indianapolis, IN). For growth factor evaluations in 1% BSA-DMEM, the following growth factors were added alone or in combination: TGF-α (R. Derynck, M. Winkler, Genentech, Inc., San Francisco, CA); TGF-β (Dr. Harold Moses, Mayo Clinic, Rochester, MN); PDGF; and EGF.

RESULTS

Cloning Efficiencies. The growth properties of the cell strains (C8157, C8161, and C822) and the cell lines (NRK 49F and M1RW5) were evaluated prior to assessment of responses to platelet sonicate. Two of these, the NRK fibroblasts and the C822 melanoma cells, had poor growth capabilities in soft agar with cloning efficiencies for colonies >60 μm in diameter of 0 and 0.6%, respectively. In contrast, under identical growth conditions (10% FBS), the other melanoma cells displayed high cloning efficiencies in soft agar for colonies >60 μm in diameter. The short-term cell strains had an average cloning efficiency of 8%, and the M1RW5 long-term cell line cloned with an average efficiency of 18%. The responses of cells from melanoma biopsies, the NRK 49F fibroblasts, and the C822 cells to platelet sonicate were therefore evaluated in 10% FBS-containing medium. The other melanoma cells were assayed in 1% FBS-containing medium which resulted in the decreased cloning efficiency of these cells and allowed for observation of growth potentiation after platelet sonicate addition.

Effect of Platelet Sonicate on Growth of NRK 49F Cells in Soft Agar in 10% FBS DMEM. The NRK cells responded to sonicated platelets by forming colonies in soft agar (Chart 1). Maximal stimulation of all colony sizes examined occurred at the

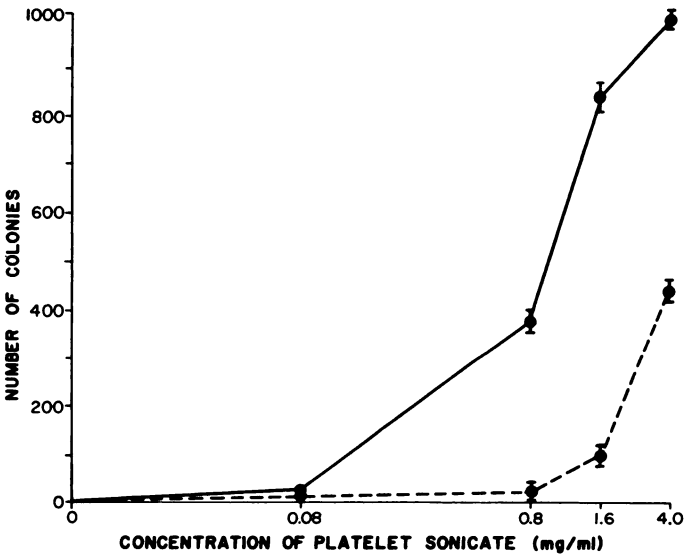

Chart 1. Effects of platelet sonicate on NRK fibroblast colony formation in soft agar. Cells were plated in 10% FBS-DMEM medium over a platelet concentration range. ——, colonies >60 μm in diameter; - - - -, colonies >124 μm in diameter; *bars*, SD.

4-mg/ml level of platelet sonicate protein, but activity was apparent at 800 μg/ml for colonies greater than 60 μm in diameter. Control plates on day 0 had 20 ± 6 counts and platelet sonicate-treated plates had 32 ± 4 counts. These counts represented 0.6% of the total cells plated and 1% of the total colonies formed. These counts confirmed that the platelet sonicate was not causing a large number of aggregates to form which could subsequently be erroneously counted as colonies.

Effect of Platelet Sonicate on Melanoma Colony-forming Cell Growth in Soft Agar. The responses of the four melanoma cell strains and one melanoma cell line are shown in Chart 2. The culture conditions were identical for all cells except that the C822 cells were assayed in medium supplemented with 10% FBS instead of 1% as for the others. All the melanoma cells responded to the platelet sonicate in a dose-dependent manner with half-maximal stimulation seen protein levels between 160 and 800 μg/ml. The cells assayed in 1% FBS were unable to form colonies greater than 60 μm in diameter, yet the addition of platelet sonicate resulted in formation of at least 600 colonies greater than 60 μm for all four cell lines. For the M1RW5 cells, the counts on day 0 were 24 ± 6 for nontreated plates and 14 ± 2 for sonicate-treated plates which represented 0.4% of total cells plated and 0.8% of total colonies formed.

Cells from ten melanoma biopsies were assayed for responses to platelets in 10% FBS-containing medium. Six of the ten patient samples did not grow, with or without platelets. Table 2 shows the data for cells from those four melanoma biopsies which grew colonies over 60 μm in diameter without platelet addition. The presence of 400 μg of platelet protein/ml increased the number of colonies by 100 to 900%. On day 0, the control and sonicate-treated plates for biopsy 8442 had the same number of counts (24 ± 6). These counts represented 0.05% of the total cells plated and 1.4% of the total colonies formed and therefore demonstrate that the sonicate did not induce aggregate formation prior to plating in soft agar where migration and aggregation are inhibited (27).

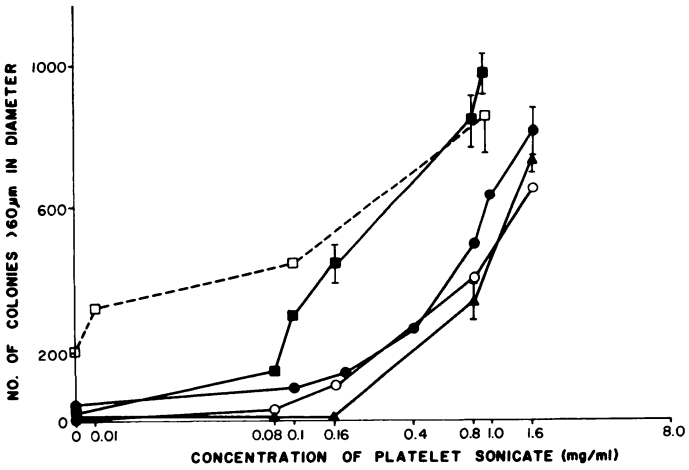

Chart 2. Effects of platelet sonicate on human metastatic melanoma colony-forming cells in soft agar. Platelet protein was added to overlayer with cells in 1% FBS-RPMI medium (——) or 10% FBS-RPMI medium (– – – –). ■, M1RW5; ●, C8161; ▲, C832C; ○, C8157; □, C822; *bars*, SE.

Table 2

Responses of cells from melanoma biopsies to platelet sonicate (400 μg/ml) for colonies >60 μm in diameter

Patient	No. of colonies: Control	No. of colonies: Plus platelets	% of increase over control
8240[a]	166 ± 60[b]	1160 ± 98	699
8414[c]	50 ± 2	452 ± 18	904
8423[c]	358 ± 28	674 ± 54	188
8442[a]	988 ± 146	1666 ± 240	169

[a] Cryopreserved sample.
[b] Mean ± SE.
[c] Fresh tumor sample.

Effect of Platelet Sonicate on Cellular Proliferation of Melanoma Colony-forming Cells. In order to examine the platelet effects on proliferation, we measured different size classes of colonies and calculated the number of cells within colonies of different sizes to enumerate the total number of cells contained in all colonies formed and thus total cellular proliferation. Table 3 summarizes the data for the melanoma cell cultures with regard to number of colonies formed and number of cells within colonies >42 μm in diameter in the control and treated plates. The three cell strains (C8157, C8161, and C832C) and one cell line (M1RW5) assayed in 1% FBS had approximately 1000 total cells within all the colonies. The addition of platelet sonicate increased the total number of cells within all colonies to over 26,000 for M1RW5, 22,900 for C8161, 39,600 for C832C, and 56,500 for C8157. The other cell strain that was evaluated in 10% FBS, C822, increased total cell numbers within colonies from 4,300 to 17,500 after platelet treatment.

Characteristics of Platelet Sonicate by Physical Treatment. Table 4 shows the sensitivity of the platelet sonicate to treatment with DTT, trypsin, and acid. DTT treatment reduced the stimulation to less than 3% of growth achieved with platelet sonicate treatment, and trypsin treatment resulted in reduction of stimulation to 4% of platelet sonicate-treated plates. This sensitivity to DTT and trypsin suggests that the sonicate activity is due to a protein. The acid treatment of the platelet sonicate resulted in the loss of stimulatory activity for human melanoma, while the activity for NRK fibroblasts was maintained and even slightly enhanced (Table 4). Acid treatment destroyed 95% of melanoma-

Table 3

Total cellular proliferation calculated for human melanoma cells in the presence and absence of platelet sonicate

Cell line or strain	Cells plated/dish	Cell diameter (μm)	Growth condition	Total colonies >42 μm diameter	Total cells[a] in colonies
M1RW5	5,000	19.8	1% FBS	122	1,028
			1% FBS + platelets (1 mg/ml)	1,556	26,331
			10% FBS	1,948	45,932
C8161	10,000	22	1% FBS	168	1,164
			1% FBS + platelets (1.6 mg/ml)	1,268	22,901
			10% FBS	1,448	24,563
C832C	10,000	17.8	1% FBS	50	645
			1% FBS + platelets (1.6 mg/ml)	1,032	39,625
			10% FBS	1,942	87,503
C8157	10,000	14.2	1% FBS	14	513
			1% FBS + platelets (1.6 mg/ml)	752	56,528
			10% FBS	1,082	99,018
C822	10,000	21.6	10% FBS	488	4,356
			10% FBS + platelets (1 mg/ml)	1,242	17,540

[a] The number of cells within specific colony size classes was calculated as described previously (15). Addition of the number of cells from each size class results in total number of cells per plate.

Table 4

Characterization of platelet sonicate by physical treatments

Cell line	Treatment	Growth condition	Colonies >60 μm in diameter
M1RW5	DTT	10% FBS-RPMI control	2840 ± 114[a]
		1% FBS-RPMI control	210 ± 12
		1% FBS-RPMI + sonicate	2076 ± 42
		1% FBS-RPMI + DTT-treated sonicate	56 ± 10
	Trypsin	10% FBS-DMEM control	1132 ± 44
		1% BSA-DMEM control	4 ± 1
		1% BSA-DMEM + sonicate	330 ± 10
		1% BSA-DMEM + trypsin-treated sonicate	14 ± 2
	Acid	10% FBS-DMEM control	1594 ± 77
		1% BSA-DMEM control	24 ± 10
		1% BSA-DMEM + sonicate	414 ± 24
		1% BSA-DMEM + acid-treated sonicate	22 ± 10
NRK	Acid	10% FBS-DMEM control	704 ± 38
		10% FBS-DMEM + sonicate	1566 ± 118
		10% FBS-DMEM + acid-treated sonicate	2604 ± 38

[a] Mean ± SE.

Table 5

M1RW5 cellular responses to growth factors in 1% FBS-containing RPMI

Factor[a]	% of Control growth (colonies > 60 μm in diameter)
EGF (100 ng/ml)	107
PDGF (1 unit/ml)	150
FGF (100 ng/ml)	140
NGF (100 ng/ml)	91
Insulin (100 ng/ml)	142
Transferrin (500 μg/ml)	200

[a] Each growth factor was plated in the upper layer of soft agar with the M1RW5 cells.

stimulatory activity but resulted in a 370% increase in colony formation by NRK fibroblasts. This indicates that the stimulation of melanoma colony-forming cells is not an effect of acid-stable activities from human platelets such as PDGF, EGF, and TGF-β.

Growth Factor Responses. In order to evaluate growth factor requirements for the expression of the anchorage-independent phenotype in human melanoma, the M1RW5 cells were plated in 1% FBS-RPMI in the presence of known growth factors. The concentration of each growth factor that gave the greatest stimulation of M1RW5 cellular growth in soft agar was derived from dose-response curves with each growth factor. Table 5 illustrates the limited responses of human melanoma to the purified growth factors tested.

The results of the more detailed studies of melanoma cellular responses to specific growth factors present in human platelets are shown in Table 6. These results were obtained from the concentration of each growth factor that gave the greatest stimulation of M1RW5 growth. For M1RW5 cells, the combination of PDGF, TGF-α, and TGF-β stimulated only 27% of the growth promotion obtained with the platelet sonicate.

In contrast, the NRK cells responded to these growth factors as described previously (3), in that maximal colony formation was observed with the combination of the three proteins PDGF, TGF-α, and TGF-β. These results suggest that the melanoma-

Table 6

Effects of known growth factors from human platelets

Factor	% of control growth[a] M1RW5	NRK
Platelet sonicate (18 μg/ml)	100	100
PDGF (1 unit/ml)	20	56
EGF (1 ng/ml)	2	45
TGF-α (5 ng/ml)	2	42
TGF-β (5 ng/ml)	2	0.8
TGF-α + TGF-β	11	115
TGF-α + TGF-β + PDGF	27	179
EGF + TGF-β + PDGF	7	138

[a] For each cell line, the control represents optimal growth in the presence of the platelet sonicate. The M1RW5 cells had 602 colonies in 1% BSA-DMEM plus sonicate, and the NRK cells had 1566 colonies in 10% FBS-DMEM plus sonicate.

stimulatory activity present in human platelets is not due to acid-stable growth factors such as PDGF, TGF-β, TGF-α, or EGF.

DISCUSSION

Human metastatic malignant melanoma cells from patient biopsies, cell strains, and a cell line responded to a sonicated human platelet preparation by forming more and larger colonies than did nontreated controls. Cells from biopsies which did not respond to platelets had no growth on the control plates, which suggests that cells must be capable of at least some colony formation in order to respond to platelet factors. The clonogenic cells that grew were able to go through extensive proliferation upon the addition of platelet sonicate to the system. A comparison of the total number of cells in colonies in control and platelet-treated cultures reflects this tremendous growth increase (Table 3). This stimulation represents a proliferative response to the platelet sonicate, because day 0 counts revealed no aggregate induction by the sonicate. The results shown here indicate that factors present in platelet sonicate stimulated the clonogenic cells to express the transformed phenotype, even in the presence of reduced FBS. The previous work of Cowan and Graham (7) suggested that a variety of human tumors were capable of responding to platelet lysate, but their method of manually counting formaldehyde-stained colonies did not allow for a precise measure of proliferative events stimulated by platelet treatment. Our work confirms the responsiveness of melanoma tumor cells to human platelets and also quantitates this proliferative response for human metastatic malignant melanoma colony-forming cells. The results demonstrate that factors within platelets stimulated increases in the cloning efficiency and proliferative capacity of human melanoma colony-forming cells.

Serum contains a complex mixture of hormones and growth factors which stimulate growth (4, 19). Growth factors are nonnutritive substances that control proliferation in a regulative manner without participating directly in biosynthesis, metabolism, or catalysis (9). Because C822 cells exhibited low cloning efficiency in soft agar in 10% FBS-supplemented medium, they must have a greater requirement for growth-regulatory molecules than those which can be supplied by 10% FBS. The growth factors contained in platelets may provide a proliferation signal for C822 cells beyond those molecules supplied by 10% FBS. This point is well illustrated by increased proliferation of these cells with the addition of platelet sonicate to 10% FBS-containing medium (Chart 2).

In contrast to C822 cells, the cells from other melanoma strains were capable of responding to the growth stimuli supplied by

10% FBS, as shown by their relatively high cloning efficiencies in soft agar. For responsive cells, the growth factors present in 10% FBS could interfere in assays for growth-regulatory molecules; therefore the growth-promoting capacity of serum was largely obviated by reducing the FBS concentration to 1% for studies using cells from C8157, C8161, C832C, and M1RW5. This approach allowed for evaluation of substances that regulated colony-forming growth in soft agar that would otherwise be masked by the growth-promoting substances present in serum (4, 6, 23). The reduction to 1% FBS does not represent nutritional deprivation, because the addition of the platelet sonicate resulted in an average of 73% of the growth observed with 10% FBS (Table 4).

The response of melanoma cells to platelet sonicate was observed over reasonably small additions of protein to the system. The FBS lot used in these studies contained approximately 4.2 mg of total protein/ml at the 10% concentration. In assays utilizing 1% FBS, the addition of platelet sonicate at the highest concentration examined resulted in a total protein concentration of 2 mg/ml, and clear responses were observed at a total protein concentration of 520 μg/ml for the M1RW5 cell line. In addition, a dose-response curve for M1RW5 using bovine serum albumin up to a concentration of 2.5% gave no stimulation of colony formation (data not shown). These results suggest that the growth stimulation observed with this platelet sonicate was not merely a nonspecific protein effect, but rather specific activity of protein(s) which play a role in expression of the transformed phenotype in soft agar.

None of the growth factors assayed individually (EGF, PDGF, NGF, FGF, insulin, or transferrin) increased the soft agar proliferation of melanoma cells to the same extent as did the platelet sonicate factor(s). The greatest stimulation on proliferation of M1RW5 cells in 1% FBS plating medium was 200% over control after plating with a high concentration of transferrin, while the platelet sonicate stimulated colony formation to 1200% over 1% control growth. This implies that the platelet sonicate contained growth factor activity which was not substituted by the purified growth factors tested. Studies of melanoma cellular responses to characterized growth factors from human platelets also revealed that these proteins (PDGF, EGF, or TGF-α-like and TGF-β) do not play a significant role in the expression of the transformed phenotype for human melanoma.

In addition, physical treatment of the platelet sonicate with DTT, trypsin, or acid resulted in decreased activity. This loss of activity with acid treatment is different from other characterized growth factor activities from human platelets. EGF, PDGF, and TGFs have all been shown to be stable under acidic conditions (2, 21, 22, 26). The acid sensitivity of the melanoma-stimulatory activity coupled with the inability of acid-stable activities such as PDGF, EGF, TGF-α, and TGF-β (alone or in combination) to stimulate melanoma colony formation in soft agar indicate that human platelets contain a neutral protein which can drive melanoma cells to express their transformed phenotype, and this factor is different from activities previously characterized from human platelets. Studies are in progress to isolate and characterize this growth factor activity for human melanoma.

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