LACK OF CORRELATION BETWEEN FIBRINOLYSIS AND THE TRANSFORMED STATE OF CULTURED MAMMALIAN CELLS[1]

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SUMMARY

Transformed cells in culture have been reported by others to exhibit high levels of extracellular proteolytic (fibrinolytic) activity due to plasminogen activation, compared to low levels from nontransformed cells. Enhanced fibrinolysis was accordingly proposed to be a reliable and general enzymatic change associated with cell transformation.

In the present study, ten different types of serially cultured, growing cells were examined for their extracellular fibrinolytic activity. The level of the fibrinolytic activity was found not to correlate with the transformed or nontransformed state of these cells.

Transformed cells in culture have been reported to exhibit high levels of extracellular protease activity, compared to low levels from nontransformed cells (1,2). This extracellular protease is capable of converting the plasma proenzyme, plasminogen, to the active protease, plasmin, which has been measured by its ability to lyse labeled fibrin (3,4). An elevated level of plasmin activity has been indicated to confer on particular cells certain characteristics associated with transformation, namely, morphological conversion, the ability to grow in soft agar, and elevated rate of cell migration into a culture wound lacking cells (5,6). Furthermore, it has been suggested that enhanced fibrinolysis is apparently the first reliable and general enzymatic change associated with cell transformation (7,8).

In the present study, we undertook to examine whether or not the proposed

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generalization applies to various nontransformed and transformed cells that had been extensively serially passaged in culture.

MATERIALS AND METHODS

Cells and Media. Swiss mouse fibroblasts 3T3 (42L), their SV-40 virus transformed 3T3 derivatives (SV74C), and the spontaneously transformed Chinese hamster ovary cells (CHO-K1) were kindly donated by Dr. LaRoy Castor of this Institute. The mouse cells had been obtained from Dr. George Todaro of the National Cancer Institute; the hamster cells came from Dr. Abraham W. Hsie of The Oak Ridge National Laboratory. Chinese hamster kidney fibroblasts (CHK-C16a), studied at the 45th to 75th transfers, an SV-40 virus transformed subline (14SVM1), and a polyoma virus transformed subline (13PYM1) were kindly provided by Dr. Vittorio Defendi of New York University. The human diploid nontransformed cells, WI-38 (studied at the 17th to 26th cell generations) and WI-26 (examined at the 27th to 30th generations), were obtained from Dr. Leonard Hayflick of Stanford University. The SV-40 virus transformed WI-38 cells (WI-38-VA13-2RA) originated at the Wistar Institute (9), and came from the American Type Culture Collection. The SV-40 virus transformed WI-26 cells, i.e., the WI-26-VA4, were kindly provided by one of their originators, Dr. Anthony Girardi of the Wistar Institute (10).

The experiments with the 3T3, SV-3T3, CHK, SV-CHK and PY-CHK fibroblasts were carried out in Dulbecco-modified Eagle medium, experiments with CHO cells were performed in Ham's F-12, and experiments with the human cells were done in BME basal diploid medium (Eagle) with twice the amount of prescribed vitamins and amino acids. All cells were propagated in media containing 10% fetal bovine serum (previously heated 30 min at 56°C), penicillin, and streptomycin in tissue culture flasks (Falcon).

Nontransformed and Transformed States of Cells. The following conventional in vitro criteria of cell nontransformation and transformation were verified and relied on in this study: (a) low vs. high saturation cell density in medium

containing 10% fetal bovine serum; (b) the dependence of growth on the concentration of fetal bovine serum (1% vs. 10%) in the case of 3T3 cells, in contrast to lack of dependence in the case of SV-3T3 cells; (c) morphological characteristics. In addition, the SV-CHK[2], PY-CHK[2], WI-38-VA13-2RA[3] and WI-26-VA4[3] cells have the ability to grow in soft agar, and their nontransformed parent cells can not do so. Also, the human diploid fibroblasts WI-26 and WI-38 undergo senescence (11), in contrast to the unlimited passageability of their SV-40 virus transformed cells (9,10).

Assay of Fibrinolysis. One or 2 days after replacement of complete medium, the cells were plated in 25 or 75 cm^2 flasks (Falcon) in their respective media containing 10% fetal bovine serum. One day later, the cell densities were 1 to 5 x 10^4 cells/cm^2. For the assays of fibrinolysis according to the method of Unkeless et al. (1), these growing subconfluent cells were washed 3 times with their respective serum-free media, and each 5 cm^2 of growth surface was overlayed with 1 ml of serum-free medium. After incubation for 18 hr in 5% CO_2 at 37°, the cell-free supernatants ("harvest media") were isolated.

In the subsequent sterile enzymatic digestions of films of bovine ^{125}I-fibrin, 2 ml of harvest media, diluted with serum-free media as indicated below, was incubated with 61 µg of purified human plasminogen (12) in ^{125}I-fibrin-coated 35 mm plastic dishes for 18 hr at 37° in 5% CO_2 atmosphere. The ^{125}I in 1 ml of the clear digest fluids was then counted. The concentration of plasminogen activator in harvest media was compared on the basis of the concentration of plasminogen activator liberated by 10^5 cells per 5 cm^2 surface per ml of medium (100% harvest medium). The relative concentration of activator was measured by the maximum dilution of the harvest medium that brought about a sizeable solubilization of the ^{125}I-fibrin, if any. Corrections (less than 2%) were made for the radioactivity released by plasminogen in fresh serum-free

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[2] Personal communication from Dr. Vittorio Defendi, New York University.

[3] Personal communication from Dr. Leonard Hayflick, Stanford University.

Table 1. Levels of extracellular fibrinolytic activity of transformed and nontransformed cells

Type	Cells	No. of Experiments	Lowest Conc. of Harvest Medium % (v/v)	Fibrin Solubilized Range, %	Fibrinolysis Rating
Transformed	PY-CHK	4	100	50-81	high
	SV-3T3	4	50-100	36-74	high
	CHO	3	40	46-73	high
	SV-CHK	3	100	< 1-2	undetected
	WI-38-VA13-2RA	4	100	< 1	undetected
	WI-26-VA4	9	100	0-3	undetected
Nontransformed	CHK	4	100	2-8	low
	3T3	4	5	52-68	high
	WI-38	4	5	78-82	high
	WI-26	5	50	80-99	high

Subconfluent monolayer cultures of cells were maintained in serum-free medium for 18 hr, after which extracellular medium was removed and centrifuged to yield cell-free "harvest medium." Densities were then 1.0 to 4.8 x 10^4 cells/cm^2. Two ml of harvest medium, diluted (v/v) as indicated, and 61 µg purified human plasminogen were added to ^{125}I-fibrin-coated 35 mm dishes, incubated for 18 hr at 37° in 5% CO_2 atmosphere, and the ^{125}I in the supernatant digest fluids was counted. The concentration of plasminogen activator in harvest medium was compared on the basis of activator liberated by 10^5 cells per 5 cm^2 per ml of medium (100% harvest medium). The table indicates the lowest % (v/v) of harvest medium that yielded the indicated ranges of significant % solubilization of ^{125}I-fibrin, if any. Details are provided in the text.

medium, and by harvest medium without plasminogen.

RESULTS

The ability of the 10 types of growing subconfluent cells to lyse fibrin, due to liberation of extracellular activator of plasminogen, is summarized in Table 1. The levels of activator concentration elaborated by the cells are indicated by the lowest % (v/v) concentrations of harvest medium that yielded any detectable significant percentage of solubilization of the ^{125}I-fibrin. The ranges of the percent of ^{125}I-fibrin solubilized by these minimal concen-

trations of harvest medium are indicated for all experiments.

The nontransformed CHK cells and their SV-40 virus transformed subline, SV-CHK, liberated little and no detected activator, respectively. These results are in contrast to the high activity of the related PY-CHK.

On the other hand, both the control 3T3 cells and the transformed SV-3T3 cells produced high levels of extracellular activator activity. Normal 3T3 cells have previously been described to have low fibrinolytic activity, and SV-3T3 to have high activity (2). However, Chou et al. (13) have reported recently that in growing cultures both have moderate activity, but that unlike confluent cultures of SV-3T3, confluent cultures of 3T3 cells have low fibrinolytic activity.

In further contrast, both kinds of human diploid nontransformed fibroblasts, WI-38 and WI-26, exhibited rather high activator activities. Furthermore, their SV-40 virus transformed derivative cells, WI-38-VA13-2RA and WI-26-VA4, produced virtually no detected activator. Mixing experiments with the harvest media of these transformed and nontransformed cells, in ratios up to 19:1 (WI-38) and 8:1 (WI-26), gave no evidence of the presence of inhibitors of fibrinolysis. In 1969 Bernik and Kwaan reported that WI-38 cells produce a significant level of plasminogen activator (14).

The spontaneously transformed CHO fibroblasts liberated a high level of plasminogen activator.

The levels of plasminogen activator activity of the various cells in serum free media, as here presented, have also been determined by the less sensitive direct assay of fibrinolysis by the same cells in 10% human serum. These and other data will be reported elsewhere.

DISCUSSION

The levels of extracellular fibrinolytic activity exhibited in vitro by extensively cultured growing cells do not correlate with their state of transformation or nontransformation. There are nontransformed cells that have low fibrinolytic activity. There are also nontransformed cells that have high

activity, a finding consistent with a recent statement (4) that enhanced fibrinolysis is not necessarily diagnostic of cell transformation. Further, there are transformed cells that exhibit high levels of fibrinolytic activity. However, there are also transformed cells (3 of 6 types examined) that have no detectable fibrinolytic activity according to the sensitive serum-free assay. Enhanced fibrinolytic activity appears therefore not to be a sine qua non of cell transformation.

If the levels of the fibrinolytic activities of the transformed cells are compared with those of their nontransformed parent cells, then every possible relative order is evident. There are transformed cells which display more activity than do their related nontransformed cells. Conversely, there are transformed cells that have less activity than have their nontransformed parent cells. Further, there are transformed and related nontransformed cells that have approximately equal and high activities. Finally, there are also transformed cells and parent nontransformed cells that exhibit approximately equal and low activity, and no detected activity. Collectively, the present findings indicate that a low level of fibrinolytic activity is not a necessary or sufficient phenotype of the apparently nontransformed state of extensively cultured cells. Likewise, neither is a high level of fibrinolytic activity a necessary or sufficient phenotype of the transformed state of extensively cultured cells. The absence of detectable activity of the transformed SV-CHK, WI-26-VA4, and WI-38-VA13-2RA cells appears to be particularly relevant in this regard.

In cells that have undergone considerable numbers of passages in vitro, enhanced fibrinolytic activity does not generally correlate with the transformed state of cells. It remains yet to be determined whether enhanced fibrinolytic activity may or may not generally apply to primary cultures of transformed cells and malignant tissues, as compared to primary cultures of normal diploid cells (4).

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