CAPACITY FOR TUMOR CELL IMPLANTATION AS A FUNCTION OF IN VITRO CELL DENSITY

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SUMMARY: Implantation properties of two melanoma cell lines, line 26 (low rate of implantation) and line 37 (high rate of implantation) were studied as a function of the cell density of the cells grown in monolayer in vitro. Sparse cultures (collected at a density of 0.8×10^3 cells cm^{-2}) of line 37 produced 7.7 times as many lung tumor foci as those of line 26. Confluent cultures (collected at a density of 40×10^3 cells cm^{-2}) resulted in greater numbers of tumor foci for both cell lines, but line 37 produced only 3.1 times as many tumor foci as did line 26 cells. Thus the high implantation line (37) has a much greater ability to implant when grown in the sparse state and injected than the low implantation line (26), but both lines have high implantation rates when injected as confluent cells.

Recently we have reported (1) on differences in cell surface properties between sparse and confluent cultures of cells which had a high potential for metastasis and implantation and comparable cell lines which showed low potential for implantation and metastasis. The cell lines used were lines 26 (low implantation) and 37 (high implantation), initially described by Fidler (2,3). When injected into C57 mice, cells from these lines produce pulmonary metastases. The cells also grow in cell culture in monolayer and retain their respective potential for high or low pulmonary metastasis. We found (1) that in all the parameters except one studied concerning the plasma membranes of cell lines 26 and 37, similarities existed when the cells were harvested at confluency (defined here at as cell density of 40×10^3 cells cm^{-2}), but striking membrane differences were present when the cells were harvested in a sparse density (0.8×10^3 cells cm^{-2}). The exception to this generalization was in the test of two phase aqueous polymer partitioning (4), in which much greater differences were found between the sparse and confluent cultures of either cell line than between the sparse culture of line 26 and that of line 37. (The partition ratios were: sparse 26, 0.361; sparse 37, 0.432; confluent 26, 0.174;

confluent 37, 0.180. For details of partitioning system, refer to reference 1.) These observations led to the present experiments, in which sparse and confluent cells were injected into C57 mice and pulmonary tumor foci were analyzed to determine whether the membrane changes found predominantly in the sparse cultures contributed to implantation rate or whether the two phase aqueous polymer partitioning data differences reflected the state of the cell surface and its ability to implant and metastasize.

MATERIALS AND METHODS

Cells and culture. The initial cell cultures were the kind gift of Dr. I. Fidler. Cell conditions and maintenance were as previously described (1-3).

Injection of cells and identification of tumor foci. Each C57 mouse was injected with 50,000 viable cells from lines 26 or 37 in 0.2 ml of saline. The cells were harvested from the monolayer by scraping and were incubated in Hank's solution for one hour at 37° before injection (Fidler, personal communication). The cells were over 95% viable by the Trypan blue exclusion test and were

Table 1. Number of tumor foci from lines 26 or 37 when injected into either sparse or confluent cell cultures

Cells were injected and tumor foci in the lung recorded three weeks later, as given in the text. N is the number of individual animals in each group from which the mean ± 1 S.D. were calculated. × represents "times greater than" for line 37 compared to line 26.

Number of lung tumor foci			
Sparse*		Confluent	
Line 26	Line 37	Line 26	Line 37
1.5 ± 0.1	11.6 ± 0.7	6.5 ± 0.2	20.1 ± 1.1
(N = 34)	(N = 32)	(N = 32)	(N = 35)
	7.7 ×		3.1 ×

*"Sparse" and "confluent" refer to cell density in vitro immediately before injection, as defined in the text.

Table 2. Percentage of tumor foci of various sizes in the lung after injection of either confluent or sparse cell cultures of cell line 26 or cell line 37

The number of animals for each experiment was 9, and the values are the means for the 9 determinations. Injection of cells, length of time in the animal, and harvesting procedure are given in the text.

Cell line		Percent of tumors with diameter of:		
		$<2 \times 10^{-4}$ m	2×10^{-4} to 6×10^{-4} m	$>6 \times 10^{-4}$ m
26	Sparse*	37	41	22
26	Confluent	27	41	32
37	Sparse	33	53	11
37	Confluent	71	12	17

* "Sparse" and "confluent" refer to cell density in vitro immediately before injection, as defined in the text.

counted on a Coulter Counter Research Model B. After three weeks the mice were killed, the lungs were removed and coded, and the number of tumor foci were determined blind.

Sizing of tumor foci. In some experiments tumor foci were sized, by diameter, using a calibrated dissecting scope.

RESULTS AND DISCUSSION

The results were unexpected and interesting (Table 1). They show: [1] that line 37 sparse gave 7.7 times as many tumor foci as line 26 sparse; [2] that line 37 confluent gave 3.1 times as many tumor foci as line 26 confluent; and [3] that *both* cell lines gave more pulmonary tumor foci in the "confluent" state than in the "sparse" state. Thus the highest number of tumor foci was found in the confluent 37 cells. Interestingly the cell line (26) with the low implantation potential produced more than 4 times as many tumor loci when confluent than when sparse. These data indicate that the membrane differences found between the sparse 26 and 37 cell lines (1) certainly contribute to the 7.7 times higher number of pulmonary tumor foci, and that the two phase aqueous polymer

partition coefficient data are probably indicative of the higher number of tumor foci in the confluent than in the sparse cultures of both cell lines.

Table 2 shows that the largest differences in size of tumor foci were between the line 37 sparse and confluent cells. Furthermore, even though the line 26 cells formed fewer tumor foci, they were in general larger than the cells of line 37 (the cell line with highest implantation potential).

The data of this communication reinforce the notion that the state of the cell in culture (i.e., cell density and cell contact) is extremely important to its ability to implant or form metastases. Furthermore, the data implicate the cell surface and particularly indicate the utility of the parameter of two phase aqueous polymer partitioning in defining the phenomena of implantation and metastasis.

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